Assessment of dose optimisation requirements in special patient populations in the field of endocrinology and infectiology

Inaugural-Dissertation

to obtain the academic degree

Doctor rerum naturalium (Dr. rer. nat.)

submitted to the Department of Biology, Chemistry, Pharmacy

of Freie Universitaet Berlin

by

Daniela Burau

2019

Die folgende Arbeit wurde von 2012 bis 2019 unter der Leitung von Frau Prof. Dr. Charlotte Kloft am Institut für Pharmazie der Freien Universität Berlin angefertigt.

1. Gutachter: Frau Prof. Dr. Charlotte Kloft

2. Gutachter: Herr Prof. Dr. Frieder Kees

Disputation am: 19.02.2021

Hiermit erkläre ich, dass ich die vorliegende Arbeit mit dem Titel: Assessment of dose optimisation requirements in special patient populations in the field of endocrinology and infectiology selbstständig und ohne unerlaubte fremde Hilfe angefertigt, keine anderenals die angegebenen Quellen und Hilfsmittel verwendet und die den verwendeten Quellen und Hilfsmitteln wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Für meine Familie

Table of contents

List of abbreviations

AAP	Acetaminophen
ACN	Acetonitrile
ADR	Adverse drug reaction
AUC	Area under the curve
$AUC_{0\text{-}24}$/MIC	Area under the curve within 24 hours divided by the mimimum inhibitory concentration
bid	Twice daily (latin: *bis in die*)
C	Concentration
CAH	Congenital adrenal hyperplasia
Cal	Calibration
CFX	Cefuroxime
C_{ISF}	Concentration in interstitial space fluid
$C_{ISF,last}$	Concentration in the last observed sampling interval in interstitial space fluid
$C_{ISF,max}$	Maximum concentration in interstitial space fluid
$C_{ISF,min}$	Minimum concentration in interstitial space fluid
C_{last}	Concentration in the last observed sampling interval
C_{max}	Maximum concentration
$C_{\mu Dialysate}$	Concentration in microdialysate
C_{nom}	Nominal concentration
C_{min}	Minimum concentration
$C_{plasma,min}$	Minimum concentration in plasma
C_{RD}	Concentration in retrodialysate
C_{RP}	Concentration in retroperfusate
C_{SM}	Concentration in surrounding medium
CV	Coefficient of variation
CZL	Cefazolin
DAC	Deutscher Arzneimittel Codex
EMA	European Medicines Agency
EP	European Pharmacopoeia
FC	Fludrocortisone
HC	Hydrocortisone
HPLC	High performance liquid chromatography

List of abbreviations

ID	Individual identifier
ISF	Interstitial space fluid
i.v..	Intravenous
IVMS	*In vitro* microdialysis system
LC-MS/MS	Liquid chromatography coupled to a mass spectrometry detector
LIN	Linezolid
LLOQ	Lower limit of quantification
µDialysate	Microdialysate
MER	Meropenem
MET	Metronidazole
MIC	Minimum inhibitory concentration
MM	Matrix mix
MQ	Milli-Q water (ultrapure water type 1)
MRSA	Methicillin-resistant Staphylococcus aureus
MWCO	Molecular weight cut-off
NOV	Novamine sulfone sodium salt
NRF	Neues Rezeptur Formularium
PAP	Perioperative antibiotic prophylaxis
PBS	Phosphate buffered saline
PD	Pharmacodynamic
PK	Pharmacokinetic
QC	Quality control
qd	Once daily (latin: *quaque die*)
R	Correlation coefficient
R^2	Coefficient of determination
RD	Retrodialysate
RE	Relative error
RF	Refinement
RR	Relative recovery
$RR_{microdialysis}$	Relative recovery obtained in microdialysis setting
$RR_{retrodialysis}$	Relative recovery obtained in retrodialysis setting
RS	Ringer's solution
RSD	Residual standard deviation

RT	Room temperature
SD	Standard deviation
SL	Stock solution
SM	Surrounding medium
SSI	Surgical site infection
TFA	Trifluoric acid
TIG	Tigecycline
tid	thrice daily (latin: *ter in die*)
ULOQ	Upper limit of quantification
VAN	Vancomycin
V	Volume
WS	Working solution

List of tables

List of figures

1 Introduction

Each drug therapy has, beside the wanted effect on a disease, always also a risk of adverse drug reactions (ADR). In general, underdosing may lead to insufficient treatment and overdosing to dose-dependent ADR. To administer an optimal dose is therefore crucial for a safe and effective therapy. However, determination of the "right dose" is challenging, especially as patients are extremely diverse. They differ e.g. regarding sex, age, organ function, body composition, comorbidities and therefore also comedication. Clinical trials are incapable of investigating all potential risk factors and combinations of patient characteristics. They only enable the investigation of efficacy and safety of new drugs in artificial situations with strict inclusion and exclusion criteria for the patients and, at least in early stages, without comorbidities. However, some diseases change e.g. body composition or metabolic capacities and subsequently influence the pharmacokinetics of drugs [1–5]. Especially in multimorbid patients, these alterations may lead to unpredictable pharmacokinetics and therefore to an increased risk of ADRs. In addition, many patients receive more than one drug enhancing the risk of pharmacokinetic (PK) and pharmacodynamic (PD) interactions und may lead to deviating drug effects and probably suboptimal dosing [6].

Aggravatingly, the vast majority of patients which are included in the trials are men between 18 and 65 years with average height and body weight. Logically, dose recommendations bases mainly on this group of patients resulting in higher risks of suboptimal dosing for women, geriatric and paediatric patients but also for e.g. obese patients [7–12]. In the last years, a rethinking could be observed leading to more diverse patient populations in clinical trials [13]. In addition, more and more clinical trials investigated drugs in special populations to better understand the variability in drug pharmacokinetics. The resulting information support rational dosing decisions and are crucial to enable patient-individual dosing although it is impossible to evaluate all potential patient groups, comorbidities and comedication.

An additional variability in administered doses is caused by small inhomogeneities in dosage forms. In general, doses in e.g. commercially produced tablets are very uniform but as soon as they were divided, doses may significantly deviate as shown by different trials [14–16]. In contemporaneously compounded dosage forms, variability up to 15% of a dose is allowed [17,18] which can, in case of drugs with a low therapeutic window, lead to different therapeutic outcomes in the same patient.

Another, very important challenge for rational dosing is the target site of the drug which equates often not the routine sampling matrix as plasma or urine. Unfortunately, target sites as cerebrospinal fluid, synovial fluids, or epithelial lining fluid are difficult to assess. However, their drug pharmacokinetics may differ tremendously from e.g. plasma as observed in the therapy of bacterial infections. Pathogens mainly reside in the interstitial space fluid (ISF) which is therefore the target site for many antibiotics [19–21]. For several antibiotics, distinct pharmacokinetic differences in plasma and ISF have been shown [22–31] demanding sample collection directly at the infection site-a recommendation also included in the recent European Medicines Agency (EMA) guideline on development of new antibacterial medicines [32]. Several methods were developed for this purpose including biopsies, skin blisters and microdialysis, all with certain advantages and limitations. Microdialysis was the method of choice in this work allowing sample collection from almost each body fluid over a certain time period without repetitive harm to the patients as in biopsies. The method is further described in Section 1.3.

In the present work, the focus was on dose assessment in contemporaneously compounded capsules containing the hormone hydrocortisone (HC) and on microdialysis investigations at a methodological level as well as to evaluate drug concentration-time courses of different antibiotics.

Diseases - Pathophysiology and treatment

1.1.1 Congenital adrenal hyperplasia

1.1.1.1 Pathophysiology

As highlighted before, optimal dosing can be very important for the treatment outcome of many diseases. One of them is congenital adrenal hyperplasia (CAH), a rare but severe endocrine disorder. Patients have an insufficient synthesis of cortisol, mainly due to a mutation in the gene cytochrome 21A2 encoding 21-hydroxylase [33,34]. The activity of the mutated enzyme is much lower, leading to high concentrations (C) of precursors and only low concentrations of cortisol. A compensatory over-activation of the hypothalamic-pituitary-adrenal axis leads to even more precursors but unfortunately also to heavily increased concentrations of steroid hormones e.g. androstenedione and testosterone [33,35] resulting in characteristic symptoms especially ambiguous genitalia at birth in female patients. Male patients do not show clinical signs at birth. Later, both sexes suffer from e.g. early puberty and androgen excess. An affection of mineralocorticoid synthesis is found in 75% of the patients resulting in low concentrations of aldosterone. Clinical symptoms are massive salt loss and therefore severe dehydration quickly leading to life-threatening conditions [34,36].

1.1.1.2 Principles and challenges in the treatment of congenital adrenal hyperplasia

Therapy consists of a substitution of lacking hormones using glucocorticoids and if necessary fludrocortisone (FC) as mineralocorticoid [36,37]. In children, HC is the glucocorticoid of choice due to its short half-life and therefore minimised risk of growth suppression. Daily doses are between 8-18 mg/m^2 [33,34,36,38] divided in 2-4 doses.

At the time of the investigation, no commercially available tablet or capsule, containing HC in paediatric doses is approved making the treatment of these patients very challenging. Several approaches were developed to provide required doses [39] e.g. division of crushed tablets [40], drug containing suspensions [41] and compounded capsules (usually provided by local pharmacies). All approaches may easily lead to incorrect doses e.g. by drug inhomogeneity in not properly mixed suspensions or unequally divided tablets as shown for several drugs [15,16,42,43] putting patients at risk. For CAH patients, resulting under- or overdosing may lead to adrenal crisis or Cushingoid effects [44], respectively. In general, current substitution therapy shows a suboptimal outcome e.g. lower final heights compared to healthy children [37].

1.1.1.3 Compounding of hydrocortisone containing capsules

Considering the small amount of HC used in the treatment of paediatric CAH patients and therefore the low ratio of drug and filling material in the compounded capsules, only method B of the capsule compounding methods of the Deutscher Arzneimittel Codex/ Neues Rezeptur Formularium (DAC/NRF) is suitable [45]. After determination of the required volume of capsule content, pure, powdered drug is mixed with filling material in a ratio up to 1:99 (v/v) to gain about 80% of the final capsule content. Subsequently, the mixture is transferred into the capsules and stocked up with filling material until the lower capsule casing is completely filled. The capsules are emptied and the content again homogenised before the capsule casings are finally refilled and closed.

Critical steps are the homogenisation process and the uniform distribution of the mixture to the capsule casings of the compounded batch easily leading to deviations in drug content and/or capsule net mass [46]. Taking the individual preparation into account, compounded capsules have to fulfil less strict requirements than commercially produces drug formulations. Quality is formally assured if the official compounding recommendations were followed and if in-process quality tests e.g. determination of bulk density and subsequently uniformity of net mass is successfully proven. Drug content uniformity and accuracy are not mandatory to evaluate, considering the expensive and time-consuming methods for quantification. Nevertheless, criteria of uniformity of net mass and content as well as uniformity of single-dosed drug formulations are provided by the European Pharmacopeia (EP) [17,18,47].

1.1.2 Surgical site infections

1.1.2.1 Pathophysiology

Surgical site infections (SSI) remain a very common problem after surgeries and account for about 17% of hospital-acquired infections [48]. Up to 7% of all patients develop a SSI after surgical procedures [49,50] leading to significant morbidity and mortality rates [36] and therefore increasing costs [52]. Main pathogens are *Staphylococcus aureus*, *Staphylococcus epidemidis*, coagulase negative staphylococci, *Pseudomonas aeruginosa* and *Enterococcus faecalis* [53,54].

The risk increases with e.g. duration and invasiveness of the surgery. For e.g. intra-abdominal surgery, the incidence of SSIs may be up to 40%. Beside the surgical site and the type of surgery, certain patient characteristics e.g. immune-suppression, diabetes and age > 70 years are known to increase the risk of SSI [50,55–57].

Obese patients have a higher risk to develop a SSI. Aggravatingly, the number of obese patients is increasing worldwide resulting in higher rates of patients with obesity-related comorbidities as diabetes [56]. In the last decade, bariatric surgery e.g. Roux-en-Y gastric bypass or gastric banding was shown to be an effective way to decrease body weight and to reduce comorbidities but of course put patients at risk of SSI. In addition, administered antibiotic drugs may have an altered pharmacokinetic behaviour e.g. due to the changes in body composition or comorbidities [4].

1.1.2.2 Prophylaxis and treatment of surgical site infections

For patients at high risk, a perioperative antibiotic prophylaxis (PAP) has shown to be beneficial [58] reducing the occurrence of SSIs by up to 80%. A crucial precondition are sufficient antibiotic concentrations at the surgical site. The Paul Ehrlich Institute recommended several antibiotics as prophylaxis treatment, mainly beta lactams and gyrase inhibitors [59]. The chosen antibiotic agent should be administered right at the start of surgery, but at maximum 1 h before. For operations with a duration below 3 h, a single dose treatment is considered to be effective, for longer procedures, drug administration usually has to be repeated [57]. As the antibiotic prophylaxis is also connected with a risk of additional ADRs caused by the antibiotic agents. Therefore, a careful risk-benefit analysis for each patient has to be performed.

1.1.3 Sepsis in infants

1.1.3.1 Pathophysiology

Sepsis is one of the main causes for morbidity and mortality in the infant's age group and accounts for around 40% of all deaths in the first 28 days after birth [60]. It is usually classified into early-onset

sepsis occurring in the first 72 h after birth and into late-onset sepsis with an occurrence to a later time point [61,62]. Clinical symptoms are very unspecific, additionally, a general definition of sepsis including preterm and term infants does not exist. Commonly, a combination of several unspecific clinical symptoms e.g. abnormal body temperature (<36° C or >38.5° C), tachycardia or bradycardia, hypotension, apnoea, lethargy and conspicuous laboratory parameters e.g. increased white blood cells, elevated C-reactive protein and procalcitonin concentrations are used to diagnose sepsis in infants [60,63]. The consequence are either untreated septic infants or treatment of healthy infants with potentially harmful antibiotics [60,64]. Considering the fast course of the disease and the fragile constitution of infants, a fast decision-making is crucial to avoid severe morbidities and death [63,65]. Different bacteria, but also fungi and viruses cause the 2 types of neonatal sepsis. About 70% of the patients with early-onset sepsis have infections with *Streptococcus agalactiae* or *E. coli*. Another less common pathogen found is *Listeria monocytogenes*. Late-onset sepsis is to 70% caused by gram-positive bacteria, mainly coagulase-negative Staphylococci as *S. epidermidis* but also by *S. aureus*, Enterococci and *S. agalactiae*. Most of the other sepsis cases are caused by gram-negative bacteria or fungi (mainly *Candida albicans*). Beside bacteria and fungi, also viruses are described as a main source for sepsis but are not well investigated [61].

1.1.3.2 Pharmacokinetic characteristics of infants

Infants are a very challenging patient population. On the one hand very vulnerable to infections due to their untrained immune system and on the other hand characterised by highly heterogeneous pharmacokinetics depending on their individual grade of maturation [66,67]. Aggravatingly, clinical trials or approved drugs for infants are rare, owing multiple reasons and despite all efforts made in the last years by the authorities [68,69]. Even more, most clinical trials in infants used dosing information from older patients and performed an extrapolation based on body-size resulting in a high rate of ADRs and trial failures because dose adjustment is usually complex and not only limited to body size or body weight [70,71]. For infants, the most important characteristic is the gestational age conditioning the maturation of e.g. liver enzymes and the gastrointestinal permeability [72,73]. In general, the body composition of infants is different compared to older children or adults e.g. due to a higher proportion of water, especially a higher volume of ISF leading to lower concentrations of hydrophilic drugs [67]. On the other side, the blood-brain barrier is still very permeable resulting in accumulation of lipophilic drugs in the central nervous system and a higher incidence of central effects. Plasma protein binding is lower elevating the unbound, pharmacologically active portion of a drug and therefore its effect and elimination. Additionally, elimination processes also underlie a maturation, e.g. several liver enzymes which are needed for metabolic reactions are not fully expressed, especially in preterms, altering the kind and magnitude of metabolites. Renal elimination is reduced due to impaired tubular function and in general lower renal blood circulation leading to a low creatinine clearance directly after birth [67]. As renal function is rising fast, creatinine clearance is an important parameter which has to be determined individually if renally excreted drugs shall be applied. Summarising, an individual therapy accounting for the specific maturation grade is of tremendous importance for infants. To allow such an individual approach, more clinical trials have to be performed in this patient group.

1.1.3.3 Treatment of septic infants

Is an early-onset sepsis suspected, treatment with ampicillin and an aminoglycoside (mainly gentamicin) is established [65]. For late-onset sepsis treatment, vancomycin (VAN) instead of ampicillin is commonly used due to the high resistance rates of *Staphylococcus epidermidis* to ampicillin but has to be dosed carefully [72,74]. Too low VAN concentrations would result in ineffective bacterial killing

and increase the risk of resistant strains, while too high concentrations may lead to oto- and nephrotoxic effects [75]. Aggravatingly, sepsis may lead to fundamental changes in drug pharmacokinetics [76] making the dose finding in infants even more challenging. Different dosing regimens are used for dose finding of VAN based on postconceptional or chronological age, on bodyweight or serum creatinine [72,77–80]. Targeted peak concentrations range in plasma from 20 to 40 mg/L, minimum concentrations (C_{min}) from 5-10 mg/L. Thus, treatment outcome is still suboptimal.

1.2 Basic principles of PK and PD in case of antibiotics and steroid hormones

1.2.1 PK/PD properties of investigated glucocorticoid hormones

1.2.1.1 Hydrocortisone

HC equates the endogenous hormone cortisol but is produced synthetically. It interacts with glucocorticoid receptors finally resulting in a change of DNA expression. As a glucocorticoid, it has anti-inflammatory immune suppressive effects and regulates glucose and fat homeostasis [81]. In addition, cortisol concentrations trigger an important feed-back mechanism for the hypothalamic-pituitary axis. Beside as short-term immunosuppressant, HC is indicated as substitution for patients with a lack of cortisol e.g. suffering from Morbus Addison and for patients with impaired cortisol synthesis mainly with CAH [82].

HC is administered orally as tablets, capsules or solutions usually in 3 (2-4) daily doses. As cortisol has a pronounced circadian rhythm, the amount of HC differs in the respective doses with the highest dose in the morning and 2 lower ones in the early afternoon and late evening [38]. A volume of distribution of 0.49 L/kg was stated [83]. Plasma protein binding of HC is concentration-dependent as the main binding partner, the corticosteroid binding hormone, has only a limited availability and albumin, which occurs in a sufficiently high concentration, has a low binding affinity. HC has a half-life of 90 min. More than 90% is metabolised into several inactive metabolites which are renally excreted.

Due to the mode of action of HC, a direct evaluation of the treatment outcome is not possible. The best surrogate parameter is 17-hydroxyprogesterone which is usually affected by the negative feed-back mechanism of cortisol. Therefore, high concentrations of 17-hydroxyprogesterone indicate insufficient HC/cortisol concentrations. In combination with clinical signs e.g. height, weight and blood pressure to detect overdosing [36] , suboptimal treatment can be assessed.

1.2.2 PK/PD properties of investigated antibiotic drugs

1.2.2.1 Cefazolin and Cefuroxime

Cefazolin (CZL) is a first generation cephalosporin, while cefuroxime (CFX) belongs to the second generation. Both bind to transpeptidases and other penicillin binding proteins and therefore inhibits the cell wall synthesis of bacteria. Their main bactericidal effect can be observed against gram-positive bacteria e.g. staphylococci and streptococci. CFX has an additional effect on gram-negative bacteria as *Escherichia coli* and *Haemophilus influenzae*.

CZL is not orally available, hence it has to be administered either intravenously (i.v.) or intramuscularly. It is administered as 0.5 to 1 h infusion every 8 to 12 h. Daily doses range depending on the type and severity of infection between 1.5 g (mild infections with gram positive strains) to 4 g (strong infections with gram-negative strains). A volume of distribution of 0.14 L/kg was reported [84] with very good

distribution into tissue including placenta and brain [84,85]. For plasma protein binding, a value of 79% [86] was stated (values between 65% and 92% are reported due to saturation processes [85]). Half-life in plasma is 2 h. As the main route of elimination is renal without prior metabolism, a dose adjustment for renally impaired patients is recommended. As PAP, 1 g CZL is infused 0.5 to 1 h prior to start of surgery followed by a 0.5 to 1 g dose after 6 to 8 h [85].

CFX can be administered both, intramuscularly and orally. Oral bioavailability is 67.9% [87]. Standard daily doses range between 2.25 and 4.5 g depending on severity and location of the infection and are usually divided into 3 doses. CFX is also indicated as PAP using an initial dose of 1.5 g and maintenance doses of 0.75 g every 8 h for up to 24 h [88]. A volume of distribution of 0.19 L/kg and a plasma protein binding of 33% were reported [89,90]. Tissue penetration is high and results in effective concentrations [88]. A half- life of 70 min was stated. As CZL, CFX is eliminated unchanged via urine and therefore needs a dose adjustment for renally impaired patients.

The effectiveness of cephalosporins depends on the time span, the drug concentration exceeds the minimum inhibitory concentration (MIC) of the respective strain and should reach 40%-50% [91–93]. Therefore, prolonged and continuous infusions were recently discussed.

1.2.2.2 Meropenem

Meropenem (MER) belongs to the carbapenems and has a similar way of action as the cephalosporins but a much broader spectrum of antibacterial activity. It includes many gram-negative bacteria but not methicillin-resistant *Staphylococcus aureus* (MRSA). It is still a reserve antibiotic agent and is only indicated in case of complicated bacterial infections including hospital-acquired pneumonia.

Administration is performed via 0.5 h infusion every 8 h with daily dosed between 1.5 and 6 g depending on location and severity of the infection [94]. MER distributes well into various tissues with a volume of distribution of 0.25 L/kg which is slightly higher than for the cephalosporins. Plasma protein binding is only 2% and thus of minor relevance. The half-life is stated with 1 h. Although the betalactam-structure can be dismantled to a microbiologically inactive form, only 28% of MER is eliminated as metabolite whereas 70% is excreted unchanged via urine. Hence, a dose adjustment for renally impaired patients is recommended.

As for the cephalosporins, time above MIC is the best index to evaluate effectiveness of MER. Concentrations should exceed MIC at least 40% of the time in a dosing interval [93].

1.2.2.3 Vancomycin

VAN is a glycopeptide antibiotic agent which mainly impairs cell wall synthesis by binding to bacterial murein molecules. In addition, it increases permeability of the bacterial cell membrane and hampers RNA synthesis. It is used as a reserve antibiotic drug. VAN is effective against gram-positive bacteria including MRSA. Thus, it is indicated for complicated infections with gram- positive bacteria including skin, joint and bone infections, hospital-acquired pneumonia and sepsis. In special cases, VAN can also be used as PAP [95].

Normal way of administration is as intravenous infusion as VAN is not adsorpted from the gastrointestinal tract. Oral administration is only performed to treat intestinal *Clostridium difficile* infections. Dosing is based on body weight for patients ≥12 years. 15 to 20 mg/kg every 8 to 12 h are recommended. For infants, different dosing regimens based on body weight, postmenstrual age or gestational age are used in clinical routine (Section 1.1.3). Therapeutic drug monitoring with a focus on C_{min} is common to avoid nephro- and ototoxicity [96]. Volume of distribution ranges from 0.4 to

1 L/kg in literature. VAN concentrations in tissues are usually much lower than in plasma. Nevertheless, relevant concentrations were found in e.g. bone and placenta. [97] A plasma protein binding between 30% and 55% and a half-life of 4 to 6 h were stated. VAN is renally eliminated, mainly without prior metabolism, hence VAN dose has to be adjusted for patients with severe renal impairment [95].

Beside minimum concentrations giving an insight into the risk of ADRs, a quotient of area under the curve (AUC) and MIC is the most effective index to evaluate efficacy of VAN dosing [98] and should at least equate 400 [93,99].

1.2.2.4 Metronidazole

Metronidazole (MET) is a nitroimidazole antibiotic. It acts as a prodrug, which is reduced under anaerobe conditions- as in anaerobe bacteria and anaerobe protozoa- to instable nitroso radicals. These radicals react with DNA and probably other macromolecules hampering their functionality. Under aerobe conditions, MET is nontoxic. Therefore, it is indicated for the treatment of infections with anaerobe bacteria, parasites and protozoa.

MET can be administered intravenously or orally (bioavailability about 82%[100]). Usually, 1.5 g MET are given either once daily or divided into 3 equal doses every 8 h. As part of PAP, an initial dose of 0.5 g MET 1 h before surgery and 2 maintenance doses in an 8 h interval are administered. A volume of distribution of 0.51 to 1.1 L/kg was found as well as good penetration into various ISFs including of brain, adipose tissue and placenta [101]. A plasma protein binding between 10% and 20% was stated and a half-life of 8 h. MET is metabolised to more than 90% and its metabolites excreted via urine to about 80%. Thus, hepatic insufficiency leads to a strong increase of half-life making a dose adjustment necessary while renal impairment has only minor impact on MET pharmacokinetics.

The PK/PD index best describing the efficacy is maximum concentration (C_{max}) divided by MIC. Target ranges depend on the respective bacterial strain [92,93].

1.2.2.5 Linezolid

Linezolid (LIN) belongs to the group of oxazolidinone antibiotics. It interacts with the 50S unit of ribosomes resulting in a hampered aggregation of the ribosomal initiation complex and therefore in an impeded protein synthesis. The antibacterial activity of LIN covers gram positive bacteria including MRSA [102]. LIN is an important reserve antibiotic and indicated for e.g. nosocomial pneumonia, skin and soft tissue infections [103].

Due to an oral bioavailability of 100%, it can be administered as infusion or orally without dose adjustment. LIN is administered as a 600 mg dose every 12 h and has a volume of distribution of 0.57 to 0.71 L/kg. It penetrates various tissues but has lower concentrations as in plasma. A plasma protein binding of 31% is reported in older publications [103,104], more recent ones found a lower one around 15% [22,105–107]. As parameters of applied ultracentifugation in newer publications e.g. temperature, better equates to body conditions, the latter value is used in the present work. Half-life is reported with 5 to 7 h. LIN is transformed to about 50% into 2 inactive metabolites which are renally excreted. Another 40% are eliminated unchanged via urine. No dose adjustment for renally or hepatically impaired patients is recommended [103].

For LIN, the best PK/PD target to assess therapeutic efficacy is AUC_{0-24}/MIC, describing the AUC in a 24 h dosing interval in relation to the MIC of the investigated bacterial strain. This ratio should be at least 50, preferably ≥100 [93,104].

1.2.2.6 Tigecycline

Tigecycline (TIG) belongs to the glycylcycline antibiotics, a subgroup of tetracycline antibiotics. It binds to the 30S subunit of ribosomes and impairs the translation process in the bacterial protein synthesis. TIG has a bacteriostatic effect on several gram-positive and gram-negative bacteria including Staphylococci, Streptococci and *E. coli* which are resistant to other tetracyclines.

It is intravenously administered as 0.5 or 1 h infusion every 12 h using an initial dose of 100 mg and maintenance doses of 50 mg. TIG has, compared to the other here mentioned drugs, a very large volume of distribution of 7 to 10 L/kg [104] demonstrating the excellent tissue penetration into all up to now investigated ISFs. A plasma protein binding between 71 and 89% is stated and seems to be concentration-dependent. Half-life is variable and very long compared to the ones of other here characterised antibiotic agents: in average 42 h. Less than 20% of TIG is metabolised. Most of the drug is eliminated via faeces (59%) and urine (33%). For patient with very severe renal or hepatic impairment, a dose adjustment is recommended [108].

Efficacy of TIG is best assessed using $AUC_{0\text{-}24}$/MIC as for LIN [93]. The respective threshold depends on the bacterial strain [104].

1.3 Microdialysis

A very powerful tool to acquire pharmacokinetic information about a drug directly at the target site, is microdialysis sampling. It was developed by Ungerstedt and Pycock in 1974 to investigate pathways of neurotransmitters [109]. For many years, microdialysis was mainly applied in neuroscience, but starting in the 1990's, this elegant approach was also used to gain target site concentrations of e.g. antiinfective or oncologic drugs [110–118].

1.3.1 Principle of microdialysis

This minimally invasive technique consists of a small catheter with a semipermeable membrane, an inlet- and an outlet-tubing; a pump and sampling vials. Microdialysis bases on diffusion of endogenous (e.g. neurotransmitters) or exogenous analytes (e.g. drugs) through a semipermeable membrane among the concentration gradient. The membrane is located in the tip of the microdialysis catheter which is implanted in the interstitial fluid (ISF) of interest. In case of *in vitro* investigations, the catheter is placed in a vial containing surrounding medium (SM). The catheter is flushed with a constant flow. The perfusion fluid, the so-called perfusate is usually a physiological, but protein-free solution e.g. Ringer's solution (RS) but can also contain different additives such as human serum albumin or cyclodextrine. In microdialysis settings, it is analyte free. In retrodialysis settings, it contains a high concentration of the analyte exceeding the expected ISF concentration. Depending on the concentration ratios of the analyte in ISF and perfusate, diffusion will be directed into the perfusate (microdialysis setting) or the ISF (retrodialysis setting) as displayed in Figure 1.1. At the catheter outlet, the perfusate/ retroperfusate is collected in certain time intervals as the so-called microdialysate (µDialysate, microdialysis setting) or retrodialysate (RD, retrodialysis setting).

The constant flow, usually with rates of 1-2 µL/min in *in vivo* investigations, is generated by special, pulsation-free working pumps. The catheter usually consists of polyurethane and has a concentric shape (Figure 1.1) with the semipermeable membrane at the catheter tip. In special cases, also linear shaped catheter are beneficial e.g. for skin tissue. The semipermeable membrane usually consists of polyarylethersulfone and has a certain molecular weight cut-off (MWCO) stating the molecular weight at which 80% of the analyte is able to cross the membrane. Molecules with a molecular weight ≤25%

of the MWCO are able to pass the membrane without any obstruction. A common MWCO is 20 kDa, leading to diffusion of only unbound and therefore pharmacologically active analyte molecules. Catheters with a MWCO of 100 kDa are available for investigations of large molecules but have an important disadvantage: analyte molecules bound to small proteins may diffuse into the perfusate making statements to unbound analyte concentrations challenging.

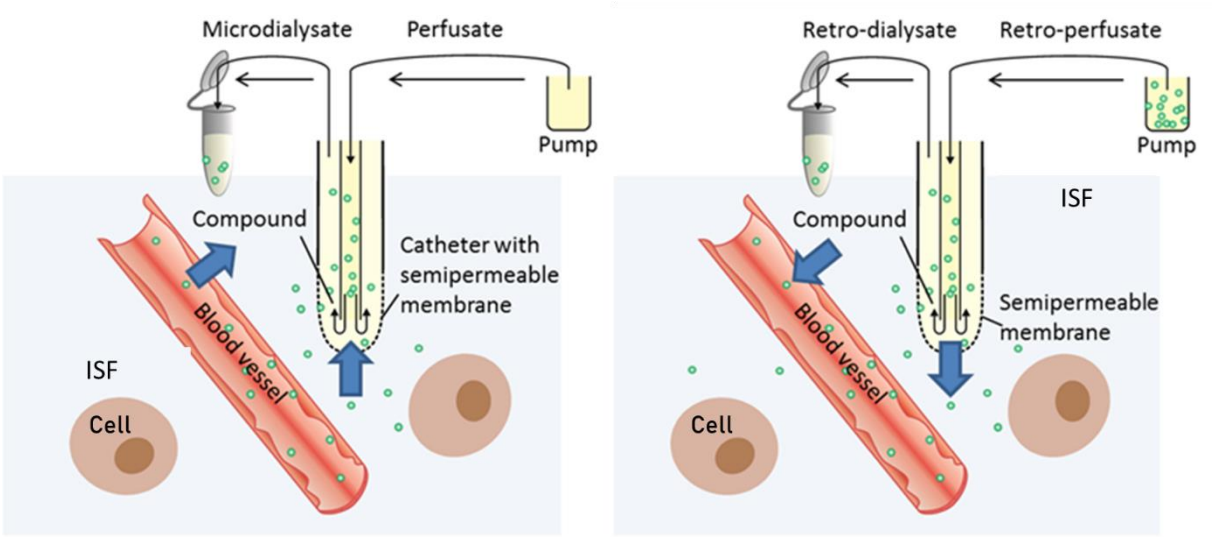

Figure 1.1 Principle of microdialysis (left side) and retrodialysis (right side)
ISF: interstitial space fluid
Blue arrows display diffusion direction

Due to the constant perfusate flow through the catheter and hence incomplete equilibrium conditions at the semipermeable membrane, only a certain percentage of the drug which is present in the ISF will diffuse through the membrane, which is referred to as 'relative recovery' (RR). The calculation of RR in microdialysis setting prerequisites a known concentration from microdialysate ($C_{\mu dialysate}$) on one hand and from ISF (C_{ISF}) or in case of *in vitro* investigations from SM (C_{SM}) on the other hand (Equation 1.1).

$$\mathbf{RR}, \% = \frac{\mathbf{C_{\mu Dialysate}}}{\mathbf{C_{ISF/SM}}} \bullet \mathbf{100} \qquad \textbf{Equation 1.1}$$

In retrodialysis setting, the fraction, which is lost from the retroperfusate into the ISF or SM is also called RR or 'relative delivery'. In this setting, analyte concentrations in the retrodialysate (C_{RD}) and the retroperfusate (C_{RP}) are known and RR can be calculated without need of assumptions (Equation 1.2). RR is used for both settings later on in this thesis stating the setting if necessary for comprehension.

$$\mathbf{RR}, \% = \mathbf{100} - \left(\frac{\mathbf{C_{RD}}}{\mathbf{C_{RP}}} \bullet \mathbf{100}\right) \qquad \textbf{Equation 1.2}$$

RR values in microdialysis and retrodialysis setting are usually equal, hence the determination of RR via retrodialysis allows subsequent calculation of ISF concentrations from microdialysate concentrations.

$$\mathbf{C_{ISF/SM}} = \frac{\mathbf{C_{\mu Dialysate}}}{\mathbf{RR}} \bullet \mathbf{100} \qquad \textbf{Equation 1.3}$$

Concentrations in ISF (*in vivo*) or SM (*in vitro*) are calculated from microdialysate concentrations as shown in Equation 1.3 using the previously determined RR value.

1.3.2 Influence factors on relative recovery and catheter calibration

A crucial prerequisite for calculation of ISF concentrations is a constant RR value, the magnitude of RR is of minor importance. Several factors influence RR permanently and need to be considered in the design of an *in vitro* investigation or a clinical trial: e.g. the membrane surface area and MWCO of the catheter, physicochemical properties of the investigated analyte as well as the characteristics of the insertion site. Adjustable parameters are flow rate, perfusate composition, temperature (at least in *in vitro* investigations) and the concentration gradient of the analyte [4,10,11].

The surface area of the semipermeable membrane and its MWCO as well as the temperature and concentration gradient are proportional influence factors: the higher the parameters, the higher is the resulting RR. The chemical and physical characteristics of the analyte have a strong impact on the resulting RR. For example, high molecular weight/ molecule size impedes diffusion, high lipophilicity often results in adsorption to tubings or membrane surface. RR may be enhanced by a suitable perfusate adjusting e.g. pH value or reducing bindings by different additives. Also drug combinations were observed to affect RR values [123] by e.g. pH value changes, complex formation or displacement from plasma protein binding. The tortuosity of the catheter surrounding tissue may impede free diffusion of analytes to and through the membrane. Human *in vivo* studies showed constant tortuosity for at least 3 days without indications of encapsulation of the catheter [124], therefore tortuosity can be considered as a static influence factor in usual microdialysis investigations. An important sampling parameter is the flow rate which has to be chosen carefully. Low flow rates result in higher RR values. As a certain volume of microdialysate is required for analysis, a low flow rate would result in long sampling intervals and hence in low temporal resolution of the obtained pharmacokinetic data. Higher flow rates on the other side lead to lower RR values und may lead to analyte concentrations close to the lower limit of quantification (LLOQ) aggravating the sample quantification [4]. Since the microdialysis catheters are handmade, each catheter has e.g. slightly deviating pore sizes, membrane surface areas et cetera. Therefore, RR has to be determined for each catheter separately and is referred to as catheter calibration. Considering the many possibilities to affect the RR value, sampling parameters should be kept constant within microdialysis sampling and calibration (Cal).

Several calibration methods as (dynamic) no-net-flux method, flow rate variation, internal standards and retrodialysis were developed [4,14,15,16]. However, the vast majority of clinical trials use retrodialysis settings. As described above, the diffusion of the analyte is directed from the perfusate into the ISF. To ensure a sufficient concentration gradient, the analyte concentration in the retroperfusate should be at least 10fold higher than in the ISF/SM [122]Even though RR in retrodialysis setting is usually equal to RR in microdialysis setting, their comparability should be investigated *in vitro* prior to clinical trials [130].

1.3.3 Benefits and limitations of microdialysis

Some research areas are predestined for microdialysis investigations: e.g. neurosciences, infectiology, oncology, dermatology, metabolic studies and partially endocrinology. For all of them, the main site of action is not plasma or another easily accessible body fluid and administered drugs show a direct, short-term effect. As already mentioned in the introduction, conventional sample collection e.g. plasma or saliva may lead to significantly different drug concentrations misleading therapeutic decisions. Hence, microdialysis sampling should be considered as a good addition to standard sample collection. In contrast to other tissue sampling methods as biopsies or skin blisters, microdialysis enables continuous measurements of analytes over a certain time period of up to several days

[112,122,124,131,132] without repetitive harm. In addition, only unbound and therefore pharmacologically active analytes are collected. On the other hand, a routine use of microdialysis is not recommendable due to the time-consuming sampling, the still invasive nature of the sample collection and the high costs of the catheters. Certain drug molecule characteristics are suboptimal for microdialysis investigations. Large molecule size or extensive plasma protein binding result in lack of diffusion through the catheter membrane[133]. High lipophilicity leads to adsorption at the tubing material and unpredictable recovery in the microdialysate [124,134,135]. However, for some clinical research questions, microdialysis is a very potent tool to close critical knowledge gaps e.g. during drug development and optimisation of therapeutic treatments [32] .

1.3.4 *In vitro* microdialysis system

Potential impact factors should ideally be assessed prior to *in vivo* microdialysis investigations under standardised conditions. Ideally, an *in vitro* microdialysis system (IVMS, see Figure 1.2) [111,113] is used minimising sources of variability and alleviating evaluation of effects. It consists of (i) a platform adjusting the height of the pumps to avoid pressure differences between different catheters, (ii) a heating block providing a constant temperature in the SM, (iii) a holder attaching vials containing the SM, adjusting catheter tips in the middle of the SM and fixing vials for collection of microdialysate/retrodialysate and (iv) a magnetic stirrer to avoid inhomogeneities in the SM.

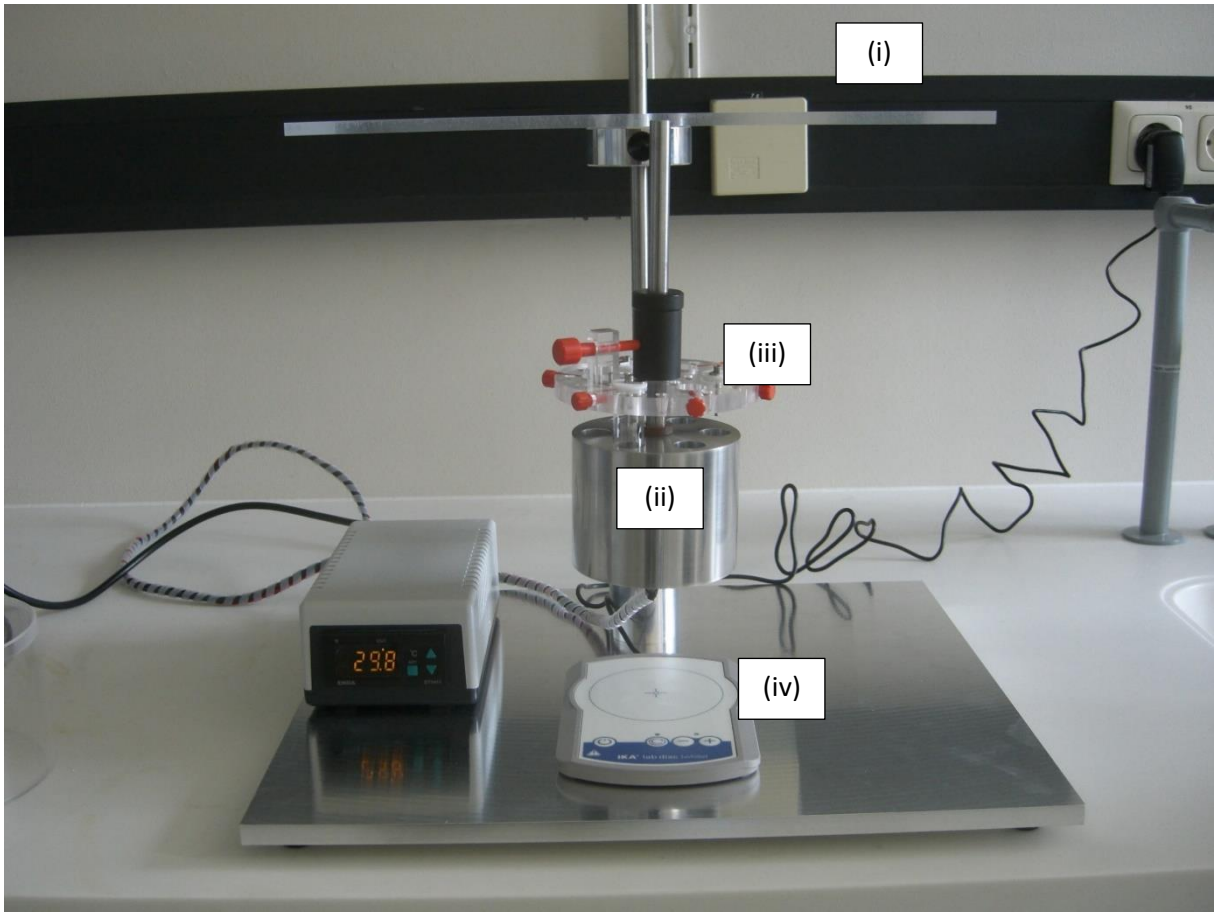

Figure 1.2 *In vitro* microdialysis system
(i) Platform for the pumps, (ii) heating block, (iii) holder attaching vials and adjusting catheters and (iv) magnetic stirrer

1.4 Objectives of this work

Rational dosing is challenging in special patient populations, often aggravated by a lack of information. The present work aimed to close some of the knowledge gaps in the field of endocrinology and infectiology, either directly by supporting clinical trials or indirectly by improving microdialysis sample collection.

Project I was planned as preparation of an upcoming clinical trial aiming to determine the pharmacokinetics of HC from compounded capsules in paediatric CAH patients. The content of manually manufactured capsules as used for paediatric HC formulations underlay a higher variability than industrially manufactured formulations. Considering that the capsule administered to the patient for pharmacokinetic investigation is not available for drug quantification, the drug content has to be estimated in a different way. Hence, the possibility to deduce this unknown drug amount from other capsules of the same capsule batch should be assessed. For this purpose, a trial was planned to gather information about the uniformity of net mass and drug content, the accuracy of drug content and finally a potential correlation of net mass and drug content of HC capsules for paediatric use which were compounded by German pharmacies. The present work contains the study sample analysis and the subsequent data analysis.

The aim of Project II was to investigate potential impact factors of microdialysis sampling on RR values to increase reliability and accuracy of the information obtained from clinical microdialysis trials. Subsequently, the findings shall be used to inform the design of a clinical microdialysis trial assessing the pharmacokinetics of different antibiotic regimens used to prevent SSI in obese patients (EudraCT Number 2012-004383-22). Using the IVMS for standardised *in vitro* microdialysis investigations, 4 hypotheses aimed to be evaluated:

(I) RR values of a certain drug may be altered by drug combinations (as used in the clinical trial) as drugs diffuse simultaneously through the semipermeable membrane potentially hampering free diffusion by e.g. blocking of pores or accumulation processes. (II) RR values of a certain drug may be altered by the microdialysis order e.g. due to adsorption of a previously microdialysed drug to the catheter membrane. (III) RR values may be altered by air in the microdialysis catheter especially if gas bubbles are located at the semipermeable membrane potentially impairing free diffusion of drugs. Gas bubbles are commonly observed and occur presumably due to temperature differences (e.g. room temperature in the tubing and body temperature in the patient) and while connecting the tubing of the microdialysis catheter with the perfusate containing syringe. (IV) Flow rate has a well-known reverse impact on RR values. Hence flow rate changes during the sampling process would most probably also impact RR values. As after each start of the used *in vivo* pumps, an automated flush program (5 min at 15 µL/min) starts before reducing to the applied flow rate, a significant deviation in the resulting RR was expected. In addition, it would be plausible that this deviation may last for some time making an equilibration period in the sampling schedule necessary. Hence, the magnitude of RR alteration as well as the duration until consistent RR values are assessed.

Project III aimed to determine VAN concentration-time courses in septic infants/neonates directly at the site of infection. As sepsis is often accompanied by soft tissue infections [136], VAN concentrations should be investigated in subcutaneous ISF using microdialysis. By now, no ISF concentrations for this patient group are available in literature, only serum concentration-time profiles. As shown by Zeitlinger et al. [137], both concentrations may differ significantly in septic patients hampering a successful treatment. In addition, monitoring of serum or plasma concentrations is connected with repetitive

harmful blood drawings and a reduction of the anyway limited blood volume in the neonatal patients whereas microdialysis allows sampling over several days without blood loss and only a single pain experience.

Therefore, a clinical trial was conducted to investigate the feasibility and safety of microdialysis sampling of VAN in infants/neonates with sepsis (see also Section 1.1.3.). Prior to the clinical trial, *in vitro* investigations should be performed Assessing potential impact factors as well as optimal microdialysis sampling and calibration parameters. Five hypotheses should be evaluated by the *in vitro* investigations: (I) the potential impact of flow rate on RR. The flow rate is a well-known impact factor on RR. Low flow rates usually lead to increased RR values and therefore higher drug concentrations in the samples facilitating the subsequent quantification. On the other side, low flow rates prolong the sampling intervals and therefore the temporal resolution of the trial as a certain sample volume is needed for bioanalysis. Hence, a compromise had to be found with regard to the trial objective and the sensitivity of the bioanalytical method. An aggravating factor was that catheters with a reduced membrane length of only 10 mm instead of 30 mm should be used lowering RR in addition. Hence, different commonly used flow rates were evaluated with regard to the resulting RR values and the necessary duration of the sampling intervals. (II) The potential impact of pH value of SM and perfusate on RR. PH value was found to reduce RR values in a previous investigation of Plock et al. [138] in a pH range of 5.4 to 6.1. Taking into account that septic patients often show decreased tissue pH values [139,140], a potential impact of a clinically plausible pH 7.0 versus pH 7.4 should be evaluated and, if necessary, considered in the final data interpretation. (III) The potential impact of VAN concentration on RR: Concentration independency is a crucial prerequisite for microdialysis investigations as it ensures consistent RR values over the whole concentration range. Plock et al. [138] showed also a concentration dependence of the VAN RR values, hence it should be reassessed in RS and in pH-adjusted phosphate buffered saline (PBS) buffer over the concentration range expected in the clinical trial. (IV) The potential impact of the diffusion direction on RR: catheter calibration should preferably be performed using retrodialysis. A prerequisite is consistent diffusion of VAN in both directions across the semipermeable membrane and ultimately equality of RR values in microdialysis and retrodialysis setting. Hence, RR values should be assessed in both settings and compared. (V) The potential impact of the perfusate composition on RR: the results of all *in vitro* investigations should be used to determine a suitable perfusate taking into account a potential dependency of RR values on pH value, concentration or setting and should finally be implemented in the design of the clinical trial.

The present work contains the *in vitro* investigations, the sample analysis (microdialysis samples from the *in vitro* investigations and the study) including the development of a suitable quantification method for *in vitro* samples and the data analysis with regard to plausibility of obtained concentration-time profiles, applicability of retrodialysis for RR determination and a potential correlation of ISF concentrations and plasma minimum concentrations.

The aim of Project IV was to quantify and compare concentration-time profiles of CFX and LIN in patient's plasma, synovial fluid and muscle ISF directly after arthroscopic surgery. As synovial fluid is often the site of infection in SSI, pharmacokinetic information about prophylactic administered antibiotics is crucial to optimise PAP treatment. But by now, no pharmacokinetic data about CFX and LIN, frequently used antibiotic agents for PAP, is available from synovial fluid although several clinical trials were performed investigating other ISF concentrations: CFX [12,13,97,98,99] and LIN [76,77,100,101,102,103,104]. Hence, a clinical microdialysis trial was conducted to close the knowledge gap. The present work contains the sample quantification from plasma as well as the data analysis for all matrices. At first, a previously established high performance liquid chromatography

(HPLC) method which enabled the quantification of CFX and LIN from microdialysate had to be extended for plasma samples by a suitable sample preparation The final method should be validated according to the respective EMA Guideline [141] and used for the bioanalysis of the plasma study samples. Finally, a comparison of the resulting concentration-time profiles with previously quantified profiles from synovial fluid and muscle ISF should be performed.

2 Methods

2.1 Chemicals and devices

2.1.1 Project I - Uniformity and accuracy of compounded capsules

Hydrocortisone	Tianjin Tianyao Pharmaceuticals Co., Ltd., Tianjin, China, batch: 12/25-N01
Fludrocortisone	Fountain Limited, Naxxar, Malta, batch FTB0255
Acetonitrile	HiPerSolv Chromanorm Acetonitrile, VWR International, Radnor, USA
Ethanol 96% (v/v), HPLC grade	Roth, Karlsruhe, Germany
Purified water (MQ)	Milli-Q®, Millipore, Molsheim, France
Pipette 10-100 µL	Research Plus®, Eppendorf, Hamburg, Germany
Pipette 100-1000 µL	Research Plus®, Eppendorf, Hamburg, Germany
Pipette 5000 µL	Research®, Eppendorf, Hamburg, Germany
Safe lock tubes, 1.5 mL	Eppendorf, Hamburg, Germany
Falcon™ conical tubes, 15 mL	BD, Franklin Lakes, USA
Nunc™ 96 well plate, round bottom	Thermo Fisher Scientific, Waltham, USA
HPLC	Ultimate 3000®, Thermo Fisher Scientific, Waltham, USA
HPLC column	Eclipse XDB-C18®, 4.6x150 mm, 5 µm, Agilent Technologies, Santa Clara, USA
HPLC guard column	LiCrospher® C18 guard column, particle size 5 mm, Merck KGaA, Darmstadt, Germany
Vortex mixer	Reax 2000®, Heidolph Instruments, Schwabach, Germany
Centrifuge	Sepatech Megafuge 1.0R®, Heraeus, Hanau, Germany
Balance	Research R 200 D®, Sartorius, Göttingen, Germany

2.1.2 Project II - Potential impact factors on relative recovery

Linezolid	Zyvoxid®, Pfizer, Karlsruhe, Germany, batch: 13I07U93
Meropenem	Meronem®, Astra Zeneca GmbH, London, United Kingdom, batch: OD142A1; for retrodialysis investigations: EP CRS, Council of Europe, Strasbourg, France, batch: 1.0 ID: 006M32
Cefazolin	Fresenius Kabi Deutschland GmbH, Bad Homburg vor der Höhe, Germany, batch: 14GI25
Tigecycline	Tygacil®, Pfizer, Karlsruhe, Germany batch: AI3U/18

Acetaminophen	Paracetamol, Fresenius Kabi Deutschland GmbH, Bad Homburg vor der Höhe, Germany, batch: 14GI22
Novamine sulfone sodium salt	Metamizol Ratiopharm GmbH, Ulm, Germany, batch: N40597
Sodium chloride	Chempur Feinchemikalien und Forschungsbedarf, Karlsruhe, Germany
Purified water (MQ)	Milli-Q®, Millipore, Molsheim, France
Pipette 10-100 µL	Research Plus®, Eppendorf, Hamburg, Germany
Pipette 100-1000 µL	Research Plus®, Eppendorf, Hamburg, Germany
Safe lock tubes, 1.5 mL	Eppendorf, Hamburg, Germany
Syringes 3 mL	Omnifix® Solo with Luer-Lock, B. Braun, Melsungen, GER
Syringes 2.5 mL	106 microdialysis syringe, M Dialysis AB, Stockholm, Sweden
Microdialysis catheters	63 catheters, membrane length 30 mm, MWCO 20 kDa, batches T23499 (microdialysis setting) and T22538 (retrodialysis setting), M Dialysis AB, Stockholm, Sweden
In vitro microdialysis system with magnetic stirrer and heating block	Stirrer: RCT basic/ IKAMAG REO, IKA, Staufen Germany; Heating blocks: P3/P5, Julabo GmbH, Seelbach, Germany
Microdialysis pump *in vivo*	CMA 107®, M Dialysis AB, Stockholm, Sweden
Microdialysis pump *in vitro*	CMA 102®, CMA Microdialysis AB, Solna, Sweden
Vortex mixer	Reax 2000®, Heidolph Instruments, Schwabach, Germany
Balance	Research R 200 D®, Sartorius, Göttingen, Germany

2.1.3 Project III - Vancomycin monitoring in septic infants via microdialysis

Vancomycin	Sigma-Aldrich, St. Luis, USA, batch LSBH8922V
Lidocaine	Lidocain Braun 1% Injektionslösung; B. Braun, Melsungen, Germany, batch: 13163011
Piperacillin/Tazobactam	Fresenius Kabi, Bad Homburg, Germany, batch: 18S0826
Tobramycin	Tobrazid®, Infectopharm, Heppenheim, Germany, batch: S081701
Meropenem	Fresenius Kabi Deutschland GmbH, Bad Homburg vor der Höhe, Germany, batch: 14GI25
Caffeine	Hospital of the University Munich, Munich, Germany, batch: A20190319-05
Acetaminophen	Paracetamol, Fresenius Kabi Deutschland GmbH, Bad Homburg vor der Höhe, Germany, batch: 14GI22
Teicoplanin	Targoci®, Sanofi-Aventis, Frankfurt, Germany, batch: A8050

Levetiracetam	Desitin, Hamburg, Germany, batch: 026690
Acetonitrile	HiPerSolv Chromanorm Acetonitrile, VWR International, Radnor, USA; HPLC grade for HPLC-UV analysis and MS-grade for Liquid chromatography coupled to a mass spectrometry detector (LC-MS/MS) analysis
2-Propanole	Roth, Karlsruhe, Germany, MS-grade
Purified water (MQ)	Milli-Q®, Millipore, Molsheim, France
Formic acid	MS-grade, VWR International, Radnor, USA
Trifluoric acid	Roth, Karlsruhe, Germany
Pipette 10-100 µL	Research Plus®, Eppendorf, Hamburg, Germany
Pipette 100-1000 µL	Research Plus®, Eppendorf, Hamburg, Germany
Pipette 5000 µL	Research®, Eppendorf, Hamburg, Germany
Safe lock tubes, 0.5 mL	Eppendorf, Hamburg, Germany
Nunc™ 96 well plate, conical bottom	Thermo Fisher Scientific, Waltham, USA
Safe lock tubes, 1.5 mL	Eppendorf, Hamburg, Germany
Nunc™ 96 well plate, conical bottom	Thermo Fisher Scientific, Waltham, USA
Syringes 3 mL	Omnifix® Solo with Luer-Lock, B. Braun, Melsungen, GER
Syringes 2.5 mL	106 microdialysis syringe, M Dialysis AB, Stockholm, Sweden
Microdialysis catheters	63 catheters, membrane length 10 mm, MWCO 20 kDa, batch T24141, M Dialysis AB, Stockholm, Sweden
In vitro microdialysis system with magnetic stirrer and heating block	IKAMAG REO, IKA, Staufen Germany; P5, Julabo GmbH, Seelbach, Germany
Microdialysis pumps in vitro	CMA 102®, CMA Microdialysis AB, Solna, Sweden
Vortex mixer	Reax 2000®, Heidolph Instruments, Schwabach, Germany
Balance	Research R 200 D®, Sartorius, Göttingen, Germany
HPLC column (HPLC-UV analysis)	Accucore C18, 100x2.1, 2.6 µm, Thermo Fisher Scientific, Waltham, USA
Guard column	Accucore C18, 10x2.1, 2.6 µm, Thermo Fisher Scientific, Waltham, USA
HPLC device (HPLC-UV analysis)	Ultimate 3000 HPLC Thermo Scientific including a HPG-3200pump, a WPS-3000TSL autosampler, a TCC-3000SD column thermostat and a DAD3000 detector, Thermo Fisher Scientific, Waltham, USA
HPLC column (LC-MS/MS analysis)	Phenyl-Hexyl Poroshell 120 (2.1x100 mm, 2.7 µm), Agilent Technologies, Santa Clara, USA

HPLC device (LC-MS/MS analysis)	1290 HPLC with 6460 Triple Quadrupole, Agilent Technologies, Santa Clara, USA

2.1.4 Project IV - Perioperative antibiotic prophylaxis in synovial fluid

Linezolid	Pfizer, Karlsruhe, Germany, batch: 00-18
Cefuroxime	Molekula GmbH, Munich, Germany, batch: 163987
Acetonitrile	HiPerSolv Chromanorm Acetonitrile, VWR International, Radnor, USA
Purified water (MQ)	Milli-Q®, Millipore, Molsheim, France
Trifluoric acid	Roth, Karlsruhe, Germany
Pipette 10-100 µL	Research Plus®, Eppendorf, Hamburg, Germany
Pipette 100-1000 µL	Research Plus®, Eppendorf, Hamburg, Germany
Safe lock tubes, 0.5 mL	Eppendorf, Hamburg, Germany
Safe lock tubes, 1.5 mL	Eppendorf, Hamburg, Germany
Nunc™ 96 well plate, round bottom	Thermo Fisher Scientific, Waltham, USA
HPLC device	Ultimate 3000®, Thermo Fisher Scientific, Waltham, USA
HPLC column	Hypersil® GOLD Phenyl column (100x4.6 mm, 3 µm), Thermo Fisher Scientific, Waltham, USA
HPLC guard column	Phenyl guard column, particle size 5 mm, Thermo Fisher Scientific, Waltham, USA
Vortex mixer	Reax 2000®, Heidolph Instruments, Schwabach, Germany
Centrifuge	Centrifuge 5430R, Eppendorf, Hamburg, Germany
Balance	Research R 200 D®, Sartorius, Göttingen, Germany

2.2 Project I - Uniformity and accuracy of compounded capsules

2.2.1 Study design

An exploratory study regarding uniformity and accuracy of contemporaneously compounded capsules containing HC was performed. The study was approved by the institutional ethics committee of the Charité Berlin (EA2/134/12). The Department of experimental paediatric endocrinology of the Charité Berlin published a newsletter to recruit German care givers of paediatric CAH patients. They were asked, after signing a written informed consent, to send in 20 capsules of the currently used, contemporaneously compounded capsule batch. HC dose in the capsules should be between 0.5 mg and 9.5 mg and may be combined with FC. All incoming batches were documented regarding nominal HC dose, capsule filling material, date of compounding and contact information. In case of missing information, especially missing dose, care givers were contacted to complete the data if possible. Each batch was visually inspected and photographed against a light screen to get a first impression of the uniformity of the capsule filling volume. Batches were anonymised by renaming them to batch 001 to 062. Subsequently, they were transferred to the Department of Clinical Pharmacy and Biochemistry at the Freie Universitaet Berlin accompanied by a form stating the available batch-related information (batch number, nominal drug content, filling material). This work describes the further evaluation of the capsules. Capsules were investigated batch-wise with respect to their net masses and the content of HC. Therefore, a suitable sample preparation method as well as an HPLC quantification method for HC in presence of FC was developed and validated. Results were assessed regarding a potential correlation of net mass and drug content, acceptable uniformity of net mass and drug content within the batches as well as for accuracy of drug content.

2.2.2 Preparation of blank, calibration and quality control samples

Blank samples consisted of 5% ethanol 96% (v/v) in MQ water. Stock solution (SL) for Cal samples contained 5 mg/mL and 15 mg/mL HC in ethanol 96% (v/v) which were diluted with ethanol 96% (v/v) to concentrations of 50 µg/mL (Cal 1) to 3000 µg/mL (Cal 7). Subsequently, 50 µL of the respective diluted SL was mixed with 950 µL MQ water to prepare Cal working solutions (WS) with final concentrations of Cal 1: 2.5 µg/mL, Cal 2: 10 µg/mL, Cal 3: 25 µg/mL, Cal 4: 50 µg/mL, Cal 5: 75 µg/mL, Cal 6: 100 µg/mL and Cal 7: 150 µg/mL. Due to the high diversity of substances in the investigated capsules, only an incomplete separation of HC from capsule filling was achieved in method development. To account for that, quality control (QC) SLs (C=5 mg/mL and 15 mg/mL) were prepared as Cal SLs but with addition of 1 g of a matrix mix (MM) containing common capsule filling ingredients(described in detail in Section 2.2.4) before vortex mixing. SLs were diluted with ethanol 96% (v/v) to final concentrations between 50 µg/mL (QC 1) and 2250 µg/mL (QC 4). As for blank and Cal samples, 50 µL of the respective diluted QC SLs were mixed with 950 µL MQ water to get WSs with resulting concentrations of: QC 1: 2.5 µg/mL, QC 2: 7.5 µg/mL, QC 3:75 µg/mL and QC 4: 112.5 µg/mL. Aliquots of SLs and diluted SLs were stored at -80° C.

2.2.3 Study sample preparation

Each of the capsules (casing and filling) were weighted with a Sartorius Research R 200D balance and subsequently placed in a 15 mL falcon tube. Batch and capsule number were labelled on the tubes to enable a correlation of mass and drug content to a specific capsule. Capsule size was determined using the length and width of a representative capsule of the particular batch. Damaged, broken or leaking capsules were excluded from further analysis to prevent potential bias.

