Closing gaps in the Y-site compatibility data of different antibiotics and analgetics with Ringer's acetate

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Summary

Ringer's acetate is a balanced crystalloid solution that is used for volume replacement at the Kantonsspital Baden (KSB). Compared to NaCl 0.9% or dextrose 5%, it carries a lower risk of inducing metabolic or dilutional acidosis while effectively maintaining blood pH and potassium levels within physiological range. The frequent use of Ringer's acetate poses particular challenges to hospital pharmacists, as stability and compatibility data is not available for many intravenous drug products that are regularly co-infused with Ringer's acetate. This represents a significant risk to medication safety. In practice, the lack of data often necessitates the interruption and flushing of Ringer's acetate infusion, or the placement of an additional venous access. The Ringer's acetate compatibility list of the KSB hospital pharmacy is frequently consulted. The aim of this thesis was to address experimentally missing data on antibiotics and analgesics of the KSB compatibility list. This included the assessment of chemical stability of selected drug products in the Ringer's acetate solution using reversed-phase high performance liquid chromatography (RP-HPLC), as well as the occurrence of potential precipitates by examination of sub-visible particles in accordance with the European Pharmacopoeia (EP). The selected drug substances encompassed piperacillin and tazobactam (Sandoz), benzylpenicillin (Penicillin "Grünenthal"®), meropenem (Meronem®) and ketorolac (Tora-dol®). Morphine was initially tested but ultimately excluded due to poor separation on the HPLC column. A multianalyte RP-HPLC-UV/VIS method was developed and validated in terms of linearity, accuracy, precision and stability. For piperacillin, tazobactam, and meropenem, the developed multianalyte method was successful. Ketorolac and benzylpenicillin followed a single-point calibration due to stability issues. To simulate co-infusion of drug product solution and Ringer's acetate via a three-way stopcock (henceforth referred to as Y-site), average and high therapeutic doses of each drug product were prepared according to the manufacturer's leaflet, mixed in equal parts with Ringer's acetate, and left at room temperature for 2.5 hours (24 hours for average-dose ketorolac). The concentrations of all drug substances remained above the limit of 95% relative to the time zero concentration, in accordance with the European Medicines Agency (EMA) guideline. The counting of sub-visible particles by light obscuration showed that all test solutions met the acceptance criteria of the EP. For solutions of maximum volume of 100 ml, the EP limits the number of particles greater than or equal to 10 µm in diameter to 6000, and that of particles greater than or equal to 25 µm to 600. Based on this data, the Y-site compatibility of Piperacillin/Tazobactam Sandoz®, Penicillin "Grünenthal"®, and Tora-dol® with Ringer's acetate was confirmed and integrated into the existing compatibility list of the KSB. Conversely, as Meronem® deviated by more than 10% from the nominal concentration, a conclusive statement could not be made despite meeting the specifications of the EMA and EP. Thus, until reliable data is available, Y-site co-infusion of Meronem® and Ringer's acetate should be avoided. The result of this work will support

doctors and nurses in establishing safe infusion regimes and thus, contribute to improved medication safety at the KSB. Furthermore, the presented analytical methods may serve other hospital pharmacies as a foundation for future compatibility studies.

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1. List of abbreviations

ACN ..... acetonitrile
D5 ..... dextrose 5%
DP ..... drug product
DS ..... drug substance
EMA ..... European Medicines Agency
EP ..... European Pharmacopoeia
FA ..... formic acid
ICU ..... Intensive Care Unit
KSB ..... Kantonsspital Baden
QC ..... quality control sample
RA ..... Ringer's acetate
RL ..... Ringer's lactate
RP-HPLC ..... reversed-phase high-performance liquid chromatography
USP ..... United States Pharmacopeia
WFI ..... water for injection

2. Introduction

2.1. Properties of