For extraction of HC, capsules were carefully opened and both, content and casing separately transferred into the falcon tube. After addition of 5 mL ethanol 96% (v/v) to each falcon tube, they were vortex mixed for 0.5 min. Finally, the tubes were centrifuged for 10 min with a Heraeus Sepatech Megafuge 1.0R centrifuge at 2000 g. From each resulting supernatant, 2 samples containing 1 mL each were transferred into Eppendorf vials. One of these samples was directly frozen and stored at -80° C for potential reanalysis, while the other one was used for HC quantification. WS for HPLC analysis was obtained by mixing 50 µL of the sample with 950 µL MQ water. As a double quantification was performed, 2 WS from the respective sample were prepared and consecutively injected into the HPLC.

2.2.4 Development and validation of the quantification method

For quantification of HC in presence of FC, a new HPLC-UV method using a Thermo Scientific Ultimate 3000 HPLC was developed. To achieve sufficient selectivity, several mobile phases of acetonitrile (ACN) and Milli-Q (MQ) water (80:20 (v/v)-20:80 (v/v)) were investigated in gradient and isocratic mode. Flow rates were assessed from 0.8 mL/min over 1.0 mL/min and 1.2 mL/min. Investigated column oven temperatures ranged from 15° C over room temperature (RT) to 40° C, respectively. The injection volume was set to 2.5 µL. To quantify HC concentrations in QC and study samples, peak areas of Cal samples were analysed with a weighted linear regression ($1/C^2$) to account for deviations at lower concentrations. The resulting final assay was fully validated in accordance to the EMA Guideline on bioanalytical method validation [141].

2.2.4.1 Recovery from matrix and matrix effects

Due to different starting material e.g. HC containing tablets (including several ingredients) or pure HC and the chosen capsule filling, the investigated capsules may have contained various, diverse matrices. Therefore, separate evaluations of all possible matrices were not feasible. Ultimately, a MM containing equal parts of common capsule and tablet filling materials was used as a combined matrix for the following investigations as well as for QC sample preparation. Compounds were mannitol, sorbitol, saccharose, lactose, glucose, potato starch, corn starch, microcrystalline cellulose, carboxymethyl cellulose, talcum, highly disperse silicon dioxide, magnesium stearate and gelatine. Matrix effects were evaluated according to the EMA Guideline using Cal 2 and Cal 6 samples (6 replicates each) with and without MM. Both sets were analysed using an unpaired, two-sided t-test with α 0.05.

2.2.4.2 Recovery from capsules and tablets

To account for potential adhesion to capsule casing and potentially incomplete extraction from crushed tablets used as starting material, recovery from capsules and tablets was additionally investigated. For recovery from capsules, 50 mg MM each were weighed in 24 lower capsule casings (hard gelatine capsules size 1). To each lower capsule casing, one out of 4 different HC amounts ranging between 0.5 and 8 mg was added resulting in 4 levels with 6 capsules each containing similar amounts. Subsequently, MM was added until the lower casing was filled to approximately half of the total capsule volume. Capsules were closed and subsequently shaken to distribute HC in the MM. Half of the capsules (3 capsules of each dose level, in total 12 capsules) were directly analysed as study samples, while the other half was stored for one day prior to analysis. For recovery from tablets, 12 tablets, 6 from Jenapharm and 6 from Hoechst, containing 10 mg HC each were pulverised using an agate mortar to minimise drug loss. Subsequently, the resulting powder was completely transferred to falcon tubes. HC quantification from both dosage forms equated to study sample analysis (Section 2.2.3).

2.2.4.3 Lower limit of quantification and linearity

LLOQ and linearity were determined using 5 QC 1 samples and 3 sets of Cal samples (Cal 1-6) prepared as described in Section 2.2.2. Signal to noise ratio, accuracy and precision of QC 1 samples were determined for LLOQ evaluation, calibration function and coefficient of determination (R^2) for evaluation of linearity.

2.2.4.4 Accuracy and precision

To assess accuracy and precision, blank samples in duplicate, Cal samples in triplicate and QC samples in quintuplicate were prepared as described in Section 2.2.2 and analysed. The resulting calibration function was used for computation of QC sample concentrations. Relative error (RE) and coefficient of variation (CV) of QC samples were calculated for assessment of within-run accuracy and precision. Investigation was repeated 2 times to evaluate between-run accuracy and precision.

2.2.4.5 Carry-over

For the assessment of a carry-over effect, 6 blank samples and 5 samples of Cal 7 were prepared as described above (Section 2.2.2) and injected alternately in the HPLC. Peak areas of HC in subsequent blank samples were investigated.

2.2.4.6 Selectivity

To evaluate selectivity, a mixture of 2 mg HC and 20 µg FC (equating to common drug doses for the treatment of CAH) were prepared and analysed analogously to study samples. Resulting peaks were evaluated regarding peak separation.

2.2.4.7 Dilution integrity

The upper limit of quantification (ULOQ) equates to the highest soluble concentration of HC in water, impeding the investigation of different dilution patterns from higher concentrations. Therefore, the investigation of dilution integrity was not necessary.

2.2.4.8 Stability

Stability investigations were performed for SL with concentrations of 0.15 mg/mL, 2.25 mg/mL and 15 mg/mL HC and WS with concentrations of 7.5 µg/mL and 112.5 µg/mL HC. SLs were further diluted to WS equating QC 2, 3 and 4, respectively. Autosampler stability was investigated for WS only, since SLs are not stored under this respective condition. All solutions were prepared in quintuplicate with and without addition of MM and analysed using a freshly prepared set of Cal samples.

Short-term stability was assessed at RT and at 4° C after 0, 4 and 24 h. Long term stability was evaluated for SL samples at -80° C after 35 months. For autosampler stability, WS were analysed directly after preparation and again after 21 h at RT in the autosampler of the HPLC device. For evaluation of freeze-thaw-stability, SL and WS samples were prepared and directly aliquoted into 3 separate samples each. One aliquot per sample was analysed immediately after preparation, one was frozen once for at least 12 h at -80° C, subsequently thawed and analysed, one underwent this freeze-thaw cycle twice.

2.2.5 Assessment of uniformity, accuracy and correlation of drug content and capsule net mass

As prerequisite of a potential correlation of net mass and drug content for the subsequent clinical trial, a general characterisation of net mass and drug content was desired. Therefore, uniformity of net mass and drug content should be determined for each batch using the respective investigations in the EP 7th edition, method 2.9.5 [47] and method 2.9.6 [17], respectively. In addition, accuracy of drug content should be assessed. As no separate test method for drug content accuracy is described in the EP, the content uniformity evaluation was adapted by using the nominal content instead of the mean content per batch to calculate deviations. Recommended thresholds from the uniformity of drug content were also applied for the accuracy assessment. The newer combined investigation 2.9.40-uniformity of dosage units [18] was not used in the present project as only net mass uniformity and drug content accuracy but not drug content uniformity is included.

To evaluate uniformity of mass, the net mass of the capsules had to be determined. Deviating from the proposed procedure in the EP, the capsule casing was not weighted separately. It was added to the HC extraction step to ensure a complete recovery of the drug for content accuracy and uniformity which was considered to be more relevant. Capsule casings have a standardised size and mass with minimal variability [45], thus determination of capsule size also leads to a mean weight of a casing which was then subtracted from the total mass of each capsule. The mean net mass and the deviation from this mean was calculated for each batch. In accordance with EP, tolerable limits were ±10% for capsules with a mean net mass of <300 mg and ±7.5% for capsules with mean net mass >300 mg. Non-tolerable limits were ±20% and ±15%, respectively. Batches with capsule numbers of 18 to 22 capsules and acceptable uniformity have a maximum of 2 capsules exceeding tolerable limits (equating to 10% of the capsules) and 0 capsules non-tolerable limits. As recommended by the DAC/NRF for in process controls, the residual standard deviation (RSD) of the net masses per batch was calculated in addition to the investigations requested by the EP. The RSD should not exceed 5% [142]. Results of the evaluations of EP and DAC/NRF were compared.

For the evaluation of uniformity of drug content, the EP suggests the use of 30 capsules in a 2-step procedure. Considering an average number of only 20 capsules per batch in the present trial, the investigation had to be adjusted to a one step assessment of all available capsules. Drug content was assessed by quantifying HC via HPLC in 2 replicates. Both results were compared and in case of a deviation above ±10%, a reanalysis of the capsule was performed. Obtained HC amount were divided by 0.968 to correct the results by the analytical recovery of 96.8% assessed during bioanalytical method validation (Section 2.2.4). The mean of each batch and the deviation from this mean were computed. Deviations exceeding ±15% were tolerable for at maximum 2 capsules (10% of the investigated 20 capsules), deviations >±25% were considered to be non-tolerable as recommended by the EP [17] for 18 to 22 capsules per batch.

Accuracy of drug content was calculated as deviation from nominal drug content per capsule and per batch. Applied thresholds equated to the ones used for assessment of the uniformity of drug content. Tolerable limits of ±15% and non-tolerable limits of 25% were used after correction of all calculated HC contents with the analytical recovery. At maximum, 10% of the capsules per batch may exceed tolerable limits, no capsules may exceed non-tolerable limits as also applied for drug content uniformity. As the DAC/NRF published a recommendation of a surcharge of 10% for HC capsule for paediatric use [143] in 2017, the results for drug content accuracy were also theoretically evaluated

with this surcharge. It was added to the calculated amount of HC after correction by the analytical recovery.

Finally, a potential correlation of capsule net mass and HC content was assessed for the subsequent clinical trial. For this purpose, a correlation coefficient (R) was calculated using all obtained net masses and HC contents per batch. For R values between 0 and ±0.3 no correlation, between ±0.3 and ±0.5 a low correlation, between ±0.5 and ±0.8 a medium correlation and above ±0.8 a high correlation was considered.

2.3 Project II - Potential impact factors on relative recovery

2.3.1 *In vitro* microdialysis investigations

For detection of potential impact factors on RR values in the clinical trial, sampling parameters for the *in vitro* investigations were chosen in agreement with the parameters used in the clinical trial. Necessary adaptions are stated in detail in the respective section. The investigations were performed with 2 IVMS using microdialysis catheters with identical batches as used in the clinical trial. In order to mimic body conditions in all investigations, SM was kept at a constant temperature of 37° C. To avoid inhomogeneities, it was stirred at 700 min^{-1}. CMA 102 *in vitro* microdialysis pumps were used allowing investigations over longer time periods than with the battery-driven *in vivo* pumps, which were used in the clinical trial. For the investigation of a potential impact of flow rate change on RR, a CMA 107 *in vivo* microdialysis pumps were used as the *in vitro* pumps have no automatic flushing sequence after pump start.

Drugs were dissolved in 0.9% sodium chloride solutions and subsequently degassed. The resulting final concentrations matched those used for catheter calibration in the clinical trial: LIN 150 µg/mL, MER 20 µg/mL, MET 5 µg/mL, CZL 10 µg/mL, TIG 500 µg/mL, acetaminophen (AAP) 10 µg/mL and novamine sulfone sodium salt (NOV) 10 µg/mL. The drug-containing solutions were either used as SM (microdialysis settings) or as retroperfusate (retrodialysis setting). A drug-free isotonic sodium chloride solution was used as perfusate (microdialysis settings) or as SM (retrodialysis settings). As in the clinical trial, flow rate was set to 2 µL/min unless stated otherwise. Resulting RR values for each antibiotic agent, each scenario and each setting were calculated using Equation 1.2.

2.3.1.1 Impact of drug combination on relative recovery

To assess the impact of drug combinations on RR, investigations with 3 catheters were simultaneously performed collecting 5 consecutive samples in both, microdialysis and retrodialysis settings. In microdialysis settings, the catheters were inserted into a drug-containing SM and perfused with drug-free perfusate. Medium samples were taken directly before and after sample collection to enable calculation of RR values and to assess whether constant drug concentrations in the SM were ensured during the investigations. In retrodialysis investigations, drug-containing perfusate was pumped through the catheters which were inserted in drug-free SM. Retroperfusate samples were collected from the syringes prior to the equilibration and at the end of the experiment.

In total, 3 different antibiotic regimens with 2 to 3 scenarios were assessed: (i) LIN and MER, (ii) CZL and MET and (iii) TIG alone. For each evaluated drug, the single antibiotic agent was used as reference (Scenario 1) and compared with the combined antibiotic regimen used in the clinical trial (Scenario 2). In addition, both antibiotics were also combined with 2 analgetic agents, AAP and NOV (Scenario 3). For Regimen (iii), only scenario (1) and (3) were evaluated as it was a single treatment. In addition, investigations with tigecycline were performed with hydrolysed NOV due to analytical interferences.

As the impact of drug combinations might also depend on the absolute RR value, an increased flow rate of 5 µL/min was investigated for the model drug LIN which was expected to result in lower RR values closer to those expected *in vivo*. Using the higher flow rate, 2 different scenarios per setting were investigated: LIN alone (reference scenario) and a combination of LIN, MER, AAP and NOV (Scenario 3).

As descriptive summary statistics, the median RR value for each catheter (n=5 samples) and for all 3 catheters (n=15 samples) was calculated per antibiotic, scenario and setting. Variability was assessed as difference between minimum and maximum RR values per catheter for all 2 to 4 scenarios ("intra-catheter variability") and between the 3 catheters for all scenarios ("inter-catheter variability"), respectively. Magnitude and variability of RR values were compared to the ones from the respective reference scenario.

In addition, David Petroff from the University of Leipzig performed a statistical analysis for the impact of drug combination on RR values using linear mixed-effects models for all antibiotics to simultaneously evaluate the potential impact factors. The logarithm of RR was used as the dependent variable, scenario and setting were fixed terms in the model using the equation:

$$\log(RR) = a0 + a1 \bullet setting + a2 \bullet scenario + catheter + \varepsilon \qquad \textbf{Equation 1.4}$$

The coefficients α1 and α2 represent the fixed effects of setting and scenario, respectively, α0 the intercept and ε an error term. The applied catheter was considered to be a random-effect term on intercept. An additive residual error term was used. Tests for the fixed effects were based on an F-statistic using Satterthwaite's approximation for the degrees of freedom, based on the lmerTest package [144] in the software R version 3.4.1 [145]. P-values ≤ 0.05 indicated a statistically significant result.

2.3.1.2 Impact of microdialysis order on relative recovery

In the evaluation of drug combinations, an additional scenario for Regimen (i) and (ii) was included investigating an impact of microdialysis order on RR: Scenario 4. It compared RR values obtained for single antibiotic agents after microdialysis/retrodialysis of the second antibiotic drug of the regimen with RR values from the reference (Scenario 1). Taking into account, that Regimen (iii) consists of a single antibiotic treatment, only Regimen (i) and (ii) were investigated for the impact of microdialysis order. Sample collection, experimental design and subsequent data analysis including the statistical analysis by David Petroff were equal to the ones performed for the assessment of an impact of drug combination on RR.

2.3.1.3 Impact of air in the microdialysis catheter on relative recovery

The investigations of LIN alone (reference scenario) and of the combination of LIN, MER, AAP and NOV (Scenario 3) assessing the impact of drug combination on RR were repeated with freshly carbonated surrounding medium and perfusate/retroperfusate. In addition, air bubbles were forced in the connection of syringe and catheter to enable the evaluation of a maximum effect of air in the catheters. Subsequently, magnitude of mean RR, intra- and intercatheter variability were calculated and compared with the ones observed in the air-free solutions of the investigation of drug combinations.

2.3.1.4 Impact of flow rate change on relative recovery

To observe a direct effect of flow rate changes on RR, investigations using again LIN as a model drug (concentration of 150 µg/mL) were performed in retrodialysis setting. Linezolid was investigated in 5 consecutive investigations using the same catheter and a 107 *in vivo* microdialysis pump with an

automated flush program. The flush program is automatically activated as soon as the pump is started and sets a flow rate of 15 µL/min for 5 min before decreasing the flow rate to the chosen final flow rate. Retrodialysate samples were collected for 2 h in 15- or 10-min intervals depending on the applied final flow rate of 1 or 2 µL/min, respectively. Retroperfusate was collected from the syringe before the pump was started and after each investigation to proof a consistent LIN concentration. The calculated median RR values per flow rate and sampling interval were evaluated focusing on the time course of RR and the necessary time span to reach constant RR values.

2.3.2 Sample analysis

Drug quantification was performed at the University of Regensburg [146]. A Prominence Modular HPLC device equipped with an SPD-20A UV detector (Shimadzu, Germany) was used. The autosampler was cooled to 6° C. The column temperature was set to 40° C. A flow rate of 0.4 µL/min was applied. Samples were injected with a volume of 2 µL each. Samples containing MER, MET and CEF were separated onto an XBridge BEH C18 column (50x3 mm, 2.5 µm), samples with LIN and TIG onto a Nucleoshell RP18 column (100x3 mm, 2.7 µm). Both columns were preceded by a Nucleoshell RP18 guard column (4x3 mm, 2.7 µm). The isocratic mobile phases consisted of sodium hydrogen phosphate buffer and ACN and, in case of TIG, also of sodium octanesulfonate. Details of the mobile phases and detection wavelengths are summarised in Table 2.1. The processed samples were stable in the autosampler for at least 24 h. Further characteristics such as limit of quantification, precision and accuracy are summarised in Table 2.2

Table 2.1 Mobile phase, retention time and detection wavelength for quantification of 5 antibiotic and 2 analgetic agents in microdialysis samples

Drug	Mobile phase	Retention time [min]	Wave-length [nm]
Meropenem	0.1 M H_3PO_4/ACN 100:8 (v/v), pH 2.9*	2.6	300
Linezolid	0.1 M NaH_2PO_4/ACN 75:25 (v/v), pH 6.1*	2.5	254
Metronidazole	0.1 M H_3PO_4/ACN 80:12 (v/v), pH 3.1*	1.5	313
Cefazolin	0.1 M H_3PO_4/ACN 80:12 (v/v), pH 3.1*	2.5-2.6	260
Tigecycline	0.1 M H_3PO_4/ACN 80:12 (v/v), pH 3.1*, 700 mg/L SOS	3.9-4.3	350

ACN: acetonitrile, SOS: sodium octane sulfonate; v: volume

*apparent pH value (adjusted with NaOH or H_3PO_4)

Table 2.2 Limit of quantification, precision and between-run accuracy obtained from in-process control samples

Drug	Lower limit of quantification [µg/mL]	Precision		Accuracy, RE, %
		Intra-assay CV, %	Inter-assay CV, %	
Linezolid	50	0.7	1.9	102
Meropenem	400	1.7	2.8	100
Cefazolin	130	1.0	2.3	101
Metronidazole	80	1.9	5.2	101
Tigecycline	120	1.0	2.9	99.8

CV: coefficient of variation, RE: relative error

A weighted linear regression ($1/C^2$) using the peak areas of Cal samples was performed accounting for deviations at lower concentrations. The resulting calibration function was subsequently used for the calculation of QC samples as well as for samples obtained in the *in vitro* investigations.

2.4 Project III - Vancomycin monitoring in septic infants via microdialysis

2.4.1 Study design

The clinical trial was performed at the Dr. von Hauner Children's Hospital in Munich (number of vote of the local ethics committee: 524-13). Included patients were <1 year with a proven or suspected infection with gram-positive bacteria and therefore an indication for VAN therapy. Parents or care givers signed a written informed consent form prior to inclusion. Patients for which an immediate start of the VAN treatment was necessary, were excluded due to the time-consuming preparation of the clinical trial (e.g. catheter insertion, equilibration). In addition, a plasma minimum concentration ($C_{plasma,min}$) right before the third dose was obtained as recommended by hospital guidelines. The general design is shown in Figure 2.1 .

After local anaesthesia with a lidocaine patch, a 63 microdialysis catheter was implanted in the subcutaneous tissue of the lateral thigh and flushed with RS at a flow rate of 1 µL/min which was provided by a 107 microdialysis pump. After 0.5 h of equilibration and another 0.5 h of baseline sampling, VAN was infused over 1 h. The VAN single dose was 15 mg/kg body weight. Sampling interval was chosen based on the patient's body weight: infants <1200 g received a VAN infusion every 24 h, infants with a body weight between 1200 and 2000 g an infusion every 12 h and heavier infants every 8 h. Microdialysis samples were collected every 0.5 h over 24 h followed by catheter calibration via retrodialysis. Therefore, the following VAN dose was delayed by about 1.5 h (0.5 h equilibration, 1 h retrodialysis). As in Project IV, 2 consecutive retrodialysate samples were obtained after 0.5 h of equilibration (Section 2.5.1). The applied retroperfusate contained 100 µg/mL VAN. Finally, 1 baseline sample, 48 microdialysis samples, 1 retroperfusate sample and 2 retrodialysate samples were collected and stored at -80° C until analysis in the Department of Biochemistry and Clinical Pharmacy at the Freie Universitaet Berlin. Plasma samples were analysed within clinical routine in the biochemistry laboratory of the Dr. von Hauner Children's Hospital using an online TDM Vancomycin gen3™ test kit (Roche Diagnostics Operations) [147].

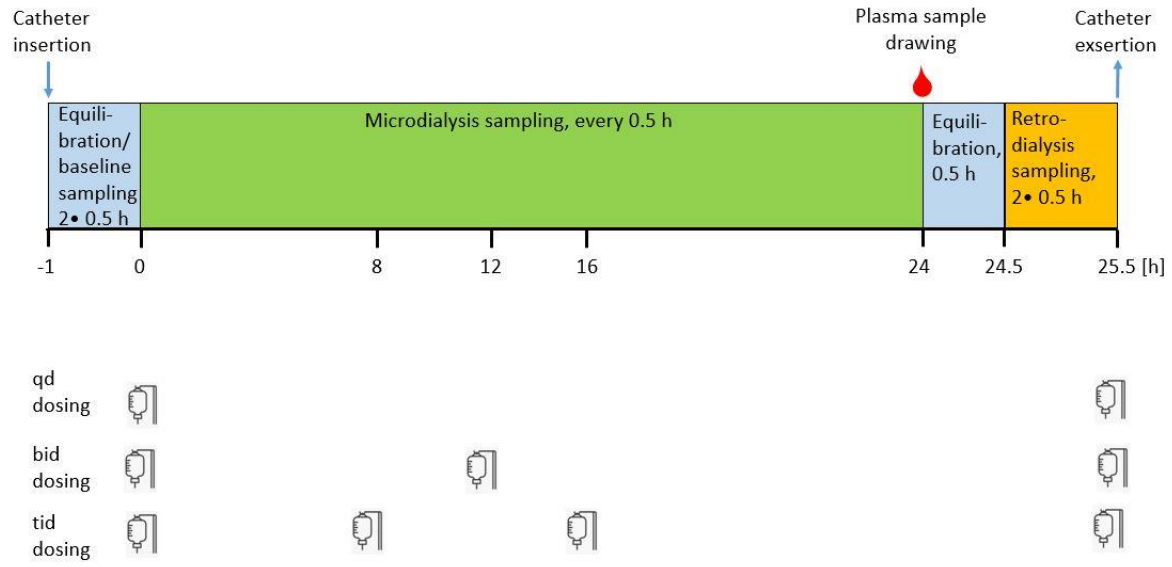

Figure 2.1 Design of the clinical microdialysis trial investigating feasibility of vancomycin sampling via microdialysis in subcutaneous tissue of septic infants

bid: twice daily, i.v. intravenous, qd: once daily, tid thrice daily

2.4.2 Preparation of calibration, quality control and blank samples for bioanalysis

Sample preparation from *in vitro* investigations consisted of a dilution step adding 20 µL MQ water to 40 µL of the sample. Blank samples contained 40 µL of RS and 20 µL of MQ water. Cal and QC samples were prepared from separate SLs containing 1 mg/mL VAN each. For Cal samples, SL was diluted with RS to concentrations of 2.50 µg/mL (Cal 1), 7.50 µg/mL (Cal 2), 20 µg/mL (Cal 3), 40 µg/mL (Cal 4), 60 µg/mL (Cal 5) and 80 µg/mL (Cal 6). Prior to quantification, 40 µL of the Cal sample was added to 20 µL MQ water, vortex mixed for 30 s and transferred to 96 well plates for analysis. QC samples were also diluted with RS to concentrations of 2.5 µg/mL (QC 1), 7.5 µg/mL (QC 2), 30 µg/mL (QC 3) and

75 µg/mL (QC 4) and further mixed with MQ water similar to Cal and blank samples. After preparation, SL, Cal and QC samples were divided in 50 µL aliquots and stored at -80° C until usage.

Blank, Cal and QC samples used for bioanalysis of clinical trial samples were diluted using a different pattern accounting also for the comedication. The blank samples consisted as before of RS. Cal and QC samples contained VAN and the concomitant drugs. A combined Cal SL was prepared from the respective single SLs resulting in concentrations of 30 µg/mL VAN, 30 µg/mL AAP, 5 µg/mL lidocaine, 20 µg/mL caffeine, 150 µg/mL MER and 300 µg/mL piperacillin. Subsequently, combined Cal samples were prepared by dilution of the SL with RS. Final VAN concentrations were 27 µg/mL (Cal 1), 81 µg/mL (Cal 2), 150 µg/mL (Cal 3), 300 µg/mL (Cal 4), 480 µg/mL (Cal 5) and 600 µg/mL (Cal 6). QC samples were prepared from a different SL containing the same drug concentrations as the combined Cal SL. They were diluted to the final QC samples with VAN concentrations of 27 µg/mL (QC 1), 81 µg/mL (QC 2), 150 µg/mL (QC 3) and 480 µg/mL (QC 4). Aliquots of all Cal and QC samples and the SLs were stored at -80° C until usage.

2.4.3 Preparation of samples for bioanalysis

For quantification of *in vitro* samples, 40 µL of the sample was diluted with 20 µL MQ and transferred to a 96 well plate. Samples from patient 1 to 5 were prepared by diluting 10 µL of the sample with 90 µL RS (10fold dilution). Concentrations above the ULOQ were reanalysed after dilution of 10 µL of the samples with 990 µL RS (100fold dilution) or, in case of retroperfusate and retrodialysate samples, with 14,990 µL RS (1500fold dilution). As various samples had to be reanalysed following this dilution scheme, sample preparation was changed for patient 6 to 9: 10fold and 100fold dilutions with RS were prepared and consecutively analysed for microdialysis samples and 100fold and 1500fold dilutions for calibration samples, respectively.

2.4.4 Development and validation of the quantification method for vancomycin from *in vitro* investigations

To quantify VAN in microdialysate samples from *in vitro* investigations, a HPLC method with UV detection was developed and validated. Several HPLC columns including Accucore C18 (100x2.1, 2.6 µm) and Hypersil® GOLD Phenyl column (100x4.6 mm, 3 µm), Eclipse XDB-C18® (4.6x150 mm, 5 µm) and LiCrospher® 100 RP18 (particle size 5 mm) were assessed. Mobile phases consisted of either methanol and dipotassium hydrogen carbonate [148] or of ACN, MQ water and trifluoric acid (TFA) in different ratios.

The final method was implemented on a Thermo Scientific Ultimate 3000 HPLC using an Accucore C18 column (100x2.1, 2.6 µm, heated to 40° C) and an Accucore C18 guard column (10x2.1, 2.6 µm). For sufficient separation, 2 mobile phases were applied in a gradient mode (Table 2.3) with a flow rate of 0.35 mL/min. Mobile phase A contained MQ water and 0.1% TFA, mobile phase B contained ACN, MQ water and TFA (40:60:0.1 (v/v/v). Samples were stored in the autosampler at room temperature. For analysis, 20 µL of the sample were injected and subsequently detected at a wavelength of 240 nm. For calculation of VAN from QC and *in vitro* samples, the peak areas of Cal samples were analysed with a weighted linear regression ($1/C^2$) enhancing accuracy for lower concentrations.

Table 2.3: Applied gradient of the bioanalytical method for quantification of vancomycin from *in vitro* investigations

Time [min]	Mobile phase B, %
0-1	10
1-7	Linear increase from 10-70
7-11	10

Validation of the final quantification method was performed in accordance with the EMA Guideline [141] regarding LLOQ, linearity, accuracy and precision as well as carry-over and stability.

For the evaluation of LLOQ, accuracy, precision and linearity, a combined investigation was performed including 2 blank samples, 5 LLOQ Cal samples, 3 Cal samples each for Cal level 2 to 6 and 5 QC samples each for QC levels 1 to 4. The resulting calibration function (weighted by $1/C^2$) was used for calculation of QC sample concentrations. For determination of LLOQ, within-run accuracy and within-run precision, RE and CV of QC samples were calculated. For between-run accuracy and precision, the investigation was performed 3 times in total. Linearity was assessed by the correlation coefficients of all Cal samples used to calculate the QC samples for accuracy and precision.

2.4.4.1 Carry-over

Cal 6 was injected 5 times alternately with blank samples to assess a potential carry-over. Peak areas of VAN in the blank samples were compared with peak areas of LLOQ samples.

2.4.4.2 Stability

Stability of VAN in RS was investigated over 24 h at room temperature, over 24 h in the autosampler, frozen over 29 months at -80° C and over up to 3 freeze-thaw cycles using 5 samples each of QC 2, QC 4 and SL. Accuracy, precision and deviation from freshly prepared QC and SL samples were calculated as recommended by the EMA [141].

2.4.5 Simultaneous quantification method for vancomycin and concomitant drugs from clinical trial samples

For the quantification of VAN in presence of the administered comedication, a previously in the working group developed and validated liquid chromatography assay with mass spectrometry detection (LC-MS/MS) was applied enabling also the determination of lidocaine, piperacillin, MER, caffeine, AAP and levetiracetam. In the present project, focus is on VAN, hence only VAN data from the method validation as well as from the clinical trial is shown here. Data of the comedication collected during the clinical trial will separately be analysed in a future project.

The analysis was performed onto an Agilent 1290 HPLC with a 6460 Triple Quadrupole. A volume of 2 µL of each sample was injected into the system. A Phenyl-Hexyl Poroshell 120 (2.1x100 mm, 2.7 µm) HPLC column was used for separation of VAN and ionic matrix ingredients. The latter ones were discarded before entering the detector. Again, 2 mobile phases were used in gradient mode (Table 2.4), mobile phase A consisted of MQ water with 0.1% FA and B of pure ACN. Flow rate was set to 0.4 mL/min, temperatures of autosampler and column oven were at 4 and 40° C, respectively. MS/MS parameters are summarised in

Table 2.5.

Table 2.6 lists mass to charge transitions for VAN in which the first transition was used for quantification, the second one for qualification. The total run time was 7 min and 10 s. Resulting peak areas of Cal samples were analysed with a weighted linear regression (1/C) enhancing accuracy for lower concentrations. The resulting calibration function was used to calculate QC and microdialysis study samples. Validation was performed following EMA recommendations [141]. Results are summarised in Table 2.7. Stability was not separately assessed as it was sufficiently proven in literature [148–150].

Table 2.4 Applied gradient of the bioanalytical method for quantification of vancomycin from clinical trial samples

Time [min]	Mobile phase B, %
0-1	2
1-4.5	Linear increase from 2-46.5
4.5-5	Linear increase from 46.5-95
5-6	95
6-6.10	Linear decrease from 95 to 2

Table 2.5 Detection parameters of the LC-MS/MS method for the quantification of vancomycin

Source parameters		Ion funnel parameters		Fragmentation parameters	
VCharging	500 C	Positive low pressure	RF 60	Fragmentor voltage	380 V
Capillary Voltage	3500 V	Negative low pressure	RF 60	Dwell time	20 ms
Sheath gas flow	12 L/min	Positive high pressure	RF 150	Cell accelerator voltage	4 V
Sheath gas heater	375° C	Negative high pressure	RF 90		
Nebulizer	25 psi				
Gas flow	13 L/min				
Gas temperature	120° C				

RF: refinement

Table 2.6 Mass to charge transitions used for identification and quantification of vancomycin

Precursor ion	Product ion	Collision energy [V]
725	144	12
725	100	24

Table 2.7 Validation parameters for vancomycin from clinical microdialysis samples

Validation parameter	Results for vancomycin
Calibration range (3 samples or 5 samples for LLOQ)	27-600 µg/mL
Within-run accuracy, RE (5 samples)	≤±5.10% (for LLOQ ≤±11.4%)
Between-run accuracy, RE (15 samples)	≤±2.49% (for LLOQ ±3.76%)
Within-run precision, CV (5 samples)	≤5.95% (for LLOQ ≤3.90%)
Between-run precision, CV (15 samples)	≤4.14% (for LLOQ ≤6.45%)
Dilution integrity, RE	±3.43%
Dilution integrity, CV	7.33%
Matrix effects, CV	≤12.7%

CV: coefficient of variation, LLOQ: lower limit of quantification, RE: relative error
Evaluations adapted from EMA Guideline for bioanalytical method validation [141]

To convert VAN concentrations from microdialysate study samples into ISF concentrations, the RR values were calculated using the mean of both retrodialysate concentrations (Equation 1.2). Missing values were replaced by the mean of all available RR values of the other patients. Resulting concentration-time courses in microdialysate and ISF were evaluated with regard to plausibility e.g. if concentrations increased after drug administration. Converted unbound maximum concentration in ISF ($C_{ISF,max}$) and unbound minimum concentration in ISF ($C_{ISF,min}$)/ last observed concentration in ISF ($C_{ISF,last}$) were compared to available literature. In addition, a potential correlation of plasma minimum concentrations ($C_{plasma,min}$) and corresponding C_{ISF} (from the sampling interval in which plasma sample was drawn) was investigated using a linear regression.

2.4.6 *In vitro* microdialysis investigations

All *in vitro* investigations were performed with the previously described IVMS (Section 1.3.1) enabling standardised investigations with limited sources of variability. SM was heated to 37° C to mimic body temperature and was additionally stirred with 700 min^{-1} to avoid inhomogeneities. For each set of sampling parameters, 3 to 5 consecutive samples were collected from 3 different 63 microdialysis catheters (membrane length 10 mm, MWCO 20 kDa) which were also used in the clinical trial. Two CMA 102 microdialysis *in vitro* microdialysis pumps were used to pump the perfusate through the catheters. Before start of sample collection, an equilibration time of at least 0.5 h was implemented in all *in vitro* investigations. RR values were calculated using Equation 1.1 and Equation 1.2. Additionally, the median RR value per catheter (n=5 samples) and for all 3 catheters (n=15 samples) were computed for each set of sampling parameters. Variability was assessed as difference between minimum and maximum RR values per catheter ("intra-catheter variability") and between the 3 catheters ("inter-catheter variability") in the respective investigation. To enable comparison of RR values from the different investigations with regard to magnitude and variability, a reference RR value obtained in RS (setting: microdialysis, VAN concentration: 50 µg/mL, flow rate: 1 µL/min) was established if not stated otherwise. Several investigations were performed assessing (I) the potential impact of flow rate on RR, (II) the potential impact of pH value of SM and perfusate on RR, (III) the potential impact of VAN concentration on RR, (IV) the potential impact of the diffusion direction on RR and (V) the potential impact of the perfusate composition on RR.

2.4.6.1 Impact of flow rate on relative recovery

3 different flow rates were evaluated in microdialysis and retrodialysis settings, 0.5 µL/min, 1 µL/min and 2 µL/min. SM and perfusate consisted of RS. The SM (microdialysis setting) or the perfusate (retrodialysis setting) were spiked with VAN in a concentration of 50 µg/mL. In microdialysis setting, 3 consecutive samples were collected per catheter and flow rate, in retrodialysis 5 consecutive samples. The mean and standard deviation (SD), intra- and inter-catheter variability (n=3/5 and n=9/15 samples, respectively) of the different flow rates were compared to each other.

2.4.6.2 Impact of pH value on relative recovery

As pH values in septic patients may be decreased, PBS buffers adjusted to either pH 7.0 or pH 7.4 were used to evaluate a potential impact of pH value on RR. Here, PBS buffer with pH 7.4 was used as reference. Investigations were performed with a flow rate of 1 µL/min and a VAN concentration of 50 µg/mL in SM (microdialysis setting) or perfusate (retrodialysis setting). 4 different scenarios were investigated: (i) unbuffered RS as perfusate and PBS buffer pH 7.0 as SM, (ii) unbuffered RS as perfusate and PBS buffer pH 7.4 as SM, (iii) PBS buffer pH 7.0 as perfusate and SM and (iv) PBS buffer pH 7.4 as perfusate and SM. Mean RR value (n=15 samples each) of Scenario (i) was compared to the mean RR value of Scenario (ii) as reference and analogously the mean RR value of Scenario (iii) with the one of Scenario (iv)) concerning magnitude as well as intra- and inter-catheter variability (n=5 and 15 samples, respectively).

2.4.6.3 Impact of drug concentration on relative recovery

For the assessment of an impact of drug concentration on RR, 3 different concentrations, 10 µg/mL, 50 µg/mL and 80 µg/mL were investigated. VAN was therefore solved in RS or PBS buffer pH 7.4 as perfusate or SM depending on the applied setting (microdialysis or retrodialysis). Flow rate was 1 µL/min. The magnitude of mean RR values (n=15 samples) as well as intra- and inter-catheter variability (n=5 and 15 samples, respectively) were calculated and compared to the abovementioned reference.

2.4.6.4 Impact of the diffusion direction on relative recovery

To evaluate a potential impact of the diffusion direction on RR, the magnitude of RR values (n=15 samples) in both settings was compared for different perfusate-SM combinations: (i) unbuffered RS as perfusate and SM, (ii) unbuffered RS as perfusate and PBS buffer pH 7.0 as SM, (iii) unbuffered RS as perfusate and PBS buffer pH 7.4 as SM, (iv) PBS buffer pH 7.0 as perfusate and SM and (v) PBS buffer pH 7.4 as perfusate and SM. A VAN concentration of 100 µg/mL was used in accordance with the catheter calibration in the clinical trial. The applied flow rate was 1 µL/min.

2.4.6.5 Impact of different perfusates on relative recovery

Three different perfusates (RS, PBS buffer with pH 7.0 and 7.4) were investigated in the previously described investigations. They were evaluated with regard of the consistency of resulting RR values with changing flow rate, VAN concentration and setting.

2.5 Project IV - Perioperative antibiotic prophylaxis in synovial fluid

2.5.1 Clinical trial

In total, 10 patients undergoing elective knee surgery were included in the trial and received CFX and LIN as PAP right after successful arthroscopy (ethics approval: EK-1063/2012, Eudra-CT Number 2012-000379-18). Drug concentrations in plasma, synovial fluid and muscle ISF were monitored subsequently for 8 h. Figure 2.2 shows the time table of drug administration and sample collection, Table 2.8 the comedication per patient

To enable the administration of CFX and LIN as short-term infusions and to allow blood sample drawing during the clinical trial, 2 i.v. cannulas were inserted in 2 different cubital veins. In addition, 2 microdialysis catheter (CMA 63, MWCO 20 kDa, membrane length 30 mm) were implanted, 1 in the synovial fluid and 1 in the ISF of the equilateral *musculus quadriceps femoris*. After an equilibration time of 0.5 h, baseline sampling was performed for another 0.5 h directly followed by 2 consecutive 0.5 h short-term infusions, 1 of 1500 mg CFX and subsequently 1 of 600 mg LIN. During the first 3 h after start of the CFX infusion, plasma and microdialysis samples were collected every 0.5 h, then every 1 h. Sample collection was finished after 8 h and followed by catheter calibration via retrodialysis. The retroperfusate, containing concentrations of 100 µg/mL of CFX and LIN each in RS, was prepared prior to the clinical trial, aliquoted and stored at -80° C until needed. For the calibration of both catheters, 1 aliquot was used. After an equilibration time of 0.5 h, 2 consecutive retrodialysate samples were collected over 0.5 h each as in Project III (Section **Fehler! Verweisquelle konnte nicht gefunden werden.**). After the calibration procedure, microdialysis catheters and i.v. cannulas were exserted.

For both, microdialysis sampling and retrodialysis calibration, a flow rate of 2 µL/min was applied. Per patient, 13-14 microdialysis samples (including equilibration time and baseline sampling) each from synovial fluid and muscular ISF, 12 plasma samples (always as duplicates), 1 retroperfusate and 2 retrodialysate samples were collected. Samples were stored at -80° C until analysis in the Department of Clinical Pharmacy and Biochemistry at the Freie Universitaet Berlin.

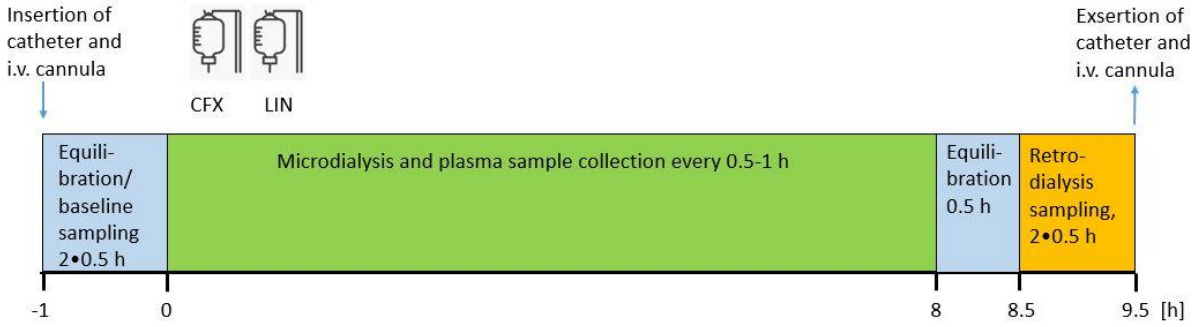

Figure 2.2 Design of the microdialysis clinical investigating concentrations of cefuroxime and linezolid in synovial fluid and muscle interstitial space fluid

CFX: cefuroxime, i.v.: intravenous, LIN: linezolid

Table 2.8 Administered comedication in the clinical trial investigating cefuroxime and linezolid after arthroscopic surgery

Patient	Comedication
ID 1	Acetaminophen, diclofenac sodium salt, enoxaparin sodium salt, esomeprazole, midazolam, propofol, tramadol
ID 2	Brotizolam, bupivacaine, diclofenac sodium salt, esomeprazole, midazolam, xylocaine
ID 3	Brotizolam, diclofenac sodium salt, enoxaparin sodium salt, fentanyl, novaminsulfone sodium salt, odansetron, pethidine hydrochloride, propofol, tramadol
ID 4	Brotizolam, diclofenac sodium salt, fentanyl, propofol
ID 5	Acetaminophen, dexamethasone, diclofenac sodium salt, enoxaparin sodium salt, fentanyl, odansetron, propofol, theophylline
ID 6	Diclofenac sodium salt, enoxaparin sodium salt, fentanyl, midazolam, novaminsulfone sodium salt, pethidine hydrochloride, trazodone hydrochloride, valproic acid sodium salt
ID 7	Diclofenac sodium salt, enoxaparin sodium salt, fentanyl, novaminsulfone sodium salt, pantoprazole, piritramide, propofol, tramadol
ID 8	Acetaminophen, enoxaparin sodium salt, fentanyl, metoclopramide, novaminsulfone sodium salt, odansetron, piritramide, propofol
ID 9	Acetaminophen, diclofenac sodium salt combined with orphenadrine citrate, enoxaparin sodium salt, fentanyl, novaminsulfone sodium salt, piritramide, propofol, tramadol
ID 10	Acetaminophen, clonidine, diclofenac sodium salt combined with orphenadrine citrate, fentanyl, nicotine, piritramide, propofol, remifentanyl, urapidil

ID: individual identifier

2.5.2 Bioanalysis of microdialysis samples

A previously developed bioanalytical method allowed the quantification of LIN and CFX from microdialysis samples with sufficient selectivity regarding the coadministered drugs in the clinical trial [151,152].

Sample preparation consisted of a dilution step adding 20 µL ACN to 30 µL of microdialysate and subsequent vortex mixing for 30 s. The diluted sample was then transferred to a 96 well plate for quantification. Blank samples were prepared by diluting 30 µL of RS with 20 µL of ACN. Similarly, Cal and QC samples were prepared using 30 µL of the respective sample. Depending on the coadministered drugs (Table 2.8), the quantification of CEFX and LIN had to be divided into 2 different methods deviating in the applied gradient and flow rate. Method I was used for patient 1-9 and Method II for patient 10, who received urapidil which hampered the selectivity of Method I. Method II allowed the determination of CFX and LIN in presence of all drugs administered to patient 10 but could not separate several drugs from the other patients, hence no general application of this method was possible. Drug separation was performed onto a Thermo Scientific Ultimate 3000 HPLC with an HPG-3200SD pump, a WPS-3000TSL autosampler, a TCC-3000SD column thermostat and a DAD3000 detector. Samples were cooled in the autosampler at 4° C and 20 µL each injected for analysis. For separation, 2 mobile phases were used in gradient mode: mobile phase A consisted of MQ water incl.

0.1% TFA and mobile phase B of MQ water and ACN 30:70 (v/v) incl. 0.1% TFA. The final gradients are summarised in Table 2.9. A column oven temperature of 35° C was chosen. Flow rates of 2 mL/min (Method I) and 1 mL/min (Method II) were used for the separation. Detection wavelengths of LIN and CFX were 251 nm and 271 nm, respectively. Peak areas of Cal samples were analysed with a weighted linear regression ($1/C^2$) increasing the accuracy of lower concentrations. QC and study sample concentrations were calculated afterwards using the resulting calibration function. Results of the successful validation according to the EMA Guideline on bioanalytical method validation [141] are summarised in Table 2.10 for CFX and in

Table 2.11 for LIN. Stability investigations were performed using Method I only as the quantification method has no impact on the analyte stability.

Table 2.9 Gradient of Method I and II for quantification of cefuroxime and linezolid from microdialysate samples

Method I		Method II	
Time table [min]	Mobile phase A, %	Time table [min]	Mobile phase A, %
0-0.5	95	0-0.5	95
0.5-10	Linear decrease to 55	0.5-2	Linear decrease to 40
10-10.5	Linear decrease to 0	2-6.5	40
10.5-15	0	6.5-8	Linear decrease to 0
15-15.5	Linear increase to 95	8-11.5	0
15.5-20	95	11.5-15	95

Table 2.10 Validation parameters for Method I and II quantifying cefuroxime from microdialysate

Validation parameter	Method I	Method II
Calibration range (3 samples or 5 samples for LLOQ)	0.30-125 µg/mL	0.30-125 µg/mL
Within-run accuracy, (5 samples)	RE ≤±4.57% (for LLOQ ≤±8.80%)	≤±7.87% (for LLOQ ≤±7.00%)
Between-run accuracy, (15 samples)	RE ≤±4.02% (for LLOQ -7.17%)	≤6.39% (for LLOQ ≤5.87%)
Within-run precision, (5 samples)	CV ≤4.55% (for LLOQ ≤6.55%)	≤4.3 7% (for LLOQ ≤4.68%)
Between-run precision, (15 samples)	CV ≤3.06% (for LLOQ ≤4.71%)	≤3.46% (for LLOQ ≤3.49%)
Short-term stab. after 24 h, (5 samples)	RE ≤±7.92%	
Autosampler stab. after 24 h, (5 samples)	RE ≤±5.65%	
Freeze-thaw stab. after 2 cycl., (5 samples)	RE ≤±12.6%	
Long-term stab. after 50 weeks, (5 samples)	RE ≤±13.2%	

CV: coefficient of variation, cycl.: cycles, LLOQ: lower limit of quantification, RE: relative error, stab.: stability
All evaluations according to EMA Guideline [141]

Table 2.11 Validation parameters for Method I and II quantifying linezolid from microdialysate

Validation parameter	Method I	Method II
Calibration range (3 samples or 5 samples for LLOQ)	0.3-25 µg/mL	0.3-25 µg/mL
Within-run accuracy, RE (5 samples)	≤±4.47% (for LLOQ ≤±9.58%)	≤±8.72% (for LLOQ ≤±8.07%)
Between-run accuracy, RE (15 samples)	≤±1.68% (for LLOQ -1.77%)	≤±5.83% (for LLOQ -4.31%)
Within-run precision, CV (5 samples)	≤4.73% (for LLOQ ≤3.47%)	≤4.57% (for LLOQ ≤4.94%)
Between-run precision, CV (15 samples)	≤3.91% (for LLOQ ≤7.27%)	≤4.91% (for LLOQ ≤6.14%)
Short-term stab. after 24 h, RE (5 samples)	≤±12.9%	
Autosampler stab. after 24 h, RE (5 samples)	≤±7.13%	
Freeze-thaw stab. after 2 cycl., RE (5 samples)	≤±4.13%	
Long-term stab. after 50 weeks, RE (5 samples)	≤±8.43%	

CV: coefficient of variation, cycl.: cycles, LLOQ: lower limit of quantification, RE: relative error, stab.: stability
All evaluations according to EMA Guideline [141]

2.5.3 Bioanalysis of plasma samples

2.5.3.1 Development of the quantification method for plasma samples

The existing bioanalytical methods for microdialysate had to be extended by a suitable sample preparation to enable the quantification of CFX and LIN from plasma samples. Therefore, different ways of protein precipitation using different ratios of plasma and ACN or methanol were evaluated regarding their extraction ability. Further steps as evaporation were included to achieve sufficient accuracy and precision for the quantification method.

2.5.3.2 Validation of the quantification method for plasma samples

The final sample preparation method was coupled with the previously described quantification and validated in accordance with the EMA Guideline [141].

2.5.3.3 Accuracy, precision, LLOQ and linearity

For the evaluation, 2 blank samples, 20 Cal samples each for CFX and LIN (5 samples for LLOQ level and 3 samples each for the higher levels of Cal 2-6) and 20 QC samples (5 samples for QC 1-4 containing LIN and CFX in combination) were analysed. Peak areas of Cal samples were analysed with a weighted linear regression ($1/C^2$). The resulting calibration function was subsequently used to calculate concentrations of QC and study samples. Cal LLOQ samples were evaluated regarding their accuracy

and precision. In addition, RE and CV of QC samples were calculated for assessment of within-run accuracy and within-run precision. For between-run accuracy and precision, the investigation was performed 3 times in total. Linearity was assessed using the correlation coefficients of all 3 runs.

2.5.3.4 Stability

Stability in plasma samples over 24 h at room temperature, over at least 12 h in the autosampler, frozen over 12 weeks and also over up to 2 freeze-thaw cycles was sufficiently demonstrated in literature for CFX [90,153–155] and LIN [105,156–160] and therefore not separately investigated.

2.5.4 Data analysis of study samples

RR values were calculated for each catheter and drug from the respective retroperfusate sample and the mean of the 2 consecutively collected retrodialysate samples using Equation 1.2. The RR values were used to enable the subsequent conversion of microdialysate concentrations in ISF concentrations according to Equation 1.3.

Considering that microdialysis samples reflect unbound concentrations, total plasma drug concentrations were converted to unbound plasma concentrations to allow a better comparison of the concentration-time profiles. For the conversion, total plasma concentrations were calculated using literature values on protein binding and the following equation.

$$C_{plasma,\ unbound} = C_{plasma,\ total} \bullet (100 - \text{plasma protein binding}/100) \qquad \text{Equation 1.5}$$

For CFX, a value of 33% was reported [89], for LIN newer publications stated a protein binding of 15% [22,106,107,161]. Concentration-time courses of microdialysate samples as well as of the converted ISF concentrations were visually inspected to describe basic pharmacokinetic parameters as C_{max}, T_{max} and C_{last} for each drug and body fluid on an individual and a population level.

2.6 Statistics

In general, statistical methods are described in the respective project and were calculated using different software as described in Section 2.7.

For assessment of central tendencies in Project I, III and IV, the arithmetic mean was used. In Project I, the assessment of uniformity and accuracy of HC in capsules, mean values were explicitly recommended by the EP. For microdialysis samples, mainly mean values are reported in literature e.g. [23,24,152,154,162–164,25,97,106,110,111,113,118,151] although sample number is usually too low to test for normal distribution. In a few publications, median was used instead or additionally to the mean [164,165]. In the present thesis, decision for mean or median as index for central tendency was made by the magnitude of RR values. In Project II, the median was used as RR values were close to 100% hampering normal distribution, in Project II and IV, values were lower making a normal distribution more plausible and enabled the comparison of present results with the ones from other publications.

Uncertainty of the mean and median were characterised by RE. Data dispersion was described by SD, RSD also called CV. The confidence interval (CI) was used as an interval estimate likely to contain the investigated parameter. To describe variability in microdialysis samples within a single catheter or from several catheters, intra- and inter-catheter ranges were used, respectively.

For linear regression of bioanalytical data, describing the relationship between drug concentration and signal height/peak area, R^2 was calculated by applying the least square method. Usually, the linear

regression was weighted by $1/C^2$. For the bioanalytical LC-MS/MS method determining VAN concentrations in clinical trial samples, a weighing of 1/C was used. Data was also graphically assessed using goodness of fit plots.

In addition, further statistical analysis was performed using appropriate statistical tests as Student's t-test or the Grubbs outlier test. A significance level of α=0.05 was applied unless otherwise stated.

2.7 Software

Microsoft® Office, 2013 and 2016	Microsoft Corporation, Redmond, Washington, USA
Chromeleon®, version 7.2 and 7.3	ThermoFisher Scientific Chromatography Data System, Waltham, USA
Mass Hunter, version B06.00	Agilent Technologies, Santa Clara, USA
R, version 2.15.3 to 3.5.3	R Core Team (2017), Vienna, Austria
R Studio, version 0.97.332 to 1.1.463	RStudio Team (2016), Inc., Boston, USA

3 Results

3.1 Project I - Uniformity and accuracy of compounded capsules

3.1.1 Development of the quantification method for hydrocortisone

The final quantification method for HC in presence of FC was able to determine HC in the range of 2.5 µg/mL to 150 µg/mL from WS. Adequate selectivity, reproducible retention times and peak areas were achieved.

Separation was performed on a Thermo Scientific Ultimate 3000 HPLC with an Agilent Eclipse XDB C18 column and a Merck LiCrospher C18 guard column using a mobile phase of MQ water and ACN 70:30 (v/v) in an isocratic mode. Flow rate was set to 0.8 mL/min and the injection volume to 2.5 µL. Temperature of autosampler and column oven were fixed to RT and 15° C, respectively. HC was detected after 7 min at a wavelength of 246 nm.

3.1.2 Validation of the quantification method for hydrocortisone

The method validation was successfully performed in accordance to the respective EMA Guideline [141]. Results of accuracy, precision and stability are displayed in Table 3.1, Table 3.2 and Table 3.3. The method was suitable for determination of HC from capsules in presence of FC.

3.1.2.1 Recovery from matrix and matrix effects

Recovery from matrix was investigated by comparison of samples containing MM versus samples containing only extraction fluid as matrix. According to the performed two-sided, unpaired t-test, no significant difference was found between both matrices (p=0.07). A small peak with a similar retention time as HC was observed in samples containing pure MM. The peak area of this interference was always below 1% of the LLOQ HC peak and was therefore considered to be irrelevant. In summary, all investigations regarding a potential impact of MM on the quantification of HC from the extraction fluid showed comparable results for samples with and without MM (maximum deviation <1% of the nominal concentration (C_{nom})) and therefore no effect of the matrix.

3.1.2.2 Recovery from capsules and tablets

A mean recovery of HC of 96.8% (range 91.8%-105%) was observed across all capsules. Results from both analysis days were comparable: 97.4% at day 1 and 96.3% at day 2. Therefore, a potential adsorption over time of HC to the capsule casing was considered to be negligible especially as the casing were added to the extraction fluid. Calculated recoveries from tablets ranged from 64.9% to 72.6%, mean recoveries were 68.4% (Jenapharm) and 66.9% (Hoechst). These results showed an incomplete extraction from tablets but not from capsules.

3.1.2.3 Lower limit of quantification and linearity

Lower limit of quantification was set to 2.5 µg/mL enabling quantification of capsules containing 0.25 mg HC which was 50% of the lowest nominal HC content observed in the trial. Signal-to-noise ratio, accuracy (RE) and precision (CV) were ≥76.4, ≤4.42% and ≤3.31%, respectively and thus in accordance with the recommendations of the EMA Guideline on bioanalytical method validation [141].

3.1.2.4 Accuracy and precision

As shown in Table 3.1, within-run accuracy was ≤±10.8% and ≤±2.42% for LLOQ and for higher QCs. Between-run accuracy was ≤±10.9% and ≤±1.19%, respectively. Within-run precision was ≤5.71% for LLOQ and ≤4.42% for QC 2-4; between-run precision was ≤5.14% and ≤2.98%, respectively. Therefore, accuracy and precision were in accordance with the EMA Guideline.

Table 3.1 Accuracy and precision obtained for the analytical method quantifying hydrocortisone in presence of fludrocortisone

QC	Accuracy, RE %		Precision, CV %	
	Maximum within run, day 1-3	Between run, day 1-3	Maximum within run, day 1-3	Between run, day 1-3
1	±10.8	-10.9	5.71	5.14
2	±1.16	-0.24	4.42	2.98
3	±2.42	-1.19	2.34	2.70
4	±2.35	-0.56	2.02	2.38

CV: coefficient of variation, QC: quality control, RE: relative error

Validation parameters chosen according to the EMA Guideline for bioanalytical method validation [141]; for calibration range n=3 samples per calibration level, for LLOQ n=5 samples; for within-run accuracy, within-run precision and stability investigations n=5 samples; and for between-run accuracy and between-run precision n=15 samples

3.1.2.5 Carry-over

No carry-over was observed. Peaks detected in blank samples after injection of a high Cal sample had maximum peak areas of 0.0028 $mAu \bullet min^{-1}$ equating to less than 1% of observed peak areas for LLOQ samples during validation.

3.1.2.6 Selectivity and dilution integrity

No overlap of HC and FC was detected using the final method, proofing sufficient selectivity. The highest Cal sample equated to the highest solvable drug concentration in water of 15 mg/mL [166], therefore no different dilution patterns from higher concentrations needed to be evaluated.

3.1.2.7 Stability

Detailed results of the stability evaluations are displayed in Table 3.2 and Table 3.3. Results of short-term stability at RT and 4° C were merged since magnitudes were in similar ranges as well as results of freeze-thaw stability for cycle 1 and 2.

Table 3.2 Short-term stability data obtained for the analytical method quantifying hydrocortisone in the presence of fludrocortisone

Investigation		Mean deviation from freshly prepared samples % (RE, %)	
	Sample	Without MM	With MM
After 4 and 24 h* at RT	SL 1 (C=0.15 mg/mL)	2.42 (1.81)	0.19 (6.31)
	SL 2 (C=2.25 mg/mL)	1.19 (-3.53)	2.92 (-2.89)
	SL 3 (C=15 mg/mL)	-2.28 (0.85)	-4.55 (2.47)
	WS 1 (C=7.5 µg/mL)	6.03 (11.1)	2.64 (8.91)
	WS 2 (C=112.5 µg/mL)	-6.07 (-10.5)	4.17 (-1.70)
After 4 and 24 h* at 4 C	SL 1 (C=0.15 mg/mL)	3.97 (5.21)	2.65 (8.41)
	SL 2 (C=2.25 mg/mL)	2.97 (-0.22)	1.82 (-2.65)
	SL 3 (C=15 mg/mL)	-2.81 (-4.84)	-4.46 (3.47)
	WS 1 (C=7.5 µg/mL)	4.26 (5.50)	2.96 (6.26)
	WS 2 (C=112.5 µg/mL)	3.96 (0.73)	3.71 (-0.84)

C: Concentration, MM: Matrix mix, RE: relative error, RT: room temperature, SL: stock solution, WS: working solution

*mean of all samples stored for 4 and 24 h

Short term stability evaluation led to mean deviations from HC concentrations in freshly prepared solutions for SL samples without MM of ≤±3.97% (C=0.15 mg/mL), ≤±2.97% (C=2.25 mg/mL) and ≤±2.81% (C=15 mg/mL) after 4 and 24 h. Mean deviations from freshly prepared SL samples with MM were in a comparable range: ≤±2.65% (C=0.15 mg/mL), ≤±2.92% (C=2.25 mg/mL) and ≤±4.55% (C=15 mg/mL). Mean inaccuracy of all SL samples was ±0.48% and ±3.33% after 4 and 24 h, respectively.

Mean deviations from freshly prepared WS samples without MM were ≤±6.03% (C=7.5 µg/mL) and ≤±6.07% (C=112.5 µg/mL) after 4 and 24 h. WS samples with MM deviated from freshly prepared WS samples at maximum by ±2.96% (C=7.5 µg/mL) and ±4.17% (C=112.5 µg/mL). Mean inaccuracy for all WS samples was ±1.42% and ±2.98% after 4 and 24 h, respectively.

Table 3.3 Autosampler, long-term and freeze-thaw stability data obtained for the analytical method quantifying hydrocortisone in the presence of fludrocortisone

Investigation		Maximum deviation from freshly prepared samples % (RE, %)	
	Sample	without MM	with MM
Autosampler stability after 21 h	WS 1 (C= 7.5 µg/mL)	13.6 (17.0)	19.9 (25.5)
	WS 2 (C= 112.5 µg/mL)	15.3 (10.8)	14.4 (-0.22)
Long-term stability after 35 months at -80° C	SL 1 (C=0.15 mg/mL)	-8.06 (-7.21)	-11.1 (-8.53)
	SL 2 (C=2.25 mg/mL)	-5.48 (-8.20)	-3.91 (-8.18)
	SL 3 (C=15 mg/mL)	4.65 (2.43)	2.19 (1.19)
Freeze-thaw stability after up to 2 freeze-thaw cycles	SL 1 (C=0.15 mg/mL)	13.1 (1.67)	14.8 (1.77)
	SL 2 (C=2.25 mg/mL)	10.6 (6.52)	12.0 (3.32)
	SL 3 (C=15 mg/mL)	11.3 (3.57)	13.6 (3.93)
	WS 1 (C= 7.5 µg/mL)	-12.5 (-13.3)	-14.5 (-14.6)
	WS 2 (C= 112.5 µg/mL)	-7.42 (-5.18)	-6.85 (-8.62)

C: Concentration, MM: Matrix mix, RE: relative error, RT: room temperature, SL: stock solution, WS: working solution

After storage of WS samples in the autosampler for 21 h, mean deviations of ±13.6% (C=7.5 µg/mL) and ±15.3% (C=112.5 µg/mL) were found for WS without MM and ±19.9% (C=7.5 µg/mL) and ±14.4% (C=112.5 µg/mL) for WS with MM. Deviations are partially exceeding the acceptance criteria of the EMA Guideline. We assumed that the high deviations were caused by evaporation through the hole produced by the injection needle of the HPLC device during first injection. Hence, all subsequently investigated samples during validation and trial were covered by a new sealing foil. In addition, 2 rows of calibration samples were subsequently used, 1 at the beginning and 1 at the end of each analysis day to ensure stability over the time period of study sample analysis. Values of both sets were compared and together used for the calculation of HC concentrations in the samples. In addition, storage of samples in the autosampler was limited to 15 h. All further investigations during validation and study analysis showed no significant difference between calibrations samples of both sets, therefore we proved a sufficient stability in the autosampler for the time span of the respective analyses.

Samples with and without MM were comparable regarding their long-term stability. Without MM, mean deviations from freshly prepared SL samples were -8.06% (C=0.15 mg/mL), -5.48% (C=2.25 mg/mL) and 4.65% (C=15 mg/mL). With MM, mean deviations of -11.1%, -3.91% and 2.19% were observed, respectively.

Results for freeze-thaw stability were in a similar range for samples with and without MM. Deviations of SL samples in HC content after up to 2 freeze-thaw cycles compared to fresh samples were 13.1%, 10.6% and 11.3% for concentrations of 0.15, 2.25 and 15 mg/mL without MM and 14.8%, 12.0% and 13.6% with MM, respectively. WS samples without MM showed deviations of -12.5% (C=7.5 µg/mL) and 7.42% (C=112.5 µg/mL), compared to of -14.5% (C=7.5 µg/mL) and -6.85% (C=112.5 µg/mL) with MM, respectively.

All stability investigations except autosampler stability were successfully performed according to the EMA Guideline. Autosampler stability was separately proven by calibration samples during validation and study sample analysis. Hence, the bioanalytical method is sufficiently reliable, accurate and precise to be used in sample analysis.

3.1.3 Clinical trial

3.1.3.1 Data basis of the clinical trial

In total, 62 batches with 1231 capsules were sent in by care givers. Of these, 54 batches with 1,079 capsules could be analysed completely. Due to several reasons, single capsules or complete batches had to be excluded as displayed in the flow chart in Figure 3.1 . The vast majority of batches were delivered in simple envelopes, therefore 7 capsules arrived broken and had to be excluded. 1 batch had a capsule filling with olive oil showing significant leakage depending on the capsule position during transport and storage. In addition, HC was not extractable from this matrix, hence it was also excluded. 1 batch consisted of HC containing sachets which therefore did not met the inclusion criteria of the clinical trial. Further 2 batches were excluded due to the very low number of capsules (both n=5) making statistical analysis or evaluation based on EP impossible. Finally, 58 batches with 1.159 capsules were analysed regarding uniformity of net mass.

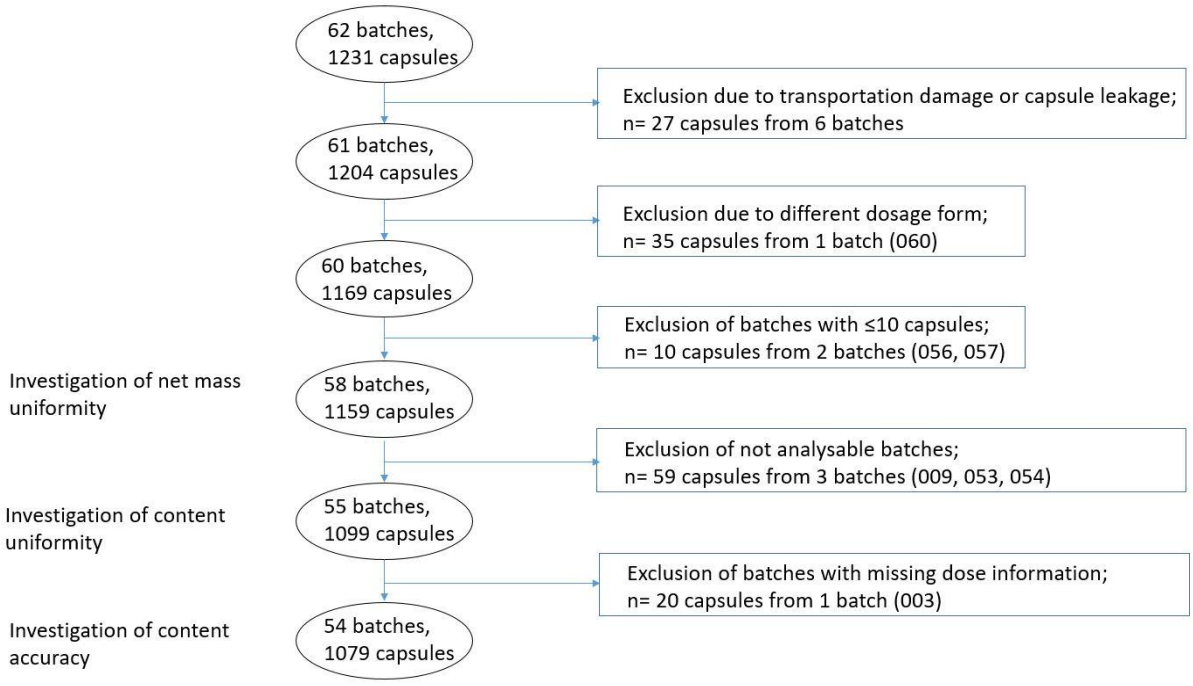

Figure 3.1 Flow chart showing the amount of excluded study samples and the underlying reasons for exclusion or all performed investigations

After HPLC analysis, 3 batches had to be excluded, 1 due to analytical problems and 2 consecutive batches, in which unknown peaks instead of HC peaks were observed. For further analysis of the unknown substance, extraction solutions (3 samples per batch) were diluted to a concentration of 100 ppm and analysed using an Agilent 6530 QTOF and the Agilent Forensic Tox database. Due to the resulting sum formula $C_{23}H_{32}O_6$, hydrocortisone acetate was assumed. To confirm the hypothesis, a reference sample of hydrocortisone acetate was also analysed with an Agilent QTOF. Spectra of reference and study samples were identical, proving hydrocortisone acetate was compounded instead of HC.

Content uniformity was successfully assessed for 55 batches with 1,099 capsules. These batches were additionally investigated regarding a potential correlation of net mass and HC content. 1 batch had no dosing information and therefore had to be excluded for investigation of drug content accuracy resulting in the evaluation of 54 batches with 1,079 capsules.

3.1.3.2 General characterisation of study samples

The following section describes all capsules and batches which were included at least for 1 investigation. Table 3.4 summarises these results. In total, 58 batches with 1159 capsules were characterised. The mean number of capsules per batch was 19.4 with a range of 18-22 capsules. For 4 batches, less than 20 capsules could be analysed due to transportation damage of single capsules as described in Figure 3.1.

Table 3.4 Characteristics of study samples included in at least 1 of the investigations

	Mean/main fraction	Range/minor fractions
Number of capsules per batch	19.4	18-22
Capsule size	1	4-000
Capsule filling material	Mannitol mixed with highly disperse silicon dioxide	Lactose, mannitol, glucose, rice starch
Total capsule mass [mg]	367	170-417
Capsule net mass [mg]	288	122-341
Calculated HC content [mg]	2.08	0.42-7.84
Nominal HC content [mg]	2.24	0.5-7

HC: hydrocortisone

Capsules for compounding have a standardised size ranging from 4 (smallest size) to 000. In the trial, capsule sizes from 4 to 0 were determined. The majority of batches had a capsule size of 1 (41 of 58 batches; 70.1%; n=816 capsules). Size 0 and 2 were used in 7 (12.1%; n=138 capsules) and 6 batches (10.3%; n=124 capsules), respectively. Capsules larger than 0 were not utilized, smaller ones i.e. size 3 and 4 were only used in 2 batches each (3.45%; n=41 and 40 capsules).

Capsule filling material was stated for 42 of 58 batches (72.4%). The majority of capsules were compounded with mannitol and highly disperse silicon dioxide (n=20, 47.6%), lactose (n=16, 38.1%), or only mannitol (n=3, 7.1%). Other capsule fillings were glucose (n=1, 2.4%) and rice starch (n=1, 2.4%).

The mean total mass of the capsules was 367 mg with a range of 170 to 417 mg and a mean net mass of 288 mg ranging from 122 to 341 mg. Calculated HC content showed a mean of 2.08 mg and a large range of 0.42 mg to 7.84 mg. Nominal HC content was in average 2.24 mg HC (range: 0.5-7 mg).

For the majority of batches, a low nominal content of 1 mg HC (21 of 61 batches, 34.4%), 2 mg HC (15 of 61 batches, 24.6%) and 3 mg HC (9 of 61 batches, 14.8%) was stated. Only 9 batches (9.8%) had a nominal content between 3.5 and 7 mg HC. As expected, doses which may alternatively be achieved by dividing the commercially available tablets containing 10 mg of HC were rare: only 1 batch (1.64%) had a nominal content of 5 mg and 4 batches (6.56%) of 2.5 mg.

3.1.4 Study results on capsule level

3.1.4.1 Uniformity of net mass

Uniformity of net mass was assessed for 1,159 capsules. For all capsules, deviation from mean net mass per batch was calculated as described in Section **Fehler! Verweisquelle konnte nicht gefunden werden.** and ranged from -18.0% to 27.6%. The majority of capsules, 1083 (93.4%) were within the tolerable limits given by the EP of ±7.5% (mean net mass >300 mg, see Figure 3.2) or ±10% (mean net mass <300 mg, see Figure 3.3). Overall, 73 capsules (6.30%) exceeded tolerable limits and 3 capsules (0.26%) non-tolerable limits of ±15% and ±20% for mean net masses of <300 mg and >300 mg, respectively.

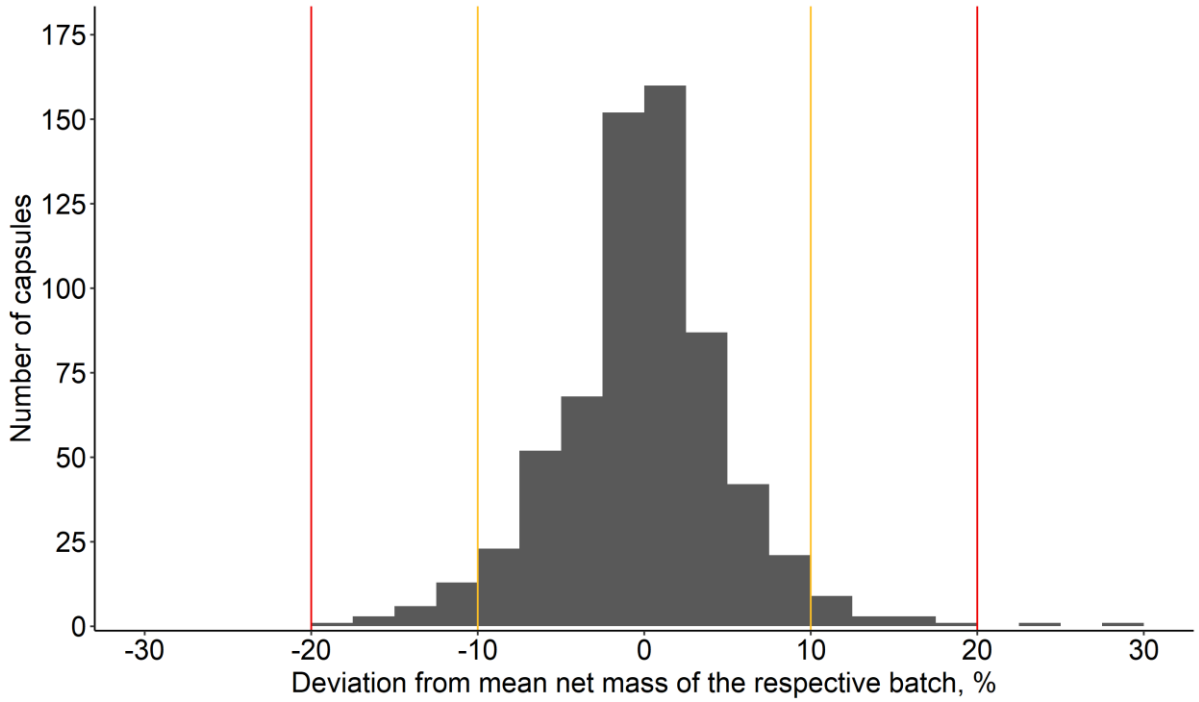

Figure 3.2 Histogram showing deviations of net mass per capsule from mean net mass of the respective batch (mean net mass <300 mg)

Yellow lines highlight tolerable limits and red lines non-tolerable limits as required by the European Pharmacopeia [47]

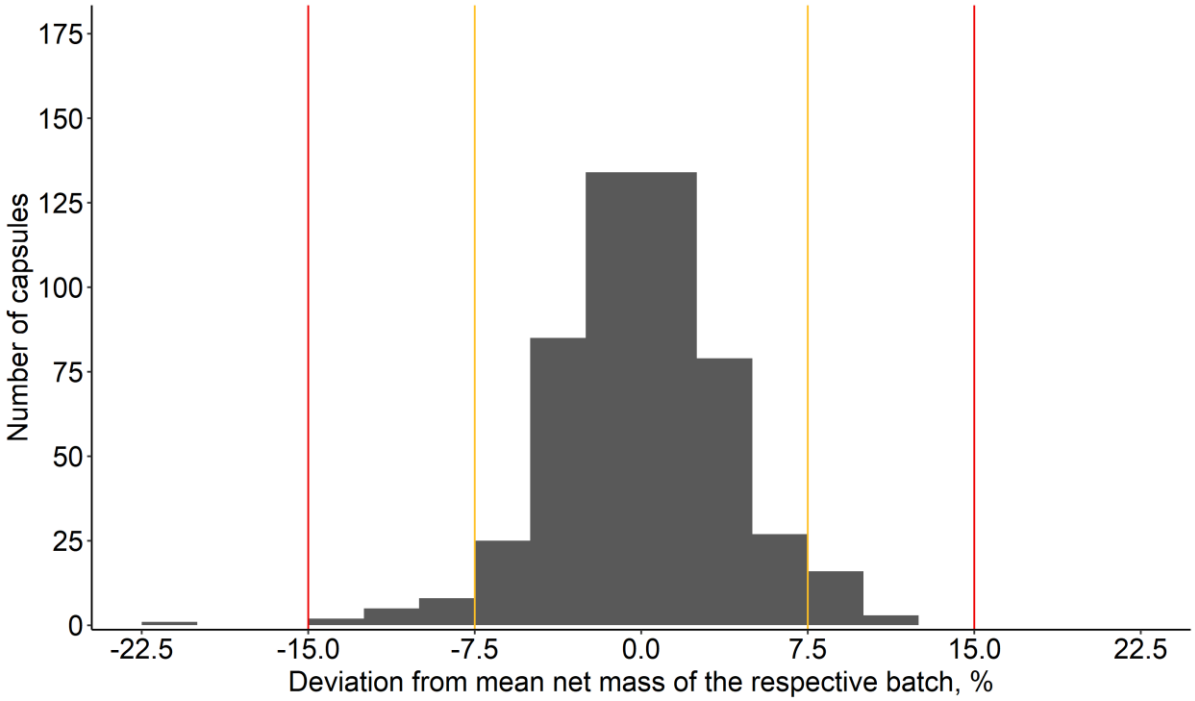

Figure 3.3 Histogram showing deviations of net mass per capsule from mean net mass of the respective batch (mean net mass of >300 mg)

Yellow lines highlight tolerable limits and red lines non-tolerable limits as required by the European Pharmacopeia [47]

3.1.4.2 Uniformity of drug content

Uniformity of drug content was successfully investigated for 1,099 capsules. All drug contents were corrected for the analytical recovery of 96.8%. Deviation of the calculated drug content from the respective mean drug content of the batch ranged from -46.7% to 203%. Overall, 1,056 of the capsules (96.1%) were within the accepted limits of ±15% deviation from mean drug content of the batch. Tolerable deviations between ±15% and ±25% were found for 21 capsules (1.91%) and non-tolerable deviations for 22 capsules (2.00%, Figure 3.4).

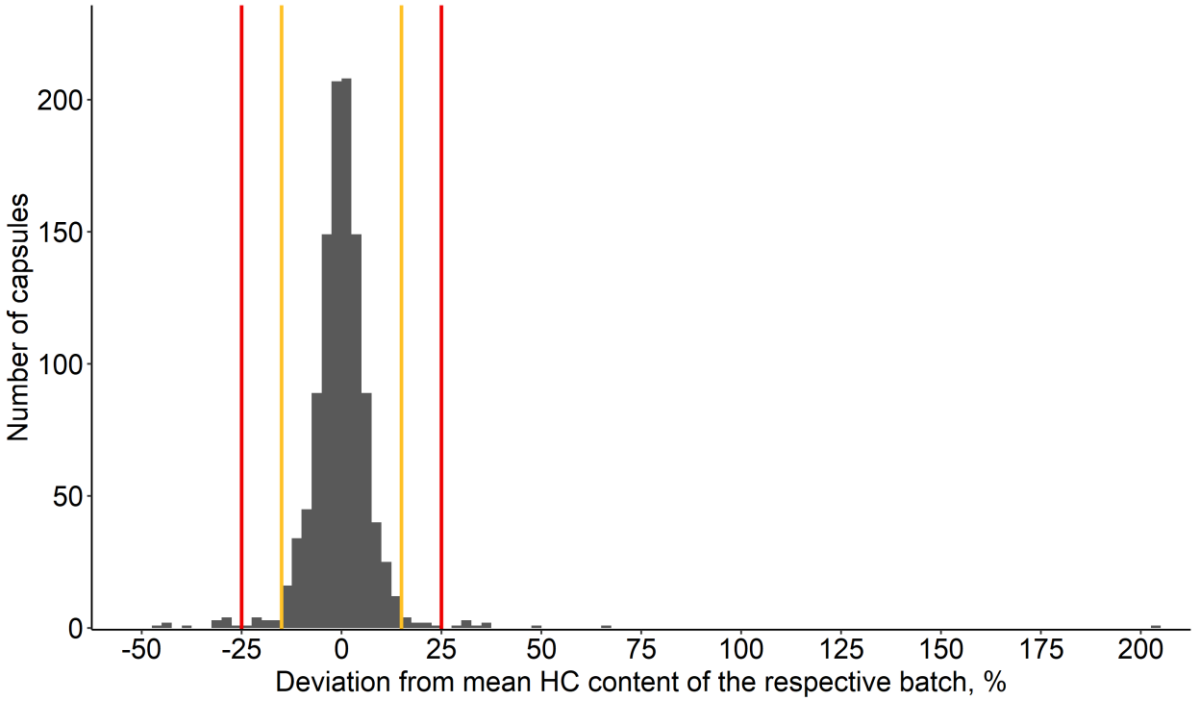

Figure 3.4 Histogram showing deviations of the investigated capsules from the mean hydrocortisone content of the respective batch

HC: hydrocortisone

Yellow lines display tolerable limits and red lines non-tolerable limits as required by the European Pharmacopeia [17]

3.1.4.3 Accuracy of drug content

For 1,079 capsules, accuracy of HC content could be determined. All drug contents were corrected by the analytical recovery and compared to their respective nominal content. Deviations range from was -46.7% to 213%. Only 704 capsules (65.2%) were within the acceptable deviation of <±15% from nominal content. For 280 capsules (25.9%), calculated deviations were between ±15 and ±25%, for 95 capsules >±25% (8.80%) and therefore outside the tolerable limits, respectively. Overall mean deviation was -10.8% which is also seen in the subgroup analysis of exceeding capsules (>±15%): only 10 capsules had a too high content but 365 capsules a too low content (Figure 3.5).

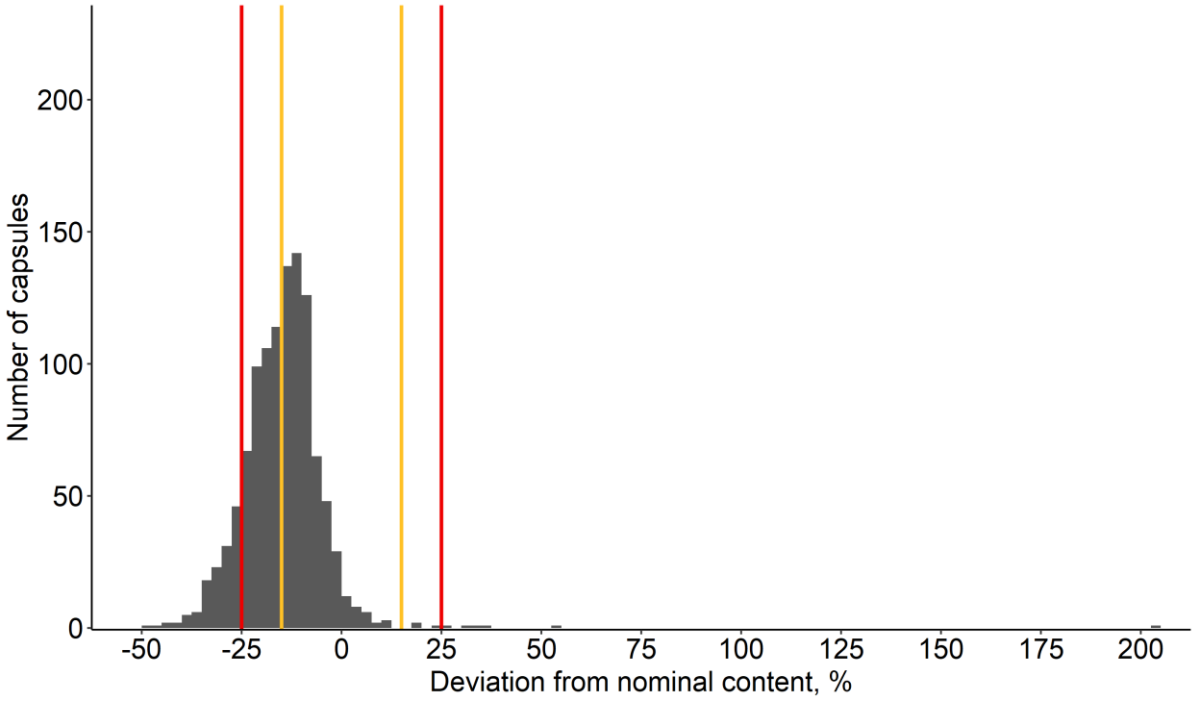

Figure 3.5 Histogram showing deviations of the investigated capsules from the nominal HC content of the respective batch

HC: hydrocortisone

Yellow lines display the applied tolerable limits and red lines non-tolerable limits

The results were also calculated using the 10% surcharge as recommended by the DAC/NRF since 2017 [143]. As expected, more capsules showed acceptable deviations. In the acceptable range of ±15% deviation from nominal content, 921 capsules (85.4%) would be obtained. Lower drug amounts would have been found in 124 capsules: 103 capsules (9.55%) with deviations between -15% and -25% and 21 capsules (1.95%) with <-25%. An increased number of capsules would show HC amounts above the nominal content. Deviations between -15% and -25% were calculated for 22 capsules (2.04%) and deviations >25% for 12 capsules (1.11%).

3.1.5 Study results on batch level

3.1.5.1 Uniformity of net mass

Uniformity of net mass was evaluated for 58 batches (Figure 3.6 and Figure 3.7) of which 47 batches (81.0%) met the acceptance criteria of the EP. Overall, 11 batches (18.9%) missed the specifications of EP. Of these batches, 5 (8.62%) had 3 or 4 capsules with a deviation between tolerable and non-tolerable limits and 3 (5.17%) had 5 to 10 capsules. The remaining 3 batches (5.17%) had either at least 1 capsule exceeding non-tolerable limits or more than 10 capsules with deviations outside tolerable limits concluding as lack of uniformity.

Using the RSD per batch as recommended by DAC/NRF, for 14 batches failed due to RSD values >5%. The results are in line with the ones from the EP. From the 14 batches failed according to DAC/NRF, 10 failed also the uniformity of mass investigation according to EP. Only 1 batch did not meet acceptance criteria of the EMA but of the DAC/NRF.

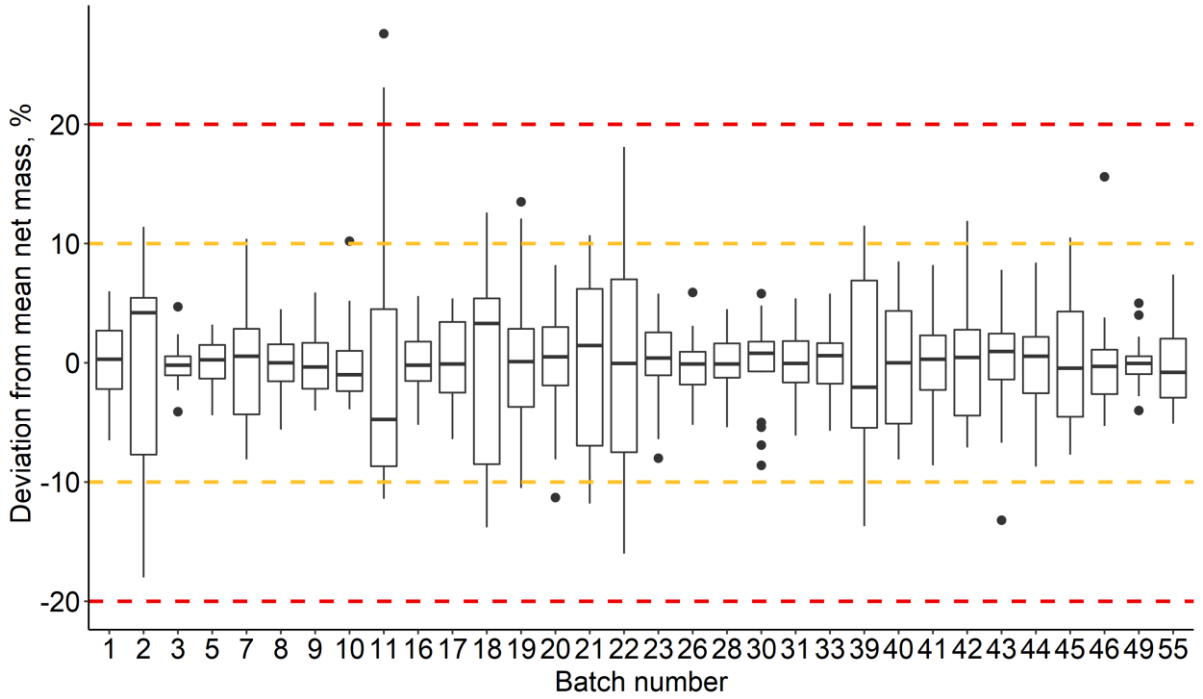

Figure 3.6 Box-Whisker plot showing deviations from mean net mass within capsule batches with a mean net mass <300 mg

Boxes: interquartile range per batch including median (bold line), whiskers: range from box to highest/lowest value within 1.5 times interquartile range, dots: data beyond ±1.5 times interquartile range, yellow lines display tolerable limits and red lines non-tolerable limits as required by the European Pharmacopeia [47]

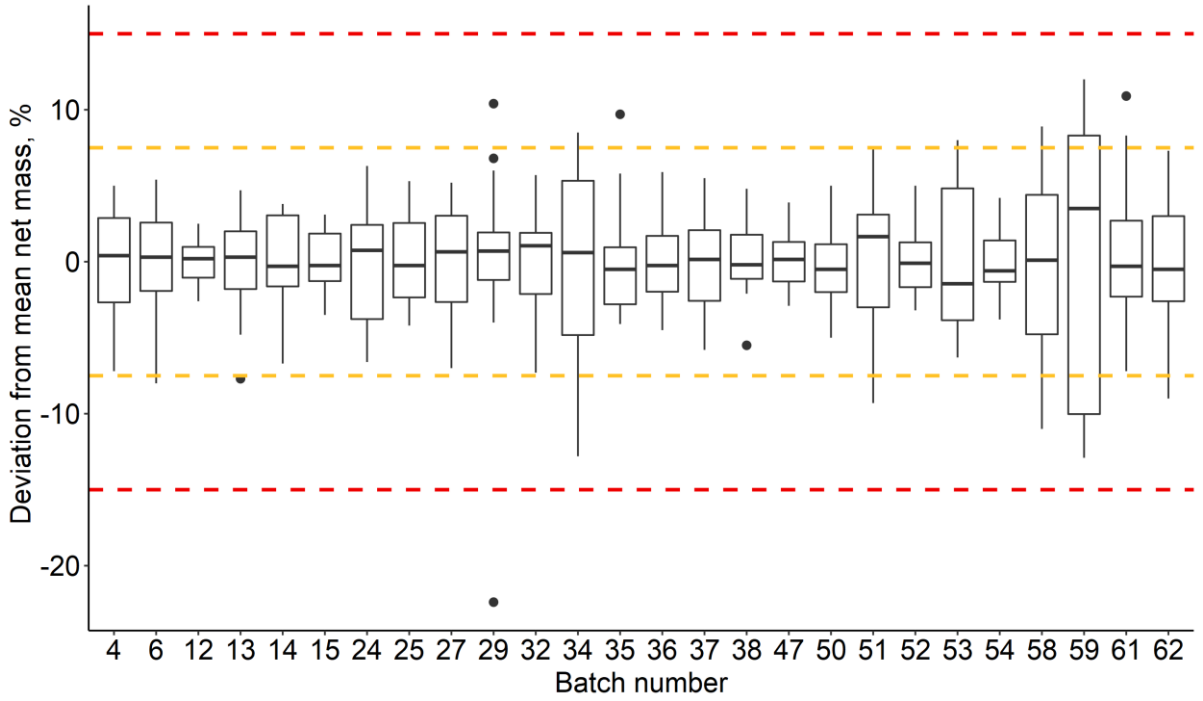

Figure 3.7 Box-Whisker plot showing deviations from mean net mass within capsule batches with a mean net mass >300 mg

Boxes: interquartile range per batch including median (bold line), whiskers: range from box to highest/lowest value within 1.5 times interquartile range, dots: data beyond ±1.5 times interquartile range, yellow lines display tolerable limits and red lines non-tolerable limits as required by the European Pharmacopeia [47]

3.1.5.2 Uniformity of drug content

For 55 batches, uniformity of drug content was assessed. Of these, 48 batches (87.3%) were in accordance with the EP (Figure 3.8), 7 batches failed. From these 7 batches, 1 batch (1.82%) had 3 capsules exceeding tolerable limits, 4 batches (7.27%) had up to 2 capsules exceeding non- tolerable limits. Another 2 batches (3.64%) exceeded tolerable limits with more than half of the capsules and also non-tolerable limits with at least 7 capsules considering no uniformity. Both batches showed the highest deviations of single capsules from their batch in the trial.

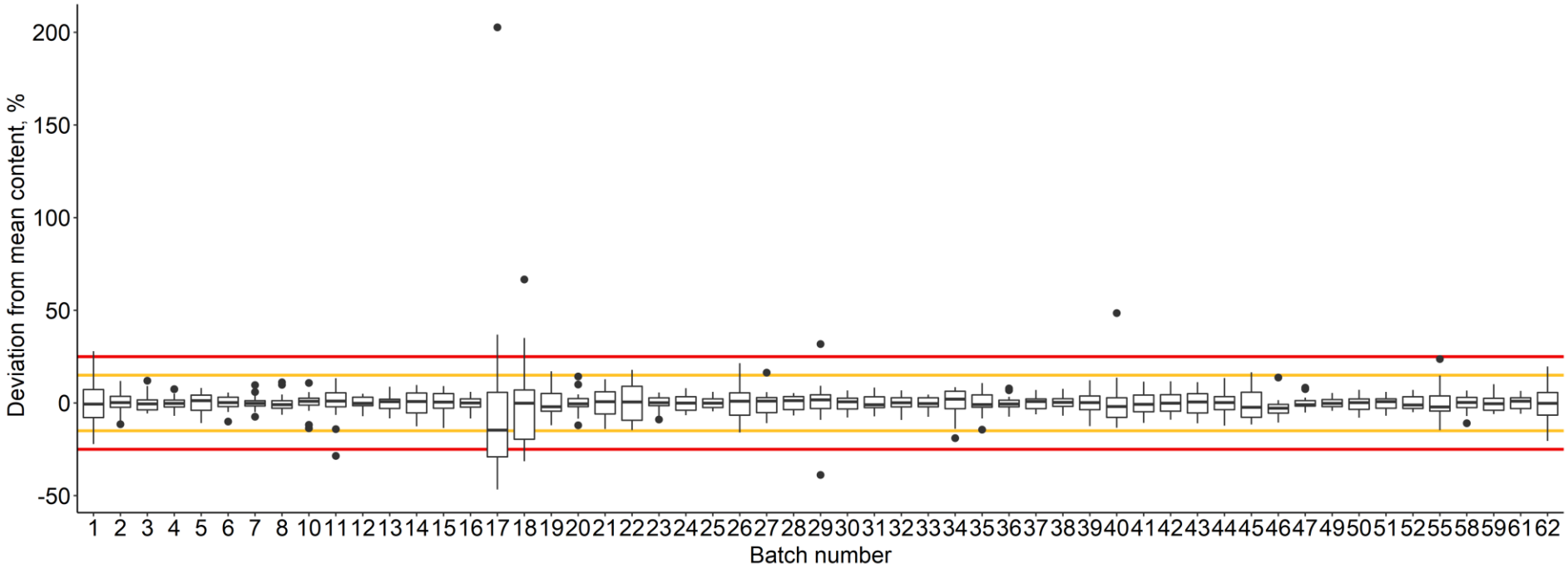

Figure 3.8 Box-Whisker plot showing deviations from mean HC content within the capsule batches

Boxes: interquartile range per batch including median (bold line), whiskers: range from box to highest/lowest value within 1.5 times interquartile range, dots: data beyond ±1.5 times interquartile range, yellow lines display tolerable limits and red lines non-tolerable limits as required by the European Pharmacopeia [17]

3.1.5.3 Accuracy of drug content

Overall, 54 batches were analysed for their correspondence of determined drug content and nominal content. Only 22 batches (40.7%) met the applied acceptance criteria (Figure 3.9). Of the 32 batches which failed, only 2 batches (3.70%) had a low count of capsules exceeding the applied limits. In 1 batch, 3 capsules exceeded tolerable limits, in the other batch 1 capsule each exceeded tolerable and non-tolerable limits. Higher counts of capsules exceeding tolerable and non-tolerable limits were found in the remaining 30 batches: ≤10 capsules per batch in 16 batches (29.6 %) and >10 capsules in the other 14 batches (25.9%).

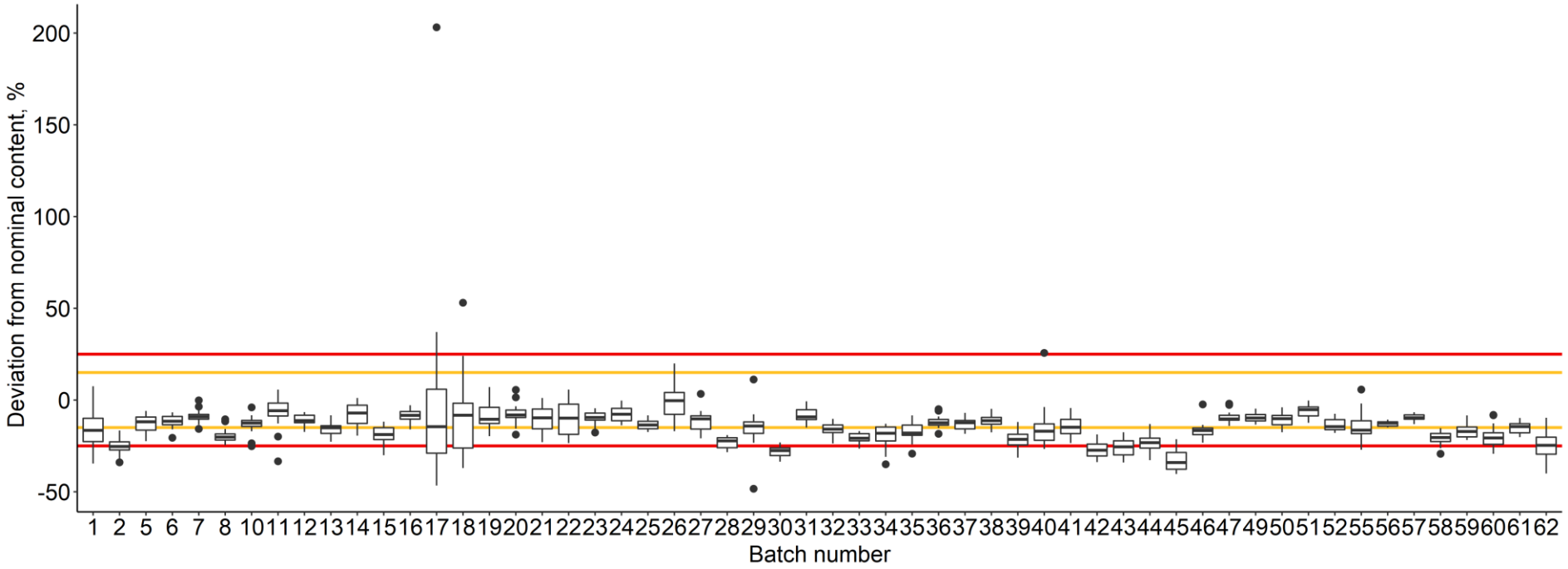

Figure 3.9 Box-Whisker plot showing deviations from nominal HC content within the capsule batches

Boxes: interquartile range per batch including median (bold line), whiskers: range from box to highest/lowest value within 1.5 times interquartile range, dots: data beyond ±1.5 times interquartile range, yellow lines display tolerable limits and red lines non-tolerable limits

Calculating the accuracy with a surcharge of 10% led to 36 batches (66.7%) with acceptable accuracy instead of 22 batches without surcharge. For 3 batches (5.56%), too many capsules exceeded the upper tolerable and non-tolerable limits and therefore result in a failure of the batch. Another 3 batches (5.56%) had capsules exceeding tolerable and non-tolerable limits in both directions. The remaining 12 batches (22.2%) failed as before due to too low drug contents.

3.1.5.4 Correlation of net mass and drug content

The correlation of net mass and drug content was evaluated for 55 batches and is shown in Figure 3.10. Only 10 of them (18.2%) showed a high correlation of R >±0.8 and 19 batches (34.5%) a medium correlation with R values between ±0.5 and ±0.8. Another 12 batches (21.8%) had R values implying a low correlation (R between ±0.3 and ±0.5) and 14 batches (25.5%) no correlation (R <±0.3).

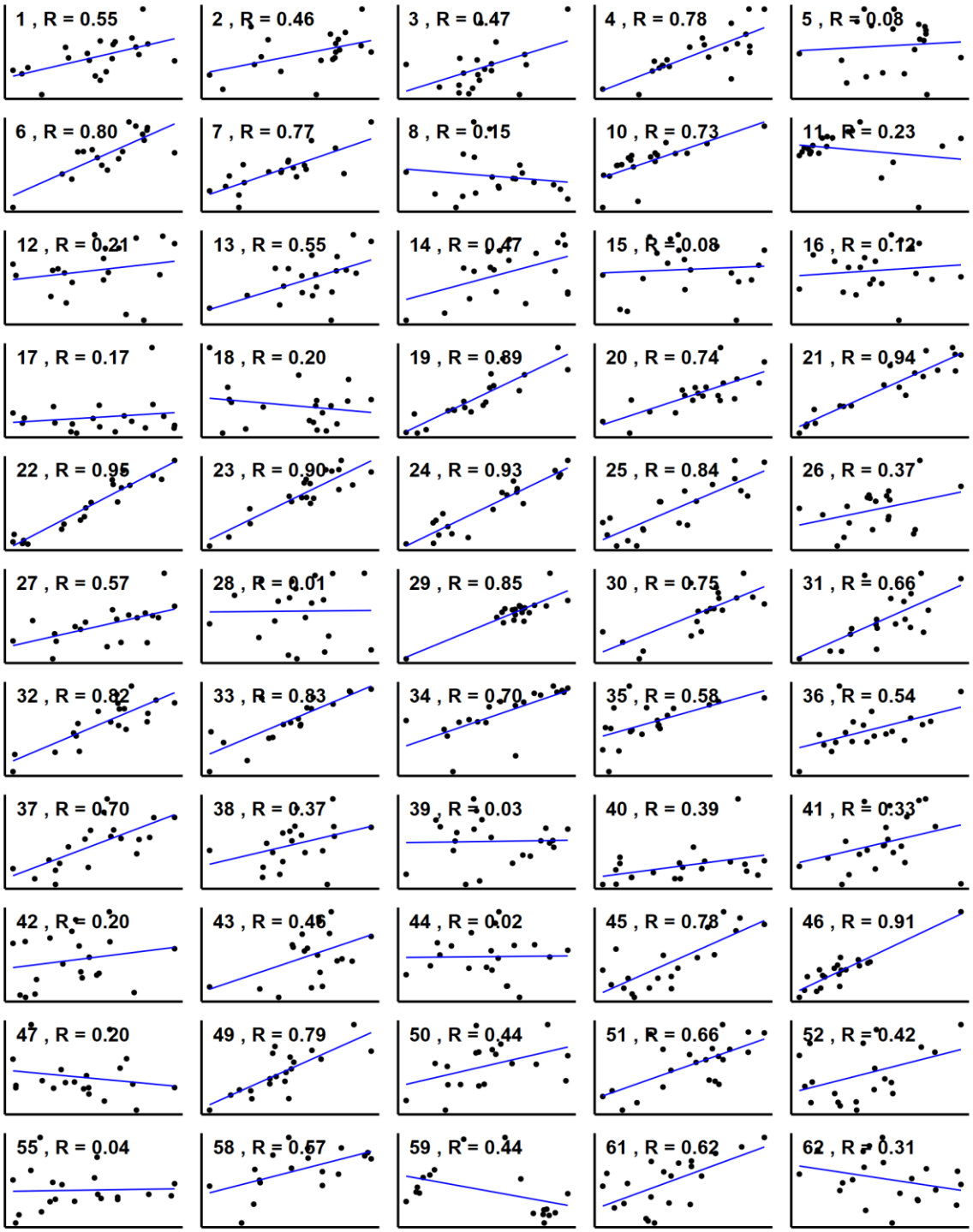

Figure 3.10 Correlation of net mass [mg] and drug content [mg] grouped per batch
Blue lines display the linear regression lines

3.2 Project II - Potential impact factors on relative recovery

3.2.1 *In vitro* microdialysis investigations

3.2.1.1 Impact of drug combinations on relative recovery

Individual RR values and descriptive summary statistics for each antibiotic alone (reference; Scenario 1) and in combination (Scenario 2 and 3) are summarised for each investigation in Figure 3.12 to Figure 3.23 as well as in Table 3.5 and Table 3.6**Fehler! Verweisquelle konnte nicht gefunden werden.**. The results of the statistical analysis using a linear mixed-effects model are presented graphically in a forest plot in Figure 3.11.

Table 3.5 Deviations in median RR values of Scenario 2, 3 and 4 from median RR values of the single drug reference obtained in the investigation regarding a potential impact of drug combination and microdialysis order on relative recovery

Regimen	Drug	Setting	Deviation of median RR value per scenario from median RR value of the reference, %		
			Scenario 2	Scenario 3	Scenario 4
i	Linezolid	Microdialysis	0.10	-7.68	-1.79
		Retrodialysis	1.06	-4.47	-1.09
		Microdialysis*	-	10.0	-
		Retrodialysis*	-	-11.8	-
	Meropenem	Microdialysis	1.83	1.74	-1.31
		Retrodialysis	-2.36	-4.60	0.46
ii	Cefazolin	Microdialysis	2.41	0.16	2.80
		Retrodialysis	-2.94	-	0.90
	Metronidazole	Microdialysis	-0.94	-0.66	2.22
		Retrodialysis	-4.01	-	-0.79
iii	Tigecycline	Microdialysis	-	-2.80	-
		Retrodialysis	-	-3.73	-

RR: relative recovery

N=15 values per scenario, 5 from each of the 3 catheters, * flow rate 5 µL/min instead of the usual 2 µL/min, for the higher flow rate only Scenario 1 and 3 were investigated; for cefazolin and metronidazole in retrodialysis setting was no data for Scenario 3 available

Table 3.6 Intra- and inter-catheter variability in RR values from the investigation regarding a potential impact of drug combination and microdialysis order on RR

Regimen	Drug	Setting (flow rate 2 µL/min)	Intra-catheter variability Delta of RR values			Inter-catheter variability Delta of RR values		
			Median	Minimum	Maximum	Median	Minimum	Maximum
i	Linezolid	Microdialysis	4.68	1.34	11.2	11.4	5.98	18.1
		Retrodialysis	1.75	0.65	11.2	7.25	2.85	16.7
		Microdialysis*	3.03	2.02	11.0	13.6	11.2	16.0
		Retrodialysis*	2.22	1.28	6.49	11.7	5.60	17.8
i	Meropenem	Microdialysis	5.09	1.38	8.61	17.1	8.20	25.0
		Retrodialysis	0.79	0.45	3.37	3.82	2.85	5.91
ii	Cefazolin	Microdialysis	3.20	1.71	7.77	10.5	7.59	23.0
		Retrodialysis	1.59	1.03	3.01	5.69	5.50	6.21
Ii	Metronidazole	Microdialysis	2.49	0.87	4.36	6.28	2.74	11.2
		Retrodialysis	1.34	0.28	1.72	3.15	2.92	4.27
iii	Tigecycline	Microdialysis	2.11	0.48	4.95	13.2	7.22	19.2
		Retrodialysis	1.11	0.36	2.20	7.09	6.06	8.13

RR: relative recovery

2-4 scenarios were compared, n=13-15 values per scenario, 4-5 from each of the 3 catheters

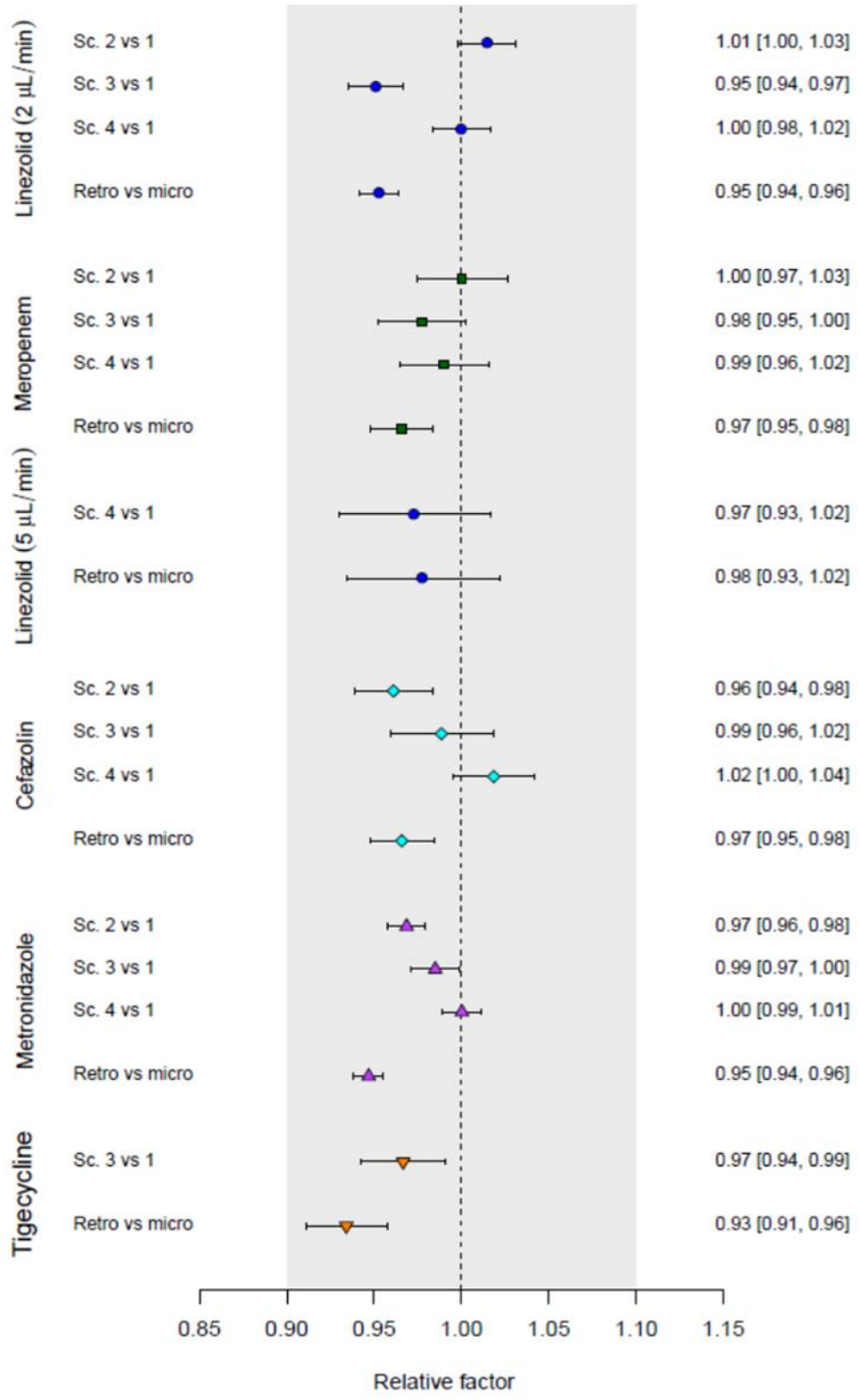

Figure 3.11 Forest plot of the statistical model

micro: microdialysis, retro: retrodialysis, Sc.: scenario

Model developed by David Petroff comparing relative recovery values obtained from Scenario 2-4 with the respective reference scenario as well as relative recovery values per drug obtained in retrodialysis setting versus microdialysis setting (reference)

Estimated relative factor as symbol with 95% confidence interval as bars. Vertical line: relative factor of 1.00 equating to no change, grey area: +10% tolerance range, adapted from [146]

Regimen (i): Linezolid (flow rate 2 and 5 µL/min) and meropenem

In Regimen (i) with a flow rate of 2 µL/min, all RR values were high for both single antibiotics: median RR values across all 13-15 samples per catheter and all 4 scenarios (including Scenario 4) were 97.2% and 90.9% for LIN; and 92.7% and 90.9% for MER in microdialysis and retrodialysis settings, respectively. For Scenario 1 of LIN in retrodialysis setting, drug concentration could only be determined in 13 samples (4 instead of 5 samples each from catheter a and b). A general trend to lower RR values in retrodialysis setting than in microdialysis setting was observed: -5.84% (LIN) and -3.17% (MER) across all 4 scenarios.

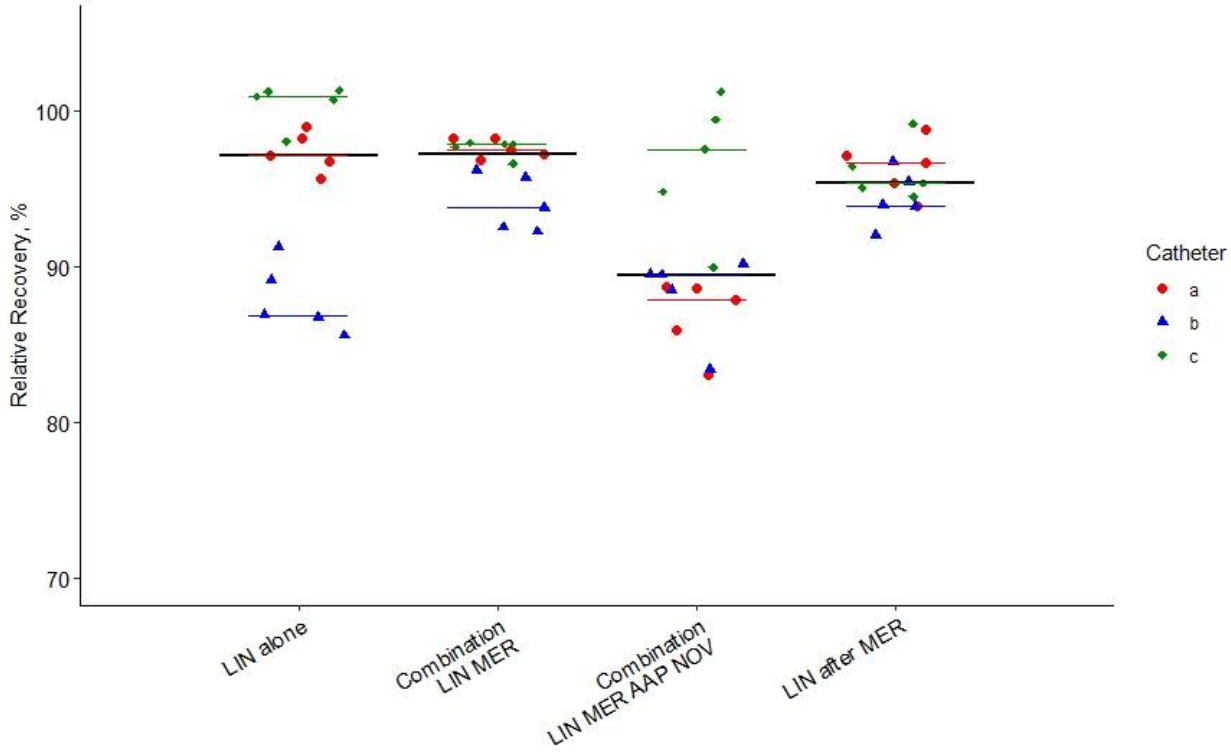

Figure 3.12 Individual relative recovery values obtained by *in vitro* microdialysis setting for linezolid investigating the impact of drug combinations and microdialysis order on relative recovery

AAP: acetaminophen, LIN: linezolid, MER: meropenem, NOV: novamine sulfone sodium salt
Horizontal lines: median relative recovery (RR) values of catheter (n=5 RR values per catheter; colour-coded) or of all 3 catheters (n=15 RR values per scenario; black), adapted from [146]; 'LIN alone' equates to Scenario 1 (reference), 'Combination LIN MER' to Scenario 2, 'Combination LIN MER AAP NOV' to Scenario 3 and 'LIN after MER' to Scenario 4

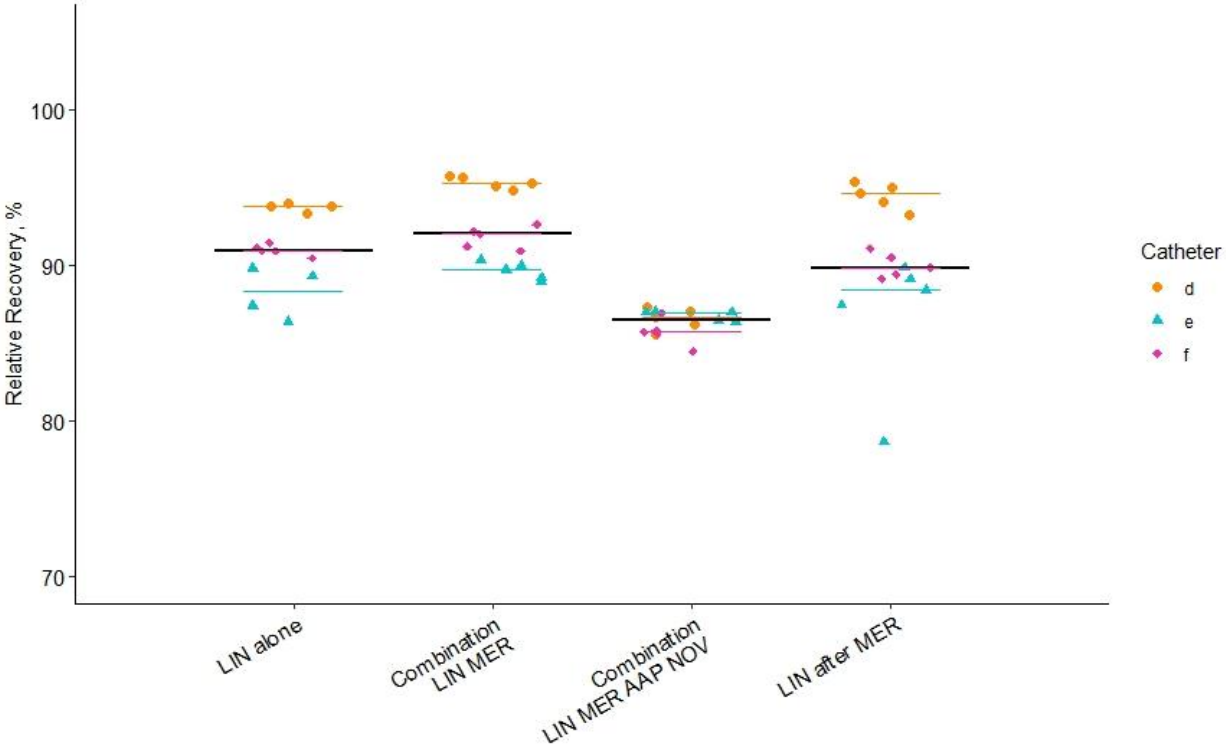

Figure 3.13 Individual relative recovery values obtained by *in vitro* retrodialysis setting for linezolid investigating the impact of drug combinations and microdialysis order on relative recovery

AAP: acetaminophen, LIN: linezolid, MER: meropenem, NOV: novamine sulfone sodium salt

Horizontal lines: median relative recovery (RR) values of catheter (n=4-5 RR values per catheter; colour-coded) or of all 3 catheters (n=13-15 RR values per scenario; black), adapted from [146], 'LIN alone' equates to Scenario 1 (reference), 'Combination LIN MER' to Scenario 2, 'Combination LIN MER AAP NOV' to Scenario 3 and 'LIN after MER' to Scenario 4

For LIN, median RR value was comparable for the reference (LIN alone) and Scenario 2 (the combination of LIN and MER), showing increases of only 0.10% (microdialysis setting) and 1.06% (retrodialysis setting) in median RR values (Table 3.5, Figure 3.12 and Figure 3.13). A different observation was made for Scenario 3, investigating the combination of LIN, MER, AAP and NOV: a noticeable decrease was found for LIN in both settings: -7.68% (microdialysis setting) and -4.47% (retrodialysis setting). Intra-catheter variability across all scenarios ranged from 1.34% to 11.2% in microdialysis setting and from 0.65% to 11.2% in retrodialysis setting (Table 3.6). As expected, inter-catheter variability ranges were higher: 5.98% to 18.1% and 2.85% to 16.7% in microdialysis and retrodialysis setting, respectively. In the microdialysis settings, inter-catheter variability was highly influenced by a single catheter (catheter b) which showed mainly lower RR values than catheter a and c, whereas in retrodialysis setting, catheter d showed generally slightly higher RR values.

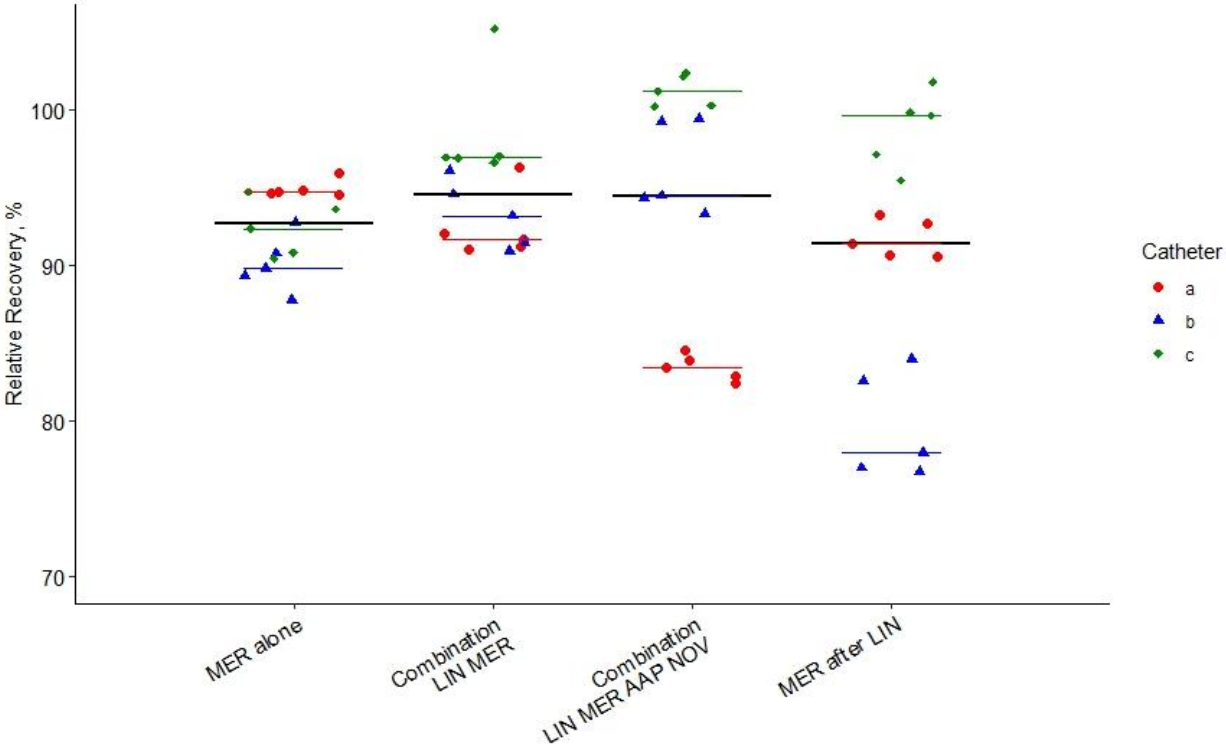

Figure 3.14 Individual relative recovery values obtained by *in vitro* microdialysis setting for meropenem investigating the impact of drug combinations and microdialysis order on relative recovery

AAP: acetaminophen, LIN: linezolid, MER: meropenem, NOV: novamine sulfone sodium salt

Horizontal lines: median relative recovery (RR) values of catheter (n=5 RR values per catheter; colour-coded) or of all 3 catheters (n=15 RR values per scenario; black), adapted from [146], 'MER alone' equates to Scenario 1 (reference), 'Combination LIN MER' to Scenario 2, 'Combination LIN MER AAP NOV' to Scenario 3 and 'MER after LIN' to Scenario 4

For MER, similar results were observed comparing Scenario 2 with the reference scenario (Table 3.5, Figure 3.14 and Figure 3.15). RR values deviated by 1.83% in microdialysis setting and -2.36% in retrodialysis setting. For the comparison of RR values of Scenario 3, the 4-drug combination, with the reference, increases of 1.74%: and -4.60% in microdialysis and retrodialysis setting, respectively, were found. For intra-catheter variability across all scenarios, a range from 1.38% to 8.61% in microdialysis setting and from 0.45% to 3.37% retrodialysis setting was observed; inter-catheter variability, ranged from 8.20% to 25.0% and of 2.85% to 5.91%, respectively (Table 3.6). Similar to the RR values of LIN, catheter b in microdialysis and catheter d in retrodialysis setting showed distinct deviations increasing inter-catheter variability.

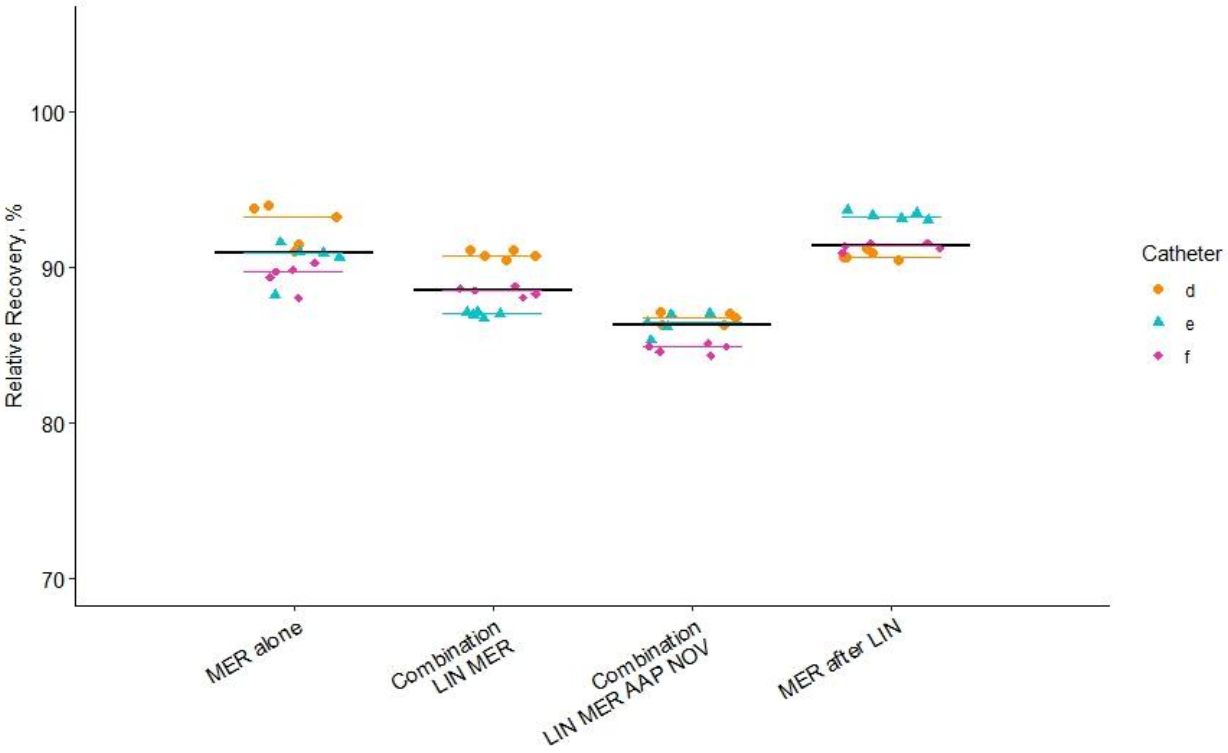

Figure 3.15 Individual relative recovery values obtained by *in vitro* retrodialysis setting for meropenem investigating the impact of drug combinations and microdialysis order on relative recovery

AAP: acetaminophen, LIN: linezolid, MER: meropenem, NOV: novamine sulfone sodium salt

Horizontal lines: median relative recovery (RR) values of catheter (n=5 RR values per catheter; colour-coded) or of all 3 catheters (n=15 RR values per scenario; black), adapted from [146], 'MER alone' equates to Scenario 1 (reference), 'Combination LIN MER' to Scenario 2, 'Combination LIN MER AAP NOV' to Scenario 3 and 'MER after LIN' to Scenario 4

The statistical model (Figure 3.11) revealed a significant but small impact of drug combination on RR: Scenario 3 led to the largest but still small decrease in RR value compared with the reference scenario of 5% [3%-6%] for LIN and 2% [0%-5%] for MER. A similar result was obtained for the impact of the setting on RR. Median RR values in retrodialysis setting were 5% [95% CI: 4%-6%; LIN] and 3% [3%-5%; MER] lower than in microdialysis setting.

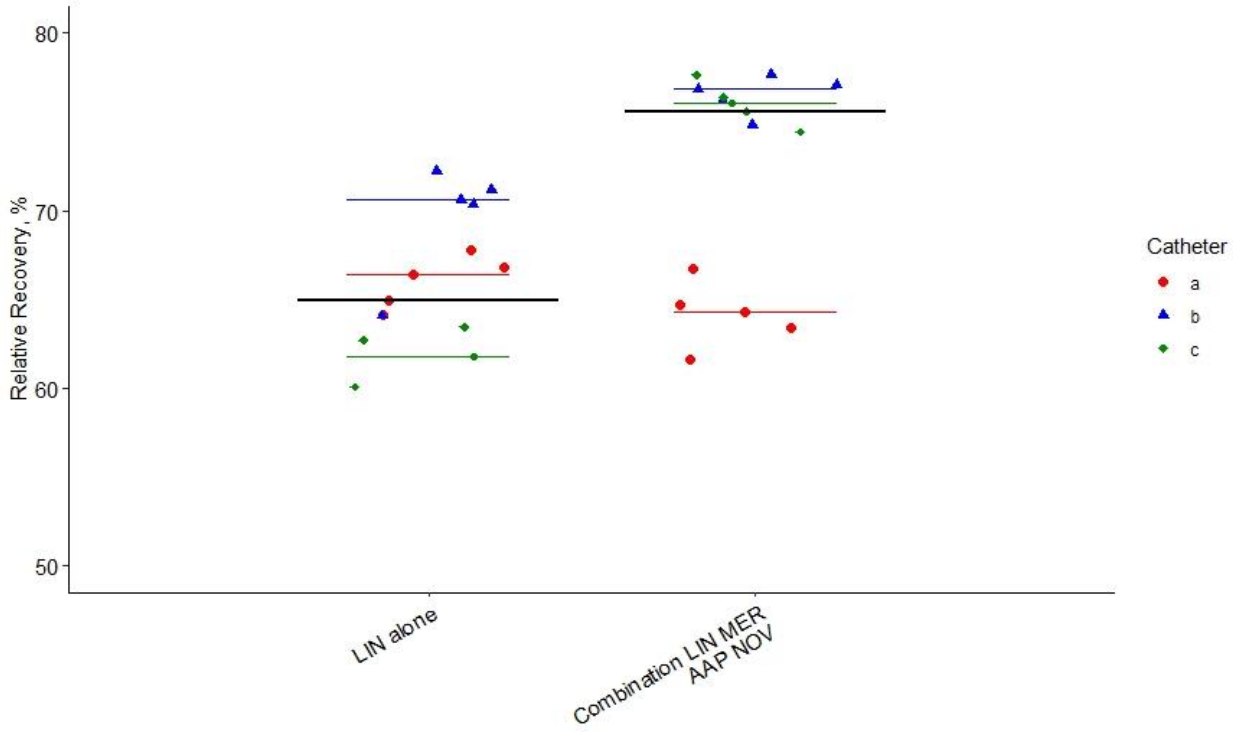

Figure 3.16 Individual relative recovery values obtained by *in vitro* microdialysis setting for linezolid investigating the impact of drug combinations on relative recovery at a flow rate of 5 µL/min

AAP: acetaminophen, LIN: linezolid, MER: meropenem, NOV: novamine sulfone sodium salt

Horizontal lines: median relative recovery (RR) values of catheter (n=5 RR values per catheter; colour-coded) or of all 3 catheters (n=15 RR values per scenario; black), adapted from [146], 'LIN alone' equates to Scenario 1 (reference), 'Combination LIN MER AAP NOV' to Scenario 3

As expected, a higher flow rate of 5 µL/min led to considerably lower RR values for linezolid compared to the flow rate of 2 µL/min (e.g. 65.6% vs. 97.2% and 72.9% vs. 90.9% for linezolid alone in microdialysis and retrodialysis setting, respectively; see Figure 3.12, Figure 3.13, Figure 3.16 and Figure 3.17). Deviating from the results obtained at the lower flow rate, scenario 3, the combination of LIN, MER, AAP and NOV, led to an increase in RR value of 10.0% in microdialysis setting compared to reference scenario investigating LIN alone (Table 3.5 and Figure 3.16). For retrodialysis, a decrease of 11.8% was found (Table 3.5 and Figure 3.17). Intra- and inter-catheter variability in both settings were comparable with the results obtained with a flow rate of 2 µL/min. Intra-catheter variability across both scenarios ranged from 1.28% to 6.49% and from 2.02% to 11.0% in microdialysis and retrodialysis setting, respectively (Table 3.6). Inter-catheter variability was 11.2% and 16.0% for Scenario 1 and 3 in microdialysis setting and 5.60% and 17.8% in retrodialysis setting.

Similar to the lower flow rate, the statistical model (Figure 3.11) resulted in a 3% lower median RR value [95% CI: 7% lower-2% higher] for Scenario 3. The median RR value during retrodialysis was 2% lower [7% lower-2% higher] than during the microdialysis setting.

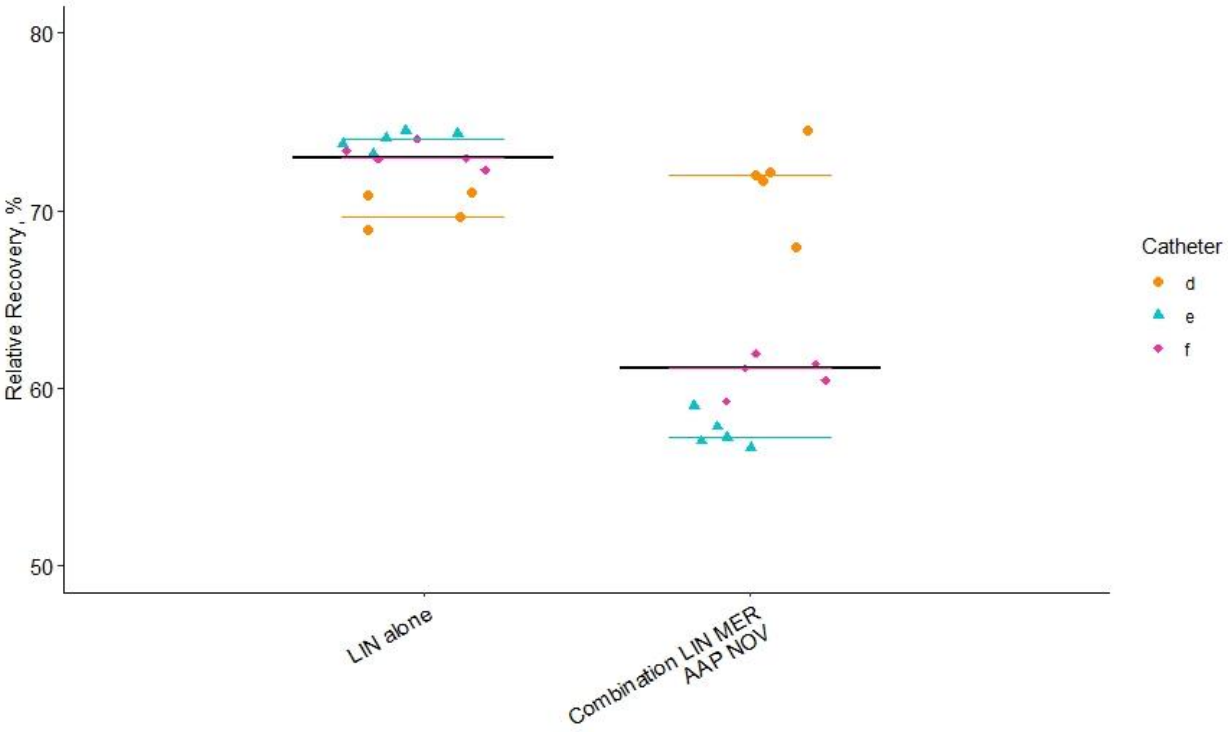

Figure 3.17 Individual relative recovery values obtained by *in vitro* retrodialysis setting for linezolid investigating the impact of drug combinations on relative recovery at a flow rate of 5 µL/min

AAP: acetaminophen, LIN: linezolid, MER: meropenem, NOV: novamine sulfone sodium salt

Horizontal lines: median relative recovery (RR) values of catheter (n= 5 RR values per catheter; colour-coded) or of all 3 catheters (n=15 RR values per scenario; black), adapted from [146], 'LIN alone' equates to Scenario 1 (reference), 'Combination LIN MER AAP NOV' to Scenario 3

Regimen (ii): Cefazolin and metronidazole

Due to analytical interferences between CZL, MET and metabolites of the instable NOV, samples collected from retrodialysis setting containing all 3 drugs could not be quantified. The subsequent analysis of samples from microdialysis setting was performed with a modified quantification method (described in Section 2.3.2) showing no interferences. Hence, only Scenario 1 and 2 were available for retrodialysis whereas all 3 scenarios from microdialysis setting could be evaluated. In addition, 1 sample with CZL of Scenario 3 in microdialysis setting as well as 1 sample with MET of Scenario 2 in retrodialysis setting could not be quantified and were therefore excluded from data analysis.

Median RR values were high across all scenarios with 91.8% and 89.2% for single CZL and 100% and 96.3% for single MET in microdialysis and retrodialysis setting, respectively. Median RR values of Scenario 2, the combination of CZL and MET, showed only a small deviation of from median RR values of the reference scenario with CZL alone: 2.41% and -2.94% in microdialysis and retrodialysis setting, respectively (Table 3.5**Fehler! Verweisquelle konnte nicht gefunden werden.**, Figure 3.18 and Figure 3.19). Deviating from results for Regimen (i), a decreased RR value of only 0.16% compared to reference was found for Scenario 3 in the microdialysis setting. Intra-catheter variability ranged from 1.71% to 7.77% and from 1.03% to 3.01% in microdialysis setting and in retrodialysis setting, respectively (Table 3.6). Inter-catheter variability in microdialysis setting was much higher than intra-catheter variability due to catheter a with comparably low RR values (Scenario 2) leading to a range of 7.69% to 23.0%. In retrodialysis setting, a lower inter-catheter range of 5.50% to 6.21% was found. As

in Regimen (i), RR values were decreased in retrodialysis setting than in microdialysis setting: 3.53% for CZL and 4.82% for MET.

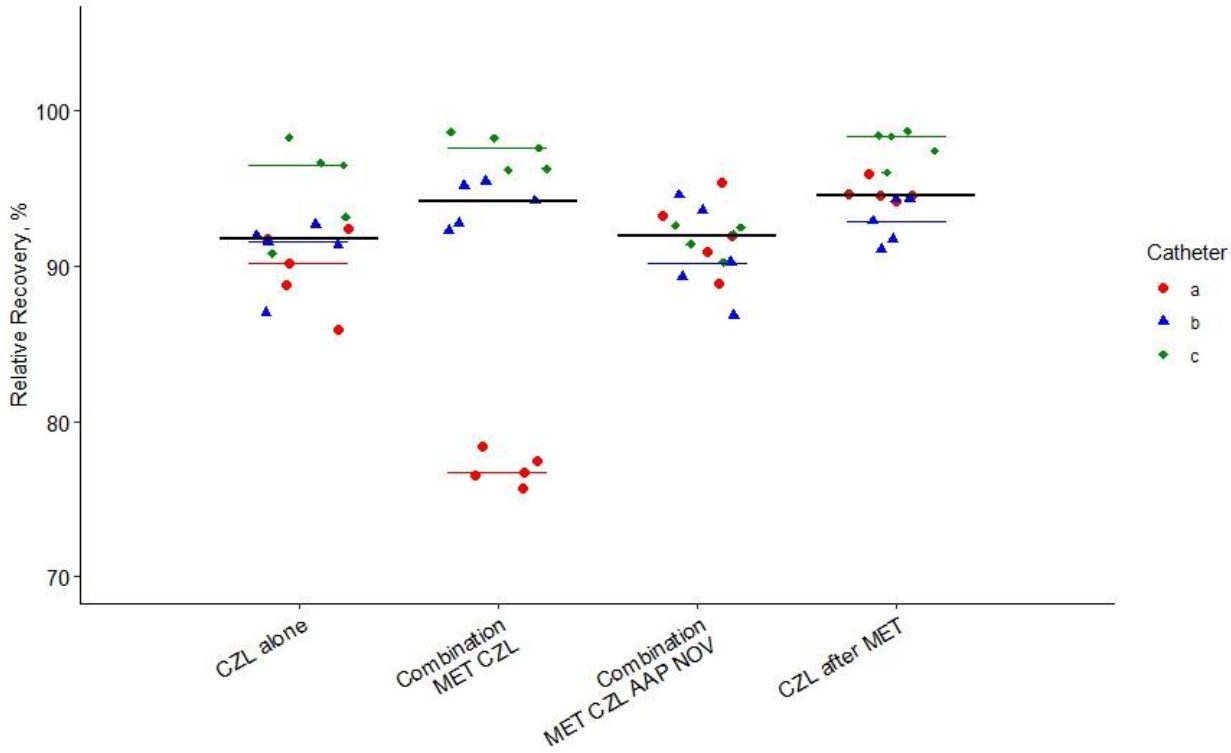

Figure 3.18 Individual relative recovery values obtained by *in vitro* microdialysis setting for cefazolin investigating the impact of drug combinations and microdialysis order on relative recovery

AAP: acetaminophen, CZL: cefazolin, MET: metronidazole, NOV: novamine sulfone sodium salt

Horizontal lines: median relative recovery (RR) values of catheter (n=4-5 RR values per catheter; colour-coded) or of all 3 catheters (n=14-15 RR values per scenario; black), adapted from [146], 'CZL alone' equates to Scenario 1 (reference), 'Combination MET CZL' to Scenario 2, 'Combination MET CZL AAP NOV' to Scenario 3 and 'CZL after MET' to Scenario 4

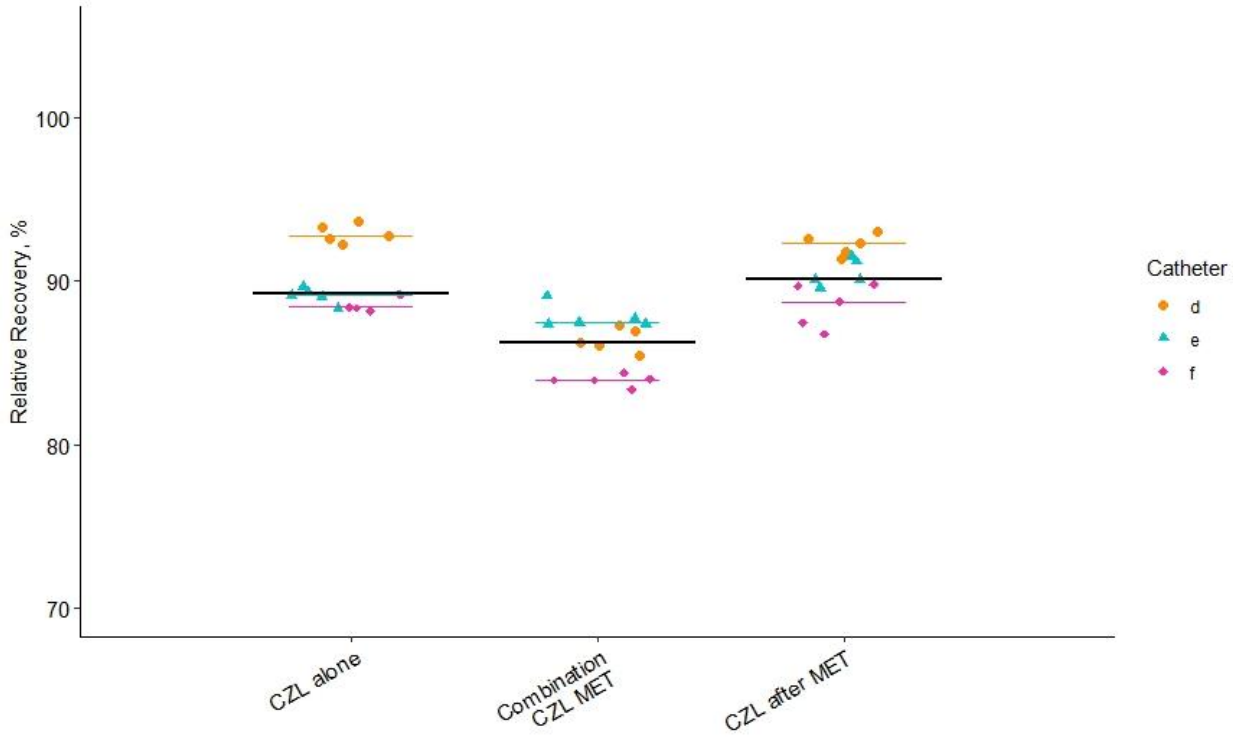

Figure 3.19 Individual relative recovery values obtained by *in vitro* retrodialysis setting for cefazolin investigating the impact of drug combinations and microdialysis order on relative recovery

AAP: acetaminophen, CZL: cefazolin, MET: metronidazole, NOV: novamine sulfone sodium salt
Horizontal lines: median relative recovery (RR) values of catheter (n=5 RR values per catheter; colour-coded) or of all 3 catheters (n=15 RR values per scenario; black), adapted from [146], 'CZL alone' equates to Scenario 1 (reference), 'Combination MET CZL' to Scenario 2 and 'CZL after MET' to Scenario 4

Comparing Scenario 2 and 3 for MET with the reference scenario, only small decreases by 0.94% and 0.66% were found in microdialysis setting, respectively. In retrodialysis setting, a higher decrease of 4.01% was found for the median RR value obtained from Scenario 2 (Table 3.5, Figure 3.20 and Figure 3.21). Intra-catheter variability of RR values showed a range from 0.87% to 4.36% and from 0.28% to 1.72% in microdialysis and retrodialysis setting, respectively, inter-catheter variability from 2.74% to 11.2% and from 2.92% to 4.27% (Table 3.6). Similar to the results of CZL, the high inter-catheter variability in microdialysis setting was mainly caused by catheter a in Scenario 2.

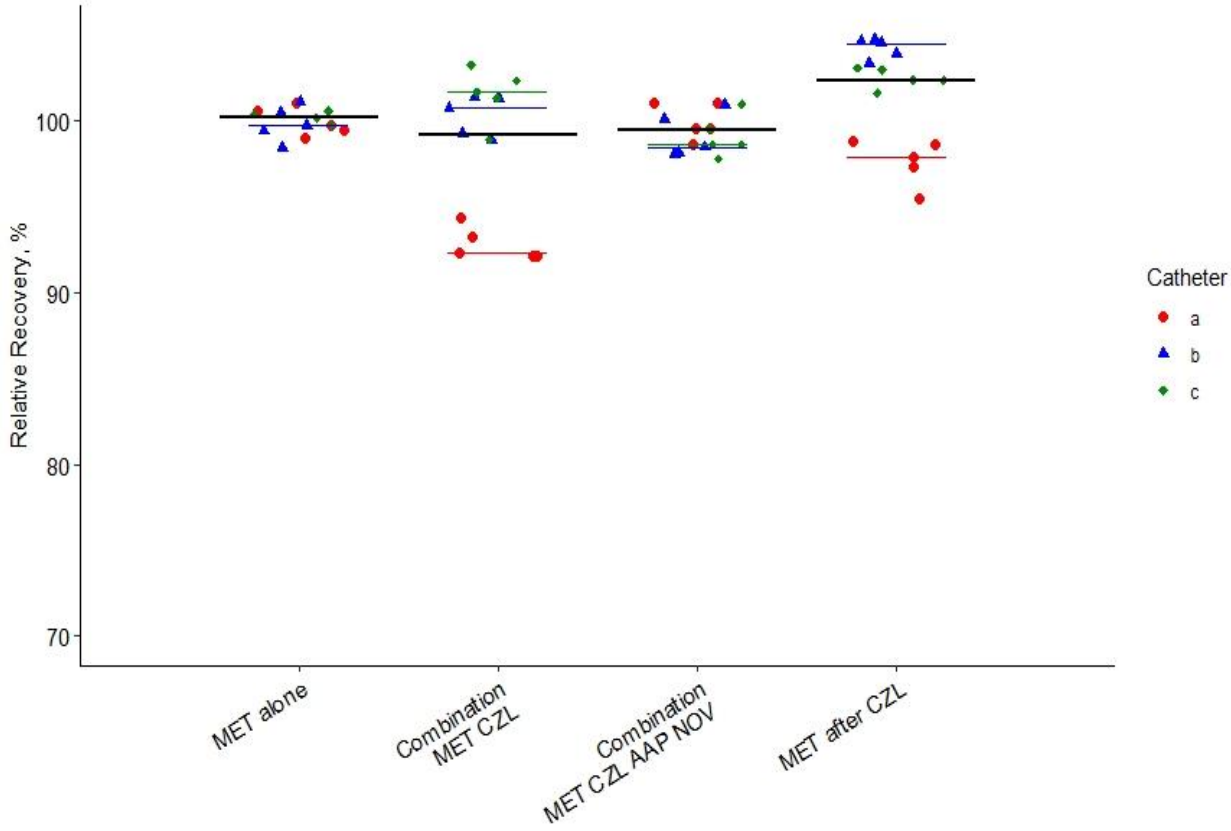

Figure 3.20 Individual relative recovery values obtained by *in vitro* microdialysis setting for metronidazole the impact of drug combinations and microdialysis order on relative recovery

AAP: acetaminophen, CZL: cefazolin, MET: metronidazole, NOV: novamine sulfone sodium salt

Horizontal lines: median relative recovery (RR) values of catheter (n=4-5 RR values per catheter; colour-coded) or of all 3 catheters (n=14-15 RR values per scenario; black), adapted from [146], 'MET alone' equates to Scenario 1 (reference), 'Combination MET CZL' to Scenario 2, 'Combination MET CZL AAP NOV' to Scenario 3 and 'MET after CZL' to Scenario 4

According to the statistical model, Scenario 2 yielded slightly lower median RR values than the remaining scenarios for both drugs, CZL (4% [95% CI: 2%-6%]) and MET (3% [2%-4%]), see Figure 3.11. Retrodialysis setting led to a reduction in median RR of 3% [2%-5%] and 5% [4%-6%] for CZL and MET, respectively, compared to microdialysis setting.

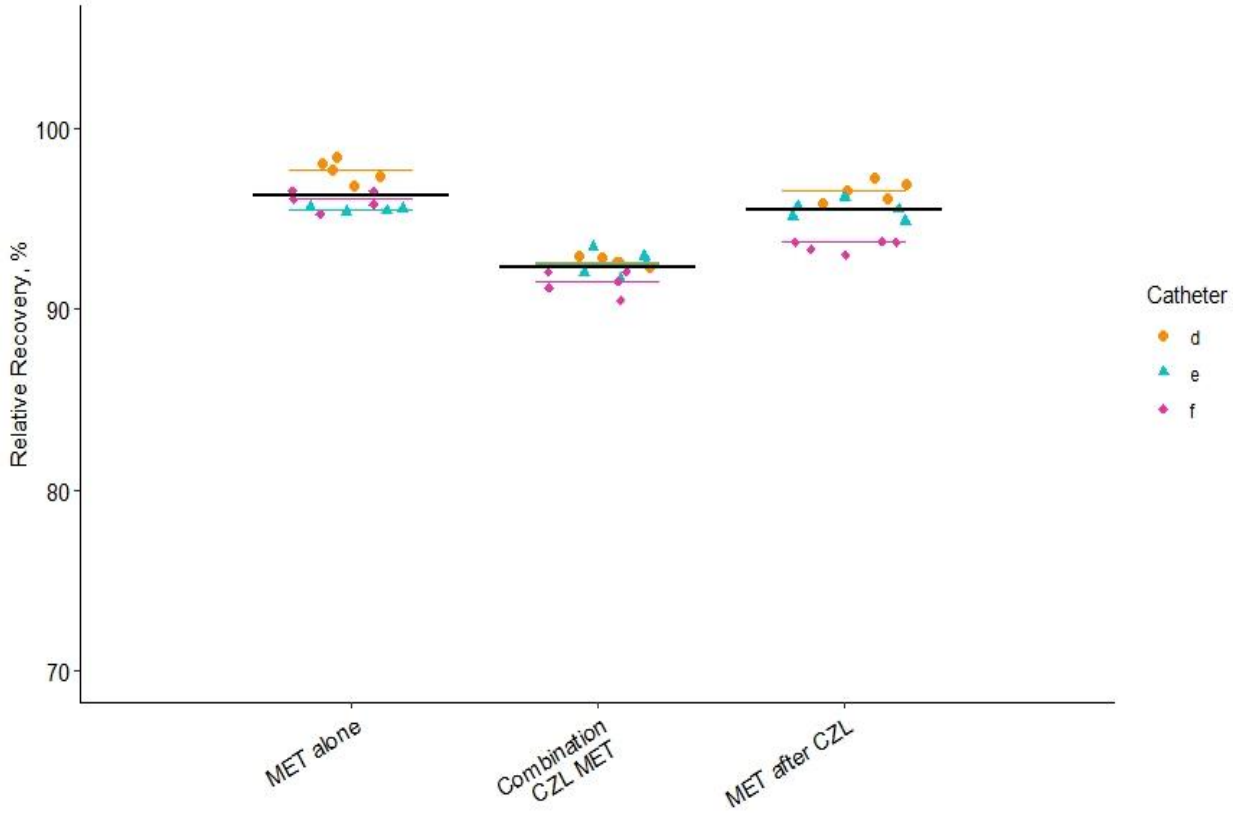

Figure 3.21 Individual relative recovery values obtained by *in vitro* retrodialysis setting for metronidazole investigating the impact of drug combinations and microdialysis order on relative recovery

AAP: acetaminophen, CZL: cefazolin, MET: metronidazole, NOV: novamine sulfone sodium salt

Horizontal lines: median relative recovery (RR) values of catheter (n=4-5 RR values per catheter; colour-coded) or of all 3 catheters (n=14-15 RR values per scenario; black), adapted from [146], 'MET alone' equates to Scenario 1 (reference), 'Combination MET CZL' to Scenario 2 and 'MET after CZL' to Scenario 4

Regimen (iii): Tigecycline

TIG showed across all scenarios and settings a slightly lower median RR value of 85.3% compared to other investigated drugs. As observed for the other drugs, median RR value in retrodialysis was 5.09% lower than in microdialysis (Table 3.5, Figure 3.22 and Figure 3.23). In Scenario 3, decreases of RR values compared with the respective reference scenario was observed: 2.80% (microdialysis) and 3.73% (retrodialysis). Intra-catheter variability ranged from 0.48% to 4.95% and from 0.36% to 2.20% in microdialysis and retrodialysis setting, respectively and is therefore similar to ranges found for the other regimens (Table 3.6). Inter-catheter variability was 7.22% to 19.2% in microdialysis setting and 6.06% to 8.13% in retrodialysis setting. The high variability in microdialysis setting was caused by the deviation of RR value magnitudes of catheter a and c.

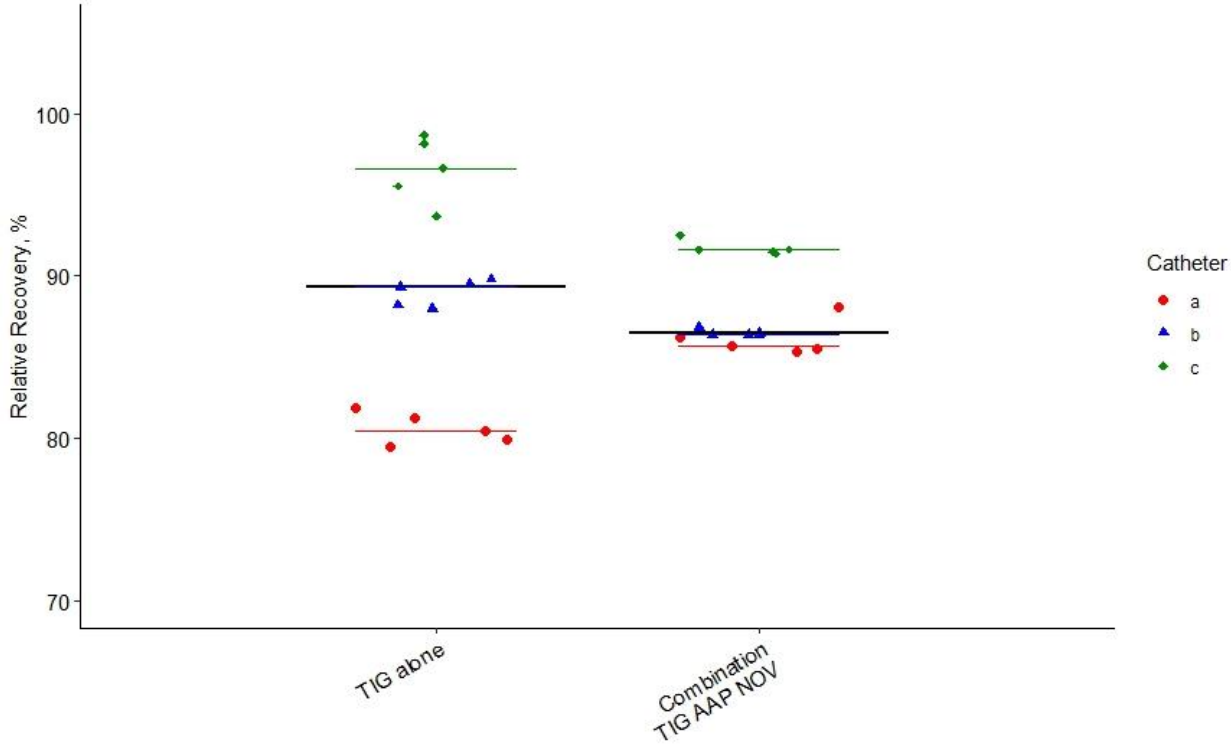

Figure 3.22 Individual relative recovery values obtained by *in vitro* microdialysis setting for tigecycline investigating the impact of drug combinations on relative recovery

AAP: acetaminophen, NOV: novamine sulfone sodium salt, TIG: tigecycline

Horizontal lines: median relative recovery (RR) values of catheter (n=5 RR values per catheter; colour-coded) or of all 3 catheters (n=15 RR values per scenario; black), adapted from [146], TIG alone' equates to Scenario 1 (reference), 'Combination TIG AAP NOV' to Scenario 3 The statistical model showed median RR values which were 3% lower [95% CI: 1%-6%] for Scenario 3 compared to the reference and 7% lower [4%-9%] for retrodialysis compared to microdialysis (Figure 3.11).

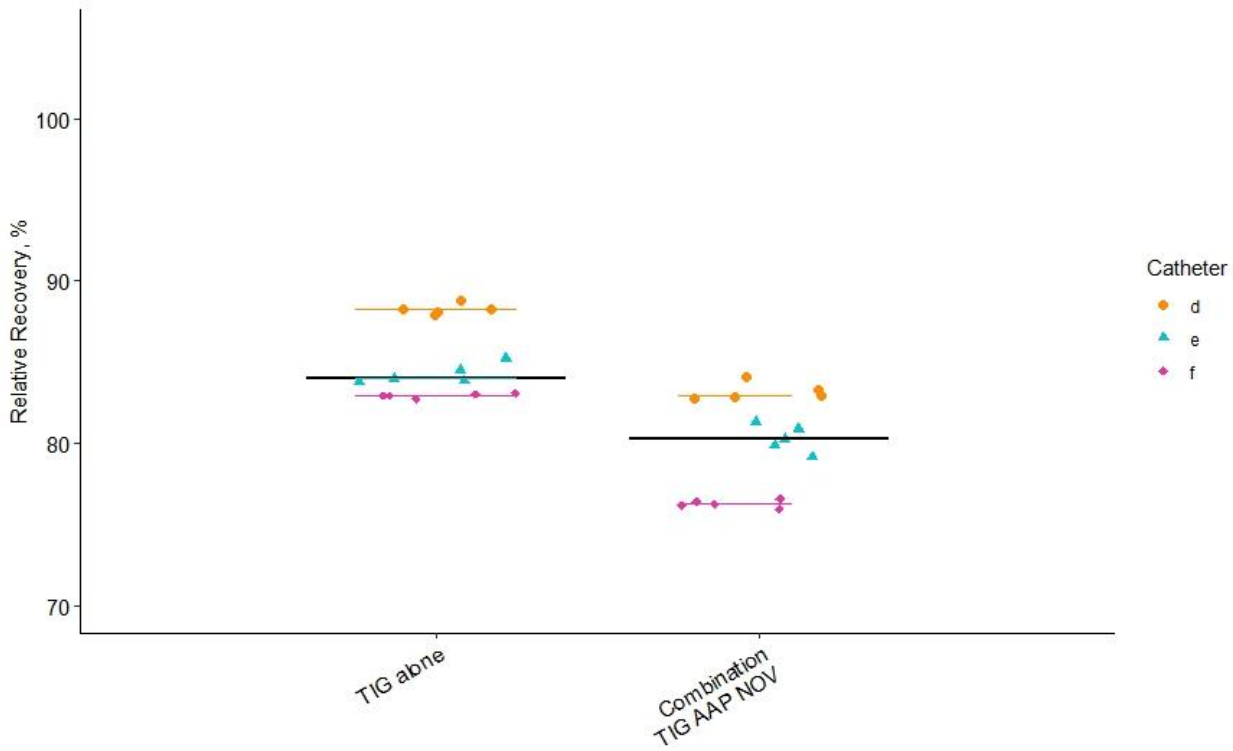

Figure 3.23 Individual relative recovery values obtained by *in vitro* retrodialysis setting for tigecycline investigating the impact of drug combinations on relative recovery

AAP: acetaminophen, NOV: novamine sulfone sodium salt, TIG: tigecycline

Horizontal lines: median relative recovery (RR) values of catheter (n=5 RR values per catheter; colour-coded) or of all 3 catheters (n=15 RR values per scenario; black), adapted from [146], TIG alone' equates to Scenario 1 (reference), 'Combination TIG AAP NOV' to Scenario 3

3.2.1.2 Impact of microdialysis order on relative recovery

Detailed RR values and descriptive summary statistics are displayed in Figure 3.12 to Figure 3.15 as well as in Figure 3.18 to Figure 3.21 as well as in Table 3.5. The results of the statistical model are graphically presented in a forest plot in Figure 3.11.

Regimen (i): Linezolid and meropenem

For LIN, Scenario 4, the reversed microdialysis order, led to only slightly deviating RR values: -1.79% (microdialysis setting) and -1.09% (retrodialysis setting) compared to reference scenario (Table 3.5, Figure 3.12 and Figure 3.13). Also according to the statistical model, no difference between reference scenario and Scenario 4 was present [CI 95%: 2% lower-2% higher]. For MER, median RR value was decreased by 1.31% in microdialysis and increased by 0.46% in retrodialysis (Table 3.5, Figure 3.14 and Figure 3.15). As expected, statistical model showed no significant impact on RR values with a decrease of 1% [4% lower-2% higher].

Regimen (ii): Cefazolin and metronidazole

Different from LIN and MER, an increase of RR value compared to reference was observed for CZL: 2.80% and 0.90% in microdialysis and retrodialysis setting, respectively (Table 3.5, Figure 3.18 and Figure 3.19). Statistical model of David Petroff showed a small, insignificant increase in median RR value of 2% [95% CI: 0%-4%].

Comparing median RR values of MET from the reference scenario and the reversed microdialysis order, an increase of 2.22% (microdialysis setting) and a decrease of 0.79% (retrodialysis setting) were observed (Table 3.5, Figure 3.20 and Figure 3.21). Statistical model revealed no difference between both scenarios: 0% [95% CI: -1%-1%].

3.2.1.3 Impact of air in the microdialysis catheter on relative recovery

Air in the microdialysis catheter had a substantial impact on the RR values of the model drug LIN (Figure 3.24). Median values from air-containing catheters were approximately 20% lower than those without air: 74.8% versus 97.2% in microdialysis and 73.6% versus 90.9% in retrodialysis settings, respectively. In the microdialysis setting, the inter-catheter variability was comparable in air-containing and non-containing catheters (14.3% vs. 15.7%). For retrodialysis setting, a high difference in inter-catheter variability of air-containing catheters compared to air-free ones was observed: 36.8% versus 7.25%. The high variability was caused by a strong trend of decreasing RR values over time in the air-containing catheters (e.g. for catheter j: 73.4% in the first sampling interval and 58.2% in the last sampling interval).

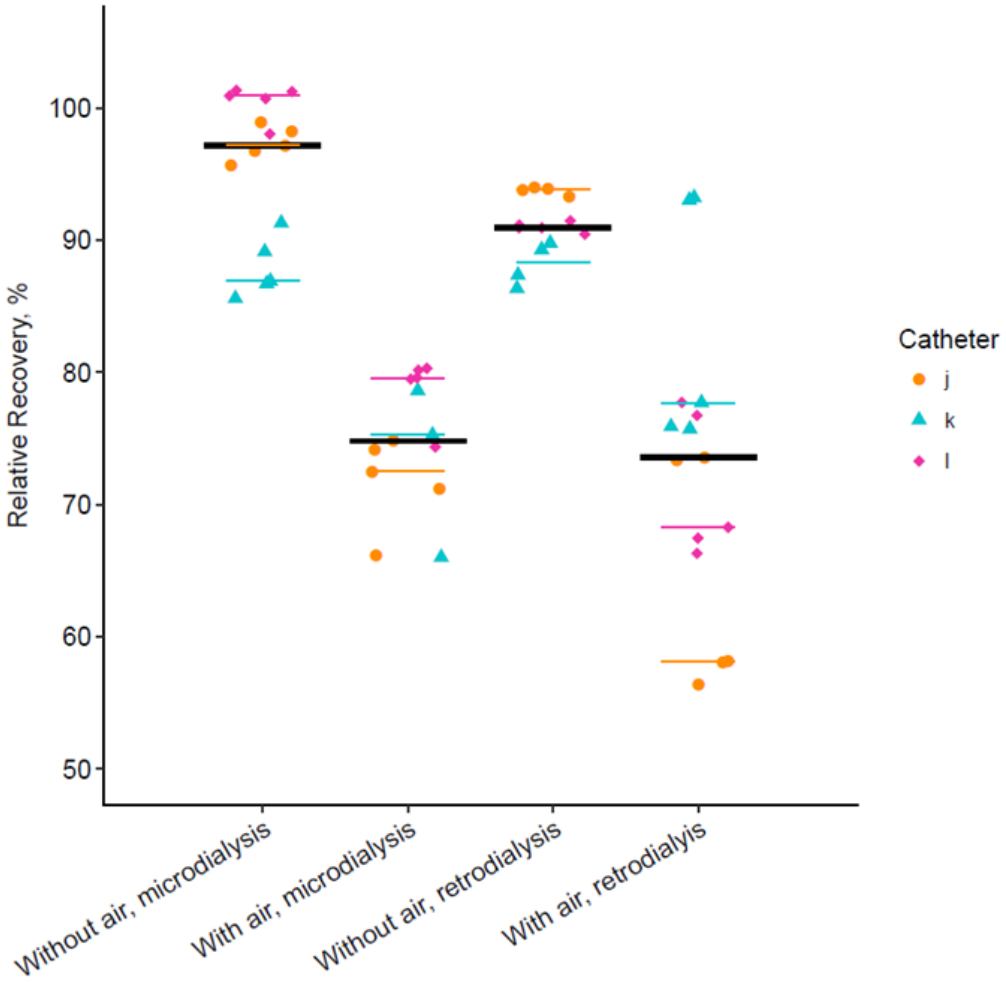

Figure 3.24 Individual relative recovery values of linezolid obtained by *in vitro* investigations with catheters of air-free and air-containing solutions

Horizontal lines: median relative recovery (RR) values of catheter (n=3-5 RR values per catheter; colour-coded) or of all 3 catheters n=13-15 RR values per scenario; black), adapted from [146]

3.2.1.4 Impact of flow rate change on relative recovery

Figure 3.25 shows the detailed results of the investigation regarding the impact of a flow rate change on RR value for the model drug LIN. The high flow rate of 15 µL/min-during the automatic flush sequence revealed initially very variable RR values ranging from 44.2% to 71.9% (median 69.8%, n=10 samples). Within 15 min after the switch to the applied final flow rate of 1 µL/min, the system adapted resulting in increased RR values ranging from 93.5% to 99.3% (n=5 samples per time interval). For investigations with a higher final flow rate of 2 µL/min, the system adapted faster. After 10 min, RR values showed a constant level with a range from 89.1% to 96.0% for all following sampling intervals (n=5 samples per time interval).

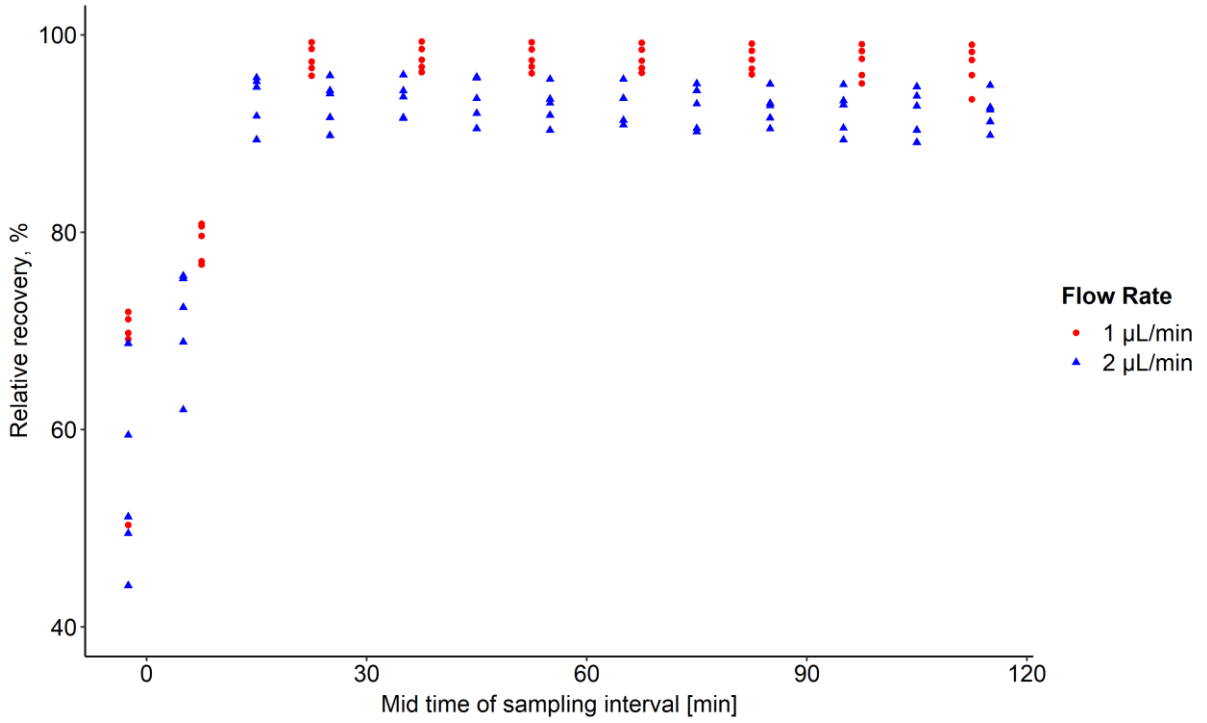

Figure 3.25 Time course of relative recovery values at standard flow rates after the automatic flush sequence at start of operation

Blue triangles: flow rate 2 µL/min, red circles: flow rate 1 µL/min, Time -5-0 min: automatic flow rate of 15 µL/min; Time 0: switch from 15 µL/min to 1 or 2 µL/min, respectively, adapted from [146]

3.3 Project III - Vancomycin monitoring in septic infants via microdialysis

3.3.1 Development and validation of the quantification method for vancomycin from *in vitro* investigations

Validation of the final quantification method was performed in accordance with the EMA Guideline [141]. Results are summarised in Table 3.7.

3.3.1.1 Lower limit of quantification and linearity

A LLOQ of 2.5 µg/mL allowed quantification with sufficient accuracy (RE=0.22%, n=5 samples) and precision (CV=2.66%). Over the calibration range of 2.5 µg/mL to 80 µg/mL, linearity was given with $R^2 \geq 0.9965$ for the 3 investigations.

3.3.1.2 Accuracy and precision

Acceptable accuracy and precision was observed for all investigated QC levels. Within-run accuracy was ≤±13.1% and ≤±13.9% for LLOQ and for QC 2-4; between-run accuracy ≤±11.49% and ≤±5.10%, respectively. For within-run precision, results of ≤7.06% (LLOQ) and ≤11.4% (higher QCs) were found and for between-run precision of ≤7.26% (LLOQ) and ≤9.68%B (QC2-4), respectively.

Table 3.7 Validation parameters for vancomycin from *in vitro* microdialysis investigations

Validation parameter	Results for vancomycin
Calibration range	2.50-80.0 µg/mL
(3 samples for QC 2-4 or 5 samples for LLOQ)	
Within-run accuracy, RE (5 samples)	≤±13.9% (for LLOQ ≤±13.1%)
Between-run accuracy, RE (15 samples)	≤±10.1% (for LLOQ 6.33%)
Within-run precision, CV (5 samples)	≤11.4% (for LLOQ ≤7.06%)
Between-run precision, CV (15 samples)	≤9.68% (for LLOQ 7.26%)
Short-term stability after 6-24 h, RE (5 samples)	≤±8.07%
Autosampler stability after 24 h, RE (5 samples)	≤±3.41%
Freeze-thaw stability after 3 cycles, RE (5 samples)	≤±13.3%
Long-term stability after 29 months, RE (5 samples)	≤±4.31%

CV: coefficient of variation, LLOQ: lower limit of quantification, RE: relative error
All evaluations according to EMA Guideline [141]

3.3.1.3 Carry-over

No VAN was detected in blank samples following an injection of a high Cal sample, therefore, carry-over effect was excluded.

3.3.1.4 Stability

All stability investigations were in accordance with EMA Guideline [141]. Short-term stability was proven over 24 h for QC 2 and 4 samples and over 6 h for SL samples with maximum deviations from freshly prepared samples of ±8.07% and ±7.07%, respectively. After storage in the autosampler for 24 h, maximum deviations of ±3.41% were observed confirming autosampler stability. Long term stability after 29 months was also successfully shown with a maximum deviation of ±4.31%. After 1-3 freeze-thaw cycles, maximum deviation of ±13.3% from freshly prepared QC and SL samples was found proving sufficient stability.

3.3.2 *In vitro* microdialysis investigations

3.3.1.5 Impact of flow rate on relative recovery

Figure 3.26 and Figure 3.27 show detailed results of the investigation of the impact of flow rate on RR. Table 3.8 summarises obtained RR values and their intra- and inter-catheter variability. As expected, flow rate had a relevant impact on RR.

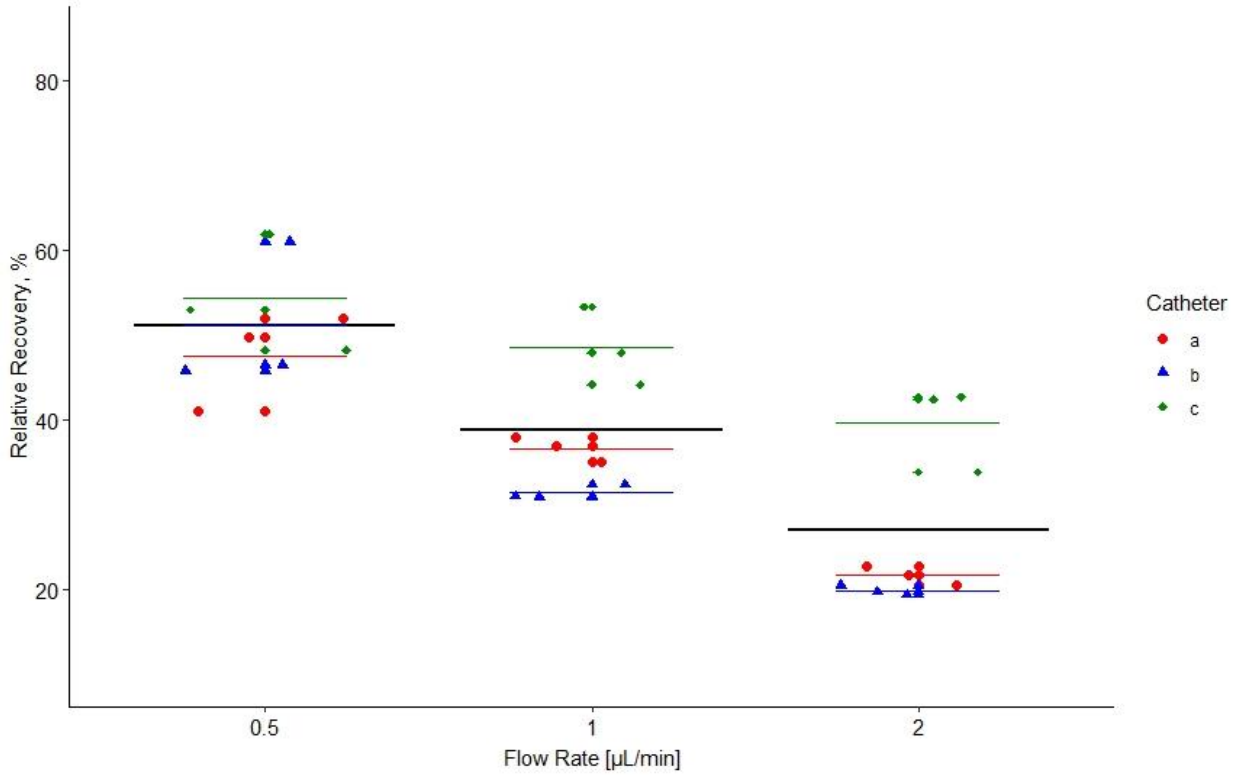

Figure 3.26 Impact of flow rate on relative recovery in microdialysis setting
Coloured horizontal lines reflect the mean of the respective catheter, black horizontal lines the mean of all 3 investigated catheters

The highest RR values were obtained at 0.5 µL/min, the lowest RR values at 2 µL/min. In the microdialysis setting, mean RR values (n=9 samples) were 51.1% (SD 6.50%), 38.9% (SD 7.50%) and 27.1% (SD 9.26%) for flow rates of 0.5, 1 and 2 µL/min, respectively. Intra-catheter variability (n=3 samples) was ≤15.2%, inter-catheter variability (n=9 samples) ≤22.4% across all flow rates. For the retrodialysis setting in general, slightly higher values with the same reverse dependency on flow rate were observed. Mean RR values (n=15 samples) were 65.5% (SD 8.24%, flow rate 0.5 µL/min), 40.7% (SD 6.30%, flow rate 1 µL/min) and 31.3% (SD 7.79%, flow rate 2 µL/min). Variability (n=5 and 15 samples, respectively) were in a similar range as in the microdialysis setting with a maximum of 10.8% (intra-catheter variability) and 25.4% (inter-catheter variability), respectively. Looking at the resulting mean RR values from the microdialysis setting with the ones obtained from the retrodialysis settings, decreases of 14.5% (flow rate 0.5 µL/min), 1.79% (flow rate 1 µL/min) and 4.17% (flow rate 2 µL/min) were calculated.

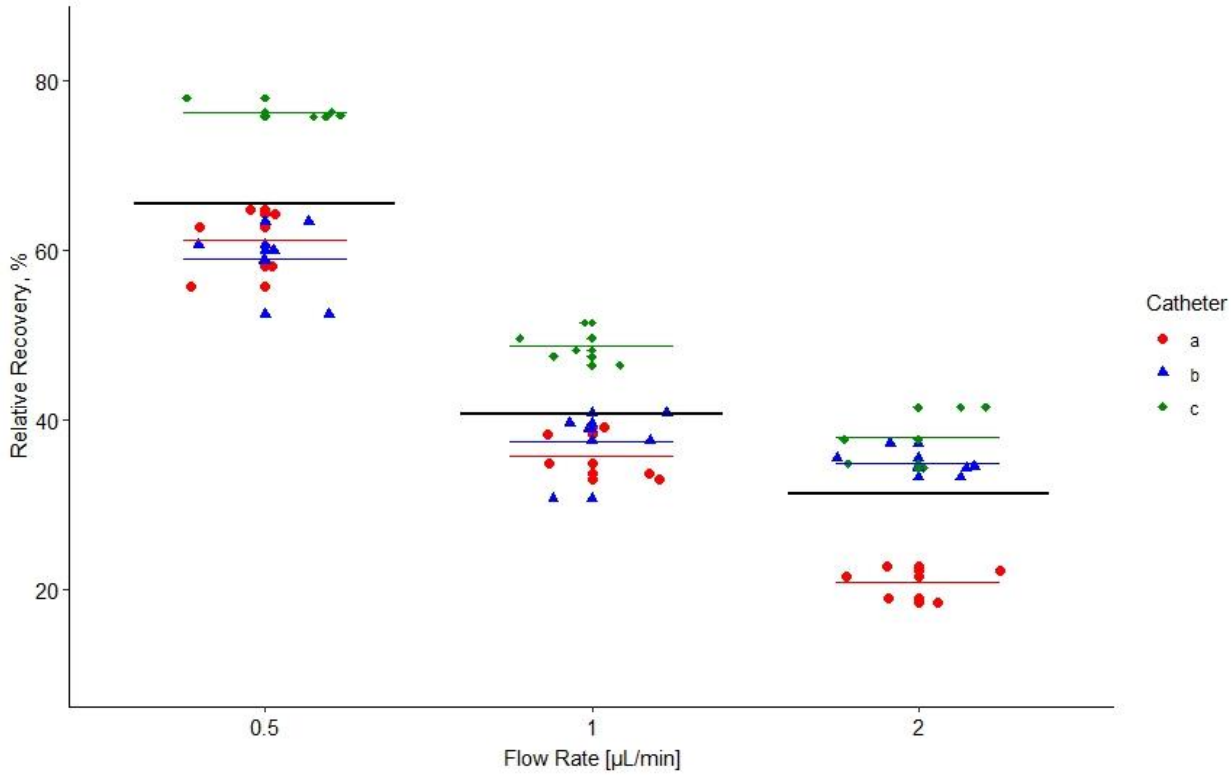

Figure 3.27 Impact of flow rate on relative recovery in retrodialysis setting
Coloured horizontal lines reflect the mean of the respective catheter, black horizontal lines the mean of all 3 investigated catheters

Table 3.8 Mean relative recovery values, intra- and inter-catheter variability obtained in the evaluation of potential impact of flow rate on relative recovery

Flow rate [µL/min]	Setting	Mean relative recovery, %	Maximum intra-catheter range, %	Inter-catheter range, %
0.5	Microdialysis	51.1	15.2	20.9
	Retrodialysis	65.5	10.8	25.4
1	Microdialysis	38.9	9.17	22.4
	Retrodialysis	40.7	10.1	20.7
2	Microdialysis	27.1	8.81	23.3
	Retrodialysis	31.3	7.17	23.0

3.3.1.6 Impact of pH value on relative recovery

Detailed plots with all single RR values including respective intra- and inter-catheter means visualising the impact of pH values on RR are shown in Figure 3.28 and Figure 3.29. A summary of the mean RR values (n=15 samples), their intra-(n=5 samples) and inter-catheter (n=15 samples) variability is given in Table 3.9.

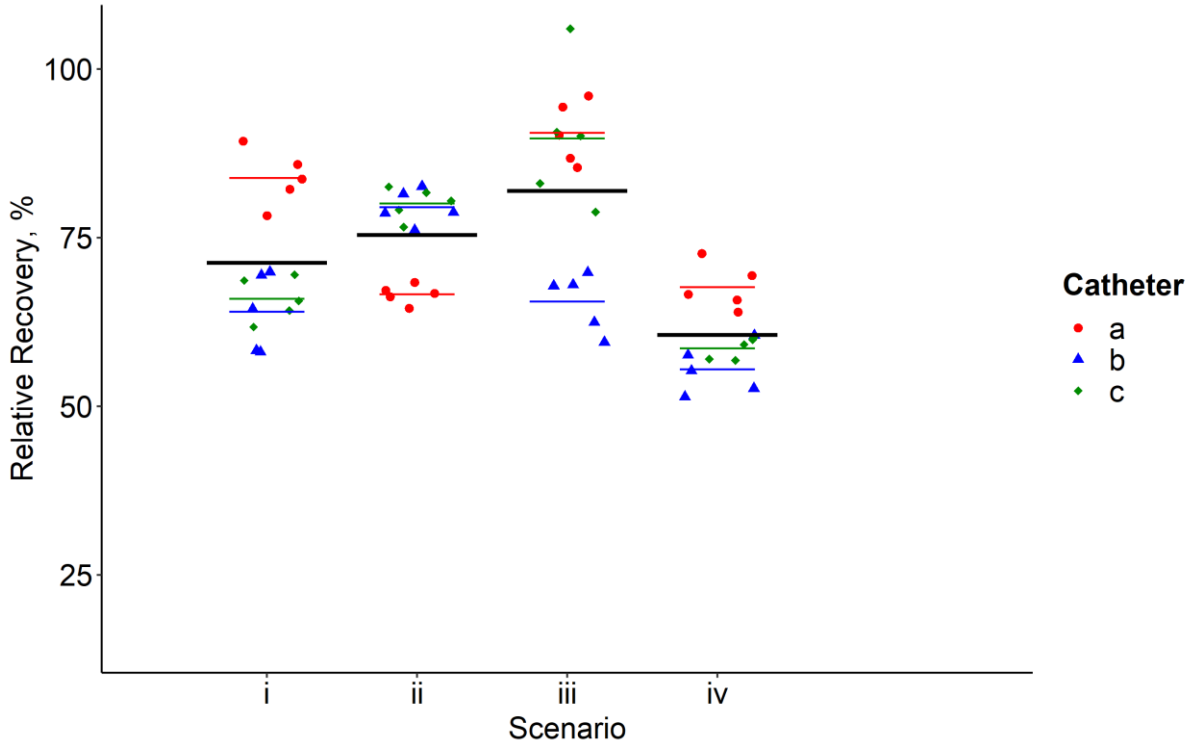

Figure 3.28 Impact of pH value on relative recovery in microdialysis setting
Coloured horizontal lines reflect the mean of the respective catheter, black horizontal lines the mean of all 3 investigated catheters, Scenario (i): unbuffered Ringer's solution (RS) as perfusate and phosphate buffered saline (PBS) buffer pH 7.0 as SM, Scenario (ii): unbuffered RS as perfusate and PBS buffer pH 7.4 as SM, Scenario (iii): PBS buffer pH 7.0 as perfusate and SM, Scenario (iv): PBS buffer pH 7.4 as perfusate and SM

Mean RR values in microdialysis setting were 71.3% (SD 9.79%), 75.4% (SD 6.52%), 81.9% (SD 13.2%) and 60.6% (SD 5.84%) for scenario (i) (unbuffered RS as perfusate and PBS buffer pH 7.0 as SM), scenario (ii) (unbuffered RS as perfusate and PBS buffer pH 7.4 as SM), scenario (iii) (PBS buffer pH 7.0 as perfusate and SM) and scenario (iv) (PBS buffer pH 7.4 as perfusate and SM). Intra-catheter variability was ≤11.9% except for scenario (iii) which had a very high variability of 27.2% in the calculated RR values of catheter c. Beside the expected large inter-catheter variability of 46.5% in scenario (iii) caused by the high intra-catheter variability, also scenario (i) showed a high inter-catheter range of 31.3% which was caused by high and very variable RR values of catheter c and low RR values of catheter b. The other scenarios had a maximum inter-catheter variability of 21.2%.

In the retrodialysis setting, mean RR values of 62.9% (SD 4.18%), 59.0% (SD 6.10%), 57.7% (SD 2.88%) and 59.9% (SD 5.66%) were found for scenario (i) to (iv), respectively and therefore generally lower RR values than in the microdialysis setting were observed. A high intra- and inter-catheter variability was not observed, maximum variabilities were 12.5% and 23.3%, respectively.

Comparing scenarios with different pH values using unbuffered RS as perfusate, pH 7.0 (scenario (i)) resulted in 4.16% lower (microdialysis setting) and 3.84% higher mean RR values (retrodialysis setting) than pH 7.4 (scenario (ii)). For PBS buffered perfusate, mean RR values for pH 7.0 (scenario (iii)) deviated by 21.3% and -2.20% from those of pH 7.4 (scenario (iv)) in microdialysis and retrodialysis settings, respectively.

Looking at mean RR values obtained from microdialysis and retrodialysis settings within a scenario, an inconsistent result was obtained: high deviations for scenario (ii) and (iii): 16.4% and -24.2%, respectively, a lower deviation of -8.40% for scenario (i) and 1 of only -0.66% for scenario (iv).

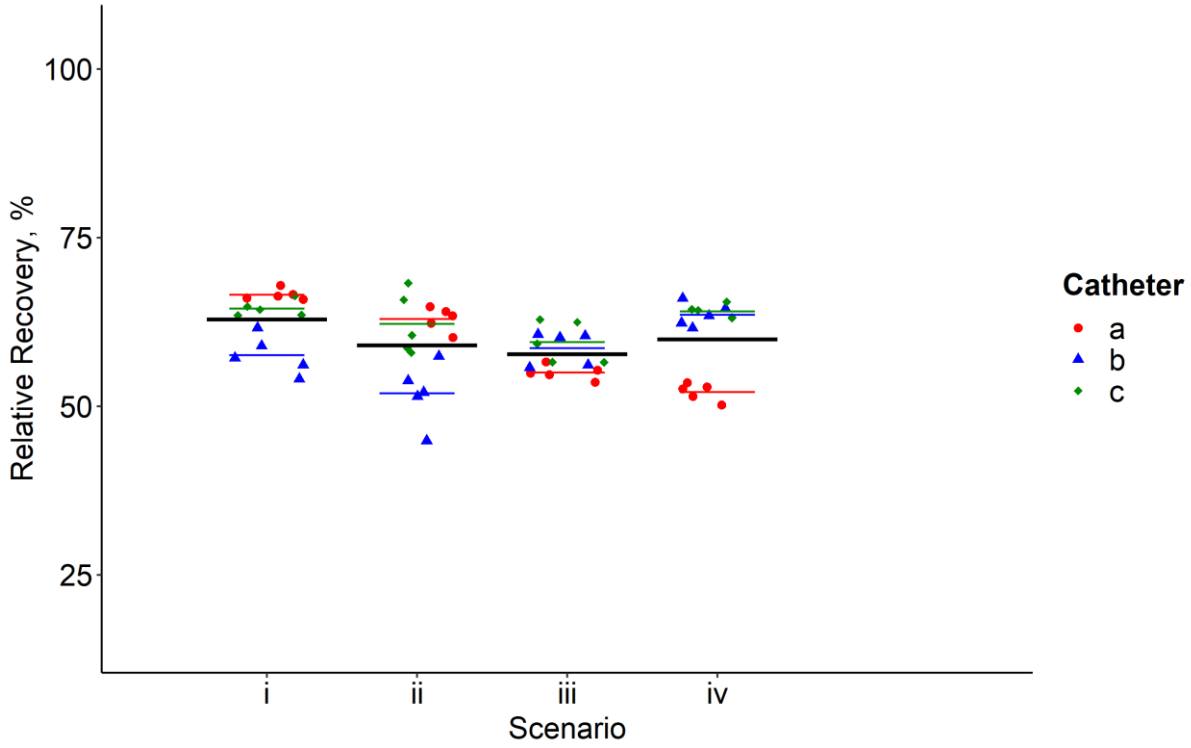

Figure 3.29 Impact of pH value on relative recovery in retrodialysis setting
Coloured horizontal lines reflect the mean of the respective catheter, black horizontal lines the mean of all 3 investigated catheters, Scenario (i): unbuffered Ringer's solution (RS) as perfusate and phosphate buffered saline (PBS) buffer pH 7.0 as SM, Scenario (ii): unbuffered RS as perfusate and PBS buffer pH 7.4 as SM, Scenario (iii): PBS buffer pH 7.0 as perfusate and SM, Scenario (iv): PBS buffer pH 7.4 as perfusate and SM

Table 3.9 Mean relative recovery values, intra- and inter-catheter variability obtained in the evaluation of potential impact of pH value on relative recovery

Scenario	Setting	Mean relative recovery, %	Maximum intra-catheter range, %	Inter-catheter range, %
(i)	Microdialysis	71.3	11.9	31.3
	Retrodialysis	62.9	7.58	13.9
(ii)	Microdialysis	75.4	6.50	18.1
	Retrodialysis	59.0	12.5	23.3
(iii)	Microdialysis	81.9	27.2	46.5
	Retrodialysis	57.7	6.31	9.28
(iv)	Microdialysis	60.6	9.12	21.2
	Retrodialysis	59.9	4.40	15.8

3.3.1.7 Impact of drug concentration on relative recovery

Detailed results obtained from the investigation of a potential impact of drug concentration on RR values are shown in Figure 3.30 to Figure 3.33. Mean RR values (n=15 samples) as well as their intra- (n=5 samples) and inter-catheter (n=15 samples) variability are summarised in Table 3.10 and Table 3.11.

In both settings, RR values in RS were in a comparable range for vancomycin concentrations of 10 µg/mL to 80 µg/mL. Mean RR values in microdialysis setting were 68.6% (SD 5.95%), 71.1% (SD 6.23%) and 62.4% (SD 5.88%) for concentrations of 10, 50 and 80 µg/mL, respectively. The slightly lower mean RR value resulting from a concentration of 80 µg/mL in microdialysis setting was caused by a lower mean RR value of catheter a which was 8.10% and 13.8% lower than mean RR value of catheter b and c, respectively. RR values of catheter a in this setting for the different concentrations showed a decrease of mean RR value of 20.6% and 24.3% for 80 µg/mL when compared to 10 and 50 µg/mL, respectively. On the contrary, catheter b and c showed similar RR values over the whole investigated concentration range. In retrodialysis setting, mean RR values were 60.1% (SD 3.76%), 50.4% (SD 8.75%) and 60.6% (SD 3.42%) for VAN concentrations of 10, 50 and 80 µg/mL. The lower RR values obtained for the VAN concentration of 50 µg/mL were only observed for catheter b and c, RR values from catheter a were in comparable range to the ones from the other VAN concentrations. Intra-catheter variability was ≤10.3% (microdialysis setting) and ≤13.8% (retrodialysis setting). Inter-catheter variability was comparable to the 1 obtained in the other investigations: 26.3% for microdialysis setting and 23.9% for retrodialysis setting.

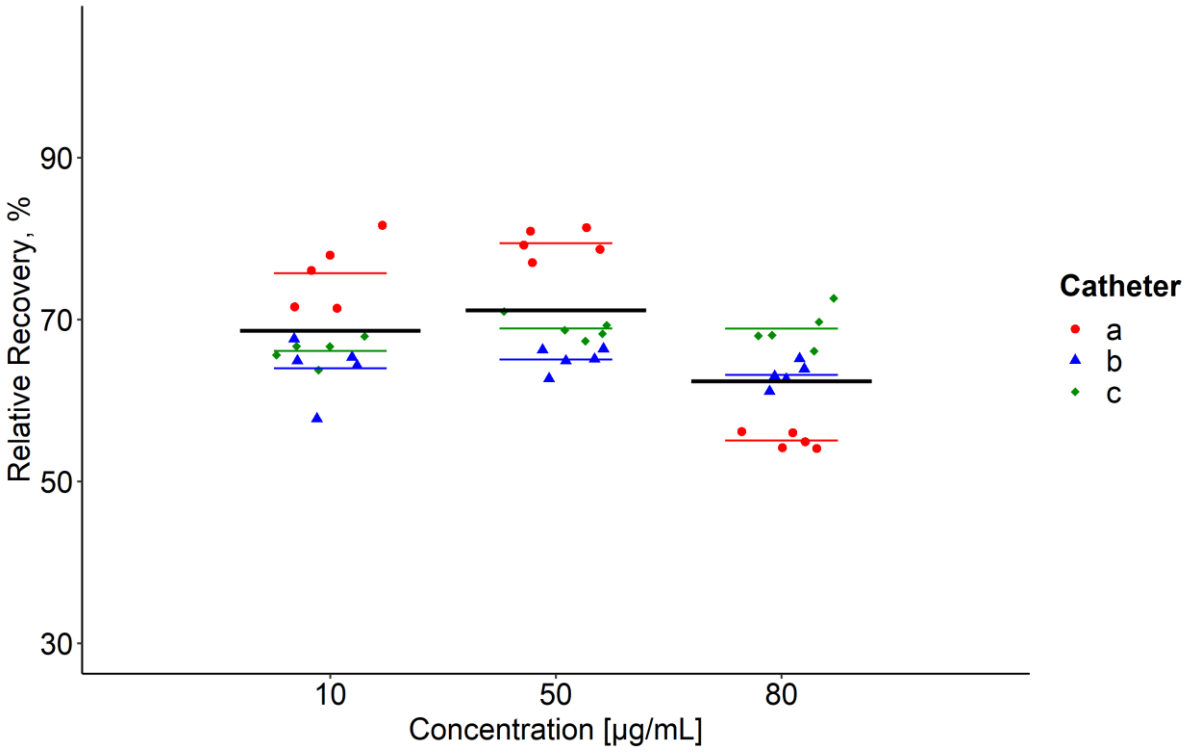

Figure 3.30 Impact of vancomycin concentration in Ringer's solution on relative recovery in microdialysis setting

Coloured horizontal lines reflect the mean of the respective catheter, black horizontal lines the mean of all 3 investigated catheters

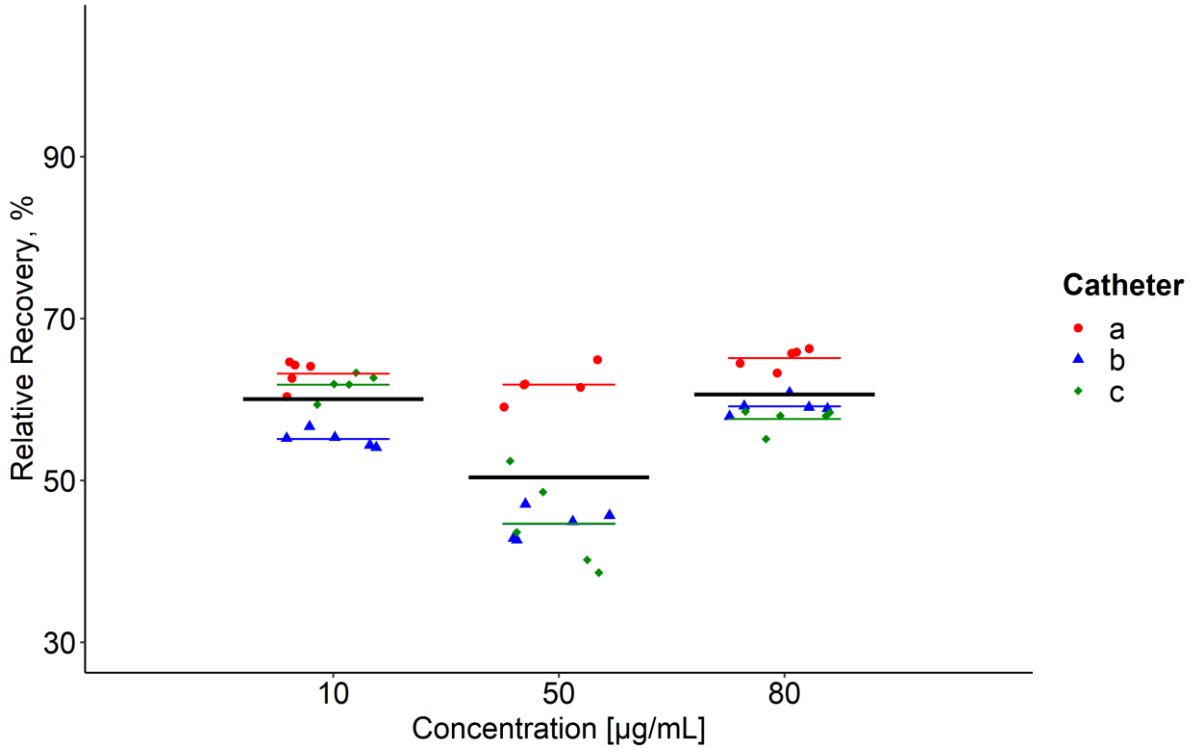

Figure 3.31 Impact of vancomycin concentration in Ringer's solution on relative recovery in retrodialysis setting

Coloured horizontal lines reflect the mean of the respective catheter, black horizontal lines the mean of all 3 investigated catheters

Table 3.10 Mean relative recovery values, intra- and inter-catheter variability obtained in the evaluation of a potential impact of vancomycin concentration in Ringer's solution on relative recovery

Vancomycin concentration [µg/mL]	Setting	Mean relative recovery, %	Maximum intra-catheter range, %	Inter-catheter range, %
10	Microdialysis	68.6	10.3	23.9
	Retrodialysis	60.1	4.29	10.6
50	Microdialysis	71.1	4.32	18.7
	Retrodialysis	50.4	13.8	26.3
80	Microdialysis	62.4	6.51	18.5
	Retrodialysis	60.6	3.42	11.2

Using PBS buffer adjusted to pH 7.4, mean RR values were all in the same range in both settings. In the microdialysis setting, mean RR values of 65.7% (SD 5.34%), 60.6% (SD 5.84%) and 71.4% (SD 6.91%) for VAN concentrations of 10, 50 and 80 µg/mL were found, respectively. In the retrodialysis setting, 58.6% (SD 5.77%), 59.9% (SD 5.66%) and 64.0% (SD 4.31%) were the resulting mean RR values for the respective concentrations. Intra-catheter variability was ≤17.2% (microdialysis setting) and ≤11.4% (retrodialysis setting). Inter-catheter variability showed comparable ranges to the ones obtained in RS: ≤26.3% and ≤19.3% in microdialysis and retrodialysis setting, respectively.

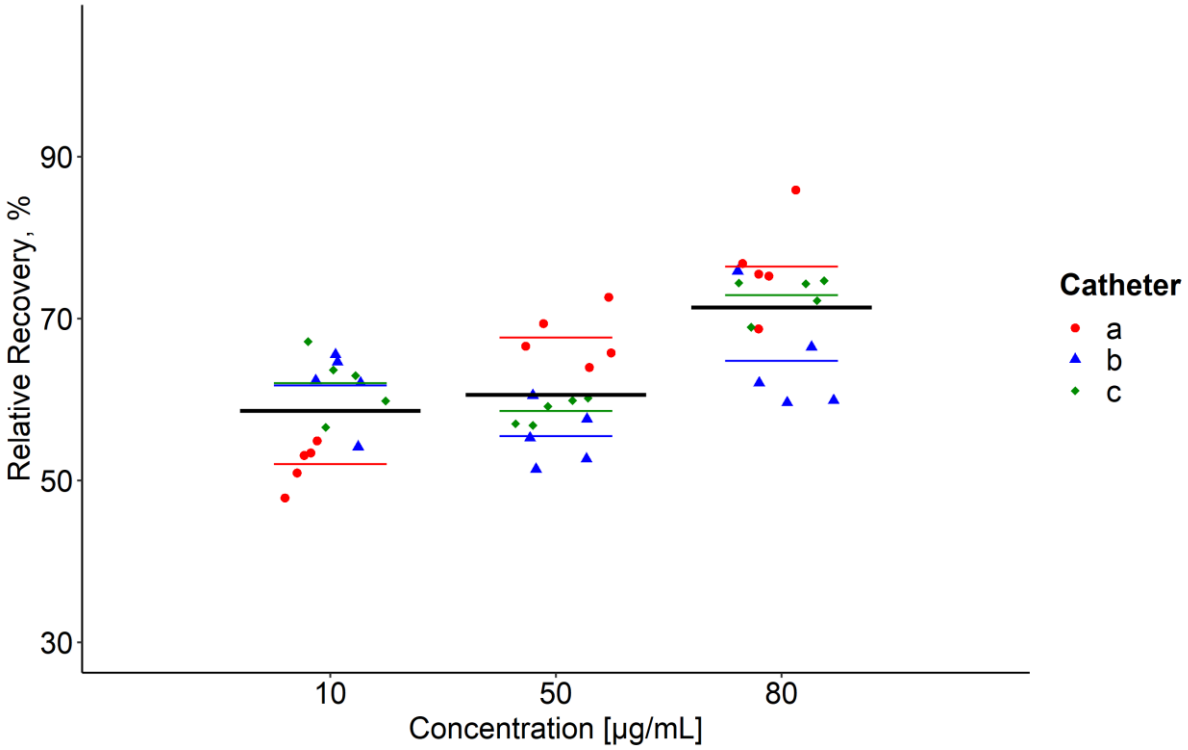

Figure 3.32 Impact of vancomycin concentration in PBS buffer pH 7.4 on relative recovery in microdialysis setting

PBS: phosphate buffered saline

Coloured horizontal lines reflect the mean of the respective catheter, black horizontal lines the mean of all 3 investigated catheters

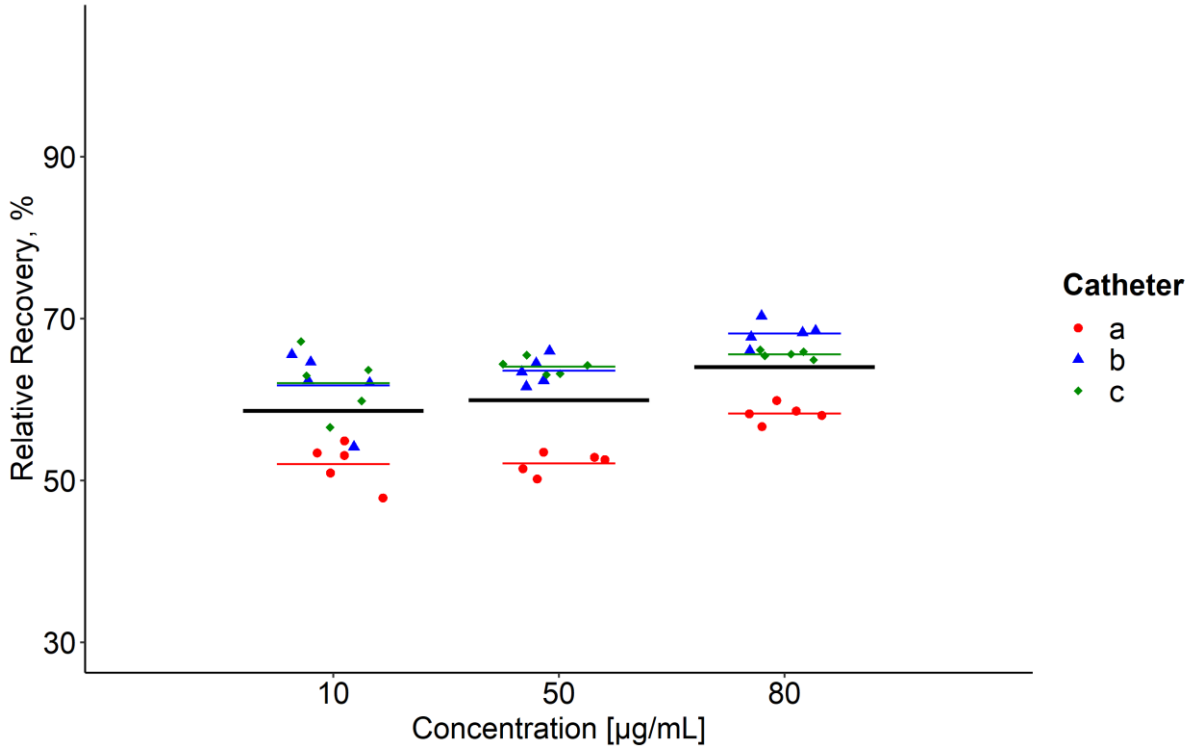

Figure 3.33 Impact of vancomycin concentration in PBS buffer pH 7.4 on relative recovery in retrodialysis setting

PBS: phosphate buffered saline

Coloured horizontal lines reflect the mean of the respective catheter, black horizontal lines the mean of all 3 investigated catheters

Table 3.11 Mean relative recovery values, intra- and inter-catheter variability obtained in the evaluation of a potential impact of vancomycin concentration in PBS buffer pH 7.4 on relative recovery

Vancomycin concentration [µg/mL]	Setting	Mean relative recovery, %	Maximum intra-catheter range, %	Inter-catheter range, %
10	Microdialysis	65.7	14.3	18.9
	Retrodialysis	58.6	11.4	19.3
50	Microdialysis	60.6	9.12	21.2
	Retrodialysis	69.9	4.40	15.8
80	Microdialysis	71.4	17.2	26.3
	Retrodialysis	64.0	4.29	13.7

PBS: phosphate buffered saline

3.3.1.8 Impact of diffusion direction on relative recovery

Figure 3.34 and Figure 3.35 display detailed results of the investigation regarding a potential impact of diffusion direction on RR. A summary of the obtained data, including mean RR values (n=15 samples), intra- and inter-catheter variability (n=5 and 15 samples, respectively) and the difference between microdialysis and retrodialysis setting are shown in Table 3.12.

Mean RR values for Scenario (i), the unbuffered RS as perfusate and SM were 36.2% (SD 13.1%) in microdialysis setting and 38.9% (SD 4.66%) in retrodialysis setting. For Scenario (ii), unbuffered RS as perfusate and PBS buffer pH 7.0 as SM, mean RR values of 60.5% (SD 8.46%) and 56.8% (SD 10.2%), were observed in microdialysis and retrodialysis setting, respectively. For Scenario (iii), unbuffered RS as perfusate and PBS buffer pH 7.4 as SM, mean RR values of 68.7% (SD 12.5%, microdialysis setting) and 62.8% (SD 6.11%, retrodialysis setting) were found. Mean RR values for Scenario (iv), PBS buffer pH 7.0 as perfusate and SM and Scenario (v), PBS buffer pH 7.4 as perfusate and SM, were 71.4% (SD 6.13%) and 63.2% (SD 6.99%) in microdialysis setting and 55.4% (SD 7.84%) and 61.7% (SD 7.57%) in retrodialysis setting, respectively. Differences between mean RR values of retrodialysis setting and microdialysis setting were small for the majority of investigated scenarios: -2.72% (Scenario (i)), 3.67% (Scenario (ii)), 5.95% (Scenario (iii)), 16.0% (Scenario (iv)) and 1.50% (Scenario (v)). Hence, except for Scenario (iv), no relevant differences of mean RR values in microdialysis and retrodialysis setting were observed using a VAN concentration of 100 µg/mL. Maximum intra- and inter-catheter variability was 20.6% and 40.2% in microdialysis setting and 17.3% and 25.9% in retrodialysis setting, respectively.

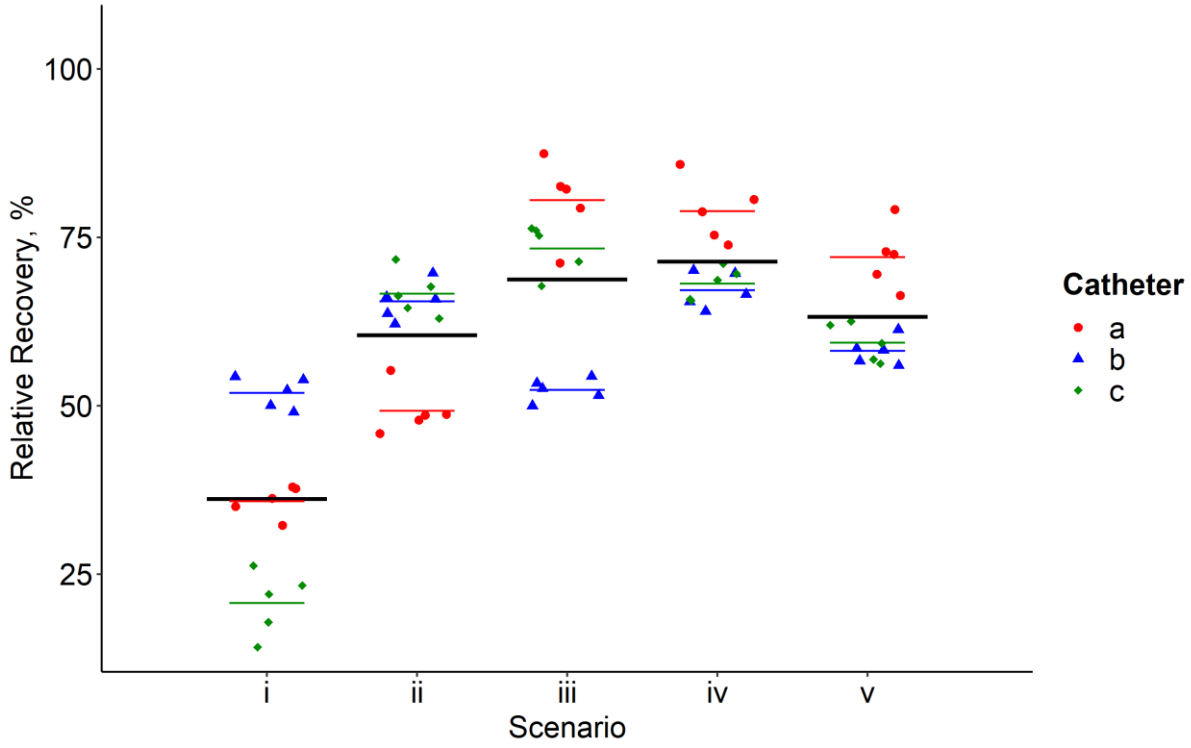

Figure 3.34 Impact of the setting on relative recovery in microdialysis setting
Coloured horizontal lines reflect the mean of the respective catheter, black horizontal lines the mean of all 3 investigated catheters, Scenario (i): unbuffered Ringer's solution (RS) as perfusate and SM, Scenario (ii): unbuffered RS as perfusate and phosphate buffered saline (PBS) buffer pH 7.0 as SM, Scenario (iii): unbuffered RS as perfusate and PBS buffer pH 7.4 as SM, Scenario (iv): PBS buffer pH 7.0 as perfusate and SM, Scenario (v): PBS buffer pH 7.4 as perfusate and SM

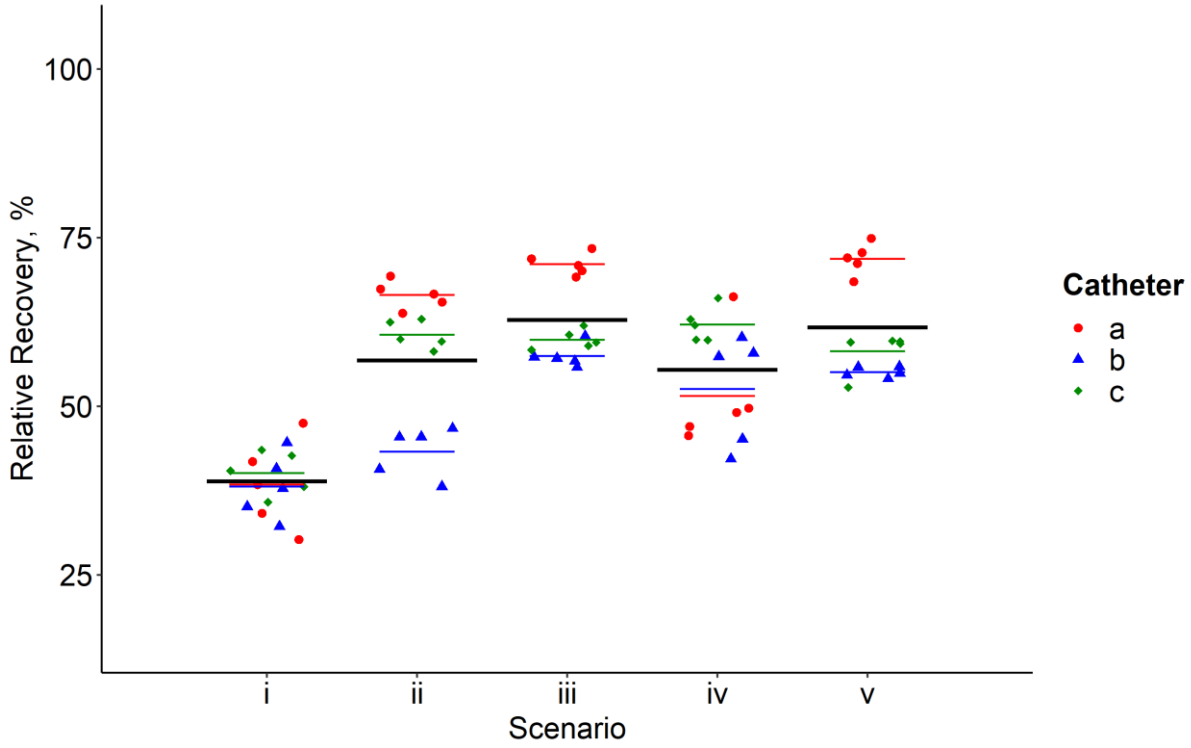

Figure 3.35 Impact of the setting on relative recovery in retrodialysis setting
Coloured horizontal lines reflect the mean of the respective catheter, black horizontal lines the mean of all 3 investigated catheters, Scenario (i): unbuffered Ringer's solution (RS) as perfusate and SM, Scenario (ii): unbuffered RS as perfusate and phosphate buffered saline (PBS) buffer pH 7.0 as SM, Scenario (iii): unbuffered RS as perfusate and PBS buffer pH 7.4 as SM, Scenario (iv): PBS buffer pH 7.0 as perfusate and SM, Scenario (v): PBS buffer pH 7.4 as perfusate and SM

Table 3.12 Mean relative recovery values, intra- and inter-catheter variability obtained in the evaluation of potential impact of the setting on relative recovery

Scenario	Setting	Mean relative recovery, %	Maximum intra-catheter range, %	Inter-catheter range, %	Difference $RR_{microdialysis}$ - $RR_{retrodialysis}$
(i)	Microdialysis	36.2	12.1	40.2	-2.72
	Retrodialysis	38.9	17.3	17.3	
(ii)	Microdialysis	60.5	11.8	25.0	3.67
	Retrodialysis	56.8	9.38	25.9	
(iii)	Microdialysis	68.7	16.2	37.4	5.95
	Retrodialysis	62.8	4.63	17.6	
(iv)	Microdialysis	71.4	20.6	24.1	16.0
	Retrodialysis	55.4	12.0	21.8	
(v)	Microdialysis	63.2	12.8	23.2	1.50
	Retrodialysis	61.7	6.91	22.1	

3.3.1.9 Impact of different perfusates on relative recovery

Based on the *in vitro* findings, unbuffered RS was proposed as perfusate of choice for the subsequent clinical trial as no impact of pH value, drug concentration or microdialysis setting was observed on the resulting RR.

3.3.3 Clinical trial

3.3.1.10 Implementation of results from *in vitro* investigations in the clinical trial design

An equilibration time of 0.5 h was included prior to microdialysis and retrodialysis sampling as described in Section 3.3.2. Based on the *in vitro* results, the applied flow rate was 1 µL/min leading to sampling intervals of 0.5 h for microdialysis and retrodialysis sampling. Unbuffered RS was used as perfusate. Catheter calibration was performed via retrodialysis with two consecutive sampling intervals. Calculation of RR was performed using the mean of both obtained RR values.

3.3.1.11 Patient characteristics

In the feasibility study, 9 infants were included, 5 females and 4 males. Their characteristics as well as vancomycin dosing and comedication is summarised in Table 3.13. Four of these infants were terms, 5 preterms. The body weight had a range of 890 to 5,890 g (mean 3,722 g). Gestational and chronological age were between 23+5 and 41+0 weeks (mean 32+4 weeks) and between 23 and 255 days (mean 93.4 days), respectively. The maximum plasma creatinine concentration was 0.5 mg/dL in all patients. Total VAN daily doses ranged from 18 to 255 mg (mean 143 mg) divided into up to 3 doses. During the clinical trial, patients received up to 10 concomitant drugs, mainly antiinfective and analgetic agents.

Table 3.13 Patient characteristics, administered vancomycin dosing regime and comedication during the study

Patient	Gestational age at birth [weeks + days]	Chrono-logical age [days]	Body weight [g]	Vancomycin daily dose [mg], dosing frequency	Comedication
ID 1	32+1	48	3030	44; tid	Lidocaine*, piperacillin/tazobactam, chloral hydrate, ursodeoxycholic acid, tobramycin, nystatin
ID 2	23+5	61	1185	18, qd	Lidocaine*, dopamine, piperacillin/tazobactam, phenobarbital, midazolam
ID 3	24+2	27	890	13, qd	Lidocaine*, nystatin, meropenem, fluconazole, caffeine, midazolam, vitamin K
ID 4	41+0	86	4500	70, tid	Lidocaine*, nystatin, meropenem, levoththyroxine, midazolam
ID 5	38+2	23	2750	43.5, tid	Lidocaine*, nystatin, piperacillin/tazobactam, ursodeoxycholic acid, vitamin K
ID 6	37+2	93	5700	85, bid	Lidocaine*, dopamine, meropenem, levetiracetam
ID 7	37+6	177	5200	78, bid	Lidocaine*, spironolactone, hydrochlorothiazide, furosemide, coumadin, levothyroxine, nystatin
ID 8	24+2	255	5890	80, tid	Lidocaine*, teicoplanin, piperacillin/tazobactam, enoxaparin, nystatin, acetaminophen
ID 9	32+2	71	4350	67.5, bid	Lidocaine*, acetylic salicylic acid, aciclovir, meropenem, phenobarbital, hydrochlorothiazide, spironolactone, metamizole, ibuprofen, propofol, nystatin

bid: twice daily, ID: individual identifier, qd: once daily, tid thrice daily

* lidocaine administered as cream (EMLA®) for local anaesthesia

3.3.1.12 Feasibility in clinical routine and safety

Microdialysis sampling did not interfere with medical or caring procedures and was well integrated into clinical routine. For 8 patients, microdialysis samples were collected as planned. In patient 2, a catheter dislocation occurred 6 h after the first VAN infusion. Based on recommendations from the local ethics committee for this feasibility study, no second catheter was inserted, hence the measurement ended and catheter calibration could not be performed. In patient 7, pump problems led to an inconsistent flow rate and ultimately to the inability of sample collection within the first 2.5 h of microdialysis sampling. Because of vital medical interventions on the patient, the measurement period was adjusted and ended after 21.5 h instead of after 24 h, the catheter calibration was performed as planned after an equilibration time of 0.5 h but by collecting only 1 retrodialysate sample. The second retrodialysate sample as well as the plasma minimum sample could not be obtained. For patient 1, retrodialysis was performed prior to first VAN administration, for all other patients retrodialysis followed the microdialysis sampling.

No severe adverse events connected to the microdialysis sampling were observed during the trial or the following hospital stay. Two patients showed minor skin bleeding after catheter insertion or during sample collection. As no sign of an infection or a contamination of microdialysate samples was observed, both events were classified as minor complication. Lidocaine might cause a methemoglobinaemia, especially in infants, therefore methemoglobin concentrations were monitored during the investigation but showed no elevation in the included patients.

3.3.4 Patient samples

Cal and QC samples used for the majority of patient samples were in accordance with the recommendations of the EMA Guideline [141]. Anyhow, some QC samples showed deviations from nominal content outside the tolerable range: at maximum of ±206%. Affected were analytical runs of samples from patient 6, 8 and 9 as well from a reanalysis of single samples from all patients which were outside the calibration range in the applied sample dilution. Cal samples met the acceptance criteria in all performed analytical runs.

3.3.1.13 Microdialysate concentrations

Individual unbound concentration-time courses are displayed in Figure 3.36, Figure 3.37 and Figure 3.38. For a summary of basic pharmacokinetic parameters see Table 3.14.

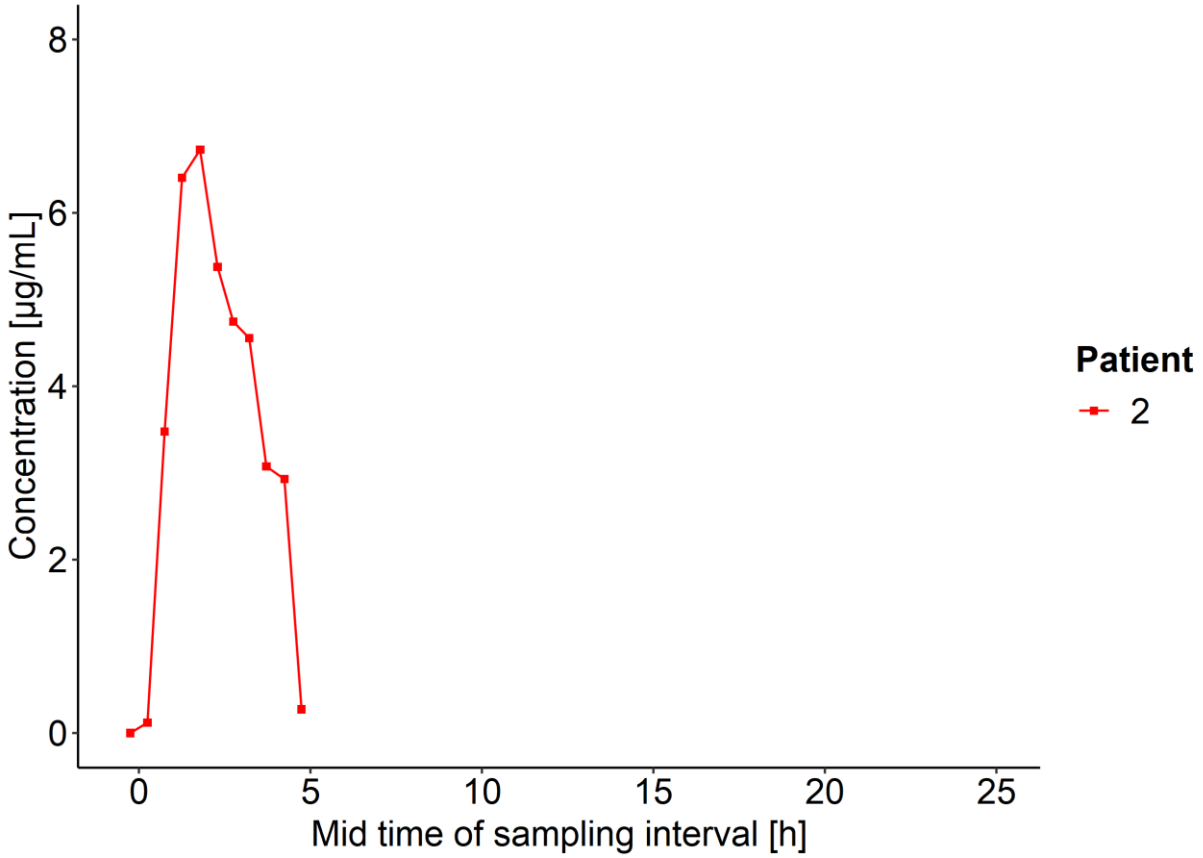

Figure 3.36 Unbound concentration-time courses of vancomycin in microdialysate samples for patients with a once daily dosing

Time represents the mid time of the sampling interval

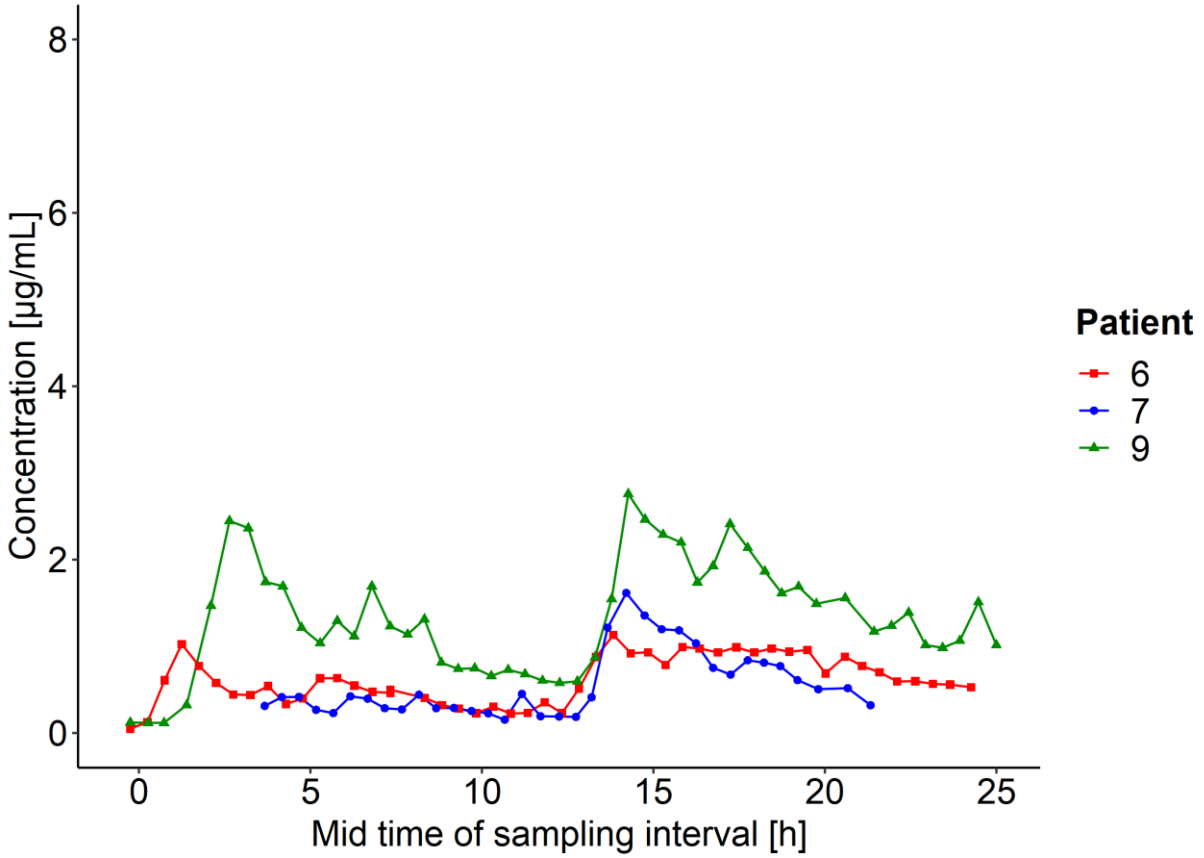

Figure 3.37 Unbound concentration-time courses of vancomycin in microdialysate samples for patients with a twice daily dosing

Time represents the mid time of the sampling interval

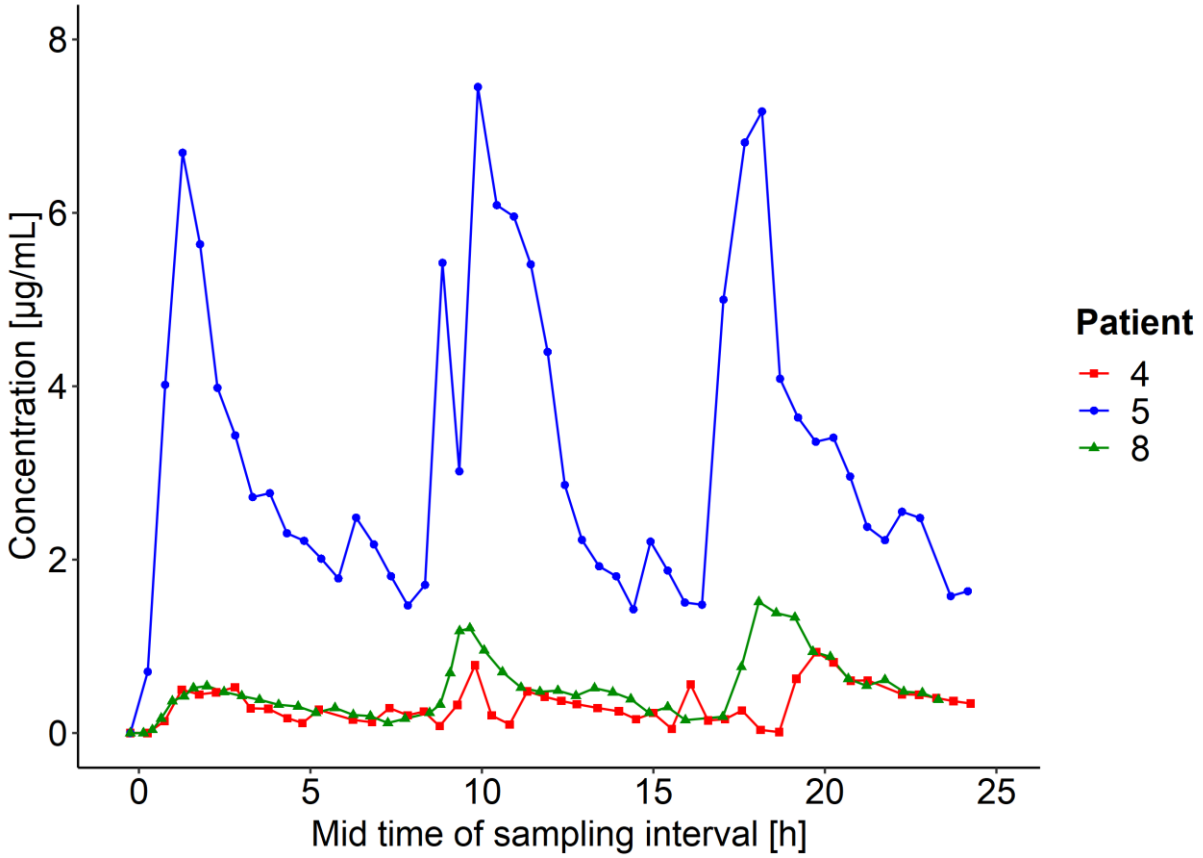

Figure 3.38 Unbound concentration-time courses of vancomycin in microdialysate samples for patients with a thrice daily dosing

Time represents the mid time of the sampling interval

Table 3.14 Basic pharmacokinetic parameters for vancomycin in microdialysate

Patient	Dosing interval [h]	Unbound C_{max} [µg/mL]	T_{max} [h]	Unbound C_{min}/C_{last} [µg/mL]
2	24	6.73	1.78	0.27
4	8	0.53, 0.78, 0.93	2.80, 9.80, 19.8	0.08, 0.01, 0.34
5	8	6.69, 7.45, 7.17	1.28, 9.88, 18.2	1.71, 1.48, 1.64
6	12	1.03, 1.13	1.25, 13.8	0.23, 0.53
7	12	1.62	14.2	0.41, 0.32
8	8	0.54, 1.21, 1.51	1.98, 9.65, 18.1	0.33, 0.19, 0.38
9	12	2.45, 2.76	2.64, 14.3	0.58, 1.01

C_{max}: maximum concentration per dosing interval, C_{min}/C_{last}: lowest concentration within a dosing interval or in case of the last dosing interval, the concentration in the last obtained sampling interval, T_{max}: mid time of the sampling interval with the highest drug concentration per dosing interval

For all patients except 1 and 3, plausible unbound concentration-time courses were observed in microdialysate characterised by an increase of unbound VAN concentrations over 2 to 4 sampling intervals starting with the 1 h infusion and a subsequent decrease. During the clinical trial, patient 2 received 1 VAN dose, patient 6, 7 and 9 received 2 doses and patient 4, 5 and 8 received 3 doses.

Unbound C_{max} had a range from 0.53 (patient 4) to 6.69 µg/mL (patient 5) in the first dosing interval. T_{max} was observed 1 to 2 h after start of infusion in all patients with plausible concentration-time courses in all dosing intervals. Similar to unbound C_{max} values, a wide concentration range was found for unbound C_{min} and unbound C_{last}. The lowest and highest values were found again in patient 4 and 5 with 0.01 µg/mL (unbound C_{min} right before the third dose) and 1.63 µg/mL (unbound C_{last}), respectively.

Patient 1 showed highly variable concentrations over the whole observation with an implausible concentration-time course. In addition, unbound concentrations ranged from 19 to 24,027 µg/mL which were up to 3fold higher than concentrations observed in other patients and for most sampling intervals also significantly above the concentration in the retroperfusate (28 µg/mL). Also for patient 3, an implausible concentration-time course was observed with increases in VAN concentration without prior drug administration and drug administrations without subsequent increase of VAN in the samples. Further investigations showed similar concentration-time courses as observed for VAN for all concomitantly quantified drugs, e.g. for lidocaine. Lidocaine was administered as a patch prior to the catheter insertion which should result in a slow and constant drug increase instead of the observed 3 peaks at the same time as for VAN. All peaks across all drugs observed in this patient showed an extremely steep decrease from C_{max} to C_{min} with a 0.5 h sampling interval which was not observed in the other patients. Finally, both patients were excluded from further data analysis assuming an analytical problem.

For the 2 samples collected from patient 2 right before the catheter dislocation, very high and strongly increasing VAN concentrations were observed. As they might already be affected by the dislocation, both concentrations were excluded as well. In patient 7, very low microdialysate volumes were obtained due to the pump problems within the first 4 h. Starting with the 8th sampling interval, a different pump was used which had to be equilibrated for another 0.5 h to ensure reliable concentrations in the microdialysate samples. Therefore the first 8 samples were excluded. In addition, microdialysis sampling had to be stopped after 21.3 h. Patient 8 had a high unbound VAN concentration of 2.10 µg/mL in the sampling interval with a mid time of 8.17 h accompanied by unbound concentrations of 0.16 µg/mL (at 7.76 h) and 0.23 µg/mL (at 8.48 h). In addition, maximum concentration in the whole investigation time was only 1.51 µg/mL. Although acknowledging the low number of samples and thus not allowing to test its prerequisites, a performed Grubbs test (α=0.05, p=<0.05) indicated an outlier, hence this concentration was excluded from further analysis.

3.3.1.14 Catheter calibration

Catheter calibration was successfully performed for all patients except for patient 2 (catheter dislocation), 1 and 3 (analytical problems), resulting RR values are displayed in Table 3.15. For patient 6 and 7, only 1 retrodialysate sample was available, for all other patients 2 retrodialysate samples as planned. For patient 7, a very low RR value of 0.88% was determined compared to RR values obtained from the other patients (range 15.4% to 36.9%). The low RR value led to extremely high ISF concentrations and was considered to be implausible. The mean RR value calculated from plausible RR values increased after exclusion of this value from 17.9% to 21.3% and was subsequently used to enable a calculation of ISF concentrations for patient 2 and 7.

Table 3.15 Relative recovery values obtained for vancomycin in the clinical trial

Patient	Relative recovery, %
1	n.a.
2	n.a.*
3	n.a.
4	16.8
5	36.9
6	19.0
7	0.88*
8	18.4
9	15.4
Mean	21.3

*Implausible or missing values were substituted by the mean relative recovery value of the other patients

3.3.1.15 ISF concentrations

Previously excluded patients and single samples were also excluded in the present section. Detailed plots can be found in Figure 3.39, Figure 3.40 and Figure 3.41, a summary of the unbound ISF concentrations including basic pharmacokinetic parameters in Table 3.16.

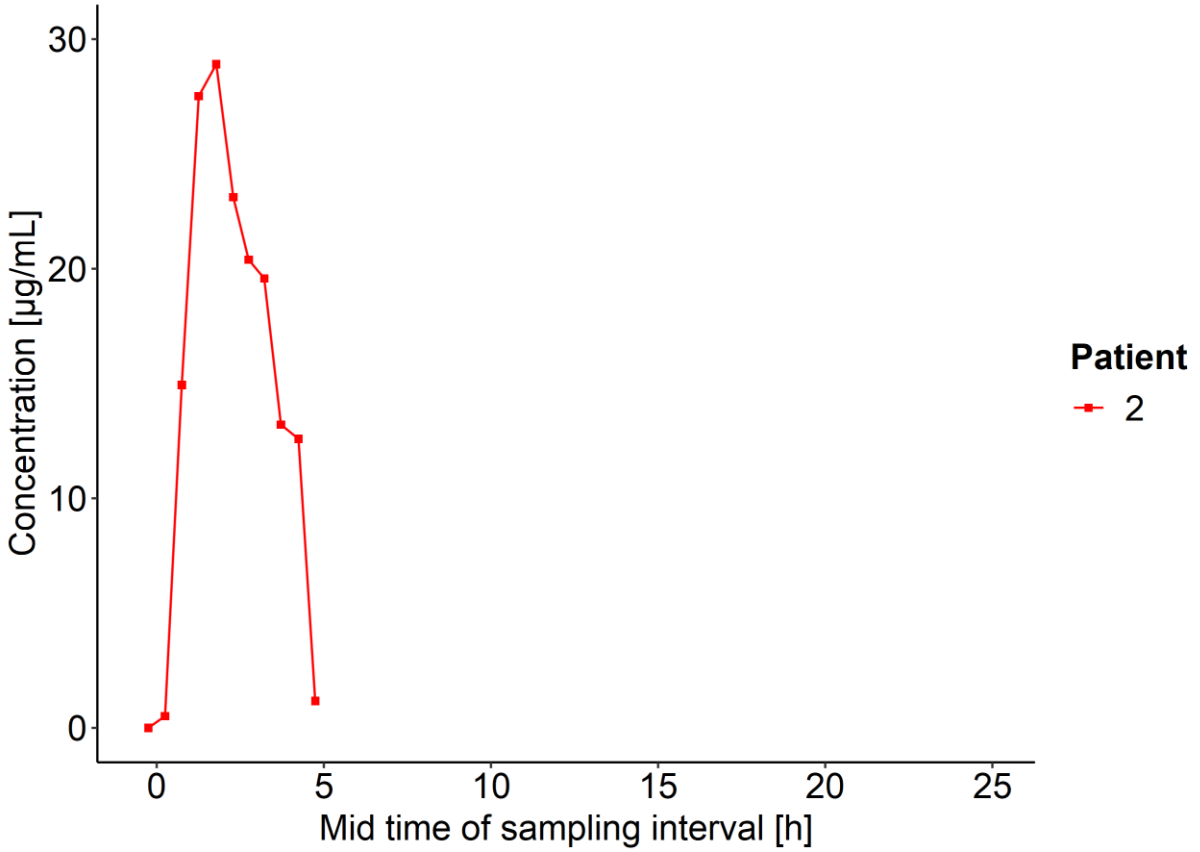

Figure 3.39 Unbound concentration-time courses of vancomycin in subcutaneous interstitial space fluid for patients with a once daily dosing

Time represents the mid time of the sampling interval

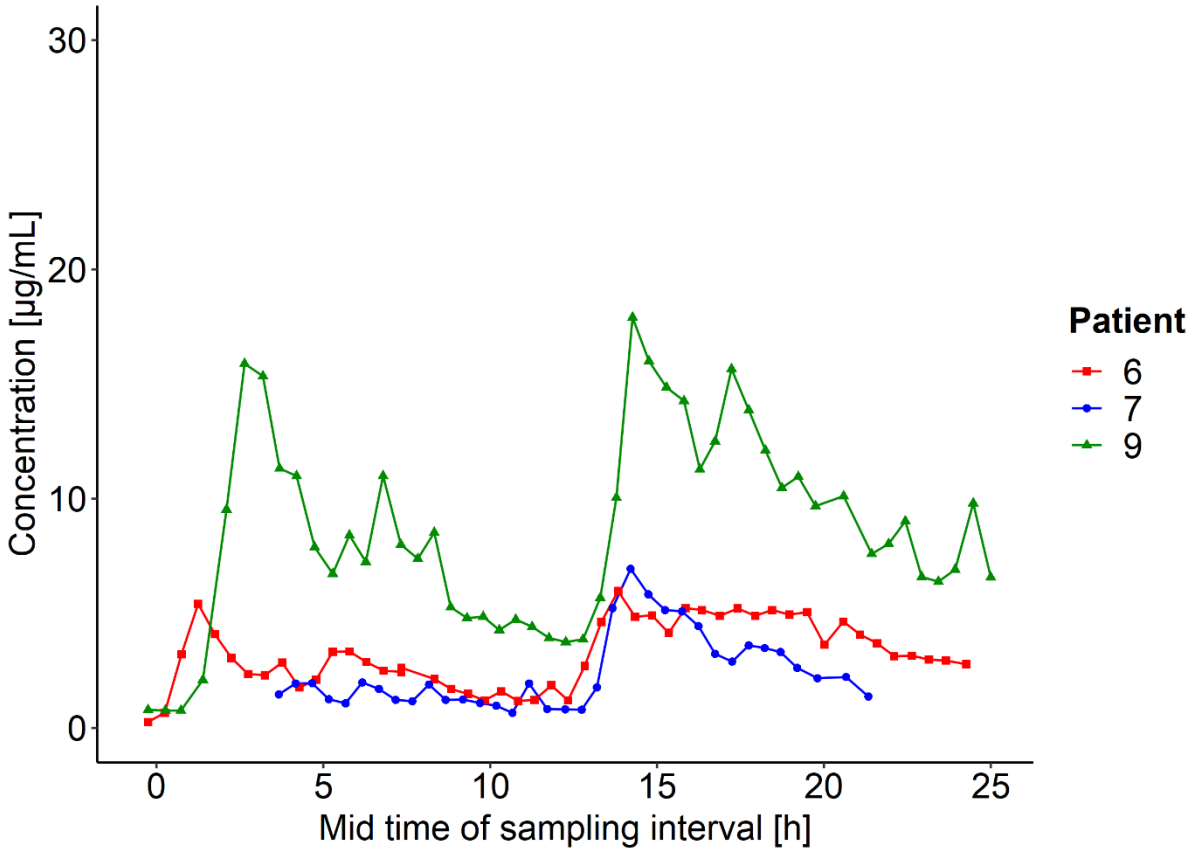

Figure 3.40 Unbound concentration-time courses of vancomycin in subcutaneous interstitial space fluid for patients with a twice daily dosing

Time represents the mid time of the sampling interval

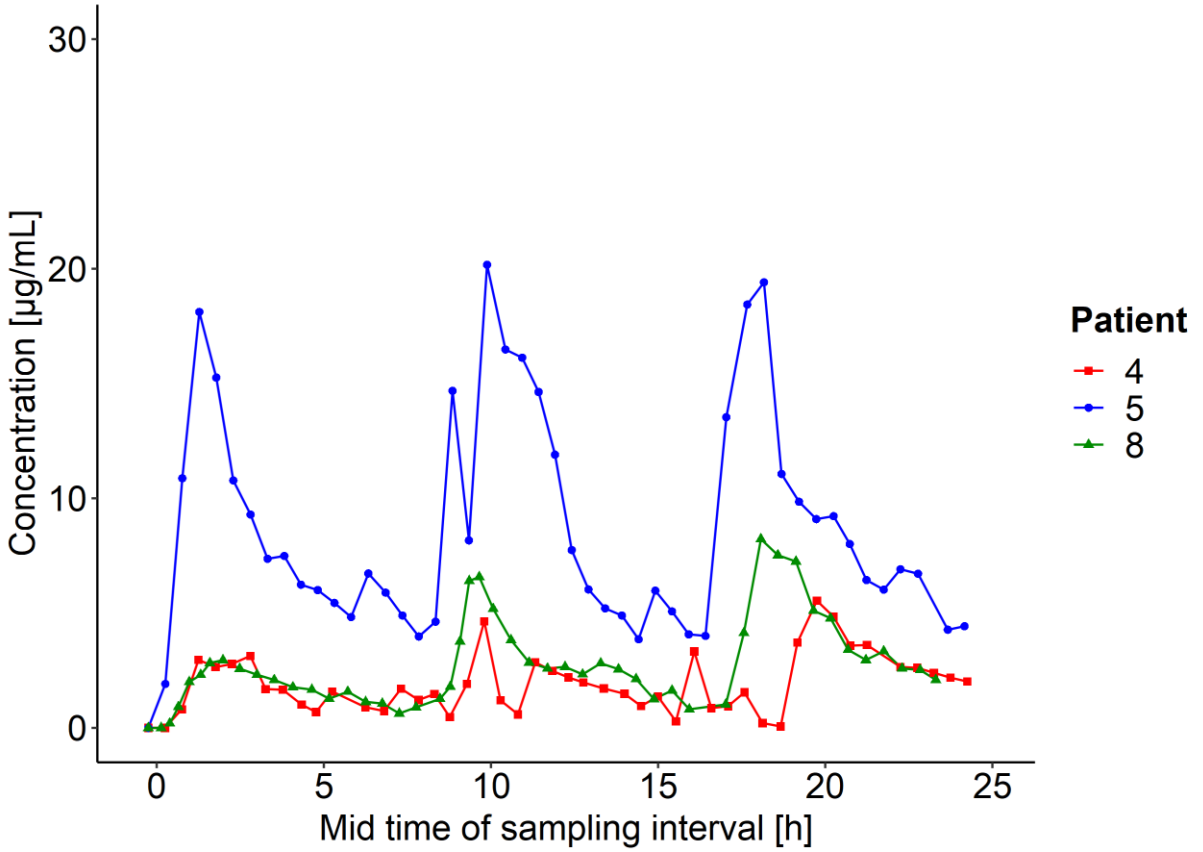

Figure 3.41 Unbound concentration-time courses of vancomycin in subcutaneous interstitial space fluid for patients with a thrice daily dosing

Time represents the mid time of the sampling interval

Table 3.16 Basic pharmacokinetic parameters for vancomycin in subcutaneous interstitial space fluid

Patient	Dosing interval [h]	Unbound C_{max} [µg/mL]	T_{max} [h]	Unbound C_{min}/C_{last} [µg/mL]
2	24	28.9	1.78	1.17
4	8	3.13, 4.64, 5.54	2.80, 9.80, 19.8	0.47, 0.06, 2.02
5	8	18.1, 20.2, 11.1	1.28, 9.88, 18.2	4.62, 4.01, 4.43
6	12	5.41, 5.97	1.25, 13.8	1.21, 2.78
7	12	6.94	14.2	1.78, 1.37
8	8	2.95, 6.57, 8.22	1.98, 9.65, 18.1	1.79, 1.06, 2.09
9	12	15.9, 17.9	2.64, 14.3	3.75, 6.58

C_{max}: maximum concentration per dosing interval, C_{min}/C_{last}: lowest concentration within a dosing interval or in case of the last dosing interval, the concentration in the last obtained sampling interval, T_{max}: mid time of the sampling interval with the highest drug concentration per dosing interval

Unbound concentration-time courses in ISF are similar to the ones described from microdialysis samples as they were converted using the RR value obtained from catheter calibration for the respective catheter. Maximum unbound ISF concentrations for the 7 included patients had a range of

2.95 µg/mL to 28.9 µg/mL. T_{max} were observed at sampling interval mid times of 1 to 2 h after start of infusion in all patients and all dosing intervals as already seen in the microdialysate profile. For unbound C_{min}/C_{last}, a range from 0.06 µg/mL to 6.58 µg/mL was found.

3.3.1.16 Plasma concentrations and their correlation to ISF concentration

Table 3.17 compares VAN concentrations in plasma with the respective unbound ISF concentrations obtained in the sampling interval of the plasma sample drawing.

Plasma concentrations were available for 8 patients. For patient 7, no sample was drawn due to the previously mentioned emergency situation. The plasma concentration for patient 3 was below lower limit of detection and could therefore not be quantified. Minimum concentrations from the remaining 7 patients had a mean of 5.56 µg/mL and a range of 3.9 to 8.7 µg/mL. Corresponding unbound ISF concentrations were calculated for 5 patients: patient 4, 5, 6, 8 and 9. The correlation coefficient was 0.018, indicating no correlation between both concentrations for this limited sample size.

Table 3.17 Plasma concentrations and respective subcutaneous interstitial space fluid concentrations for vancomycin in the clinical trial

Patient	Plasma concentration [µg/mL]	Unbound ISF concentration [µg/mL]
1	4.6	n.a.
2	3.9	n.a.
3	<1,3	n.a.
4	4.1	7.54
5	7	9.38
6	5.9	0.65
7	n.a.	n.a.
8	4.7	1.04
9	8.7	1.42

ISF: interstitial space fluid

3.4 Project IV - Perioperative antibiotic prophylaxis in synovial fluid

3.4.1 Bioanalysis of plasma samples

3.4.1.1 Development of plasma sample preparation method

At first, 100 µL of the plasma samples were precipitated using 100-400 µL ACN or methanol, subsequently vortex mixed for 30 s and centrifuged at 13,800 g for 15 min. This sample preparation failed as the obtained supernatant had too low drug concentrations to be quantified by the HPLC methods. Therefore, 300 µL of the supernatants obtained from the centrifugation were additionally evaporated until dryness and resolved in 50 µL MQ water containing 4% ACN (equating to the composition of the mobile phase in the HPLC system at injection time). For homogenisation, samples were vortex mixed for 30 s and transferred to a 96 well plate for HPLC analysis. Finally, comparable accuracy and precision were achieved using 400 µL ACN or methanol for the protein precipitation. ACN

was favoured as it was part of the mobile phase in the quantification method. This final sample preparation method for plasma samples was successfully implemented in the quantification of CFX and LIN.

3.4.1.2 Sample preparation

All blank, Cal and QC samples were prepared with pooled plasma from at least 6 different donors. As described above, each sample was mixed with ACN and centrifuged. The supernatant was evaporated until dryness and resolved with MQ water incl. 4% ACN. Blank samples contained only pooled plasma. Cal samples were prepared for CFX and LIN separately, QC samples contained both drugs in combination. For LIN, Cal levels were 0.3 µg/mL (Cal 1/LLOQ), 1 µg/mL (Cal 2), 5 µg/mL (Cal 3), 10 µg/mL (Cal 4), 15 µg/mL (Cal 5) and 25 µg/mL (Cal 6). Cal levels for CFX had a larger range: 0.3 µg/mL (Cal 1/LLOQ), 1 µg/mL (Cal 2), 20 µg/mL (Cal 3), 50 µg/mL (Cal 4), 100 µg/mL (Cal 5) and 120 µg/mL (Cal 6). QC 1 and QC 2 contained 0.3 and 0.9 µg/mL of both drugs, respectively. QC 3 contained 10 µg/mL LIN and 50 µg/mL CFX and QC 4 contained 20 µg/mL LIN and 100 µg/mL CFX. Plasma study samples were thawed, vortex mixed for 30 s and equally prepared as Cal and QC samples.

3.4.1.3 Method validation

The extended quantification method, enabling the determination of CFX and LIN in plasma and microdialysis samples, was successfully revalidated regarding linearity, calibration range, accuracy and precision in accordance to EMA Guideline [141]. Carry-over was neither found for plasma samples nor for microdialysis samples. Retention times of the drugs were comparable to the ones of microdialysate, hence no separate selectivity investigation was performed. Stability was not evaluated as it was already performed for the original method. Results are summarised in Table 3.18 and Table 3.19 for CFX and LIN, respectively.

3.4.1.4 Accuracy, precision, LLOQ and linearity

Table 3.18 Validation parameters for cefuroxime from plasma for Method I and II

Validation parameter	Method I	Method II
Calibration range (3 samples or 5 samples for LLOQ)	0.30-125 µg/mL	0.30-125 µg/mL
Within-run accuracy, RE (5 samples)	≤±10.4% (for LLOQ ≤±15.4%)	≤±9.72% (for LLOQ ≤±15.4%)
Between-run accuracy, RE (15 samples)	≤±3.14% (for LLOQ ≤5.56%)	≤±6.28% (for LLOQ -1.23%)
Within-run precision, CV (5 samples)	≤6.59% (for LLOQ ≤7.40%)	≤4.21% (for LLOQ ≤3.51%)
Between-run precision, CV (15 samples)	≤7.39% (for LLOQ 8.41%)	≤1.84% (for LLOQ 3.19%)

CV: coefficient of variation, LLOQ: lower limit of quantification, RE: relative error
All evaluations according to EMA Guideline [141]

Table 3.19 Validation parameters for linezolid from plasma for Method I and II

Validation parameter	Method I	Method II
Calibration range (3 samples or 5 samples for LLOQ)	0.30-25.0 µg/mL	0.30-25.0 µg/mL
Within-run accuracy, RE (5 samples)	≤±9.85% (for LLOQ ≤±13.5%)	≤±10.9% (for LLOQ ≤±15.2%)
Between-run accuracy, RE (15 samples)	≤±1.89% (for LLOQ -1.29%)	≤±10.4% (for LLOQ 7.35%)
Within-run precision, CV (5 samples)	≤5.88% (for LLOQ ≤4.72%)	≤3.83% (for LLOQ ≤3.99%)
Between-run precision, CV (15 samples)	≤8.51% (for LLOQ 10.3%)	≤3.10% (for LLOQ 6.17%)

CV: coefficient of variation, LLOQ: lower limit of quantification, RE: relative error
All evaluations according to EMA Guideline [141]

Within-run accuracy for both drugs and methods was ≤±15.4% for LLOQ and ≤±10.9% for higher QC samples. Between-run accuracy was ≤±7.35% and ≤±10.4%, respectively (Table 3.18 and Table 3.19). A within-run precision of ≤7.40% for LLOQ and ≤6.59% for higher QC samples was observed. Comparably, between-run precision was ≤10.3% and ≤8.51%. The coefficients of determination were $R^2 \geq 0.995$ except for one day at which R^2 was 0.955 for LIN. Hence, all parameters were in accordance with the EMA Guideline [141].

Patient samples

3.4.1.5 Microdialysate concentrations

The mean unbound concentrations of CFX and LIN in both, synovia and muscle ISF, are displayed in Figure 3.42 and Figure 3.43. Individual unbound concentration-time courses are shown in Figure 3.44 and Figure 3.45 for CFX and in Figure 3.46 and Figure 3.47 for LIN. Time is always stated as mid of the sampling interval calculated from start of the respective drug infusion (0 h). A summary of basic pharmacokinetic parameters for both drugs is listed in Table 3.20 for CFX and in Table 3.21 for LIN. In total, 6 of 277 samples were missing, 3 from synovial fluid (mid time -0.25 and 0.75 h in patient 4 and 7.5 h in patient 8) and 3 from muscle ISF (mid time 0.75 h in patient 3 and 0.25 h and 7.5 h in patient 4. Furthermore, 2 consecutive samples from muscle ISF of patient 8 (sampling interval mid time of 3.5 and 4.5 h) showed no LIN at all and were excluded due to plausibility reasons.

Table 3.20 Basic pharmacokinetic parameters for cefuroxime obtained from microdialysate samples

Patient	Synovial fluid			Muscle interstitial space fluid		
	T_{max} [h]	Unbound C_{max} [µg/mL]	Unbound C_{last} [µg/mL]	T_{max} [h]	Unbound C_{max} [µg/mL]	Unbound C_{last} [µg/mL]
1	0.75, 1.75	12.8, 13.7	2.20	0.75	9.82	0.71
2	1.25, 2.25	3.44, 3.29	1.26	0.75	8.02	1.11
3	0.75	15.0	1.17	1.25, 2.25	6.04, 3.01	0.91
4	1.75, 2.75	6.77, 8.77	1.86	0.75	5.02	1.42*
5	0.75	12.2	1.32	0.75	9.36	1.07
6	0.75	10.9	1.13	0.75	1.93	0.67
7	0.75	5.22	0.80	0.75	9.91	0.84
8	0.75	6.7	0.83*	0.75	13.0	0.85
9	0.75, 1.75	9.01, 9.20	1.09	0.75	6.21	1.10
10	1.25	1.41	0.15	0.75	7.53	0.15

C_{max}: highest observed drug concentration in a sampling interval, C_{last}: drug concentration obtained in the last sampling interval of the trial, T_{max}: mid time of the sampling interval showing the highest drug concentration calculated from start of cefuroxime infusion

Adapted from [152], * C_{last} not available, therefore the previously collected concentration at time 6.5 h is stated

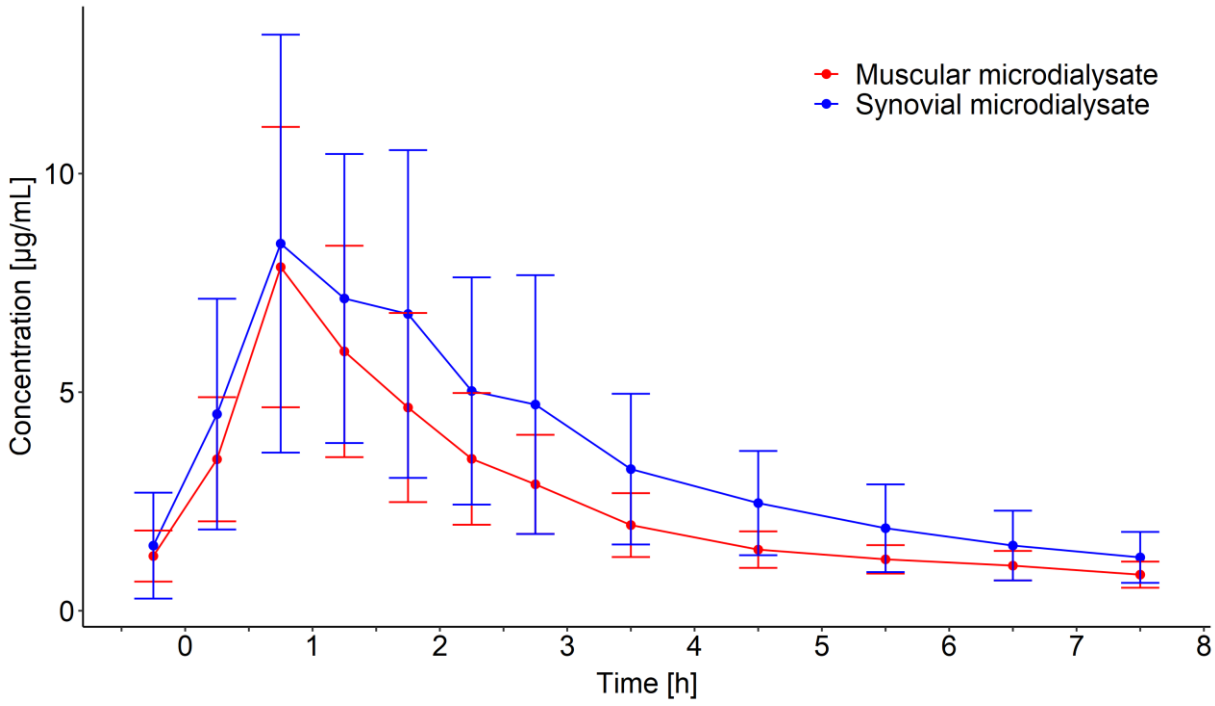

Figure 3.42 Mean unbound concentration-time courses of cefuroxime in microdialysate samples collected from synovial fluid and from muscle interstitial space fluid

Standard deviation is implemented as error bars in the respective colour, 0 h equates to the start of drug infusion, adapted from [152]

Table 3.21 Basic pharmacokinetic parameters for linezolid obtained from microdialysate samples

Patient	Synovial fluid			Muscle interstitial space fluid		
	T_{max} [h]	Unbound C_{max} [µg/mL]	Unbound C_{last} [µg/mL]	Tmax [h]	Unbound C_{max} [µg/mL]	Unbound C_{last} [µg/mL]
1	1.25, 2.25	2.65, 2.68	1.13	0.75	2.35	0.70
2	1.75, 6	0.74, 0.68	0.49	1.25	1.99	0.63
3	0.75	3.88	1.32	2.25	1.43	0.40
4	1.25, 2.25	1.77, 2.56	1.28	1.75	1.42	0.64*
5	1.25, 2.25, 4.00	1.30, 1.45, 1.37	0.58	1.25, 3.00	2.48, 1.68	0.23
6	1.25	2.55	0.99	1.25	0.64	0.26
7	0.75	1.08	0.33	0.75	2.73	0.71
8	0.75	1.59	0.45*	0.75, 2.25	2.81, 2.32	0.59
9	1.25	2.45	0.94	1.25	1.53	0.87
10	1.75	0.36	0.14	0.75	1.93	0.29

C_{max}: highest observed drug concentration in a sampling interval, C_{last}: drug concentration obtained in the last sampling interval of the trial, T_{max}: mid time of the sampling interval showing the highest drug concentration calculated from start of linezolid infusion

Adapted from [152], * C_{last} not available, therefore the previously collected concentration at time 6.5 h is stated

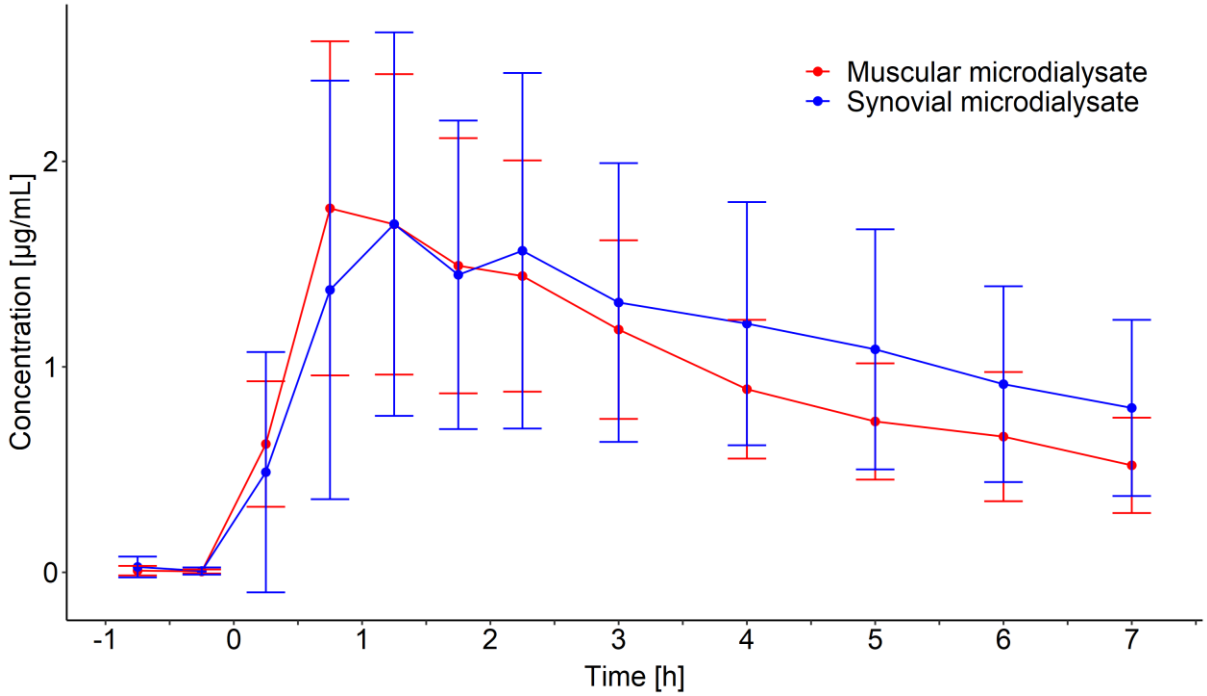

Figure 3.43 Mean unbound concentration-time courses of linezolid in microdialysate samples collected from synovial fluid and from muscle interstitial space fluid

Standard deviation is implemented as error bars in the respective colour, 0 h equates to the start of drug infusion, adapted from [151]

Cefuroxime concentrations in synovial fluid

For CFX in synovia, mean unbound concentration in the first sampling interval prior to CFX infusion was 1.49 µg/mL (SD 1.21 µg/mL). The mean unbound C_{max} of 8.40 µg/mL (SD 4.78 µg/mL) was reached in the sampling interval with a mid time of 0.75 h after start of infusion. The maximum unbound concentrations in this sampling interval widely deviated among the patients ranging from 1.41 µg/mL to 15.0 µg/mL (Figure 3.42, Figure 3.44 and Table 3.20). Although mean unbound C_{max} was within this sampling interval, it only comprised 5 individual unbound C_{max} values. Patient 1 and 9 showed a second slightly higher unbound peak concentration in the interval with a mid time of 1.75 h. For patient 2 and patient 4, also 2 sampling intervals with local maximum unbound concentrations were found: 1.25 and 2.25 h as well as 1.25 and 2.75 h, respectively. The first local maximum was slightly higher for patient 2 and the second 1 for patient 4. Patient 10 had a very flat unbound concentration-time course, thus a clear peak at T_{max} (mid time of 0.75 h) cannot be observed. Mean unbound concentration in the last sampling interval with a mid time of 7.5 h after start of CFX infusion was 1.22 µg/mL (SD 0.58 µg/mL) with a range of 0.15 µg/mL to 2.20 µg/mL.

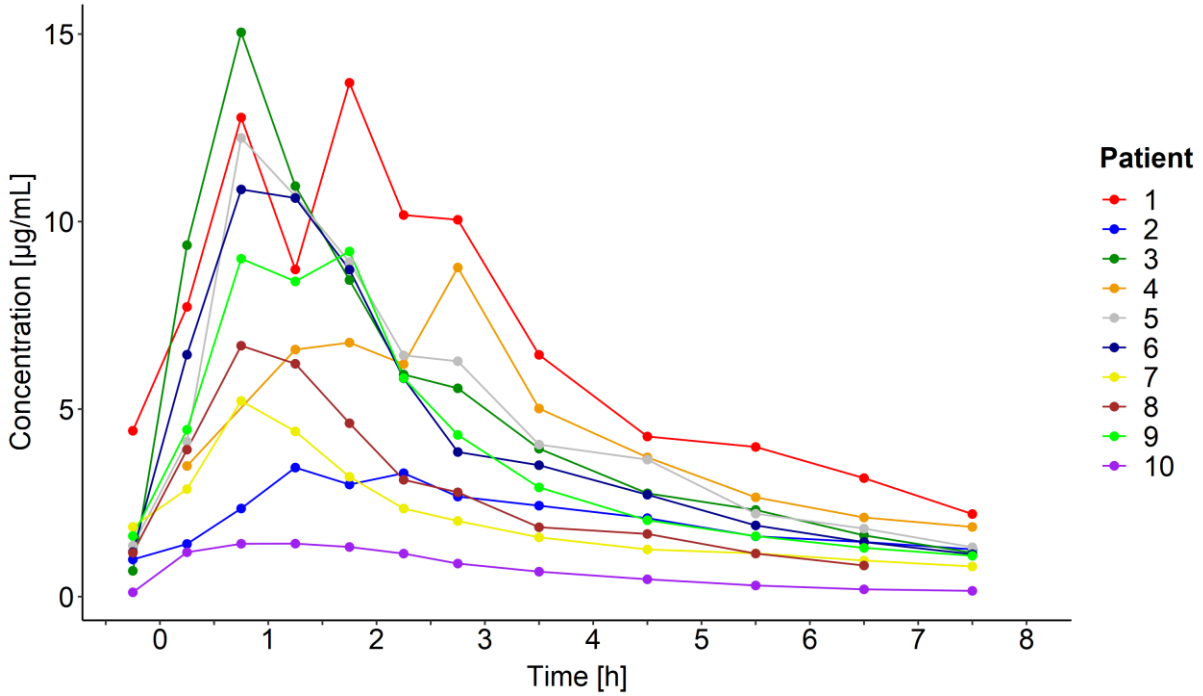

Figure 3.44 Patient-individual unbound concentration-time courses of cefuroxime in microdialysate samples from synovial fluid

0 h equates to the start of drug infusion, adapted from [152]

Cefuroxime concentrations in muscular interstitial space fluid

A similar unbound mean concentration-time course was found for CFX in muscle ISF. During baseline sampling, a mean unbound concentration of 1.25 µg/mL (SD 0.58 µg/mL) was obtained. As in synovia, mean unbound C_{max} of 7.86 µg/mL was reached in the interval with a mid time of 0.75 h. The range was also comparable to the results of synovial fluid with values from 1.93 to 13.0 µg/mL (Figure 3.42, Figure 3.45 and Table 3.20). T_{max} was observed in the sampling interval with a mid time of 0.75 h in all patients except in patient 3 who had a CFX concentration below LLOQ in that sampling interval. For patient 3, unbound C_{max} was observed at 1.25 h (mid time of the sampling interval) and a second, smaller peak at 2.25 h. At the mid of the last sampling interval, an unbound concentration range of 0.15 to 1.11 µg/mL with a mean of 0.82 µg/mL (SD 0.30 µg/mL) was determined.

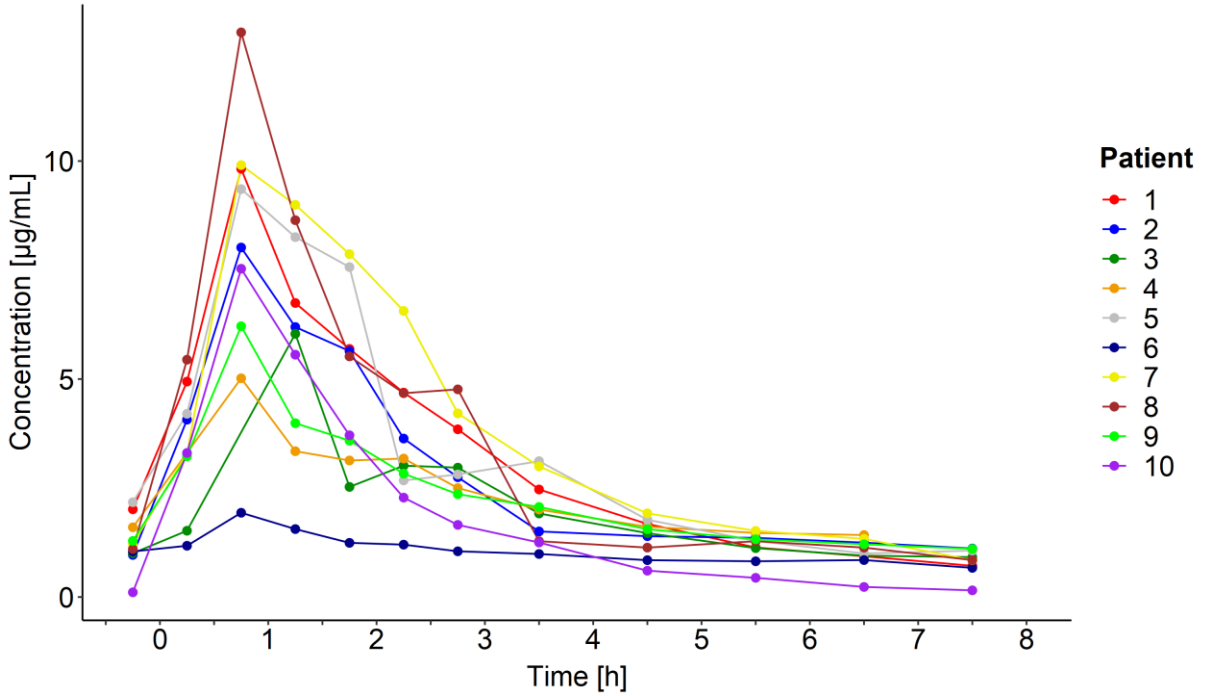

Figure 3.45 Patient-individual unbound concentration-time courses of cefuroxime in microdialysate samples from muscular interstitial space fluid

0 h equates to the start of drug infusion, adapted from [152]

Linezolid concentrations in synovial fluid

For LIN in synovia, the resulting mean unbound concentration-time courses were delayed by 0.5 h compared to CFX due to the study design. Hence, 2 baseline samples were available showing no LIN concentrations in synovial fluid except for patient 9 and 10 who had already very low unbound concentrations at this time. A mean unbound C_{max} of 1.69 µg/mL (SD 0.93 µg/mL) was attained in the interval with a mid time of 1.25 h after start of LIN infusion. Individual unbound C_{max} values ranged from 0.36 to 3.88 µg/mL (Figure 3.43, Figure 3.46 and Table 3.21). T_{max} was very diverse and in addition, 4 of 10 patients showed multiple local maxima. The 6 patients with a single maximum had a T_{max} at a sampling interval with a mid time of 0.75 (3 patients), 1.25 (2 patients), or 1.75 h (1 patient) after start of LIN infusion. The latter one was difficult to determine due to a very flat unbound concentration-time profile as already seen for the CFX concentrations in this matrix. For 3 patients with multiple peaks, the maxima were found in the sampling intervals with mid times of 1.25, 2.25 and 4 h.

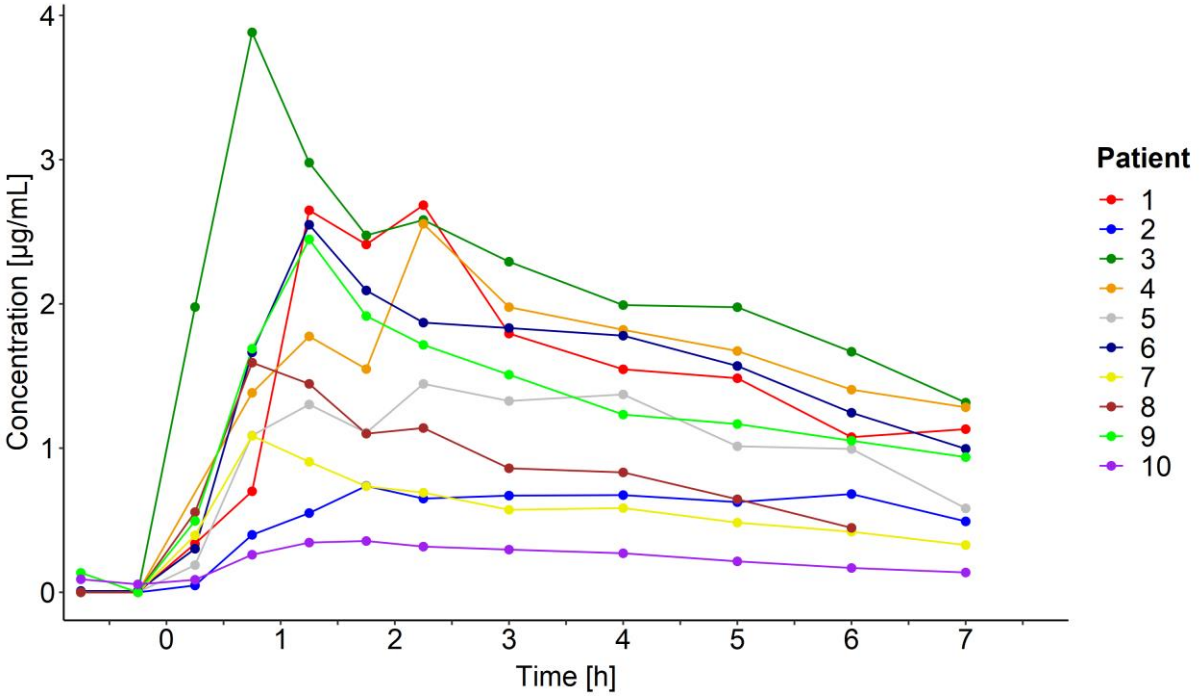

Figure 3.46 Patient-individual unbound concentration-time courses of linezolid in microdialysate samples from synovial fluid

0 h equates to the start of drug infusion, adapted from [151]

Linezolid concentrations in muscular interstitial space fluid

In the baseline samples from muscle ISF, no LIN concentrations were found except for patient 10 who had also measurable concentrations at baseline in synovial fluid. Mean unbound C_{max} was 1.77 µg/mL (SD 0.81 µg/mL) in the sampling interval with a mid time of 0.75 h after start of LIN infusion. It ranged from 0.62 to 2.81 µg/mL (Figure 3.43, Figure 3.47 and Table 3.21). Overall, for 8 patients a single maximum was observed which was found either in the sampling interval with a mid time of 0.75 (3 patients), 1.25 (3 patients), 1.75 (1 patient), or 2.25 h (1 patient). An additional local maximum was found for 2 patients: patient 5 in the intervals with a mid time of 1.25 and 3 h, patient 8 in the intervals with a mid time of 0.75 and 2.25 h. Unbound C_{last} was 0.52 µg/mL (SD 0.23 µg/mL) ranging from 0.23 to 0.87 µg/mL.

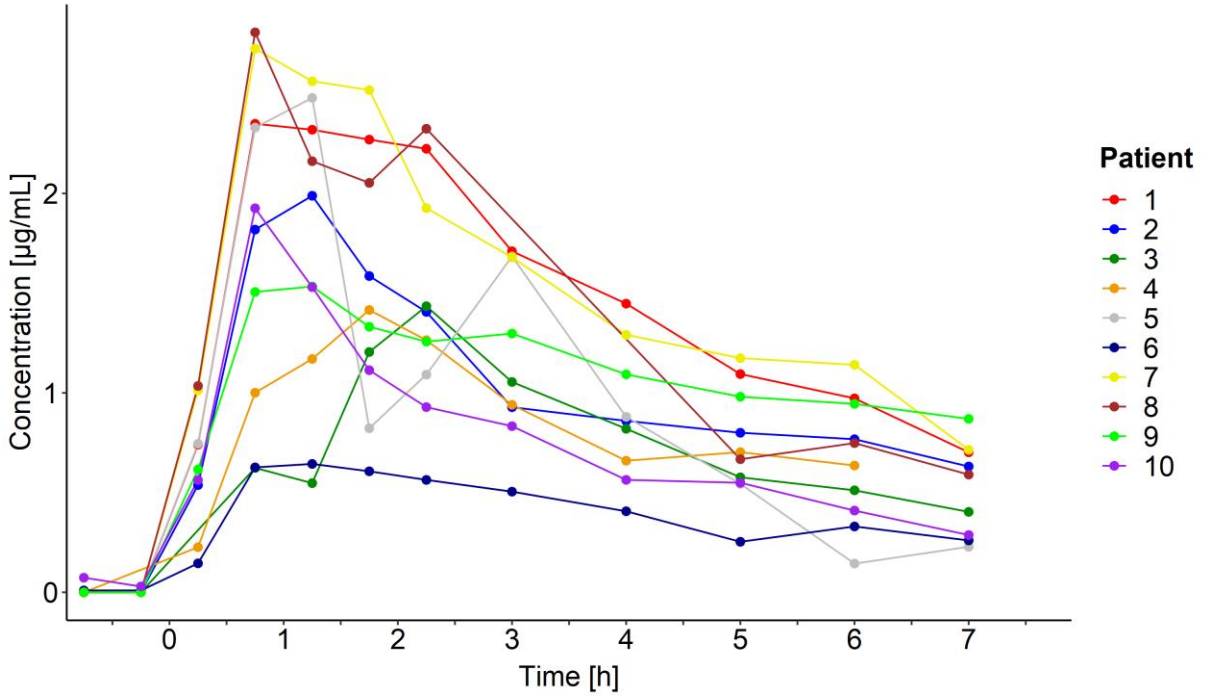

Figure 3.47 Patient-individual unbound concentration-time courses of linezolid in microdialysate samples from muscular interstitial space fluid

0 h equates to the start of drug infusion, adapted from [151]

3.4.1.6 Catheter calibration

The determination of a reliable RR value for the respective drug and catheter (Section 1.3.1) is crucial to enable conversion of microdialysate concentrations into ISF concentrations. To enhance the accuracy of the RR values, 2 retrodialysate samples were collected consecutively after the investigation period in the clinical trial. Retroperfusate and retrodialysate concentrations as well as resulting RR values for both drugs are summarised in Table 3.22, Table 3.23 and Table 3.24.

Table 3.22 Retroperfusate and retrodialysate concentrations of cefuroxime used for calibration of catheters inserted in synovial or muscular interstitial space fluid

Patient	Synovial fluid				Muscle interstitial space fluid			
	C_{RP} [µg/mL]	C_{RD} 1 [µg/mL]	C_{RD} 2 [µg/mL]	Mean RD [µg/mL]	C_{RP} [µg/mL]	C_{RD} 1 [µg/mL]	C_{RD} 2 [µg/mL]	Mean RD [µg/mL]
1	111	89.1	84.4	86.8	99.1	77.6	80.1	78.8
2	114	97.1	92.7	94.9	110	77.7	84.6	81.1
3	60.1	41.0	39.3	40.2	57.8	54.2	57.3	55.8
4	90.0	56.0	57.2	56.3	115	106	97.9	102
5	89.8	82.8	74.7	78.8	67.1	76.0	88.7	82.3
6	58.6	97.0	87.6	92.5	94.7	113	96.7	105
7	75.5	85.2	79.1	82.1	73.7	75.8	78.5	77.2
8	99.1	92.0	81.6	86.8	85.9	87.7	83.1	85.4
9	94.1	85.9	84.9	85.4	95.0	83.7	91.1	87.4
10	104	101	103	102	105	91.2	99.4	95.3

C_{RD}: concentration in retrodialysate, C_{RP}: concentration in retroperfusate, RD: retrodialysate
Adapted from [152]

Table 3.23 Retroperfusate and retrodialysate concentrations of linezolid used for calibration of catheters inserted in synovial or muscular interstitial space fluid

Patient	Synovial fluid				Muscle interstitial space fluid			
	C_{RP} [µg/mL]	C_{RD} 1 [µg/mL]	C_{RD} 2 [µg/mL]	Mean RD [µg/mL]	C_{RP} [µg/mL]	C_{RD} 1 [µg/mL]	C_{RD} 2 [µg/mL]	Mean RD [µg/mL]
1	94.0	69.4	73.1	71.3	100	78.5	77.4	77.9
2	94.9	54.1	61.2	57.7	99.1	74.4	75.3	74.9
3	66.1	51.2	56.2	53.7	67.9	40.1	39.9	40.0
4	98.6	72.1	70.5	71.3	86.5	46.4	48.4	47.4
5	73.1	59.4	72.5	66.0	83.9	67.1	62.3	64.7
6	84.0	90.4	78.9	84.7	56.5	77.8	71.6	74.7
7	75.7	67.2	71.7	69.4	74.1	82.1	77.1	79.6
8	86.1	73.1	70.7	71.9	96.8	81.5	73.3	77.4
9	91.6	67.8	75.8	71.8	90.5	76.4	77.2	76.8
10	93.3	71.2	80.0	75.6	93.4	81.6	85.1	83.4

C_{RD}: concentration in retrodialysate, C_{RP}: concentration in retroperfusate, RD: retrodialysate
Adapted from [151]

Table 3.24 Obtained RR values for cefuroxime and linezolid from catheter calibration via retrodialysis

Patient	RR CFX, synovial fluid, %	RR CFX, muscle ISF, %	RR LIN, synovial fluid, %	RR LIN, muscle ISF, %
1	21.8	20.5	22.2	24.3
2	16.7	26.4	24.5	39.3
3	33.2	3.51	41.1	18.7
4	37.3	11.2	45.3	27.7
5	12.3	-22.7*	22.8	9.79
6	-57.9*	-10.8*	-32.3	-0.75
7	-8.84*	-4.72*	-7.36	8.32
8	12.4	0.56	20.0	16.5
9	9.30	8.04	15.2	21.6
10	2.43	9.37	10.7	18.9
Mean	20.4	15.1	25.2	20.6

CFX: cefuroxime, ISF: interstitial space fluid, LIN: linezolid, RR: relative recovery
Adapted from [152] and [151], * Implausible values treated like missing values and replaced by the mean of all plausible values of the respective drug and matrix

Retroperfusate concentrations of CFX had a mean of 89.6 µg/mL (C_{nom}=100 µg/mL) for catheters in synovial fluid and of 90.4 µg/mL in muscle ISF and showed a wide range from 58.6 to 114 µg/mL and from 57.8 to 115 µg/mL in synovial and muscular implanted catheters, respectively. Compared to the ISF concentrations in the last sampling interval, resulting concentration ratios between retroperfusate and ISF were as least 10.6 (synovia) and 9.54 (muscle ISF) and ensuring sufficient concentration gradients. For LIN, retroperfusate concentrations had similar magnitudes and ranges. Mean concentrations were 84.9 and 85.8 µg/mL in synovial fluid and muscular ISF, respectively. Observed individual concentrations ranged from 56.5 to 100 µg/mL in synovia and from 66.1 to 98.6 µg/mL in muscle ISF. Comparing ISF concentrations of LIN in the last sampling interval with retroperfusate concentrations, the latter ones showed ≥14.3-fold and ≥30.7-fold higher concentrations enabling unhampered diffusion.

Mean CFX concentrations in retrodialysate samples were 80.6 and 85.0 µg/mL in synovial fluid and muscle ISF, respectively. Similar to the retroperfusate concentrations, a wide range of 39.3 to 103 µg/mL was found for catheters in synovial fluid and of 54.2 to 113 µg/mL for catheters in muscle ISF. Mean LIN concentrations in retrodialysate showed a similar magnitude and range. Retrodialysate concentrations from synovial catheters had a mean of 69.7 µg/mL (range 39.9 to 85.1 µg/mL) and from muscular catheters a mean of 69.3 µg/mL (range 51.2 to 90.4 µg/mL).

For CFX, the resulting RR values showed a range of -57.9% to 37.3% (synovial fluid) and from -22.7% to 26.4% (muscle ISF). Negative RR values were observed for patient 6 and 7 from catheters implanted in synovial fluid and in patient 5, 6 and 7 from catheters in muscle ISF. In addition, very low RR values (≤5%) were found for patient 10 (RR 2.43%) from synovia as well as patient 3 (RR 3.51%) and patient 8 (0.56%) from muscle ISF. Negative or very low values were considered to be implausible and were

therefore replaced by the mean RR value obtained from plausible RR values of the respective matrix. Plausible RR values for CFX had a range of 9.30% to 37.3% with a mean of 20.4% in synovia and a range of 8.0% to 26.4% with a mean RR value of 15.1% in muscle ISF.

For LIN, mean RR values per patient ranged from -32.3% to 45.3% and from -0.75% to 39.3% in synovial fluid and muscle ISF, respectively. Negative RR values were observed in patient 6 and 7 for synovial fluid and in patient 6 for muscle ISF. As for CFX, implausible RR values were replaced by mean of the plausible LIN RR values in the respective matrix. Plausible RR values had a range of 10.7% to 45.3% with a mean RR value of 25.2% (synovia) and of 8.32% to 39.3% with a mean of 20.6% (muscular ISF).

3.4.1.7 ISF concentrations

Mean concentrations of the drugs in both matrices are displayed in Figure 3.48 and Figure 3.49,**Fehler! Verweisquelle konnte nicht gefunden werden.** individual concentration-time courses in Figure 3.50**Fehler! Verweisquelle konnte nicht gefunden werden.** and Figure 3.51 for CFX as well as in Figure 3.52 and Figure 3.53 **Fehler! Verweisquelle konnte nicht gefunden werden.**for LIN. A summary of basic pharmacokinetic parameters for both drugs is shown in Table 3.25 and Table 3.26. As drug concentrations in synovial fluid were calculated from the respective microdialysate concentrations using Equation 1.3, resulting concentration-time courses showed identical T_{max} but deviating magnitudes of C_{max} and C_{last}. Accordingly, missing values were identical to the ones in microdialysate.

Table 3.25 Basic pharmacokinetic parameters for cefuroxime from unbound plasma and calculated interstitial space fluid concentrations

Patient	Plasma			Synovial fluid			Muscle ISF		
	T_{max} [h]	Unbound C_{max} [µg/mL]	Unbound C_{last} [µg/mL]	T_{max} [h]	Unbound C_{max} [µg/mL]	Unbound C_{last} [µg/mL]	T_{max} [h]	Unbound C_{max} [µg/mL]	Unbound C_{last} [µg/mL]
1	0.5	64.5	1.12	0.75, 1.75	58.6, 62.8	10.1	0.75	48.0	3.51
2	0.5	54.8	1.67	1.25, 2.25	20.6, 19.8	7.57	0.75	30.4	4.22
3	0.5	61.0	0.74	0.75	45.3	3.53	1.25, 2.25	172, 85.9	6.06
4	0.5	40.9	0.46	1.75, 2.75	18.1, 23.5	4.98	0.75	44.8	12.7*
5	0.5	89.1	1.17	0.75	99.1	10.7	0.75	91	7.06
6	0.5	72.8	0.53	0.75	74.7	5.53	0.75	18.8	4.44
7	0.5	89.2	1.34	0.75	35.9	3.93	0.75	96.3	5.59
8	0.5	82.0	1.30	0.75	53.9	6.69*	0.75	126	5.61
9	0.5	81.6	0.03	0.75, 1.75	96.9, 99.0	11.7	0.75	77.3	13.7
10	0.5	54.0	0.32	1.25	58.2	0.75	0.75	80.4	1.64

C_{last}: drug concentration obtained in the last sampling interval of the observation, C_{max}: highest observed drug concentration in a sampling interval, ISF: interstitial space fluid, T_{max}: sampling time (plasma)/ mid time of the sampling interval (synovia fluid/ muscle ISF) showing the highest drug concentration

Adapted from [152], * C_{last} not available, therefore the previously quantified concentration was stated

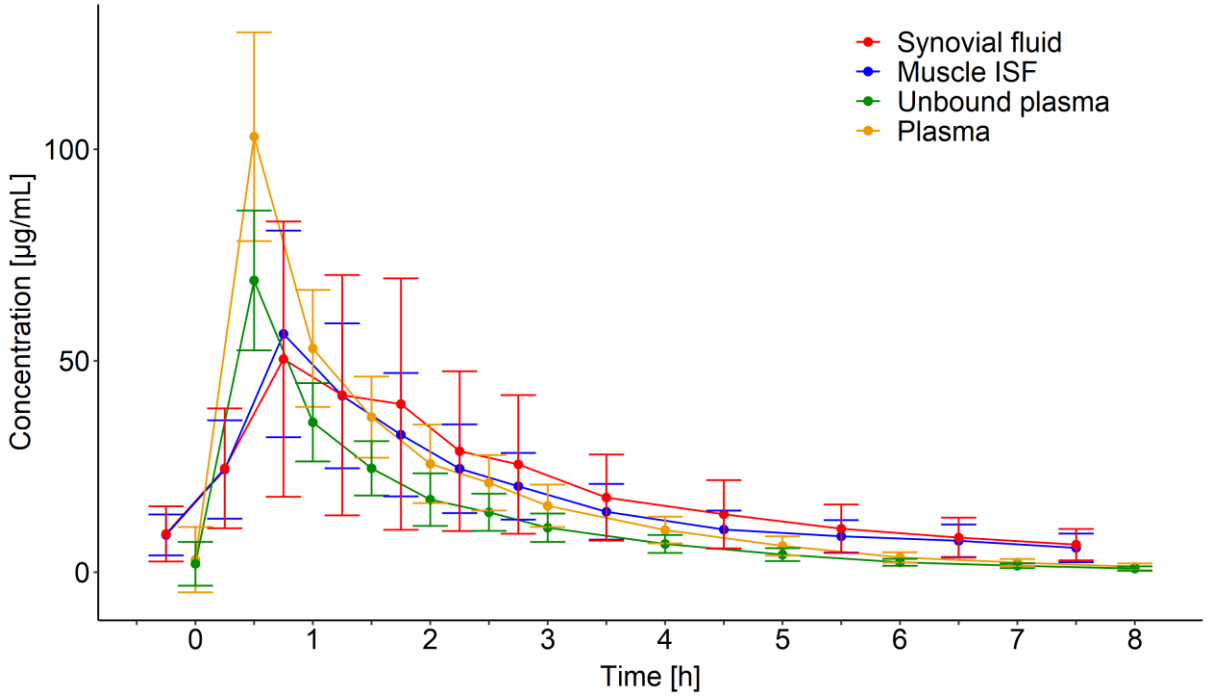

Figure 3.48 Mean concentration-time courses of cefuroxime from plasma, unbound plasma, unbound synovial fluid and unbound muscular interstitial space fluid

Error bars show the respective standard deviation, time is the either the time point of sample drawing (total and unbound plasma) or the mid of the sampling interval (synovial fluid and muscular interstitial space fluid) , 0 h equates to the start of drug infusion, adapted from [152]

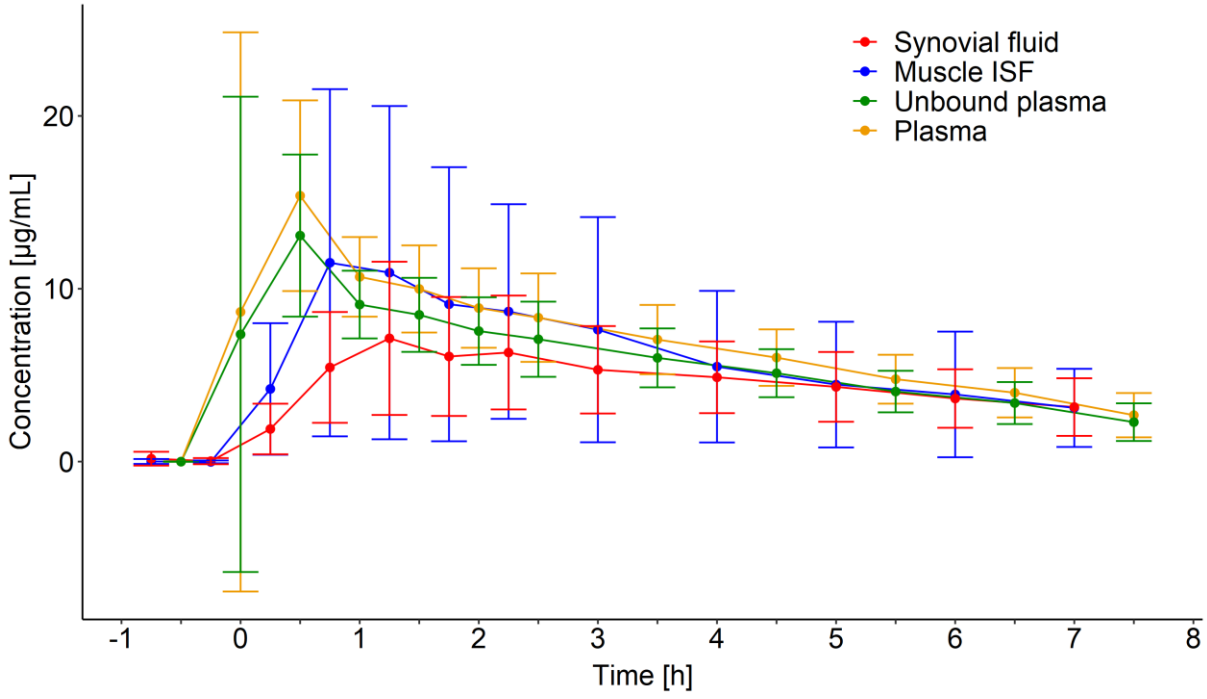

Figure 3.49 Mean concentration-time courses of linezolid from plasma, unbound plasma, unbound synovial fluid and unbound muscular interstitial space fluid

Error bars show the respective standard deviation, time is the either the time point of sample drawing (total and unbound plasma) or the mid of the sampling interval (synovial fluid and muscular interstitial space fluid) , 0 h equates to the start of drug infusion, adapted from [151]

Table 3.26 Basic pharmacokinetic parameters for linezolid from unbound plasma and calculated interstitial space fluid concentrations

Patient	Plasma			Synovial fluid			Muscle ISF		
	T_{max} [h]	Unbound C_{max} [µg/mL]	Unbound C_{last} [µg/mL]	T_{max} [h]	Unbound C_{max} [µg/mL]	Unbound C_{last} [µg/mL]	T_{max} [h]	Unbound C_{max} [µg/mL]	Unbound C_{last} [µg/mL]
1	1.5, 2.5	10.3, 10.6	3.62	1.25, 2.25	11.9, 12.1	5.09	0.75	9.66	2.89
2	0.5	8.58	2.19	2.25	3.01	2.01	1.25	5.06	1.61
3	0.5	12.2	2.40	0.75	9.46	3.20	2.25	7.65	2.15
4	0.5	7.55	1.64	1.25, 2.25	3.93, 5.65	2.83	1.75	5.11	2.30*
5	0.5	13.4	2.37	1.25, 2.25, 4	5.70, 6.33, 6.01	2.55	1.25, 3	25.3, 17.2	2.33
6	0.5	22.2	3.45	1.25	11.3	3.94	1.25	3.13	1.27
7	0.5	18.4	3.22	0.75	4.81	1.30	0.75	30.3	8.59
8	0.5	13.6	2.61	0.75	7.96	2.23*	0.75, 2.25	17.0, 14.1	3.58
9	0	11.7	-0.05	1.25	16.1	6.18	1.25	7.11	4.03
10	0	31.7	1.33	2.25	3.32	1.28	0.75	10.2	1.52

C_{last}: drug concentration obtained in the last sampling interval of the observation, C_{max}: highest observed drug concentration in a sampling interval, ISF: interstitial space fluid, T_{max}: sampling time (plasma)/ mid time of the sampling interval (synovia fluid/ muscle ISF) showing the highest drug concentration
Adapted from [151], * C_{last} not available, therefore the previously quantified concentration was stated

Cefuroxime concentrations in synovial fluid

For CFX in synovia, unbound drug concentrations during baseline sampling had a mean of 9.06 µg/mL (SD 6.52 µg/mL). Mean unbound C_{max} was 50.4 µg/mL (SD 32.6 µg/mL) and ranged in the sampling interval of 0.75 h from 6.90 to 99.1 µg/mL (Figure 3.48, Figure 3.50 and Table 3.25). Unbound C_{last} was 7.49 µg/mL (SD 2.64 µg/mL) ranging from 0.75 to 11.7 µg/mL.

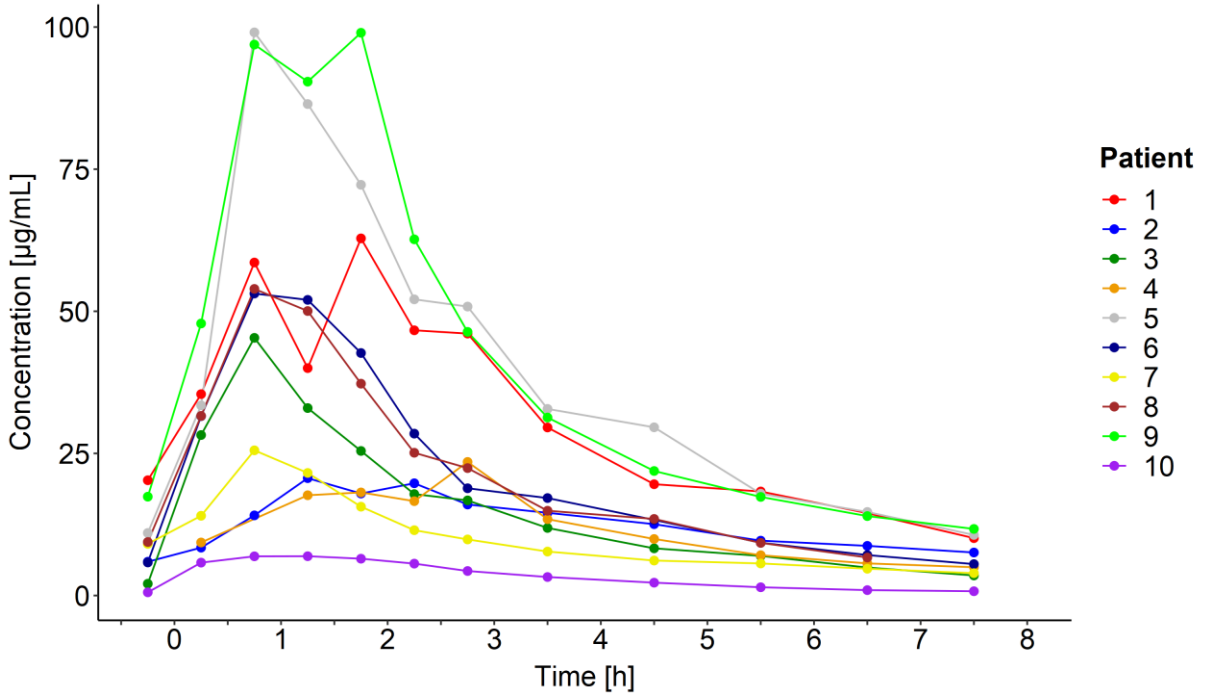

Figure 3.50 Calculated individual unbound concentration-time courses of cefuroxime in synovial fluid

0 h equates to the start of drug infusion, adapted from [152]

Cefuroxime concentrations in muscular interstitial space fluid

CFX in muscle ISF showed a mean unbound concentration of 8.83 µg/mL (SD 4.84 µg/mL) in baseline samples. The mean unbound C_{max} was 56.4 µg/mL (SD 24.4 µg/mL) and showed a smaller range than in synovia with 12.8 to 85.9 µg/mL (Figure 3.48, Figure 3.51 and Table 3.25). A mean C_{last} of 9.51 µg/mL (SD 7.00 µg/mL) with a range of 1.64 to 13.7 µg/mL was observed.

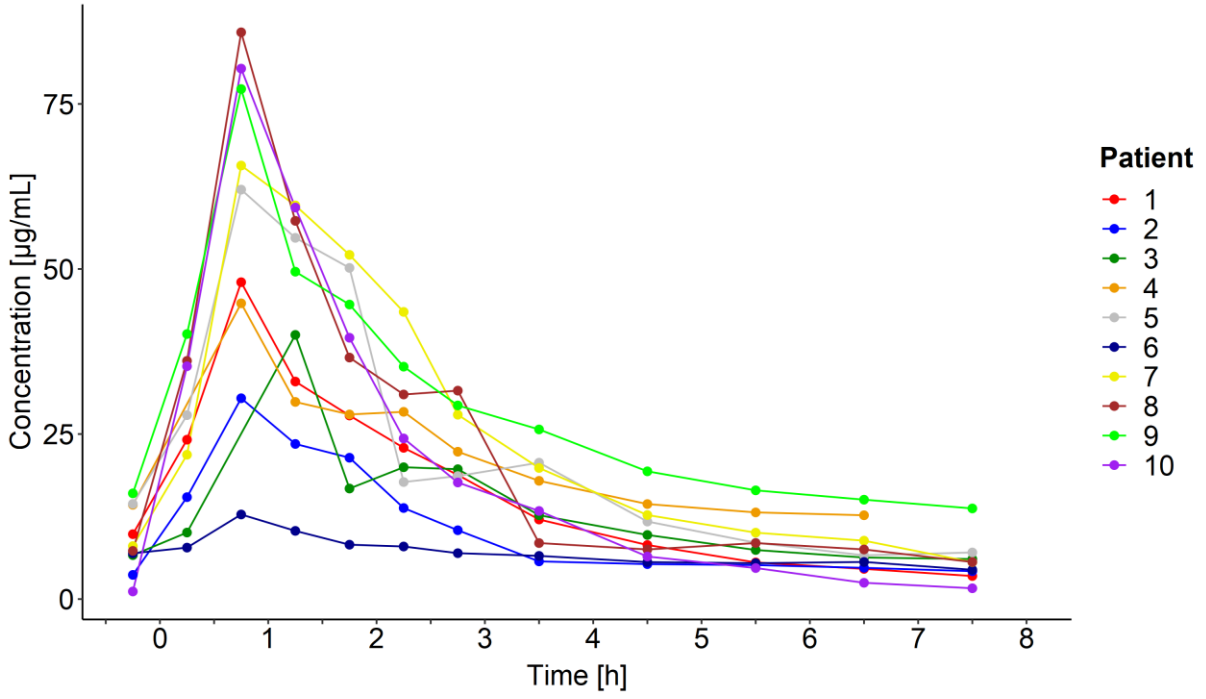

Figure 3.51 Calculated concentration-time courses of cefuroxime in muscular interstitial space fluid

0 h equates to the start of drug infusion, adapted from [152]

Linezolid concentrations in synovial fluid

For LIN in synovial fluid, mean unbound profile started with a low baseline concentration of 0.59 µg/mL (SD 0.48 µg/mL). Unbound C_{max} had a mean of 7.13 µg/mL (SD 4.43 µg/mL) ranging from 3.32 to 16.1 µg/mL were observed (Figure 3.49, Figure 3.52 and Table 3.26). Mean C_{last} was 3.13 µg/mL (SD 1.62 µg/mL) with a range of 1.28 to 6.18 µg/mL.

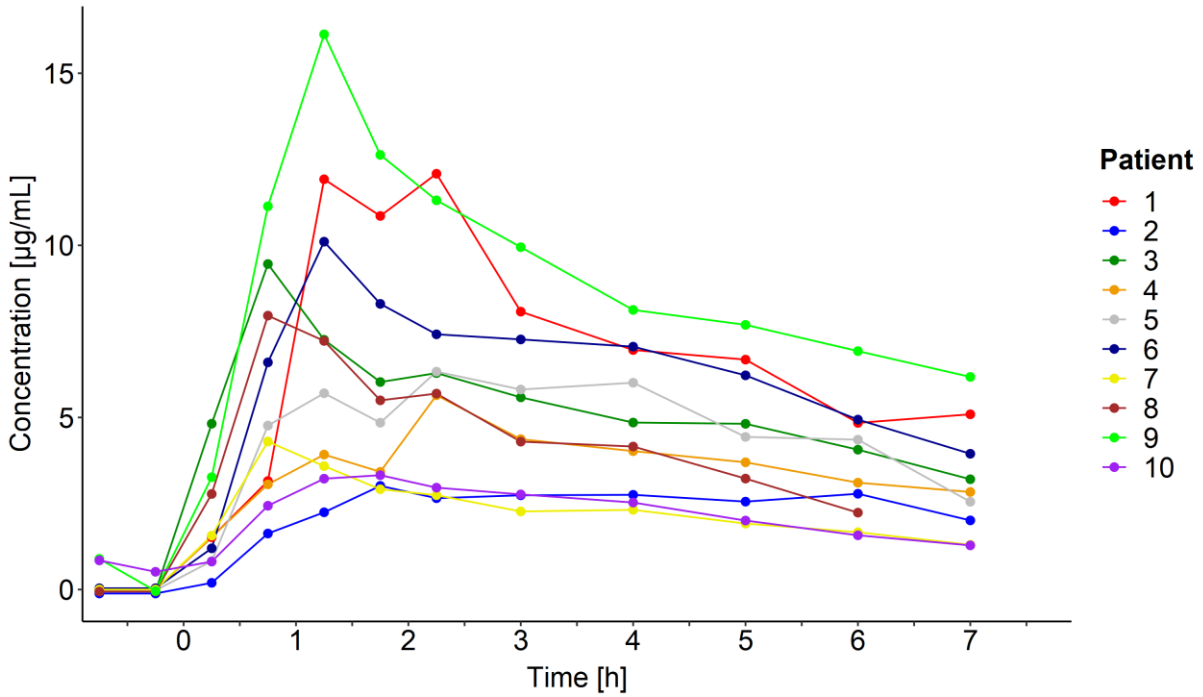

Figure 3.52 Calculated concentration-time courses of linezolid in synovial fluid
0 h equates to the start of drug infusion), adapted from [151]

Linezolid concentrations in muscular interstitial space fluid

As in synovial fluid, unbound LIN concentrations in baseline samples were only obtained for patient 10 and had a very low magnitude. Mean unbound C_{max} was 11.5 µg/mL (SD 10.0 µg/mL) with a range of 3.13 to 25.3 µg/mL (Figure 3.49, Figure 3.53**Fehler! Verweisquelle konnte nicht gefunden werden.** and Table 3.26). C_{last} ranged from 1.27 to 8.59 µg/mL with a mean of 2.68 µg/mL (SD 1.30 µg/mL).

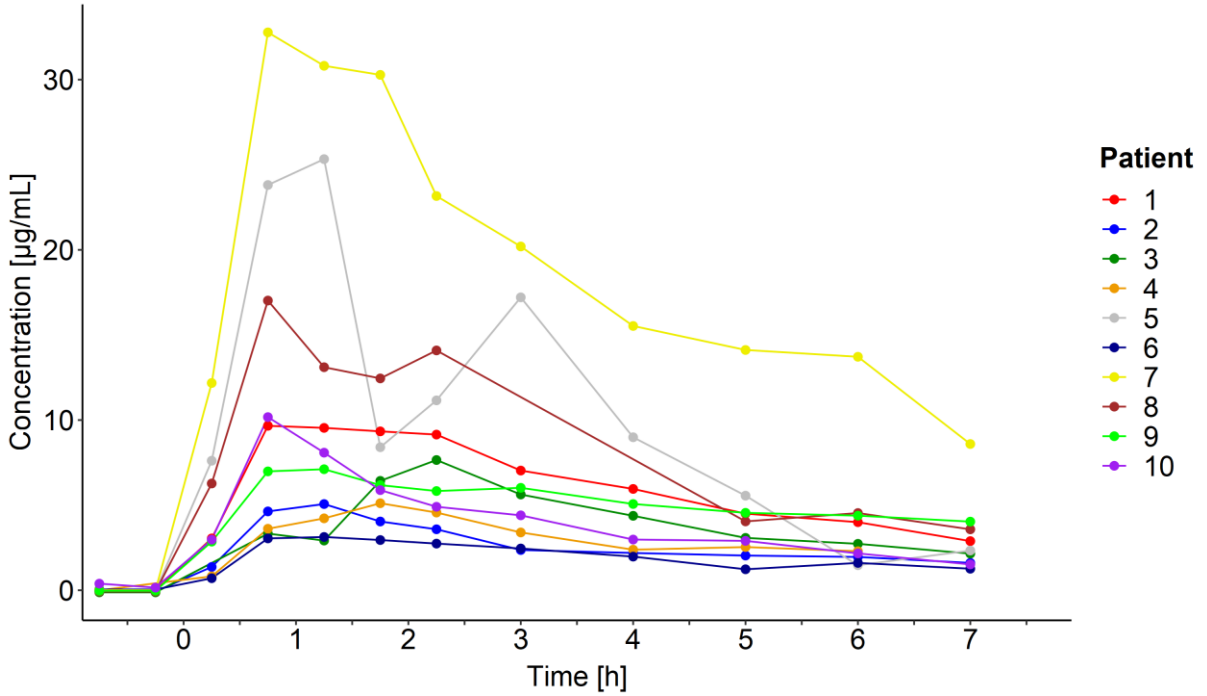

Figure 3.53 Calculated unbound concentration-time courses of linezolid in muscular interstitial space fluid

0 h equates to the start of drug infusion, adapted from [151]

3.4.1.8 Total and unbound plasma concentrations

Mean observed total and calculated unbound plasma concentrations of both drugs are shown in Figure 3.48 and Figure 3.49**Fehler! Verweisquelle konnte nicht gefunden werden.**, patient-individual unbound concentration-time courses in Figure 3.54 and Figure 3.55.

Basic pharmacokinetic parameters for both drugs are summarised in Table 3.25 and Table 3.26. Three plasma samples were missing and therefore excluded from the analysis: the samples of 0 and 2.5 h for patient 9 and the sample of 0 h for patient 10.

Cefuroxime plasma concentrations

Most of the CFX samples showed plausible total plasma concentration-time courses with low CFX concentrations at baseline and a steep increase over the duration of the short-term infusion. For patient 1, a high concentration of 22.1 µg/mL was determined at baseline compared to 0.25 µg/mL in all other patients. Nevertheless, maximum CFX concentration in this patient was obtained in the subsequent sampling interval and therefore similar to all other patients. The mean total C_{max} was 103 µg/mL (SD 24.7 µg/mL) and showed a wide range of 61.0 to 133 µg/mL (Figure 3.48, Figure 3.54 and Table 3.25). Total C_{last} had a mean concentration of 1.29 µg/mL (SD 0.79 µg/mL) ranging from 0.05 to 2.49 µg/mL. Unbound plasma concentrations showed a mean C_{max} of 69.0 µg/mL (SD 16.5 µg/mL) and a mean C_{last} of 0.87 µg/mL (SD 0.53 µg/mL).

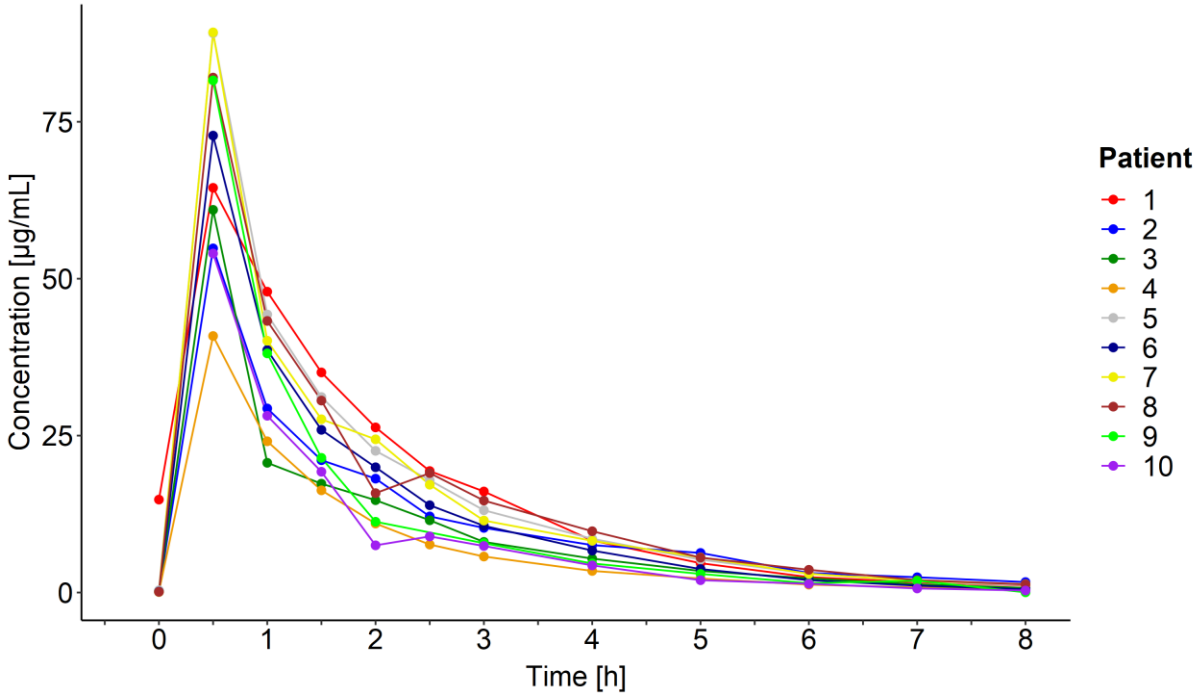

Figure 3.54 Calculated concentration-time courses of unbound cefuroxime in plasma
0 h equates to the start of drug infusion, adapted from [152]

Linezolid plasma concentrations

For LIN, similar concentration-time courses as for CFX were observed (Figure 3.49, Figure 3.55 and Table 3.26). Most of the patients showed a strong increase in drug concentration after start of infusion, resulting in mean C_{max} of 15.4 µg/mL (SD 5.52 µg/mL) at 0.5 h, i.e. the end of infusion. As already observed for CFX, a wide range of C_{max} was found for LIN (8.88 to 26.1 µg/mL). However, 2 concentration-time courses deviated from this trend. Patient 10 showed total plasma C_{max} at start of infusion. Patient 1 had a much shallower concentration-time course impeding the determination of C_{max} and T_{max}. Total plasma concentrations in the last sample had a mean of 2.68 µg/mL (SD 1.30 µg/mL) and ranged from LLOQ to 4.26 µg/mL. Mean C_{max} and C_{last} for unbound plasma concentrations were 13.1 µg/mL (SD 4.69 µg/mL) and 2.28 µg/mL (SD 1.10 µg/mL), respectively.

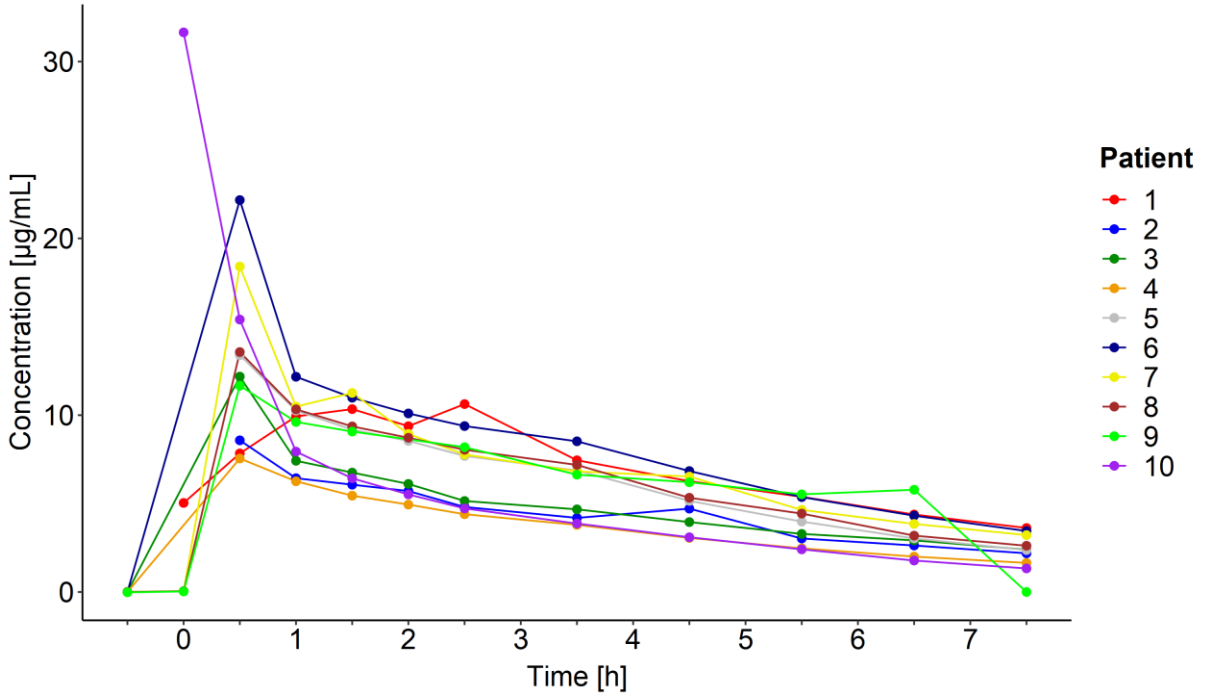

Figure 3.55 Calculated concentration-time courses of unbound linezolid in plasma
0 h equates to the start of drug infusion, adapted from [151]

3.4.1.9 Comparison of plasma and ISF concentrations

Mean concentration-time courses from microdialysate samples (unbound fraction), ISF concentrations (unbound fraction) and plasma samples (total and unbound fraction) of both drugs are shown in Figure 3.48 and in Figure 3.49**Fehler! Verweisquelle konnte nicht gefunden werden.**.

Comparison of the mean concentration-time courses showed that increase and decrease of drug concentrations were generally faster in unbound plasma than in synovial fluid and muscle ISF. Maximum unbound CFX concentrations in synovial fluid and muscle ISF were in a similar range and equated to 98.7% and 86.4% of the maximum unbound plasma concentration, respectively. For LIN, unbound C_{max} of synovial fluid and muscle ISF equated to 51.3% and 74.7% of unbound plasma concentrations, demonstrating different penetration abilities.

Cefuroxime concentrations

Deviating from most of the plasma samples, microdialysate samples showed detectable CFX concentrations during baseline. Mean unbound C_{max} in plasma was reached directly at the end of infusion. Whereas C_{max} in muscular ISF and synovial fluid was found in the sampling interval with a mid time of 0.75 h and therefore in the interval following the end of infusion. On a patient-individual level, T_{max} was more variable in synovia than in muscle ISF. Mean unbound C_{max} of CFX was comparable in plasma and in muscular ISF (69.0 µg/mL and 68.1 µg/mL) and slightly lower in synovial fluid (59.6 µg/mL). Unbound C_{last} in synovial fluid and muscle ISF concentrations were comparable (6.53 µg/mL and 5.76 µg/mL), but much higher than unbound plasma concentrations (0.87 µg/mL) at this time. High concentrations in one sample matrix did not necessarily occur also in other matrices. No general trend regarding the ratio of concentrations in plasma, synovia and muscle ISF was found. For patient 8, CFX concentrations were very high in plasma and muscle ISF but only half the concentration in synovia. Other patients, e.g. 7 and 9, showed similarly high concentrations in both microdialysate samples but almost no CFX in plasma. And, e.g. patient 5, had no similarities between the concentrations in the different matrices.

Linezolid concentrations

For LIN compared to CFX, stronger differences in the concentration-time profiles from the 3 matrices were observed. The steepest increase was observed in plasma, followed by muscular ISF and synovial fluid resulting in different time points and magnitudes for unbound C_{max}: 0.5 h (15.4 µg/mL), 0.75 h (11.5 µg/mL) and 1.25 h (7.30 µg/mL), respectively. Fluctuating concentrations in the drug decline were observed in all matrices, in plasma for 3 patients, in muscle ISF for 4 patients and in synovial fluid for 3 patients. Unbound C_{last} of synovia and muscular ISF were comparable (3.15 and 3.11 µg/mL, respectively) but higher than of unbound plasma (2.28 µg/mL). As observed for CFX, the ratios of concentrations in plasma, synovia and muscle ISF were very diverse on the patient-individual level and followed no general trend

4 Discussion

4.1 Project I - Uniformity and accuracy of compounded capsules

Most of the investigated capsules showed a good uniformity of capsule net mass, drug content and/or accuracy of drug content on capsule as well as on batch level. However, only 24 batches (44.4%) met acceptance criteria for all 3 investigations mainly due to too low drug contents. In addition, a good or medium correlation of net mass and drug content was only observed for 34.5% of the batches and seemed to be inappropriate for application in the planned clinical trial.

Capsule characteristics

As expected, most of the capsules were compounded in capsule size 1, the standard size for capsule compounding, which is most probably available in all German pharmacies. Only 7 batches were compounded in size 0, which is larger than size 1. Smaller capsule sizes (2-4) were used for 10 batches. Taking into account that most children are not able to swallow the capsules, it is most likely that the care givers will open the capsules and administer the content together with beverages or food. From our experience, larger capsules are easier to open and therefore should be preferred. On the other hand, it might be beneficial to use small capsule sizes to reduce the daily intake of filling material which might cause gastrointestinal side effects in e.g. very small children. Hence, the experience and wishes of the care takers should be considered in the choice of the capsule size.

The capsule filling material consisted mainly of mannitol and highly disperse silicon dioxide or lactose, which are the recommended filling materials of the DAC/NRF [45]. Other batches contained glucose, mannitol or starch which is also common. As already mentioned, 1 batch was excluded from the study as it was compounded with olive oil as filling material. Hard gelatine capsules are not supposed to contain liquids, hence a massive leakage of the oil and probably solved/ suspended HC resulted. Capsule content which did not leak out, adhered to the casings making a complete pouring of the content into food or beverages nearly impossible. In addition, opening of the capsule casing was more challenging as the leakage made the casing slippery. Hence, the only option for the patient would be to swallow the whole capsule, which might be difficult depending on the patient's age. Finally, the intake of HC in oil might alter the resorption of HC after administration. Overall, the use of olive oil cannot be recommended.

As expected, most capsule batches had nominal HC contents between 0.5 and 7 mg which were not commercially available during study enrolment. In theory, at least nominal doses of 2.5 mg or 5 mg could be easily achieved by dividing usual tablets containing 10 mg HC in halves or quarters.

Unfortunately, several studies [15,16] found bad accuracy and drug content uniformity in divided tablets, therefore this seems to be no suitable option for a drug with a small therapeutic window as HC.

Uniformity of net mass

Uniformity/accuracy of drug content was valued higher with regard to the upcoming clinical trial than uniformity of net mass, therefore the capsule investigation regarding uniformity of net mass recommended by the EP [47]was modified. Usually, capsules would be emptied and the capsule casing weighted. The obtained mass would be subtracted from the total capsule mass to calculate the capsule net mass. For the present project, the capsule casings were added to the capsule filling and underwent as well the drug extraction to ensure complete recovery of the contained HC. During the extraction process, casings were often broken, impeding a complete recovery and hence also mass determination. Therefore, a different approach was applied: the capsule size was determined and the mean mass of standard casing mass for the respective size was subtracted from the respective total mass of the capsules to determine the capsule net mass. This method added a potential error of 1.5%-3.5% depending on the capsule size and the mass of the compounded mixture of drug and filling material [142] to the results of the uniformity of net mass evaluation but prevented a potential drug loss of 1%-2% due to residuals of capsule filling in the casings as shown by Grauls [46]. Ultimately, the slightly decreased accuracy of the results in the uniformity of net mass investigation was reasonable and did not change the main findings.

On the capsule level, uniformity of net mass was very good with 93.4% of all capsules showing deviations from their batch mean within tolerable limits and only 0.26% exceeding non-tolerable limits. 1 reason for the very good result might be that many pharmacies test mass uniformity directly after compounding according to DAC/NRF recommendations. Determination of drug content uniformity and accuracy is much more complicated and time-consuming and is therefore not routinely investigated.

Looking at the batch level, uniformity of net mass was achieved in about 80% of the investigated batches which is in agreement to the findings of Grauls et al. [46] and Markman et al [167]. Further 9% of the batches almost met the acceptance criteria but 10% showed either non-tolerable deviations of single capsules and/or deviations exceeding tolerable limits in at least 5 different capsules of the respective batch. Considering that the evaluation of net mass uniformity is mandatory after compounding capsules in pharmacies, the percentage of batches missing acceptance criteria of the EP is high. At least for the batches exceeding non-tolerable limits or tolerable limits for ≥5 capsules (equating to ¼ of capsules in the investigated batches), a successful evaluation of net mass uniformity by the compounding pharmacies is unlikely.

Uniformity of net mass was additionally assessed as recommended by the DAC/NRF using RSDs. 24% of batches had a RSD >5% and therefore failed in this evaluation. Comparing the results of net mass uniformity according to EP and to DAC/NRF, a high agreement in the assessment of the batches was observed although the threshold of the DAC/NRF seemed to be slightly more rigorous.Bioanalysis

To enable HC quantification, a bioanalytical HPLC method was developed. The subsequent validation of the method fulfilled all requirements given by the EMA Guideline except for autosampler stability [141]. After adaptation of the storage time, sufficient stability was proven for all subsequent analytical runs. HC recovery from capsules compounded from HC tablets was incomplete (around 67.7%, probably depending on the company), therefore only capsules from pure HC could be investigated properly. As HC is a common drug which is regularly compounded, it should be available in the vast majority of pharmacies. Also, capsule compounding from tablets is more time-consuming due to the

additional steps of tablet crushing and grinding. Therefore, it was assumed that most of the batches were prepared from pure HC. As an incomplete recovery from tablets would led to high negative mean deviations from the nominal content of a batch, such batches were identified as a potential source of bias. Recovery from capsules compounded with pure HC as starting material was also incomplete (96.8%), therefore a correction factor was included for the investigation of drug content accuracy.

Due to the relatively high deviations allowed by the EMA Guideline [141] for accuracy and precision (up to ±20% for LLOQ, up to ±15% for QC 2-4) of the HPLC method, double measurements of each sample were performed. The 2 concentrations were compared. In the rare case of ≥10% difference between both results, a reanalysis of the sample was performed to ensure reliable conclusions. The resulting potential inaccuracy is low enough to sufficiently assess the overall uniformity and accuracy.

Uniformity of drug content

Regarding uniformity of drug content on the capsule level, the vast majority of capsules (98%) had acceptable deviations from mean drug content of the respective batch. Only 2% of the capsules were outside non-tolerable limits. Noticeable was the magnitude of deviations outside non-tolerable limits: -47% to +203% of the respective batch mean were calculated which is far more than expected. Most of the exceeding capsules (25 of 43) including the ones with the highest deviations in both directions were observed in only 2 consecutively numbered batches. As also capsule size and filling material are equal, the assumption was made that they might be compounded by the same pharmacy.

A lack of uniformity is caused by an insufficient homogenisation procedure. Several reasons may have an impact and should be further investigated: duration of homogenisation, ratio of drug and filling material, physicochemical characteristics of the filling material and agglutination of the drug [168–170]. Conditioned by the study design, we had no information about the compounding method, the duration of homogenisation or the ratio of HC and the respective filling material. Anyhow, it is plausible to assume higher percentages of filling material during compounding of capsules with larger capsule sizes. Considering that HC is a white powder which is triturated with a white filling material, homogenisation is hard to assess during compounding, therefore addition of an inert colour pigment e.g. iron oxide, as recommended by DAC/NRF [142], might improve uniformity and should be further investigated [169]. None of the here investigated capsules contained such a substance.

Looking at the batch level, uniformity of drug content was very good in the vast majority of batches: 87% (48 batches) were accepted by EP standards. Nevertheless, 6 of 7 failed batches had at least 2 capsules exceeding non-tolerable limits. As already observed on the capsule level, 2 batches showed maximum deviations of -47% to 203% which is far outside the non-tolerable limits. In addition, more than half of the capsules from these 2 batches exceeded tolerable limits. In other words, a child receiving capsules from these batches would deal with doses between 1.11-6.30 mg HC instead of the claimed 2 mg and with doses between 1.95-4.77 mg instead of 3 mg, respectively. Especially for such a potent drug as HC with its narrow therapeutic window, a dose variation in such a magnitude is a serious threat for the respective patient.

Correlation net mass and drug content

For the planned clinical trial, the most important investigation is the correlation of net mass and drug content. Unfortunately, only about 20% of the batches showed a sufficient correlation enabling any conclusions concerning the HC content from the capsule net mass as also seen for other drugs formulations [171]. Therefore, a different study medication was proposed using special capsules in which the desired drug amount is directly weighed into the respective capsule.

Accuracy of drug content

As no single investigation of the drug content accuracy is available in the EP, the method for evaluation of drug content uniformity was adapted by using the nominal content instead of the mean content per batch. Due to a lack of a suitable threshold in the EP, ±15% and 25% as tolerable and non-tolerable limits were used as acceptance criterion similar to the ones used for the uniformity of drug content investigation.

In contrast to the uniformity investigations, sufficient accuracy of drug content was only observed for about 2/3 of the capsules. Similar to the content uniformity investigation, the range of deviations from the nominal drug content was unexpectedly high: -46% to 215%. Mean drug content in the capsules was almost 11% below the nominal content. The results are in agreement with other studies, describing a general drug loss during capsule compounding of up to 20% depending on the drug and the filling material [172,173]. D'Hondt reported an overall drug loss for HC of 8%-9% depending on the compounded doses and also investigated potential causes of drug loss e.g. adhesion to capsule casing, to pestle and mortar, capsulation device and other equipment used in capsule compounding. About 1%-2% of the drug-filling material mixture was observed to remain in the capsule casing which is especially important for paediatric patients. Due to their widespread inability to swallow whole capsules, it is common to open capsules and administer only the content with food or beverages while discarding the casings. Anyhow, as HC was extracted from capsule content as well as from casings in the analysis of the present study samples, residuals in the casings are no suitable explanation for the observed drug loss. According to the findings of D'Hondt et al., the main drug decrease of up to 77% of the total loss occurs during the homogenisation at the mortar, the pestle and the plastic card. Here, the magnitude of the decrease is influenced by the mortar material and its status of usage. An agate mortar tended to cause less drug loss than one from e.g. porcelain, a new mortar less than an extensively used one. The drug amount found on other surfaces and devices was below 1% of the total drug amount and therefore considered to be irrelevant.

As expected from the literature [172,173] and the results on capsule level, many batches missed the applied thresholds for drug content accuracy, only 41% were accepted. Incomplete recovery due to usage of HC tablets as starting material instead of pure HC would be possible. At maximum, 5 batches (15% of all exceeding batches) were found showing deviations between -30.2% and -22.5% which is in a similar range as the decrease found for tablet originating capsules in the method development. As only the used filling material and not the starting material was stated by the care givers, no further confirmation was possible. Anyhow, the main findings of this trial remain unchanged if the 5 batches would be excluded.to account for this potential bias. As HC is usually compounded as micronised powder, the loss during homogenisation in the mortar as described by Grauls [46] is most probably the main cause of drug reduction in the capsules.

To compensate the drug decrease, the Zentrallaboratorium Deutscher Apotheker recommends a surcharge of 10% for some drugs since 2017, also for HC in paediatric doses [143]. Considering the lack of specific recommendations at the time of the study enrolment, it was assumed that such a surcharge was not performed for the investigated capsules. As no data regarding the accuracy of drug content using a surcharge is available by now, we simulated such a surcharge in our data by adding 10% to the calculated drug content. 85% instead of 65% of the capsules and 67% instead of 41% of the batches would show acceptable accuracy using the surcharge. Only a small number of capsules (1%) would exceed the upper tolerable- and non-tolerable limits. Hence, the surcharge will most probably lead to

much less capsule batches failing accuracy of drug content. Anyhow, an investigation of the effect needs to be performed.

Comparison with literature data

Single evaluations of already compounded drug formulations were performed by different authorities but allow just a small insight and do not reflect the general quality of these products. Furthermore, the Zentrallaboratorium Deutscher Apotheker e.V. evaluates preselected compounded formulations on a yearly basis called Ringversuch. As many German pharmacies voluntarily participate in this evaluation, it allows a good insight into potential quality issues. A disadvantage of this approach is that the compounder knows about the assessment and possibly works more carefully than during a routine compounding. Hence, the results might be biased. For the present trial, the patients care givers sent in a part of their medication which would otherwise be administered. By my knowledge, this is the first investigation of HC capsules provided directly by patients not by compounders. Disadvantages of the chosen study design were the low number of available capsules per batch and the limited knowledge about respective compounding processes e.g. filling material, starting material or method of compounding. Additionally, several batches with different drug contents were sent in by the same care givers and were most probably produced in the same pharmacy by the same person. Nevertheless, the enrolled number of batches/capsules is high enough to draw conclusions. The trial enabled a realistic, still large-scale insight into the variability and accuracy of contemporaneously compounded HC capsules which was neither achieved by the single investigations nor by the annual Ringversuch.

Conclusion

Summarising, the uniformity of the investigated capsules was good but the accuracy was insufficient due to a general drug loss. A correlation of net mass and drug content was only obtained for a minority of capsules, therefore a conclusion from a net mass to an unknown drug content as planned for the upcoming clinical trial would not be possible. The exact dose of HC should be weighed and directly administered to the patients without using a capsule as vehicle.

The recommended surcharge of 10% HC during compounding is reasonable and should be included. Furthermore, a pigment enabling evaluations concerning drug distribution in the filling material should be added. Both approaches should be further investigated regarding their effectiveness to increase accuracy and uniformity of contemporaneously compounded HC capsules.

The present investigation enabled a broad, realistic insight into the quality of contemporaneously compounded capsules raising also attention of the EMA for the needs of paediatric CAH patients. Among with subsequent clinical trials, it supported the approval of a paediatric formulation in 2017 [174] which enables administration of HC containing microgranules in capsules to be opened by the care givers leading to a strongly enhanced dose uniformity and accuracy.

4.2 Project II - Potential impact factors on relative recovery

The *in vitro* microdialysis investigations showed no relevant impact of drug combinations or reversed microdialysis order on RR values but for air in the microdialysis catheter and flow rate changes.

Impact of drug combination on relative recovery

In general, *in vitro* RR values obtained in the assessment of a potential impact of drug combinations on RR values were very high in all investigated regimens and scenarios. The intra- and inter-catheter variabilities were comparable to published values although rarely reported for *in vitro* investigations [113,123]. Drug combination versus single drug investigations led to both, slight increases as well as

slight decreases in RR values with an overall trend toward marginally lower RR values for drug combinations, especially for combinations including also the analgetic agents. Given the larger inter-catheter variability, these small changes were deemed to be negligible which is also supported by the statistical analysis performed by David Petroff.

Flow rate has an important impact on RR values. Here, a commonly used flow rate of 2 µL/min was applied for most of the evaluations equating to the flow rate used in the clinical trial. As described in Section 1.2.2, higher flow rates lead to lower RR values. This was also observed in the present investigation: values for LIN sampled with the higher flow rate of 5 µL/min were substantially lower and in typical range of *in vivo* RR values for LIN [22,151,161]. For both flow rates, no relevant impact of drug combination on RR values was observed. Hence, for clinical trials assessing ISF concentrations of drugs with high RR values, calibration via retrodialysis can be performed either for each drug separately or using the respective drug combination without major impact on RR values. The results allow no conclusions about potential impact factors for drugs with very low RR values (<60%), therefore these conditions should be evaluated in future *in vitro* investigations.

The present results for the investigation of the drug combinations are very much in line with the findings described by MacVane et al. [123]. The authors compared *in vitro* RR values from single drugs and 2-drug combinations of 4 different beta-lactams as well as VAN in microdialysis and retrodialysis settings at a flow rate of 2 µL/min. An impact of drug combinations was defined as a deviation of ≥+20% in RR values compared to ones of the respective single drugs. In microdialysis settings, no impact of drug combinations was observed. Also, in retrodialysis settings, the majority of investigated drug combinations showed no impact on RR values except for combinations with VAN, a drug with a high molar mass.

Although in the present work only 2-drug antibiotic combinations were investigated, an extrapolation to a regimen with more antibiotics seems justified as also the 4-drug combinations with both analgetic drugs did not reveal a relevant influence (maximum deviation of 5% according to the statistical analysis by David Petroff, see Figure 3.11). For drugs with 'problematic' microdialysis characteristics (e.g. high molar mass, high lipophilicity) further *in vitro* microdialysis investigations of impacting factors on RR should be performed.

Impact of microdialysis order on relative recovery

The microdialysis order of single antibiotics did not show any impact on RR values allowing further flexibility in study design of antibiotic combinations e.g. if the first administered antibiotic has a prolonged half-life (in a patient with e.g. elimination organ failure) and thus more time is required to decrease to the low concentrations necessary for retrodialysis, the calibration for the second combination antibiotic could also be performed first.

Impact of microdialysis setting on relative recovery

Taking into account that different sets of catheters were used for microdialysis (a, b, c) and retrodialysis (d, e, f) investigations, the overall trend of about 5% lower RR values [95% CI: 9% maximum] from retrodialysis settings might be explained by catheter-specific differences in e.g. surface area of the semipermeable membranes and their pore sizes leading to deviations in RR values. Yet, the difference between the RR values in both settings is clinically irrelevant given the overall inter-catheter variability of microdialysis. Hence, retrodialysis can be recommended as feasible method for catheter calibration in a clinical trial for the investigated drugs. Nevertheless, further investigations of the impact of the applied setting on RR should be performed using the same catheter to exclude catheter as bias source.

Impact of air in the microdialysis catheter on relative recovery

Comparing LIN in air-containing solutions with degassed ones, approximately 20% lower RR values and, in case of retrodialysis setting, highly inconsistent values were observed (inter-catheter variability up to 37% in air-containing catheters). As RR values were substantially lower after 3 sampling intervals, the assumption was made, that air bubbles accumulated at that time at the semipermeable membrane hampering free drug diffusion through the pores. Additionally, RR values were in general drastically decreased in both settings compared to investigations with degassed solutions demonstrating the impact of impaired drug diffusion due to air in the system. The effect of air was observed for the whole investigation time span of at least 130 min (0.5 h equilibration and 5 consecutive sampling intervals of 0.33 h each).

Accurate RR values are crucial for reliable calculation of ISF concentrations from microdialysate samples. Therefore, suitable precautions should be implemented to avoid the occurrence of air in the catheter. Each solution should be degassed after preparation and the number of perfusate changes minimised to avoid inclusion of air in the connection between pump and catheter. For microdialysis equipment of other manufacturers, the same situation is expected but should be investigated experimentally. Nevertheless, perfusate changes should preferably be performed by an experienced person. As air bubbles in the microdialysis system also occur due to temperature changes, cooling of prepared solutions or any other procedure leading to a significant alteration in temperature should be avoided. Finally, interventions e.g. flushing should be evaluated with regard of their potential to remove air from the microdialysis catheter.

Impact of flow rate changes on relative recovery

The impact of flow rate changes on RR values was necessary to investigate as a flow rate change inherently occurs at each start of the *in vivo* microdialysis pump and at the end of each flush sequence. As expected after end of flush sequence, a fast increase of RR values was observed resulting in constant values within 10 to 15 min depending on the applied final flow rate of either 1 or 2 µL/min, respectively. Thus, after flushing or flow rate change, an equilibration time of at least 10-15 min should be included in the microdialysis sampling schedule. Otherwise, there is a high risk of falsely low RR values and ultimately falsely high calculated ISF concentrations. The lower the applied final flow rate, the longer should be the equilibration time. In the *in vitro* investigations, common flow rates of 1 and 2 µL/min [175] were assessed; if other flow rates are used in a clinical trial, further investigations should be performed prior to a recommendation concerning a sufficient equilibration time to ensure reliable RR values especially using lower flow rates.

Conclusion

The systematic *in vitro* microdialysis investigations evaluated the impact of relevant clinical trial conditions on RR values for 5 important antibiotics of a variety of drug classes covering a broad range of bacterial infections including SSIs. The reliable determination of the RR values of each catheter is crucial to convert the measured microdialysate concentration into the clinically meaningful ISF concentration (representing a relevant target site of infection) of a patient. Hence, our results have major impact on the design and performance of future microdialysis trials. They were already successfully applied to optimise the clinical trial investigating PAP regimens in obese and non-obese patients by implementing an equilibration time of 0.33 h prior to the catheter calibration and actions to avoid air in the catheters. In addition, the findings are a prerequisite to evaluate sources of variability in the data analysis of clinical trials.

4.3 Project III - Vancomycin monitoring in septic infants via microdialysis

The present project was able to show the feasibility and safety of determining VAN concentration-time courses in septic infants using microdialysis sampling in subcutaneous tissue. Therefore, it added a valuable insight into the pharmacokinetics of VAN in potential target sites. As a crucial prerequisite, a bioanalytical method was developed quantifying VAN from *in vitro* investigations which were performed to inform and optimise the study design of the feasibility trial. The *in vitro* investigations allowed the identification of impact factors, although a direct translation of *in vitro* to *in vivo* findings is not possible. Here, neither pH value, concentration nor setting were found to impact RR values.

In vitro investigations

As expected, *in vitro* investigations showed a reversed impact of flow rate on RR. Considering the infusion duration of 1 h and an expected half-life of only a few hours [77], time resolution should be as high as possible. Therefore a flow rate of 1 µL/min was chosen enabling sampling intervals of 0.5 h in the clinical trial. In addition, only a slight difference of mean RR values comparing microdialysis and retrodialysis was found at that flow rate which is a crucial prerequisite to perform catheter calibration using retrodialysis.

Evaluating the impact of pH value of SM and perfusate on RR, a maximum deviation of ±4.16% between scenarios with either pH 7.4 or 7.0 was found. Only in microdialysis setting using pH adjusted PBS buffer as perfusate and SM (Scenario (iii) and (iv)), a much higher difference in mean RR values of 21.3% was observed. In addition, RR values from Scenario (iii) showed a high intra- and inter-catheter variability in this setting: while for catheter b mean RR value was in the range of the values from other scenarios, catheter a and c showed higher values than in the other scenarios. As no impact of the pH value on RR was found in retrodialysis setting and in microdialysis setting only in 1 very variable scenario, no impact was concluded. Hence, no pH value adjustment in the perfusate was necessary and the feasibility to use RS was shown for the clinical trial. Comparing the present results with the ones of Plock et al. [138,148], who found an impact of pH value on RR, the different investigated pH ranges must be highlighted. Here, a pH range of 7.0 to 7.4 was assessed as expected in ISF of septic infants compared to the pH range of 5.4 to 6.1 as reported by Plock et al. [138,148]. Hence, an impact of pH values in a certain pH range is possible and was demonstrated by Plock et al. but did not occur in the expected pH range in septic patients. The chosen pH range from the *in vitro* investigations was supported by routine pH measurements in capillary blood of the patients during the clinical trial stating the lowest pH value with 7.1. Considering the *in vitro* findings, unbuffered RS as perfusate is suitable for *in vivo* microdialysis of VAN with respect to pH value.

To evaluate a potential impact of VAN concentration on RR, mean RR values for three different concentrations were compared in both, RS and PBS buffer (pH 7.4). They showed similar magnitudes over the whole concentration range of 10 µg/mL to 80 µg/mL in microdialysis and retrodialysis setting. In general, RR values obtained from catheter b and c were lower than from catheter a except for the microdialysis investigation using 80 µg/mL. Here, RR values of catheter a were about 10% lower than of catheter b and c resulting in a slight decrease of mean RR values at that concentration. A potential explanation might be a small gas bubble at the semipermeable membrane impeding drug diffusion as demonstrated in Section 3.2.1. Thus, no impact of VAN concentration on RR values could be shown.

A potential impact of the setting on RR values had to be excluded to enable retrodialysis sampling as method for catheter calibration. For all investigated scenarios except Scenario (iv) determining RR

values in PBS buffer pH 7.0 as perfusate and SM, maximum deviation in mean RR values of both settings was ±5.95% and therefore not relevant considering the typical intra- and inter-catheter variability. Scenario (iv) showed a difference of about 16% mainly caused by much lower RR values obtained from catheter a, leading to a difference in mean RR value of 27.4% compared to catheter b and c with 14.6% and 6.01% difference between mean RR values of both settings, respectively. As Scenario (iv) is the only 1 showing a relevant decrease of RR values in retrodialysis setting compared to microdialysis setting, it was concluded that the setting has no impact on RR.

The perfusate with the most reliable results throughout the *in vitro* investigations was PBS buffer with a pH value of 7.4. Unfortunately, PBS is not approved for human use, therefore non-inferiority of RS compared to PBS buffer had be proven. Although the variability of obtained RR values using RS was higher than using PBS buffer, no additional dependencies were observed. Hence, the application of RS as perfusate as well as catheter calibration via retrodialysis was feasible.

In summary, the results of the *in vitro* investigations had a relevant impact on the design of the clinical trial and guided the decision concerning flow rate, perfusate and calibration method. A special focus was on pH values, taking into account that septic patients as the infants included in the subsequent clinical trial often have decreased pH values in plasma [139,140] and certainly also in ISF. The proof of pH value independency of RR values was therefore important to ensure a good quality of the obtained patient data. Results regarding a sufficient equilibration time from Project II were also implemented. The applied equilibration time of 0.5 h each prior to microdialysis and retrodialysis was sufficient taking into account the flow rate of 1 µL/min. Hence, reliable microdialysis sampling and catheter calibration was enabled.

Bioanalysis

Both bioanalytical methods were successfully validated (Section 3.1.2 and 2.4.5) and enabled the quantification of VAN in samples from the *in vitro* investigations as well as from the clinical trial. Anyhow, an unknown analytical problem resulted in QC sample concentrations exceeding the acceptance criteria for several patients (6, 8 and 9) during the analysis of microdialysis study samples. Numerous but finally unsuccessful attempts were made to find the source of inaccuracy in QC samples, e.g. systematic dilution errors, sample instability, changed device parameters. No systematic deviations were observed, hence a falsely prepared stock solution (SL) or sample preparation could be excluded. As Cal samples were all accepted although similarly prepared from a different SL and stored under identical conditions, instability of QC samples was excluded. Function of the device was normal and changes in the detection method were not observed. As concentration-time courses were plausible for all 3 affected patients, only QC samples were assumed to be erroneous. Ultimately, the remaining patient data which were in accordance with the EMA recommendations regarding QC samples would be sufficient to confirm feasibility of subcutaneous microdialysis sampling in infants. Therefore, further data description was performed for 7 patients (excluded patients see Section 3.3.4).

Patient characteristics

Patients enrolled in the clinical trial showed a wide range of body weight, gestational and chronological age and do well reflect the heterogeneity of the neonatal population [67,72]. Concomitant drugs in the trial may interact with VAN [176], but a single therapy with VAN is not possible in such fragile, severely suffering patients which are in need of critical care medicine e.g. analgetics to reduce painful conditions or caffeine for stimulation of the respiratory function. The microdialysis sampling was well integrated into clinical routine without interfering with medical or caring procedures in the infants and tolerated with only little discomfort and minor complications as also shown by other trials [177–179].

Therefore, the trial demonstrated the safety of subcutaneous microdialysis sampling in this patient group.

Vancomycin concentrations in microdialysate, ISF and plasma

Concentrations of VAN in microdialysate and subcutaneous ISF were plausible for all patients except patient 1 and 3. As the present clinical trial is the first 1 investigating VAN in neonatal subcutaneous tissue, no comparison of the obtained concentration-time courses with literature was possible. A literature search revealed 2 publications reporting VAN concentrations in adults, Housman et al. [24] in patients with lower limb infections and Bue et al. [97] in patients with total knee replacement surgery. Housman et al. reported for vancomycin $C_{ISF, min}$ at steady state of 6.0 µg/mL ±2.6 µg/mL in ISF near the infection after administration of a mean dose of 12.9 mg/kg (mainly every 12 hours). After three doses of vancomycin, the highest $C_{ISF, min}$ in the present data was 1.64 mg/mL which is much lower; yet, several important differences in the studies have to be acknowledged: first, the very different populations (adults vs. infants/neonates) with associated differences in pharmacokinetic properties due to e.g. incomplete organ maturation, second despite the similar mg/kg doses, due to body weight difference the very different absolute doses and third steady state may not have been reached in the present study. Bue et al. reported a C_{max} of 6.6 µg/mL (range 3.4 to 9.8 µg/mL) after administration of 1 g vancomycin as 100 min short-term infusion. The mean C_{max} from the present study was 10.8 µg/mL and therefore higher than in the adults. Given the differences discussed above (here also a different infusion duration), a direct comparison of the study results is not justified. These differences and their impact need further investigation. Special focus should be given to two factors: vancomycin clearance being highly dependent on renal function, which is usually decreased in infants, hence decreasing vancomycin elimination. Additionally, the sepsis of the included infants/neonates may lead to altered renal clearance impacting again vancomycin concentrations [180]. In addition, the concomitantly administered drugs in the study may have led to pharmacokinetic interactions with vancomycin altering its concentration-time course.

Regarding the microdialysis performance, the risk of a catheter dislocation like in patient 2 is always present especially in patients like infants moving without taking care for the inserted catheters. Also pump problems as in patient 7 or the appearance of small gas bubbles impeding sample collection may occur in microdialysis trials. However, technical feasibility was generally given.

For both, patient 2 and 7, no plausible RR value could be calculated. Therefore, the mean RR value of patient 4, 5, 6, 8 and 9 was used to calculate ISF concentrations. This is a common way to overcome missing RR values [27,181].

Only serum minimum concentrations in term and preterm infants were published by Sinkeler et al. [78] and Dersch-Mills et al. [77], hence no direct comparison to the here obtained plasma concentrations was possible. Sinkeler et al. reported a mean $C_{serum, min}$ of 10.6 µg/mL right before the fifth dose which is 2-fold higher than the 5.56 µg/mL observed in the present study. As discussed above, several aspects need to be considered comparing both studies: the applied dosing regimen used by Sinkeler et al. was chosen according to gestational or postnatal age resulting in higher daily doses for term infants as in the present study. In addition, neither duration of intravenous administration nor time point of trough sample collection were stated impeding the comparison with the here obtained data and the use of serum instead of plasma samples.

Dersch-Mills et al. stated a mean $C_{serum, min}$ of 8.7 µg/mL right before the third vancomycin dose. Dosing regimen (always 15 mg/kg vancomycin) based on body weight and postnatal age. Minimum concentrations for patients with a chronological age of ≤7 days showed concentration ranges of

6.8 µg/mL (body weight <1200 g) to 11.5 µg/mL (body weight >2000 g) and were therefore similar or higher which is plausible considering the different dosing regimen in the present study and the 1 of Dersch-Mills et al.

Given the small number of time points for simultaneous target-site (0.7 – 9.4 µg/mL) and plasma (4.1 – 8.7 µg/mL) concentrations (n=5), the here performed investigation suggested no direct correlation similar to Hamada et al. [164] for adult diabetic patients. Considering the ongoing discussion about effective PK/PD targets for VAN and the still suboptimal therapeutic efficacy, this might be 1 reason and is crucial to be investigated further with a higher number of simultaneous measurements in ISF and plasma.

Conclusion

In summary, the present trial demonstrated the safety and feasibility of VAN monitoring using microdialysis which is the prerequisite for a larger, pharmacokinetic trial investigating influential factors of VAN pharmacokinetics in infants. In addition, an analysis of the quantified concomitant drugs from the present study and the plausibility of their concentration-time profiles is planned as 1 of the next steps.

4.4 Project IV - Perioperative antibiotic prophylaxis in synovial fluid

Bioanalysis

The extended bioanalytical methods for CFX and LIN in plasma and microdialysate were suitable for a precise and accurate drug quantification in presence of the administered comedication. Both methods were successfully applied in the bioanalysis of the clinical trial.

Pooled plasma was used to develop and validate the extended methods including plasma samples. Ideally, it should be gained from a high number of different individuals to exclude any source of potential bias by patient individual factors e.g. medication, electrolyte and blood composition. Here, at least six different persons donated plasma. In addition, all drugs, the donors took within 7 days before plasma collection were documented and included in a selectivity investigation. Most of the donors were women taking contraceptive drugs containing levonorgestrel and ethinylestradiol. Detection wavelengths of both drugs (241 nm for levonorgestrel [182] and 281 nm for ethinylestradiol [183]) differed from the wavelengths used to quantify CFX and LIN (271 nm and 251 nm, respectively). In addition, no peaks were observed in blank samples from the pooled plasma, hence it was concluded that no interference with the investigated drugs had to be expected and the pooled plasma was suitable for method development, validation and analysis of clinical trial samples.

Catheter calibration

To allow conversion of microdialysis samples of the clinical trial into ISF concentrations, retrodialysis was performed for each catheter to determine RR. In both matrices, negative RR values were observed for CFX as well as for LIN resulting from lower concentrations in retroperfusate than in the mean of both retrodialysate samples. In close contact with the clinical investigators, numerous attempts were made to explain these implausible values, e.g. sample mix-up, analytical errors/imprecision, or drug degradation, but ended unsuccessfully.

In general, retroperfusate concentrations of both drugs widely ranged although retroperfusate was prepared only once for all patients and stored deep frozen in aliquots until usage. As 1 aliquot of the

retroperfusate was used for the calibration of both catheters in a patient, retroperfusate concentrations used in synovia and muscle ISF were supposed to be in the same range. Nevertheless, relevant differences between both retroperfusate samples were observed: at maximum ±36.1 µg/mL (mean ±11.8 µg/mL) and ±27.5 µg/mL (mean ±7.58 µg/mL) for CFX and LIN (Table 3.22 and Table 3.23), respectively. Thus, even the low drug concentrations in retroperfusate resulted in sufficient concentration gradients between retroperfusate and corresponding ISF concentration right before retrodialysis performance (ratio ≥10.6 (synovia) and ≥9.54 (muscle ISF) for CFX and ≥14.3 (synovia) and ≥30.7 (muscle ISF) for LIN). Therefore, diffusion through the semipermeable membrane should be unhampered[122]. As retroperfusate concentrations of CFX and LIN were supposed to be equal, also the difference between the concentrations of both drugs was assessed: ≤±14.7 µg/mL (mean 6.30 µg/mL) in synovial fluid and ≤±16.5 µg/mL (mean 7.91 µg/mL) in muscle ISF (Table 3.22 and Table 3.23) indicating suboptimal homogeneity in the retroperfusate samples.

Retrodialysate concentrations also showed a high variability but mainly without correspondence to the respective retroperfusate concentrations. Hence, the resulting RR values were partially negative or implausibly low. According to the results from Project II (Section 3.2.1), the applied equilibration time was sufficient to enable a reliable determination of RR values, hence retrodialysate concentrations obtained in the 2 consecutively collected samples should be comparable. Nevertheless, for CFX, differences between both samples were at maximum ±10.4 µg/mL (mean ±4.94 µg/mL) in synovial fluid and ±16.4 µg/mL (mean ±7.31 µg/mL) in muscle ISF (Table 3.22). A similar result was observed for LIN, differences were ≤±8.13 µg/mL (mean ±3.28 µg/mL) and ≤±13.1 µg/mL (mean ±6.58 µg/mL, Table 3.23), respectively. As no trend to increasing or decreasing concentrations in the consecutively collected samples could be found, systematic errors as too short equilibration time or air in the catheter were excluded.

As no general explanation for the negative and very low RR values could be found, the respective RR values were excluded and replaced by the mean RR value of the other catheters for the respective drug and matrix as recommended in literature [22,27,181]. In total, 2 catheters inserted into synovial fluid showed implausible values for both drugs. In muscle ISF, 4 catheters were affected for CFX but only 1 of them had also an implausible RR value for LIN. Hence, the majority of catheters had plausible RR values. Most of the ISF concentrations calculated with the respective mean RR value showed a magnitude comparable with other patients. Only for CFX in muscle ISF, concentration-time courses of patient 6 and 8 deviated from the other obtained concentration-time courses. However, the concentration-time courses were similar in microdialysis samples in these patients, patient 6 with the lowest profile and patient 8 with the highest one, therefore resulting profiles were considered to be plausible concerning their magnitudes. The plausible mean RR values were in comparable range with reported literature values. For CFX, RR values between 10.4% in muscle ISF [27] and 31.7% in brain ISF [154] were calculated which is in line with the here found 20.4% and 15.1% in synovial fluid and muscle ISF, respectively. LIN had higher mean RR values compared to CFX of 25.2% (synovia) and 20.6% (muscle ISF) but lower than literature values ranging from 36% in inflamed subcutaneous ISF [107] to 59.1% in muscle ISF [161]. As all of the reported values were obtained using lower flow rates, the RR values computed for the present trial are plausible. For both drugs, plausible mean RR values in synovial fluid seemed to be a little higher than in muscle ISF which might be caused by the lower tortuosity at the insertion site: 5.35% and 4.65% for CFX and LIN, respectively.

Drug concentrations in the different matrices

Looking at the individual microdialysate concentrations and the converted ISF concentrations collected from synovial fluid, several patients showed 2 or 3 local maximum concentrations for both drugs. In microdialysate from muscle ISF, only 1 patient each had 2 peak concentrations of CFX or LIN. The first C_{max} was always in the same sampling interval as C_{max} of the other patients in this matrix. The additional local C_{max} was therefore observed in the elimination phase. A similar phenomenon was often described in literature for several drugs including CFX and LIN in different target tissues e.g. [22,27,84,107,162,163,184–187]. Probably, these fluctuations of drug concentration in the elimination phase are caused by distribution processes between plasma and the investigated tissue ISF. Thus, the fluctuations were more pronounced in synovial fluid than in muscle ISF which is, from a pharmacokinetic point of view, more hampered in its distributional abilities.

In all patients, CFX was already detected during baseline sampling although only patient 10 received CFX during surgery. In this patient, also LIN was found in a small concentration prior to the infusion. It was assumed that the order of drug administration and sample drawing might led to measurable ISF concentrations: if drug infusion was started first, followed by the plasma sample drawing and afterwards the exchange of microdialysis sampling vial, time might be sufficient to enable small drug concentrations in the baseline samples of synovial fluid and muscle ISF. But as the corresponding plasma concentrations were much lower, the theory was discarded

For CFX from unbound plasma and muscle ISF, maximum concentrations showed similar individual ranges as stated in the available literature. In total, 3 publications were found reporting ISF concentrations obtained via microdialysis. Barbour et al. [27] investigated CFX in muscle ISF and subcutaneous ISF, Hosmann et al. [154] in brain ISF and Mand'ák et al. [28] in brain and muscle ISF. All of them compared ISF concentrations with simultaneously obtained unbound plasma concentrations. Catheter calibration was performed using retrodialysis. Barbour et al. used CMA 60 catheters which are similar to the 63 catheters used in the present study with respect to membrane length, membrane material and MWCO. They calculated a mean RR value of 19.8% for catheters inserted in subcutaneous ISF and 10.4% in muscle ISF. Mand'ák et al. used also CMA 60 catheters but found a much higher RR value of 30%. Similarly, Hosmann et al. stated a much higher mean RR value of 30.7% ±10.3% but used a different catheter which is especially designed for brain microdialysis and has a different membrane material and length. Mean C_{max} in muscle ISF reported by Barbour was 60.1 µg/mL which equates to the C_{max} of 56.4 µg/mL from the present study. Mand'ák et al. stated a much higher C_{max} of 148 µg/mL resulting from the doubled dose administered. Assuming dose proportionality in C_{max}, this data is well in line with the here presented concentrations.

Unbound maximum plasma concentrations for CFX were 69.0 µg/mL and therefore comparable to mean C_{max} reported by Hosmann et al. [154]. They investigated patients after aneurysmal subarachnoidal haemorrhage who received CFX as postoperative antibiotic prophylaxis and found a mean C_{max} of 60.1 µg/mL right after the end of a 1 h short-term infusion containing 1500 mg CFX. As in the present trial an infusion duration of only 0.5 h was used to administer the same dose, the slightly lower values of Hosmann et al. are plausible. Barbour et al. performed dose administration as in the present clinical trial but led to lower mean C_{max} values in unbound plasma of 44.8 µg/mL. As they investigated CFX in obese patients, patient-specific factors were assumed to cause the observed lower concentrations. Bertholee et al. [153] and Mand'ák et al. reported about 2-fold higher maximum unbound plasma concentrations with mean values of 156 µg/mL and 120 µg/mL, respectively. Both clinical trials investigated patients undergoing cardiopulmonary bypass surgery and therefore applied

a different dosing scheme. In the clinical trial conducted by Bertholee et al., an initial dose of 1500 mg CFX was administered followed by another 1500 mg at the beginning of the cardiopulmonary bypass, whereas Mand'ák et al. administered 3 doses, an initial 1 of 3000 mg CFX at the time of anaesthesia induction, a second dose of 1500 mg after the cardiopulmonary bypass and a third 1 with 1500 mg 8 h later. Neither duration of i.v. administration nor time between first and second dose was stated in both publications and may vary due to clinical routine impeding a comparison with the here presented data.

For LIN, several publications were found in literature stating plasma concentrations and the corresponding ISF concentrations in muscle ISF or subcutaneous ISF. Dehghanyar et al. [188] and Buerger et al. [161] reported both concentrations in muscle ISF and subcutaneous ISF, Eslam et al. [107] and Wiskirchen et al. [106] published only subcutaneous ISF concentrations. In all publications, standard doses of 600 mg were administered via 0.5 h short-term infusion or in the trial published by Wiskirchen et al. via 1 h short-term infusion. In case of multiple dosing, dosing intervals of 12 h were used. Catheters in the trials were either CMA 60 or 63 microdialysis catheters (as in the present trial) which are comparable in their design and membrane characteristics. In each of these references, calibration was performed using retrodialysis. Deviating from the present trial for which a flow rate of 2 µL/min was used, the applied flow rate in all comparable trials was 1.5 µL/min. Dehghanyar et al. found a mean RR value for both catheter sites of 40.4%, Buerger et al. of 59.1% in muscle ISF and 53.1% in subcutaneous ISF. Wiskirchen et al. reported RR values of 39.3% in non-inflamed subcutaneous ISF and 46.4% in wound ISF. Eslam et al. who used the 63 microdialysis catheter as in the present study, calculated a RR value of 37% for catheters inserted in non-inflamed subcutaneous ISF and 36% in inflamed subcutaneous ISF. Hence, all reported RR values were higher than the here found 25.2% in synovia and 20.6% in muscle tissue. The higher flow rate in the present trial is most probably one reason for the lower RR values.

Mean ISF concentration in muscle tissue was 11.5 µg/mL in the here described clinical trial which is in a similar range as the 13.5 µg/mL published by Dehghanyar et al. However, Buerger et al. found a mean concentration of only 7.5 µg/mL which might be caused by pharmacokinetic deviations in the critically ill patient population investigated in the respective trial. For synovial fluid concentrations, no literature was found but Dehghanyar et al. and Buerger et al. clearly demonstrated the inhomogeneous permeation abilities of LIN in different body fluids as it was observed in the present work. Unbound C_{max} in plasma was in comparable ranges for the present study and the trials reported by Dehghanyar et al., Buerger et al. and Eslam et al.. Only Wiskirchen et al. stated slightly lower values caused by the prolonged infusion time.

Study limitations

Limitations of the clinical trial are, as common in microdialysis trials, the low number of patients making a further analysis of the observed interpatient variability, e.g. by non-linear mixed effects pharmacokinetic modelling, challenging.

Conclusion

The results of the present trial nicely demonstrated the different abilities of CFX and LIN to distribute into muscle ISF and synovial fluid and fundamentally supported the evaluation of therapeutic drug concentrations at the target site in patients with joint infections. They may guide to potential implications for optimal dosing and clinical application. C_{max} concentrations for CFX in synovia and muscle ISF was almost as high as in unbound plasma due to a good and homogenous drug distribution while LIN showed in average a lower C_{max} in synovial fluid than in unbound plasma and muscle ISF. The faster increase of the concentrations of both drugs in plasma was plausible from a pharmacokinetic

point of view as drug distribution occurs from plasma into the respective ISFs. Interestingly, all ISF concentrations obtained after C_{max} in the respective matrix up to the end of the investigation were higher than unbound plasma concentrations (except for LIN in synovial fluid) showing again the good ISF distribution of CFX and partly LIN. For CFX, the percentage of the dosing interval that the unbound concentration exceeds the MIC is more related to clinical efficacy than C_{max} [92,93,98], therefore the slower increase and decrease in synovia and muscle ISF might be even beneficial. For LIN, the most important PK/PD index is the ratio of AUC and MIC [93,104], therefore potential inferiority of tissue concentration-time courses has to be further investigated. In addition, an analysis of interpatient variability was planned potentially leading to optimised dose recommendation in the therapy of joint infections.

5 Abstract

Drug therapy is always at risk of over- or underdosing resulting in inefficient treatment or adverse drug reactions. To find an optimal dose of a drug is therefore crucial. However, determination of the "right dose" is challenging due to several reasons as patient-individual factors impacting the pharmacokinetics or pharmacodynamics of a drug (e.g. obesity) or due to the still present knowledge gaps mainly in special patients (e.g. infants) or special conditions (e.g. hemofiltration). Aggravatingly, the available pharmacokinetic information comprises mainly easily accessible matrices as plasma, which is often not the target site for drugs and may differ significantly with regard of the concentration-time courses of a drug. In addition, small inhomogeneities in the dosage forms change the amount of administered drug which may also influence the therapy outcome.

The objective of Project I was to evaluate uniformity of dug content and net mass as well as accuracy of content in contemporaneously compounded hydrocortisone capsules as preparation of a subsequently planned clinical trial. Uniformity and accuracy investigations were performed in adaption of testing methods provided by the European Pharmacopeia using a suitable quantification method developed for this project. The vast majority of capsules showed a good uniformity within the tolerable limits but on the other side, some batches contained capsules with maximum deviations of ±27.6% (net mass) and even ±203% (content) from the respective batch mean which would most probably lead to suboptimal treatment. The accuracy investigation revealed across all capsules and batches a by about 11% decreased dose with similar deviations as found for uniformity of content. In addition, a potential correlation of net mass and drug content was assessed but could be shown in just a minority of batches.

The aim of Project II was to investigate potential impact factors of microdialysis sample collection on relative recovery values to inform the study design of a clinical trial investigating antibiotic combinations in obese patients. Several *in vitro* investigations were performed using a standardised *in vitro* microdialysis system. Combinations of up to 4 drugs and the order in which drugs were microdialysed did not impact relative recovery, but air in the system led to much lower and highly inconsistent relative recovery values. Hence, air should urgently be avoided to obtain reliable and consistent results from microdialysis sampling. Additionally, time until consistency of obtained relative recovery values after a flow rate change was assessed and resulted in recommendations for equilibration times of at least 10 to 15 min depending on the applied flow rate.

Also in Project III, *in vitro* investigations were performed to evaluate potential impact factors using the *in vitro* microdialysis system. The investigations showed neither an impact of the pH value in surrounding medium and perfusate, nor of diffusion direction (microdialysis or retrodialysis setting) or vancomycin concentration on resulting relative recovery values. As expected, an impact of flow rate on relative recovery values was observed. The results of the *in vitro* investigations were successfully implemented in the design of a clinical trial determining the feasibility of vancomycin quantification in infants via subcutaneous microdialysis. Study samples were quantified using a previously in the Department developed LC- MS/MS method. The resulting concentrations showed plausible time courses. In addition, no increased risk of adverse events was observed and therefore feasibility of microdialysis sampling in the infants was proven.

Project IV aimed to extent a previously developed HPLC method for the quantification of cefuroxime and linezolid from microdialysate to also plasma samples. The final method was applied for the quantification of both drugs in a clinical trial investigating both drugs as perioperative antibiotic

prophylaxis treatment. The plasma concentration-time courses were subsequently compared to previously determined microdialysate and interstitial fluid concentrations showing a very good tissue penetration of cefuroxime and an impaired 1 for linezolid, especially in the synovial fluid of the knee.

All here presented projects helped to close knowledge gaps in the treatment of endocrine disorders or bacterial infections. Project I supported the subsequent clinical trial and ultimately the approval of a commercial paediatric dosage form lowering the variability of administered hydrocortisone doses and therefore increasing the therapeutic outcome. The *in vitro* investigations of Project II and III answer basic research questions in the field of microdialysis and increased the quality of obtained data from the clinical trials by optimising the study designs. Finally, the analysis of clinical trial samples in Project III and IV gave a valuable insight into target site pharmacokinetics.

6 Zusammenfassung

Arzneimitteltherapien bergen immer das Risiko einer Über- oder Unterdosierung des Arzneistoffes und dadurch bedingt entweder einer ineffektiven Therapie oder von unerwünschten Arzneimittelwirkungen. Es ist daher unabdingbar, die richtige Dosierung des Arzneistoffes zu finden, was teilweise schwierig ist. Mehrere Gründe lassen sich dafür finden. Zum einen verändern Patienten-individuelle Faktoren beispielsweise Fettleibigkeit die Pharmakokinetik und Pharmakodynamik von Arzneistoffen, zum anderen gibt es noch immer viele Wissenslückeninsbesondere bei speziellen Patientengruppen wie Neugeborenen oder bei Patienten in besonderen Umständen wie der Hämofiltration. Erschwerend kommt hinzu, dass die vorhandenen pharmakokinetischen Informationen meist nur aus Daten von leicht zugänglichen Körperflüssigkeiten wie Plasma stammen, die oft aber nicht dem Wirkort der Arzneistoffe entsprechen und sich die Pharmakokinetik teils beträchtlich unterscheidet. Des Weiteren können auch kleine Dosisabweichungen in der Arzneiform selbst die Therapiegüte beeinflussen, da so die verabreichte Dosis variiert.

Das Ziel von Projekt I war dementsprechend auch eine Untersuchung der Gleichförmigkeit von Arzneistoffgehalt und Masse des Kapselinhaltes sowie die Richtigkeit des Gehalts in Patienten-individuell hergestellten Hydrocortison-Kapseln zur Vorbereitung einer geplanten klinischen Studie. Gleichförmigkeit und Richtigkeit wurden mit an die entsprechenden Testmethoden des Europäischen Arzneibuchs angelehnt. Dabei wurde eine eigens entwickelte Quantifizierungsmethode verwendet. Ein sehr großer Teil der untersuchten Kapseln zeigte eine gute Gleichförmigkeit innerhalb der vom Arzneibuch tolerierten Grenzen, jedoch enthielten einige Chargen auch Kapseln mit starken Abweichungen vom jeweiligen Chargenmittelwert. In Bezug auf die Masse des Kapselinhaltes wurden Abweichungen von -18.0% bis 27.6% und für den Gehalt an Hydrocortison sogar Spannbreiten von -46.7% bis 203% festgestellt. Derartige Abweichungen würden den Therapieerfolg höchstwahrscheinlich negativ beeinflussen. Die Untersuchung zur Richtigkeit zeigte über alle Kapseln und Chargen hinweg eine Verminderung des Gehaltes um etwa 11% mit ähnlichen Maximalabweichungen wie in der Untersuchung zur Gehaltsgleichförmigkeit (-46.7 bis 213%). Die erhoffte Korrelation von der Masse des Kapselinhaltes und des Gehaltes konnte nur in einer Minderheit der Chargen beobachtet werden.

In Projekt II wurden potenzielle Einflussfaktoren auf das Sammeln von Proben mittels Mikrodialyse geprüft. Die gewonnenen Erkenntnisse sollten in das Design einer nachfolgenden klinischen Studie zur Untersuchung von Antibiotika-Kombinationen in fettleibigen Patienten einfließen. Dafür wurden verschiedene *in vitro* Versuche mit Hilfe eines standardisierten *In-vitro*-Mikrodialyse-System durchgeführt. Kein Einfluss auf die relative Wiederfindung wurde von Kombinationen aus bis zu vier Arzneistoffen oder der Reihenfolge, in der die Arzneistoffe mikrodialysiert wurden, beobachtet. Dagegen führte Luft im Mikrodialyse-System zu stark reduzierten und zusätzlich sehr variablen relativen Wiederfindungen und sollte daher so weit wie möglich vermieden werden. Zusätzlich wurde die Zeitspanne untersucht bis nach einer Flussratenänderung wieder konsistente Werte für die relative Wiederfindung erhalten werden. Die Ergebnisse zeigten, dass Equilibrierzeiten von mindestens 10 bis 15 min, je nach verwendeter Flussrate, nötig sind.

Auch in Projekt III wurden wieder Untersuchungen mit dem *In-vitro*-Mikrodialyse-System zu möglichen Einflussfaktoren auf die relative Wiederfindung durchgeführt. Sie zeigten weder einen Einfluss des pH-Wertes im Umgebungsmedium oder Perfusat noch der Diffusionsrichtung (Mikrodialyse oder Retrodialyse) oder der Vancomycin-Konzentration auf die relative Wiederfindung. Wie erwartet

konnte jedoch der Einfluss der Flussrate auf die relative Wiederfindung beobachtet werden. Die Ergebnisse der *In vitro* Untersuchungen wurden erfolgreich verwendet um das Design einer klinischen Studie zu optimieren, die die Machbarkeit von Mikrodialyse-Untersuchungen in der Subcutis von Neugeborenen zeigen wollte. Die dabei anfallenden Studienproben wurden mit Hilfe einer zuvor in der Abteilung entwickelten LC- MS/MS Methode quantifiziert. Die resultierenden Konzentrationen zeigten plausible Zeitverläufe. Auch wurden keine schwerwiegenden unerwünschten Wirkungen auf die Neugeborenen beobachtet, daher konnte die Machbarkeit der Mikrodialyse in dieser Patientengruppe gezeigt werden.

In Project IV sollte eine bestehende Quantifizierungsmethode für Cefuroxim und Linezolid aus Mikrodialysat erweitert werden um auch die Bestimmung der Arzneistoffe aus Plasma zu ermöglichen. Die finale Methode wurde genutzt um Proben einer klinischen Studie zu messen, in der beide Arzneistoffe im Rahmen einer perioperativen antibiotischen Prophylaxe eingesetzt wurden. Die ermittelten Konzentrations-Zeit-Verläufe wurden mit zuvor berechneten Verläufen aus Mikrodialysat bzw. Interstitialflüssigkeit verglichen und zeigten eine sehr gute Gewebegängigkeit für Cefuroxim und eine gehinderte für Linezolid, insbesondere in Bezug auf die untersuchte Synovialflüssigkeit des Knies.

Alle hier vorgestellten Projekte helfen dabei Wissenslücken in der Behandlung von endokrinologischen Krankheiten oder bakteriellen Infektionen zu schließen. Projekt I unterstützte die nachfolgende klinische Studie und konnte schlussendlich zur Zulassung einer kommerziell hergestellten Dosierungsform für Kinder beitragen, was die Variabilität der verabreichten Hydrocortison-Dosen stark vermindert und den Therapieerfolg verbessert. Die *in vitro* Versuche aus Projekt II und III konnten grundlegende Forschungsfragen auf dem Gebiet der Mikrodialyse beantworten und erhöhten die Qualität der aus den klinischen Studien erhaltenen Daten durch die Optimierung der Studiendesigns. Zu guter Letzt, ermöglichte die Analyse der Studienproben in Projekt III und IV einen wertvollen Einblick in die Pharmakokinetik der Arzneistoffe an ihrem Wirkort.

7 References

[1] P. De Paepe, F.M. Belpaire, W.A. Buylaert. Pharmacokinetic and pharmacodynamic considerations when treating patients with sepsis and septic shock. Clin. Pharmacokinet., 41:.: 1135–1151 (2002).

[2] P.J. Bergen, J.B. Bulitta, C.M.J. Kirkpatrick, *et al.* Substantial impact of altered pharmacokinetics in critically Ill patients on the antibacterial effects of meropenem evaluated via the dynamic hollow-fiber infection model. Antimicrob. Agents Chemother., 61:.: e02642-16 (2017).

[3] M. Dostalek, F. Akhlaghi, M. Puzanovova. Effect of Diabetes Mellitus on Pharmacokinetic and Pharmacodynamic Properties of Drugs. Clin. Pharmacokinet., 51:.: 481–499 (2012).

[4] M.J. Hanley, D.R. Abernethy, D.J. Greenblatt. Effect of obesity on the pharmacokinetics of drugs in humans. Clin. Pharmacokinet., 49:.: 71–87 (2010).

[5] D.M. Roberts, J. Sevastos, J.E. Carland, *et al.* Clinical pharmacokinetics in kidney disease-application to rational design of dosing regimens. Clin. J. Am. Soc. Nephrol., 13:.: 1254–1263 (2018).

[6] A.F. Macedo, C. Alves, N. Craveiro, *et al.* Multiple drug exposure as a risk factor for the seriousness of adverse drug reactions. J. Nurs. Manag., 19:.: 395–399 (2011).

[7] A.A. Elzagallaai, M.J.E. Greff, M.J. Rieder. Adverse Drug Reactions in Children: The Double-Edged Sword of Therapeutics. Clin. Pharmacol. Ther., 101:.: 725–735 (2017).

[8] A.H. Lavan, P. Gallagher. Predicting risk of adverse drug reactions in older adults. Ther. Adv. Drug Saf., 7:.: 11–22 (2016).

[9] Y. Yu, J. Chen, D. Li, *et al.* Systematic Analysis of Adverse Event Reports for Sex Differences in Adverse Drug Events. Sci. Rep., 6:.: 24955 (2016).

[10] F. Franconi, I. Campesi. Sex and gender influences on pharmacological response: An overview. Expert Rev. Clin. Pharmacol., 7:.: 469–485 (2014).

[11] A.K. Beery, I. Zucker. Sex bias in neuroscience and biomedical research. Neurosci. Biobehav. Rev., 35:.: 565–572 (2011).

[12] R.M. Martin, P.N. Biswas, S.N. Freemantle, *et al.* Age and sex distribution of suspected adverse drug reactions to newly marketed drugs in general practice in England: Analysis of 48 cohort studies. Br. J. Clin. Pharmacol., 46:.: 505–511 (1998).

[13] M.A. Simon, E.E. de La Riva, R. Bergan, *et al.* Improving diversity in cancer research trials: The story of the cancer disparities research network. J. Cancer Educ., 29:.: 366–374 (2014).

[14] D.A. Van Riet-Nales, M.E. Doeve, A.E. Nicia, *et al.* The accuracy, precision and sustainability of different techniques for tablet subdivision: Breaking by hand and the use of tablet splitters or a kitchen knife. Int. J. Pharm., 466:.: 44–51 (2014).

[15] J. Madathilethu, M. Roberts, M. Peak, *et al.* Content uniformity of quartered hydrocortisone tablets in comparison with mini-tablets for paediatric dosing. BMJ Paediatr. Open, 2:.: e000198 (2017).

[16] Å.C. Andersson, S. Lindemalm, S. Eksborg. Dividing the Tablets for Children – Good or Bad? Pharm. Methods, 7:.: 23–27 (2016).

[17] European Pharmacopoeia (7th Edition). 2.9.6 Uniformity of content of single-dose preparations. (2010).

[18] European Pharmacopoeia (7th Edition). 2.9.40 Uniformity of dosage units. (2010).

[19] D.M. Ryan. Pharmacokinetics of antibiotics in natural and experimental superficial

compartments in animals and humans. J. Antimicrob. Chemother., 31:.: Supplement D 1-16 (1993).

[20] U. Theuretzbacher. Tissue penetration of antibacterial agents: how should this be incorporated into pharmacodynamic analyses? Curr. Opin. Pharmacol., 7:.: 498–504 (2007).

[21] F. Van Bambeke, M. Barcia-Macay, S. Lemaire, *et al.* Cellular pharmacodynamics and pharmacokinetics of antibiotics: Current views and perspectives. Curr. Opin. Drug Discov. Dev., 9:.: 218–230 (2006).

[22] I.K. Minichmayr, A. Schaeftlein, J.L. Kuti, *et al.* Clinical Determinants of Target Non-Attainment of Linezolid in Plasma and Interstitial Space Fluid: A Pooled Population Pharmacokinetic Analysis with Focus on Critically Ill Patients. Clin. Pharmacokinet., 56:.: 617–633 (2017).

[23] P. Matzneller, Z. Österreicher, B. Reiter, *et al.* Tissue pharmacokinetics of telavancin in healthy volunteers: A microdialysis study. J. Antimicrob. Chemother., 71:.: 3179–3184 (2016).

[24] S.T. Housman, A.A. Bhalodi, A. Shepard, *et al.* Vancomycin tissue pharmacokinetics in patients with lower-limb infections via *in vivo* microdialysis. J. Am. Podiatr. Med. Assoc., 105:.: 381–388 (2015).

[25] D. Frasca, C. Dahyot-Fizelier, C. Adier, *et al.* Metronidazole and hydroxymetronidazole central nervous system distribution: 1. Microdialysis assessment of brain extracellular fluid concentrations in patients with acute brain injury. Antimicrob. Agents Chemother., 58:.: 1019–1023 (2014).

[26] J. Karjagin, S. Lefeuvre, K. Oselin, *et al.* Pharmacokinetics of meropenem determined by microdialysis in the peritoneal fluid of patients with severe peritonitis associated with septic shock. Clin. Pharmacol. Ther., 83:.: 452–459 (2008).

[27] A. Barbour, S. Schmidt, W.R. Rout, *et al.* Soft tissue penetration of cefuroxime determined by clinical microdialysis in morbidly obese patients undergoing abdominal surgery. Int. J. Antimicrob. Agents, 34:.: 231–235 (2009).

[28] J. Mand'ák, M. Pojar, J. Maláková, *et al.* Tissue and plasma concentrations of cephuroxime during cardiac surgery in cardiopulmonary bypass - A microdialysis study. Perfusion, 22:.: 129–136 (2007).

[29] F. Tomaselli, A. Maier, V. Matzi, *et al.* Penetration of meropenem into pneumonic human lung tissue as measured by *in vivo* microdialysis. Antimicrob. Agents Chemother., 48:.: 2228–2232 (2004).

[30] J. Karjagin, R. Pähkla, J. Starkopf. Perioperative penetration of metronidazole into muscle tissue: A microdialysis study. Eur. J. Clin. Pharmacol., 59:.: 809–813 (2004).

[31] C. Joukhadar, N. Klein, P. Dittrich, *et al.* Target site penetration of fosfomycin in critically ill patients. J. Antimicrob. Chemother., 51:.: 1247–1252 (2003).

[32] Committee for Human Medicinal Products (CHMP). Guideline on the use of pharmacokinetics and pharmacodynamics in the development of antibacterial medicinal products. (2015).

[33] D.P. Merke, S.R. Bornstein. Congenital Adrenal Hyperplasia. Lancet, 365:.: 2125–2136 (2005).

[34] D. El-Maouche, W. Arlt, D.P. Merke, *et al.* Congenital Adrenal Hyperplasia. Lancet, 390:.: 2194–2210 (2017).

[35] P.C. White, P.W. Speiser. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev, 21:.: 245–291 (2000).

[36] P.W. Speiser, R. Azziz, L.S. Baskin, *et al.* Congenital Adrenal Hyperplasia Due to Steroid 21-Hydroxylase Deficiency : An Endocrine Society Clinical Practice Guideline. J. Clin. Endocrinol. Metab., 95:.: 4133–4160 (2010).

[37] L. Fleming, M. Van Riper, K. Knafl. Management of Childhood Congenital Adrenal Hyperplasia—An Integrative Review of the Literature. J. Pediatr. Heal. Care, 31:.: 560–577 (2017).

[38] E. Charmandari, D.R. Matthews, A. Johnston, *et al.* Serum cortisol and 17-hydroxyprogesterone interrelation in classic 21-hydroxylase deficiency: is current replacement therapy satisfactory? J. Clin. Endocrinol. Metab., 86:.: 4679–85 (2001).

[39] E. Kuriata, W. Sawicki. Evaluation of cases with the usage of commercially available tablets in the pediatric formula. Acta Pol. Pharm. - Drug Res., 72:.: 551–558 (2015).

[40] R.H. Richey, U.U. Shah, M. Peak, *et al.* Manipulation of drugs to achieve the required dose is intrinsic to paediatric practice but is not supported by guidelines or evidence. BMC Pediatr., 13:.: 81–89 (2013).

[41] J.P. Fawcett, D.W. Boulton, R. Jiang, *et al.* Stability of hydrocortisone oral suspensions prepared from tablets and powder. Ann. Pharmacother., 29:.: 987–990 (1995).

[42] F.Z. Stanczyk, C. Niu, C. Azen, *et al.* Determination of estradiol and progesterone content in capsules and creams from compounding pharmacies. Menopause, 26:.: 966–971 (2019).

[43] A. Heuermann, M. Heuermann. Qualität häufig unzureichend. Pharm. Ztg.,: epub (2015).

[44] J.E. Barillas, D. Eichner, R. Van Wagoner, *et al.* Iatrogenic cushing syndrome in a child with congenital adrenal hyperplasia: Erroneous compounding of hydrocortisone. J. Clin. Endocrinol. Metab., 103:.: 7–11 (2018).

[45] Bundesvereinigung Deutscher Apothekerverbände. Kapseln. In: *Deutscher Arzneimittel Codex/ Neues Rezeptur Formularium*. : I.9 (2010).

[46] E. Grauls. Api loss during capsule preparation. (2013).

[47] European Pharmacopoeia (7th Edition). 2.9.5 Uniformity of mass of single-dose preparations. (2010).

[48] European Centre for Disease Prevention and Control (ECDC). Systematic review and evidence-based guidance on perioperative antibiotic prophylaxis. (2013).

[49] W.G. Cheadle. Current perspectives on antibiotic use in the treatment of surgical infections. Am. J. Surg., 164:.: 44S-47S (1992).

[50] J.L. Alfonso-Sanchez, I.M. Martinez, J.M. Martín-Moreno, *et al.* Analyzing the risk factors influencing surgical site infections: The site of environmental factors. Can. J. Surg., 60:.: 155–161 (2017).

[51] J. Ferrand, Y. El Samad, B. Brunschweiler, *et al.* Morbimortality in adult patients with septic arthritis: A three-year hospital-based study. BMC Infect. Dis., 16:.: 239–248 (2016).

[52] K.B. Kirkland, J.P. Briggs, S.L. Trivette, *et al.* The Impact of Surgical-Site Infections in the 1990s: Attributable Mortality, Excess Length of Hospitalization, And Extra Costs. Infect. Control Hosp. Epidemiol., 20:.: 725–730 (1999).

[53] B. Söderquist. Surgical site infections in cardiac surgery: microbiology. J. Pathol. Microbiol. Immunol., 115:.: 1008–1011 (2007).

[54] D. Jonkers, T. Elenbaas, P. Terporten, *et al.* Prevalence of 90-days postoperative wound infections after cardiac surgery. Eur. J. Cardio-thoracic Surg., 23:.: 97–102 (2003).

[55] Dachverband der wissenschaftlichen medizinischen Fachgesellschaften (Arbeitskreis Krankenhaus- und Praxishygiene). Perioperative Antibiotikaprophylaxe (PAP) : Allgemeine Prinzipien und der Sonderfall beim arthroskopischen Operieren. https://euprevent.eu/wp-content/uploads/2017/01/AWMF-Leitlinie-Perioperative-Antibiotikaprophylaxe.pdf. [accessed 2018 Oct 27]: 1–7 (2012).

[56] T. Chopra, J.J. Zhao, G. Alangaden, *et al.* Preventing surgical site infections after bariatric surgery: Value of perioperative antibiotic regimens. Expert Rev. Pharmacoeconomics Outcomes Res., 10:.: 317–328 (2010).

[57] S. Hagel, H. Scheuerlein. Perioperative antibiotic prophylaxis and antimicrobial therapy of intra-abdominal infections. Visz. Gastrointest. Med. Surg., 30:.: 310–316 (2014).

[58] J. Meehan, A.A. Jamali, H. Nguyen. Prophylactic antibiotics in hip and knee arthroplasty. J. Bone Jt. Surg. - Ser. A, 91:.: 2480–2490 (2009).

[59] Paul-Ehrlich-Gesellschaft für Chemotherapie e. V. Perioperative Antibiotika-Prophylaxe. Empfehlung einer Expertenkomission. Chemother J,: 70 – 84 (2010).

[60] J.L. Wynn. Defining neonatal sepsis. Curr. Opin. Pediatr., 28:.: 135–140 (2016).

[61] H. Dong, H. Cao, H. Zheng. Pathogenic bacteria distributions and drug resistance analysis in 96 cases of neonatal sepsis. BMC Pediatr., 17:.: 44–49 (2017).

[62] I. Mihatov Stefanovic. Neonatal sepsis. Biochem. Medica, 21:.: 276–281 (2011).

[63] D.C. De Souza, E.R. Barreira, L. Santana Faria. The epidemiology of sepsis in childhood. Shock, 47:.: 2–5 (2017).

[64] R.A. Polin, L.A. Papile, J.E. Baley, *et al.* Management of Neonates with Suspected or Proven Early-Onset Bacterial Sepsis. Pediatrics, 129:.: 1006–1015 (2012).

[65] F. Cortese, P. Scicchitano, M. Gesualdo, *et al.* Early and Late Infections in Newborns: Where Do We Stand? A Review. Pediatr. Neonatol., 57:.: 265–273 (2016).

[66] K. Allegaert, J. Van den Anker. Neonatal Drug Therapy: The First Frontier of Therapeutics for Children. Clin. Pharmacol. Ther., 98:.: 288–297 (2015).

[67] K. Allegaert, J. Van den Anker. Clinical pharmacology in neonates: small size, huge variability. Neonatology, 105:.: 344–349 (2014).

[68] S. Conroy, J. McIntyre, I. Choonara, *et al.* Drug trials in children: Problems and the way forward. Br. J. Clin. Pharmacol., 49:.: 93–97 (2000).

[69] I. Boots, R.N. Sukhai, R.H. Klein, *et al.* Stimulation programs for pediatric drug research - Do children really benefit? Eur. J. Pediatr., 166:.: 849–855 (2007).

[70] J. Wang, D. Avant, D. Green, *et al.* A Survey of Neonatal Pharmacokinetic and Pharmacodynamic Studies in Pediatric Drug Development. Clin. Pharmacol. Ther., 98:.: 328–335 (2015).

[71] J.D. Momper, Y. Mulugeta, G.J. Burckart. Failed Pediatric Drug Development Trials. Clin. Pharmacol. Ther., 98:.: 245–251 (2015).

[72] G.M. Pacifici, K. Allegaert. Clinical pharmacokinetics of vancomycin in the neonate: A review. Clinics, 67:.: 831–837 (2012).

[73] K. Allegaert, M. Van de Velde, J. Van den Anker. Neonatal clinical pharmacology. Paediatr Anaesth., 24:.: 30–44 (2014).

[74] V. Fanos, A. Dall'Agnola. Antibiotics in neonatal infections. Drugs, 58:.: 405–427 (1999).

[75] B.S. Moffett, J. Morris, C. Kam, *et al.* Vancomycin associated acute kidney injury in pediatric patients. PLoS One, 13:.: e0202439 (2018).

[76] J.A. Roberts, M.H. Abdul Aziz, J. Lipman, *et al.* Challenges and Potential Solutions- Individualised Antibiotic Dosing at the Bedside for Critically Ill Patients: a structured review. Lancet Infect. Dis., 14:.: 498–509 (2014).

[77] D. Dersch-Mills, T. Bengry, A. Akierman, *et al.* Assessment of initial vancomycin dosing in neonates. Paediatr. Child Heal., 19:.: e30–e34 (2014).

[78] F.S. Sinkeler, T.R. de Haan, C.J. Hodiamont, *et al.* Inadequate vancomycin therapy in term and preterm neonates: A retrospective analysis of trough serum concentrations in relation to minimal inhibitory concentrations. BMC Pediatr., 14:.: 193–199 (2014).

[79] R.M. de Castro Romanelli, L.M. Anchieta, J.C. Abreu Fernandes, *et al.* Serum levels of vancomycin: is there a prediction using doses in mg/kg/day or m2/day for neonates? Brazilian J. Infect. Dis., 20:.: 451–456 (2016).

[80] D. Hwang, N.-C. Chiu, L. Chang, *et al.* Vancomycin dosing and target attainment in children. J. Microbiol. Immunol. Infect., 50:.: 494–499 (2017).

[81] J.S. Goodwin, D. Atluru, S. Sierakowski, *et al.* Mechanism of action of glucocorticosteroids. Inhibition of T cell proliferation and interleukin 2 production by hydrocortisone is reversed by leukotriene B4. J. Clin. Invest., 77:.: 1244–1250 (1986).

[82] Mibe GmbH Arzneimittel. Fachinformation Hydrocortison 10 mg Jenapharm Tabletten. (2017).

[83] E. Charmandari, A. Johnston, C.G.D. Brook, *et al.* Bioavailability of oral hydrocortisone in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J. Endocrinol., 169:.: 65–70 (2001).

[84] A. Douglas, A.A. Udy, S.C. Wallis, *et al.* Plasma and tissue pharmacokinetics of cefazolin in patients undergoing elective and semielective abdominal aortic aneurysm open repair surgery. Antimicrob. Agents Chemother., 55:.: 5238–5242 (2011).

[85] Fresenius Kabi. Fachinformation Cephazolin Fresenius 1 g Pulver zur Herstellung einer Infusionslösung. (2018).

[86] S. Van Kralingen, M. Taks, J. Diepstraten, *et al.* Pharmacokinetics and protein binding of cefazolin in morbidly obese patients. Eur. J. Clin. Pharmacol., 67:.: 985–992 (2011).

[87] C.C. Lang, T.A. Moreland, P.G. Davey. Bioavailability of cefuroxime axetil: Comparison of standard and abbreviated methods. J. Antimicrob. Chemother., 25:.: 645–650 (1990).

[88] Fresenius Kabi. Fachinformation Cefuroxim Fresenius 1500 mg Pulver zur Herstellung einer Infusionslösung. (2018).

[89] R.D. Foord. Cefuroxime: human pharmacokinetics. Antimicrob. Agents Chemother., 9:.: 741–747 (1976).

[90] J.W.L. Nascimento, M.J. Carvalho Carmona, T.M. Varejão Strabelli, *et al.* Perioperative cefuroxime pharmacokinetics in cardiac surgery. Clinics, 62:.: 257–260 (2007).

[91] W.A. Craig. Pharmacokinetic/Pharmacodynamic Parameters: Rationale for Antibacterial Dosing of Mice and Men. Clin. Infect. Dis., 26:.: 1–10 (1998).

[92] E. Asín-Prieto, A. Soraluce, I.F. Trocóniz, *et al.* Population pharmacokinetic models for cefuroxime and metronidazole used in combination as prophylactic agents in colorectal surgery: Model-based evaluation of standard dosing regimens. Int. J. Antimicrob. Agents, 45:.: 504–511 (2015).

[93] E. Asín-Prieto, A. Rodríguez-Gascón, A. Isla. Applications of the pharmacokinetic/pharmacodynamic (PK/PD) analysis of antimicrobial agents. J. Infect. Chemother., 21:.: 319–329 (2015).

[94] Hospira Deutschland. Fachinformation: Meropenem Hospira 500 mg/- 1 g Pulver zur Herstellung einer Injektions- oder Infusionslösung. (2011).

[95] Riemser Pharma GmbH. Fachinformation Vancomycin “Lederle” 1000 mg. (2018).

[96] M. Rybak, B. Lomaestro, J.C. Rotschafer, *et al.* Therapeutic monitoring of vancomycin in adult patients: A consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. Am.

J. Heal. Pharm., 66:.: 82–98 (2009).

[97] M. Bue, M. Tøttrup, P. Hanberg, *et al.* Bone and subcutaneous adipose tissue pharmacokinetics of vancomycin in total knee replacement patients. Acta Orthop., 89:.: 95–100 (2018).

[98] E.I. Nielsen, O. Cars, L.E. Friberg. Pharmacokinetic/Pharmacodynamic (PK/PD) indices of antibiotics predicted by a semimechanistic PKPD model: A step toward model-based dose optimization. Antimicrob. Agents Chemother., 55:.: 4619–4630 (2011).

[99] C. Giuliano, K.K. Haase, R. Hall. Use of vancomycin pharmacokinetic-pharmacodynamic properties in the treatment of MRSA infections. Expert Rev. Anti. Infect. Ther., 8:.: 95–106 (2010).

[100] T. Bergan, O. Leinebo, T. Blom-Hagen, *et al.* Pharmacokinetics and bioavailability of metronidazole after tablets, suppositories and intravenous administration. Scand. J. Gastroenterol. Suppl., 19:.: 45–60 (1984).

[101] K.C. Lamp, C.D. Freeman, N.E. Klutman, *et al.* Pharmacokinetics and pharmacodynamics of the nitroimidazole antimicrobials. Clin. Pharmacokinet., 36:.: 353–373 (1999).

[102] S.M. Swaney, H. Aoki, M.C. Ganoza, *et al.* The oxazolidinone linezolid inhibits initiation of protein synthesis in bacteria. Antimicrob. Agents Chemother., 42:.: 3251–3255 (1998).

[103] Pfizer. Fachinformation Zyvoxid. (2017).

[104] K.S. Estes, H. Derendorf. Comparison of the pharmacokinetic properties of vancomycin, linezolid, tigecyclin, and daptomycin. Eur. J. Med. Res., 15:.: 533–543 (2010).

[105] C. Buerger, C. Joukhadar, M. Müller, *et al.* Development of a liquid chromatography method for the determination of linezolid and its application to *in vitro* and human microdialysis samples. J. Chromatogr. B Anal. Technol. Biomed. Life Sci., 796:.: 155–164 (2003).

[106] D.E. Wiskirchen, A. Shepard, J.L. Kuti, *et al.* Determination of tissue penetration and pharmacokinetics of linezolid in patients with diabetic foot infections using *in vivo* microdialysis. Antimicrob. Agents Chemother., 55:.: 4170–4175 (2011).

[107] R.B. Eslam, A. Burian, G. Vila, *et al.* Target site pharmacokinetics of linezolid after single and multiple doses in diabetic patients with soft tissue infection. J. Clin. Pharmacol., 54:.: 1058–1062 (2014).

[108] Ratiopharm. Fachinformation Tigecyclin-ratiopharm 50 mg Pulver zur Herstellung einer Infusionslösung. (2017).

[109] U. Ungerstedt, C. Pycock. Functional correlates of dopamine neurotransmission. Bull. Schweiz. Akad. Med. Wiss., 30:.: 44–55 (1974).

[110] H.H. Alsulimani, J. Kim, S.N. Sani. Microdialysis-directed intra-tumor pharmacokinetic modeling of methotrexate in mice and humans. J. Pharm. Pharm. Sci., 19:.: 239–251 (2016).

[111] C. Kirbs, C. Kloft. *In vitro* microdialysis recovery and delivery investigation of cytokines as prerequisite for potential biomarker profiling. Eur. J. Pharm. Sci., 57:.: 48–59 (2014).

[112] C. Joukhadar, H. Derendorf, M. Müller. Microdialysis: A novel tool for clinical studies of anti-infective agents. Eur. J. Clin. Pharmacol., 57:.: 211–219 (2001).

[113] F. Simmel, C. Kloft. Microdialysis feasibility investigations with the non-hydrophilic antifungal voriconazole for potential applications in nonclinical and clinical settings. Int. J. Clin. Pharmacol. Ther., 48:.: 695–704 (2010).

[114] M. Brunner, H. Derendorf, M. Müller. Microdialysis for *in vivo* pharmacokinetic/pharmacodynamic characterization of anti-infective drugs. Curr. Opin. Pharmacol., 5:.: 495–499 (2005).

[115] M. Lindberger, T. Tomson, L. Ståhle. Microdialysis sampling of carbamazepine, phenytoin and phenobarbital in subcutaneous extracellular fluid and subdural cerebrospinal fluid in humans: An *in vitro* and *in vivo* study of adsorption to the sampling device. Pharmacol. Toxicol., 91:.: 158–165 (2002).

[116] M.C. Pigatto, B. Verlindo de Araujo, B.G. Silva Torres, *et al.* Population Pharmacokinetic Modeling of Etoposide Free Concentrations in Solid Tumor. Pharm. Res., 33:.: 1657–1670 (2016).

[117] M. Gynther, T.M. Kääriäinen, J.J. Hakkarainen, *et al.* Brain pharmacokinetics of ganciclovir in rats with orthotopic BT4C glioma. Drug Metab. Dispos., 43:.: 140–146 (2015).

[118] P. Arora, C. Huff Adams, G. Gudelsky, *et al.* Plasma and brain pharmacokinetics of letrozole and drug interaction studies with temozolomide in NOD-scid gamma mice and sprague dawley rats. Cancer Chemother. Pharmacol., 83:.: 81–89 (2019).

[119] J.A. Stenken, C.E. Lunte, M.Z. Southard, *et al.* Factors that influence microdialysis recovery. Comparison of experimental and theoretical microdialysis recoveries in rat liver. J. Pharm. Sci., 86:.: 958–966 (1997).

[120] E.C.M. De Lange, A.G. De Boer, D.D. Breimer. Methodological issues in microdialysis sampling for pharmacokinetic studies. Adv. Drug Deliv. Rev., 45:.: 125–148 (2000).

[121] J.P. Galea, P.J. Tyrrell, H.P. Patel, *et al.* Pitfalls in microdialysis methodology: An *in vitro* analysis of temperature, pressure and catheter use. Physiol. Meas., 35:.: N21–N28 (2014).

[122] N. Plock, C. Kloft. Microdialysis - Theoretical background and recent implementation in applied life-sciences. Eur. J. Pharm. Sci., 25:.: 1–24 (2005).

[123] S.H. MacVane, S.T. Housman, D.P. Nicolau. *In vitro* microdialysis membrane efficiency of broad-spectrum antibiotics in combination and alone. Clin. Pharmacol. Adv. Appl., 6:.: 97–101 (2014).

[124] F. Simmel, C. Kirbs, Z. Erdogan, *et al.* Pilot investigation on long-term subcutaneous microdialysis: Proof of principle in humans. AAPS J., 15:.: 95–103 (2013).

[125] M.I. Davies, J.D. Cooper, S.S. Desmond, *et al.* Analytical considerations for microdialysis sampling. Adv. Drug Deliv. Rev., 45:.: 169–188 (2000).

[126] O. Schwalbe, C. Buerger, N. Plock, *et al.* Urea as an endogenous surrogate in human microdialysis to determine relative recovery of drugs: Analytics and applications. J. Pharm. Biomed. Anal., 41:.: 233–239 (2006).

[127] C.S. Chaurasia. *In vivo* microdialysis sampling: Theory and applications. Biomed. Chromatogr., 13:.: 317–332 (1999).

[128] L. Ståhle, P. Arner, U. Ungerstedt. Drug Distribution Studies With Microdialysis III: Extracellular concentration of caffeine in adipose tissue in man. Life Sci., 49:.: 1853–1858 (1991).

[129] K.L. Snyder, C.E. Nathan, A. Yee, *et al.* Diffusion and calibration properties of microdialysis sampling membranes in biological media. Analyst, 126:.: 1261–1268 (2001).

[130] L. Melgaard, K.J. Hersini, P. Gazerani, *et al.* Retrodialysis: A review of experimental and clinical applications of reverse microdialysis in the skin. Skin Pharmacol. Physiol., 26:.: 160–174 (2013).

[131] W.F. Elmquist, R.J. Sawchuk. Application of Microdialysis in Pharmacokinetic Studies. Pharm. Res., 14:.: 267–288 (1997).

[132] J.W. Mouton, U. Theuretzbacher, W.A. Craig, *et al.* Tissue concentrations: do we ever learn? J. Antimicrob. Chemother., 61:.: 235–237 (2008).

[133] G.F. Clough. Microdialysis of large Molecules. 7:.: E686–E692 (2005).

[134] F. Traunmüller, I. Steiner, M. Zeitlinger, *et al.* Development of a high-performance liquid

chromatography method for the determination of caspofungin with amperometric detection and its application to *in vitro* microdialysis experiments. J. Chromatogr. B, 843:.: 542–46 (2006).

[135] Y. Zhou, J.-M. Wong, O.S. Mabrouk, *et al.* Reducing Multifunctional Adsorption to Improve Recovery and *In Vivo* Detection of Neuropeptides by Microdialysis with LC-MS. Anal. Chem., 87:.: 9802–9809 (2015).

[136] F.B. Mayr, S. Yende, D.C. Angus. Epidemiology of severe sepsis. Virulence, 5:.: 4–11 (2014).

[137] M.A. Zeitlinger, P. Dehghanyar, B.X. Mayer, *et al.* Relevance of Soft-Tissue Penetration by Levofloxacin for Target Site Bacterial Killing in Patients with Sepsis. Antimicrob. Agents Chemother., 47:.: 3548–3553 (2003).

[138] N. Plock. Target Site Pharmacokinetics of Antiinfectives in the Treatment of Serious Gram-positive Infections. (2006).

[139] I. Vasileiadis, M. Kompoti, N. Rovina, *et al.* Buffering Capacity in Sepsis: A Prospective Cohort Study in Critically Ill Patients. J. Clin. Med., 8:.: 1759 (2019).

[140] K. Ganesh, R. Sharma, J. Varghese, *et al.* A profile of metabolic acidosis in patients with sepsis in an Intensive Care Unit setting. Int. J. Crit. Illn. Inj. Sci., 6:.: 178–181 (2016).

[141] European Medicines Agency. Guideline on bioanalytical method validation. (2012).

[142] Bundesvereinigung Deutscher Apothekerverbände. Kapselhüllen. In: *Deutscher Arzneimittel Codex/ Neues Rezeptur Formularium*. : K145 (2011).

[143] Zentrallaboratorium Deutscher Apotheker. Kapselherstellung in der Apotheke Wirkstoffzuschlag. https://www.zentrallabor.com/pdf/ZL-Wirkstoffzuschlag.pdf. [accessed 2018 Oct 29] (2017).

[144] A. Kuznetsova, P.B. Brockhoff, R.H.B. Christensen. Tests in Linear Mixed Effects Models. J. Stat. Softw., 82:.: 1–26 (2017).

[145] R Foundation for Statistical Computing. R: A language and environment for statistical computing. : 2017 (2017).

[146] D. Burau, D. Petroff, P. Simon, *et al.* Drug combinations and impact of experimental conditions on relative recovery in *in vitro* microdialysis investigations. Eur. J. Pharm. Sci., 127:.: 252–260 (2019).

[147] Food and drug administration. Review memorandum: Substantial Equivalence determination, Decision summary. https://www.accessdata.fda.gov/cdrh_docs/reviews/K152245.pdf. [accessed 2019 Sep 10] (2012).

[148] N. Plock, C. Buerger, C. Kloft. Successful management of discovered pH dependence in vancomycin recovery studies: Novel HPLC method for microdialysis and plasma samples. Biomed. Chromatogr., 19:.: 237–244 (2005).

[149] D. Farin, G.A. Piva, I. Gozlan, *et al.* A modified HPLC method for the determination of vancomycin in plasma and tissues and comparison to FPIA (TDX). J. Pharm. Biomed. Anal., 18:.: 367–372 (1998).

[150] K.H. Abu-Shandi. Determination of vancomycin in human plasma using high-performance liquid chromatography with fluorescence detection. Anal. Bioanal. Chem., 395:.: 527–532 (2009).

[151] R. Schwameis, S. Syré, K. Sarahrudi, *et al.* Penetration of linezolid into synovial fluid and muscle tissue after elective arthroscopy. J. Antimicrob. Chemother., 72:.: 2817–2822 (2017).

[152] R. Schwameis, S. Syré, D. Marhofer, *et al.* Pharmacokinetics of cefuroxime in synovial fluid. Antimicrob. Agents Chemother., 61:.: e00992–e009917 (2017).

[153] D. Bertholee, P.G.J. Ter Horst, M.L. Hijmering, *et al.* Blood concentrations of cefuroxime in

cardiopulmonary bypass surgery. Int. J. Clin. Pharm., 35:.: 798–804 (2013).

[154] A. Hosmann, L.C. Ritscher, H. Burgmann, *et al.* Concentrations of cefuroxime in brain tissue of neurointensive care patients. Antimicrob. Agents Chemother., 62:.: e02164-17 (2018).

[155] M. Carlier, V. Stove, J.J. De Waele, *et al.* Ultrafast quantification of β-lactam antibiotics in human plasma using UPLC-MS/MS. J. Chromatogr. B Anal. Technol. Biomed. Life Sci., 978–979:.: 89–94 (2015).

[156] L.M. Boak, J. Li, R.L. Nation, *et al.* High-performance liquid chromatographic method for simple and rapid determination of linezolid in human plasma. Biomed. Chromatogr., 20:.: 782–786 (2006).

[157] S. Swoboda, M. Ober, K. Anagnostakos, *et al.* A simple isocratic HPLC assay to determine linezolid concentrations in different biomatrices for *in vivo* and *in vitro* studies. Clin. Chem. Lab. Med., 45:.: 1019–1022 (2007).

[158] L. Baietto, A. D'Avolio, A. Ariaudo, *et al.* Development and validation of a new UPLC-PDA method to quantify linezolid in plasma and in dried plasma spots. J. Chromatogr. B Anal. Technol. Biomed. Life Sci., 936:.: 42–47 (2013).

[159] S. Barco, R. Bandettini, A. Maffia, *et al.* Quantification of piperacillin, tazobactam, meropenem, ceftazidime, and linezolid in human plasma by liquid chromatography/ tandem mass spectrometry. J. Chemother., 27:.: 343–347 (2015).

[160] V. Ferrone, M. Carlucci, R. Cotellese, *et al.* Development and validation of a fast micro-extraction by packed sorbent UHPLC-PDA method for the simultaneous determination of linezolid and ciprofloxacin in human plasma from patients with hospital-acquired pneumonia. Talanta, 164:.: 64–68 (2017).

[161] C. Buerger, N. Plock, P. Dehghanyar, *et al.* Pharmacokinetics of unbound linezolid in plasma and tissue interstitium of critically ill patients after multiple dosing using microdialysis. Antimicrob. Agents Chemother., 50:.: 2455–2463 (2006).

[162] C.C. Bulik, D.E. Wiskirchen, A. Shepard, *et al.* Tissue penetration and pharmacokinetics of tigecycline in diabetic patients with chronic wound infections described by using *in vivo* microdialysis. Antimicrob. Agents Chemother., 54:.: 5209–5213 (2010).

[163] M.J.E. Brill, A.P.I. Houwink, S. Schmidt, *et al.* Reduced subcutaneous tissue distribution of cefazolin in morbidly obese versus non-obese patients determined using clinical microdialysis. J. Antimicrob. Chemother., 69:.: 715–723 (2014).

[164] Y. Hamada, J.L. Kuti, D.P. Nicolau. Vancomycin serum concentrations do not adequately predict tissue exposure in diabetic patients with mild to moderate limb infections. J. Antimicrob. Chemother., 70:.: 2064–2067 (2015).

[165] S.S. Tyvold, T. Dahl, S. Dragsund, *et al.* Bronchial microdialysis monitoring of inflammatory response in open abdominal aortic aneurysm repair; an observational study. Physiol. Rep., 5:.: e13348 (2017).

[166] M.J. O'Neil. The Merck Index - An Encyclopedia of Chemicals, drugs, and Biologicals. Whitehouse Station, 13th ed. (2001).

[167] B.E.O. Markman, P.C.P. Rosa, M.R.W. Koschtschak. Assessment of the quality of simvastatin capsules from compounding pharmacies. Rev. Saude Publica, 44:.: 1055–1062 (2010).

[168] M. Georgarakis, P. Hatzipantou, J.E. Kountourelis. Effect of particle size, content in lubricant, mixing time and storage relative humidity on drug release from hard gelatin ampicillin capsules. Drug Dev. Ind. Pharm., 14:.: 915–923 (1988).

[169] K.D. Capehart. A laboratory exercise in capsule making. Am. J. Pharm. Educ., 72:. (2008).

[170] R. Voigt. Pharmazeutische Technologie für Studium und Beruf 10th Edition. (2006).

[171] L. Barnscheid, J. Kakkanattuparambil, H. Yildirim, *et al.* Kapsel-Herstellung auf dem Prüfstand. Pharm. Ztg., 51:.: 2–5 (2006).

[172] M. D'Hondt, E. Wynendaele, K. Vandercruyssen, *et al.* Investigation of active pharmaceutical ingredient loss in pharmaceutical compounding of capsules. J. Pharm. Biomed. Anal., 96:.: 68–76 (2014).

[173] M. Sivén, S. Kovanen, O. Siirola, *et al.* Challenge of paediatric compounding to solid dosage forms sachets and hard capsules – Finnish perspective. J. Pharm. Pharmacol., 69:.: 593–602 (2017).

[174] European Medicines Agency. Assessment Report Alkindi. https://www.ema.europa.eu/en/medicines/human/EPAR/alkindi. [accessed 2019 May 18] (2017).

[175] S. Marchand, A. Chauzy, C. Dahyot-Fizelier, *et al.* Microdialysis as a way to measure antibiotics concentration in tissues. Pharmacol. Res., 111:.: 201–207 (2016).

[176] M.B. Goetz, J. Sayers. Nephrotoxicity of vancomycin and aminoglycoside therapy separately and in combination. J. Antimicrob. Chemother., 32:.: 325–334 (1993).

[177] K. Risby, M.B. Ellebak, M.S. Jakobsen, *et al.* Intraperitoneal microdialysis in the postoperative surveillance of infants undergoing surgery for congenital abdominal wall defect: a pilot study. J. Pediatr. Surg., 50:.: 1676–1680 (2015).

[178] M.E. Pedersen, M. Dahl, N. Qvist. Intraperitoneal microdialysis in the postoperative surveillance after surgery for necrotizing enterocolitis: A preliminary report. J. Pediatr. Surg., 46:.: 352–356 (2011).

[179] A. Hack, V. Busch, K. Gempel, *et al.* Subcutaneous microdialysis for children - Safe biochemical tissue monitoring based on a minimal traumatizing no touch insertion technique. Eur. J. Med. Res., 10:.: 419–425 (2005).

[180] S. Beck, S.G. Wicha, C. Kloft, *et al.* Pharmakokinetik und Pharmakodynamik der Antibiotikatherapie. Anaesthesist, 63:.: 775–782 (2014).

[181] A. Schaeftlein, I.K. Minichmayr, C. Kloft. Population pharmacokinetics meets microdialysis: Benefits, pitfalls and necessities of new analysis approaches for human microdialysis data. Eur. J. Pharm. Sci., 57:.: 68–73 (2014).

[182] T.M. Jackanicz. Levonorgestrel and estradiol release from an improved contraceptive vaginal ring. Contraception, 24:.: 323–339 (1981).

[183] S. Klein, A.E. James, M.M. Tuckerman. Spectrophotometric Assay for Combinations of Ethinyl Estradiol and Methyltestosterone. J. Am. Pharm Assoc., 49:.: 314–316 (1960).

[184] T. Gee, R. Ellis, G. Marshall, *et al.* Pharmacokinetics and tissue penetration of linezolid following multiple oral doses. Antimicrob. Agents Chemother., 45:.: 1843–1846 (2001).

[185] K. Skhirtladze-Dworschak, D. Hutschala, G. Reining, *et al.* Cefuroxime plasma and tissue concentrations in patients undergoing elective cardiac surgery: Continuous vs bolus application. A pilot study. Br. J. Clin. Pharmacol., 85:.: 818–826 (2019).

[186] A.A. Bhalodi, S.T. Housman, A. Shepard, *et al.* Tissue pharmacokinetics of cefazolin in patients with lower limb infections. Antimicrob. Agents Chemother., 57:.: 5679–5683 (2013).

[187] J.M. Varghese, P. Jarrett, R.J. Boots, *et al.* Pharmacokinetics of piperacillin and tazobactam in plasma and subcutaneous interstitial fluid in critically ill patients receiving continuous venovenous haemodiafiltration. Int. J. Antimicrob. Agents, 43:.: 343–348 (2014).

[188] P. Dehghanyar, C. Buerger, M. Zeitlinger, *et al.* Penetration of linezolid into soft tissues of

healthy volunteers after single and multiple doses. Antimicrob. Agents Chemother., 49:.: 2367–2371 (2005).

8 Publications

Original papers

D. Burau, D. Petroff, P. Simon, L. Ehmann, C. Weiser, C. Dorn, A. Kratzer, H. Wrigge, C. Kloft.
Drug combinations and impact of experimental conditions on relative recovery in in vitro microdialysis investigations.
Eur J Pharm Sci. 127: 252-260 (2019)
doi: 10.1016/j.ejps.2018.10.030

R. Schwameis, S. Syré, D. Marhofer, A. Appelt, D. Burau, K. Sarahrudi, C. Kloft, M. Zeitlinger.
Pharmacokinetics of cefuroxime in synovial fluid.
Antimicrob. Agents Chemother. 61: e00992-17 (2017).
doi: 10.1128/AAC.00992-17

R. Schwameis, S. Syré, K. Sarahrudi, A. Appelt, D. Marhofer, D. Burau, C. Kloft, M. Zeitlinger.
Penetration of linezolid into synovial and muscle tissue after elective arthroscopy.
J. Antimicrob. Chemother. 72: 2817-2822 (2017).
doi: 10.1093/jac/dkx219

U. Neumann, D. Burau, S. Spielmann, M.J. Whitaker, R.J. Ross, C. Kloft, O. Blankenstein.
Quality of compounded hydrocortisone capsules used in the treatment of children.
Eur. J. Endocrinol. 177: 239-242 (2017).
doi: 10.1530/EJE-17-0248

Presentations

D. Burau
Ein Arzneimittel- unterschiedliche Wirkung. Welche Rolle hat das Geschlecht auf die Wirkung von Arzneimitteln?
Lange Nacht der Wissenschaften, Berlin, Deutschland, 11 June 2016.

D. Burau, S. Schröpf
Microdialysis for therapeutic drug monitoring in infants.
8th International Symposium on Microdialysis, Uppsala, Schweden, 25-27 May 2016.

D. Burau, C. Weiser, C. Kloft.
Erreichen Patienten effektive Konzentrationen von Antiinfektiva am Wirkort? Ein multidisziplinärer Beitrag zur Arzneimitteltherapiesicherheit bei Infektionskrankheiten.
Jahrestagung der Deutschen Gesellschaft für Klinische Pharmazie (DGKlinPha), Leipzig, Deutschland, 14-15 November 2015.

D. Kauzor
Are paediatric CAH patients sufficiently treated with individually compounded capsules?.
6th Conference of the European Paediatric Formulation Initiative (EUPFI), Athen, Griechenland, 18 September 2014.

Conference abstracts

F. Kluwe, M. Ansin, R. Schwameis, D. Burau, W. Huisinga, M. Zeitlinger, C. Kloft.

Joint model for the characterisation of cefuroxime pharmacokinetics in synovial fluid, interstitial fluid of muscle tissue and plasma.

27th Population Approach Group Europe (PAGE), Montreux, Schweiz, 29 May-01 June 2018.

PAGE 27: 8668 [www.page-meeting.org/default.asp?abstract=8668], (2018).

L. Ehmann, P. Simon, D. Petroff, I.K. Minichmayr, D. Burau, M. Zeitlinger, C. Dorn, W. Huisinga, H. Wrigge, C. Kloft.

Is a pooled population pharmacokinetic model predictive of plasma and microdialysate pharmacokinetics of linezolid in obese and non-obese patients?

26th Population Approach Group Europe (PAGE), Budapest, Ungarn, 06-09 June 2017.

PAGE 26: 7322 [www.page-meeting.org/default.asp?abstract=7322], (2017).

D. Burau, P. Simon, L. Ehmann, D. Petroff, H. Wrigge, C. Kloft.

Is relative recovery influenced by drug combinations.

8th International Symposium on Microdialysis, Uppsala, Schweden, 25-27 Mai 2016.

P. Simon, D. Petroff, D. Burau, L. Ehmann, C. Nestler, A. Dietrich, A.-W. Reske, C. Kloft, M. Zeitlinger, F. Kees, H. Wrigge.

Agreement on double measurements when determining soft tissue concentrations of linezolid in normal weight and morbidly obese patients by microdialysis.

8th International Symposium on Microdialysis, Uppsala, Schweden, 25-27 Mai 2015.

D. Burau, C. Weiser, C. Kloft.

Erreichen Patienten effektive Konzentrationen von Antiinfektiva am Wirkort? Ein multidisziplinärer Beitrag zur Arzneimitteltherapiesicherheit bei Infektionskrankheiten.

Jahrestagung der Deutschen Gesellschaft für Klinische Pharmazie (DGKlinPha), Leipzig, Deutschland, 14-15 November 2015.

D. Burau, M.-A. Fürtig, S. Schröpf, C. Kloft.

Optimisation of microdialysis of vancomycin in neonates by in vitro experiments.
DPhG Jahrestreffen Düsseldorf, Deutschland, 23-25 September 2015.

M.-A. Fürtig, D. Burau, C. Kloft.

Development of an LC-MS/MS method to investigate the pharmacokinetics of antibiotics

and concomitant drugs given to neonates suffering from sepsis.

DPhG Jahrestreffen Düsseldorf, Düsseldorf, Deutschland, 23-25 September 2015.

R. Schwameis, P. Matzneller, Z. Oesterreicher, A. Appelt, D. Burau, C. Kloft, M. Zeitlinger.

Synovial fluid concentrations of cefuroxime and linezolid after surgical interventions.

25th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Kopenhagen, Dänemark, 25-28 April 2015.

www.escmid.org/escmid_library/online_lecture_library/material/?mid=23649, (2015).

Z. Oesterreicher, M. Zeitlinger, D. Burau, C. Kloft, R. Schwameis.

Plasma und Synovialflüssigkeit Pharmakokinetik von Cefuroxim und Linezolid in Patienten mit elektiver Kniearthroskopie nach intravenöser single dose Applikation- eine explorative Studie.

9. Österreichischer Infektionskongress, Saalfelden, Österreich, 15-18 April 2015.

[http://www.oeginfekt.at/download/9-oeik_programm_5.pdf]

C. Weiser, D. Kauzor; H. Brosig, F. Kees, M. Zeitlinger, C. Kloft.

HPLC method development and validation for the determination of anidulafungin in microdialysis samples of healthy volunteers and intensive care patients.

DPhG Jahrestreffen, Frankfurt, Deutschland, 24-26 September 2014.

Abstractbook, 148-149, (2014).

A.K. Appelt, D. Kauzor, S.G. Wicha, H. Brosig, M. Zeitlinger, C. Kloft

HPLC method development for the determination of linezolid and cefuroxime in synovial fluid site microdialysis samples of arthritis patients.

DPhG Jahrestreffen, Frankfurt, Deutschland, 24-26 September 2014.

Abstractbook, 170, (2014).

D. Kauzor, S. Schröpf, M. Fürtig, D. Ruhe, C. Kloft.

In vitro microdialysis characterising vancomycin as precondition for an upcoming trial in neonates.

DPhG Jahrestreffen, Frankfurt, Deutschland, 24-26 September 2014.

Abstractbook, 149-150.

D. Kauzor, S. Spielmann, H. Brosig, O. Blankenstein, C. Kloft.

Medication safety study investigating hydrocortisone in individually compounded capsules for paediatric use in congenital adrenal hyperplasia

6th Conference of the European Paediatric Formulation Initiative (EUPFI), Athen, Griechenland, 17-18 September 2014

D. Kauzor, S. Schröpf, C. Kloft.

In vitro characterisation of vancomycin as a prerequisite for a microdialysis study in neonates.

24th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Barcelona, Spanien, 10-13 May 2014.

D. Kauzor, S.Spielmann, C. Kloft, O.Blankenstein

Medication safety study investigating hydrocortisone individually and extemporaneously compounded capsules for paediatric use in congenital adrenal hyperplasia .
European Conference of Endocrinology (ECE), Breslau, Polen, 3-7 May 2014.
(2014).

D. Kauzor, O. Blankenstein, S. Spielmann, C. Kloft

HPLC method development for the determination of hydrocortisone from capsules contributing to a drug medication safety study.

DPhG Jahrestreffen, Freiburg, Deutschland,09.-11.10.2013.

Tagungsband S. 154, (2013).

9 Curriculum Vitae

Der Lebenslauf ist aus Datenschutzgründen in der Online-Version nicht enthalten.

10 Acknowledgements

Zuallererst und vor allem möchte ich meiner Familie danken: meinem Opa Klaus, der sich immer sicher war, dass ich klug und neugierig genug bin um alles zu schaffen und zu erforschen was ich mir in den Kopf setze und mich von klein auf bestärkt hat alles zu hinterfragen und nichts als gegeben hinzunehmen. Ich wünschte, du könntest das Ergebnis jetzt sehen.

Ich danke meiner Mama, die immer gekämpft hat um das Beste für mich zu erreichen, gegen alle Widerstände. Das hätten nicht viele geschafft und ich bin dir jeden Tag dafür dankbar. Zum Glück habe ich deinen Ergeiz und deine Sturheit geerbt... Ich danke meinem Bruder und meinem Stiefpapa, die immer für Unterstützung und Ablenkung zur Verfügung stehen. Schön, dass es euch gibt.

Natürlich hätte ich diese Arbeit niemals ohne meinen geliebten Mann und meine wundervollen Söhne zustande bekommen. Stephan, nicht nur, dass du mir unglaublich viel Kraft und Liebe gegeben hast, du hast mich auch mit leckerem Essen und schönen Feierabenden zum Durchhalten gebracht und mir über diverse Nervenzusammenbrüche und Motivationskrisen geholfen. Danke Paul für die schöne Kuschel- und Bastelzeit, die der perfekte Ausgleich zum wissenschaftlichen Arbeiten ist. Bald mehr Zeit mit euch zu verbringen, ist die beste Motivation, diese Arbeit zu beenden, die ich mir denken kann.

Ich möchte mich natürlich auch bei meiner Doktormutter, Frau Prof. Kloft für die Möglichkeit bedanken an derart vielfältigen und relevanten Forschungsfragen arbeiten zu können. Ich habe in der Promotionszeit viel gelernt, über wissenschaftliches Arbeiten und Lehre aber auch über die Zusammenarbeit mit Menschen und anderen Professionen. Auch habe ich in dieser Zeit viel über mich, meine Stärken und Grenzen gelernt.

Danke an den Arbeitskreis für viele, tolle Mittagspausen, Meetings und die ein oder andere Feier. Ich habe viel von euch gelernt und unsere interkulturellen Diskussionen sehr genossen. Danke v.a. an Helena, Johanna und Zinnia für lange Gespräche über Gleichberechtigung und Lebensrealitäten. Ihr habt meinen Horizont sehr erweitert. Danken möchte ich auch Andrea und Lisa, die auch jetzt noch immer da sind und ein offenes Ohr haben, wenn ich zweifle oder nicht weiß, wie ich ein Problem angehen soll. Ohne euch wäre ich nicht fertig geworden! Und Andrea, für deine Unterstützung hättest du Mittag essen für die nächsten 20 Jahre verdient! Danke auch an Claudia und Christine, die Mikrodialyse-Tanten, für ewige Fachdiskussionen und 90er Jahre Musik im Labor sowie an Sebastian, Eva und Johanna für die analytischen Diskussionen. Ihr habt mir immer gute Denkansätze gegeben und mir geholfen mich nicht allzu sehr zu verzetteln.

Ohne die Unterstützung im Labor von Dr. Holger Brosig, Tania Fuhrmann-Selter und Dr. Werner Herrmann sowie meine beiden DiplomandInnen Ann-Katrin Appelt und Marc Alexander Fürtig wäre es nicht möglich gewesen, an so vielfältigen Studien arbeiten zu können. Ich danke euch für die unermüdliche Hilfe und die tollen Gespräche im Labor, die mir so viele andere Ansichten nähergebracht haben.

Meine Forschungsprojekte wären ohne engagierte Kooperationspartner nicht möglich gewesen. Daher ein großes „Danke!“ an Sebastian Schröpf, Markus Zeitlinger und seine Arbeitsgruppe in Wien, Dr. Blankenstein und seine Mitarbeiter an der Charité Berlin, Prof. Herrmann Wrigge und Dr. Philipp Simon vom Universitätsklinikum Leipzig sowie Prof. Kees und Dr. Christoph Dorn von der Universität Regensburg.

Vielen Dank an Andrea, Lisa, Franzi, Christine und Dr. Ingo Siebenbrodt für die Erstkorrektur meiner Dissertation, die mir viele wertvolle Hinweise gebracht hat.

Acknowledgements

Ich möchte mich auch bei meinen Studierenden und PraktikantInnen bedanken. Über die Semester habe ich viele Ideen mit euch ausprobiert und immer wieder wertvolles Feedback bekommen. Ich hatte viel Freude daran, euch mein Wissen näher zu bringen und hoffe, dass ihr es gut einsetzen könnt.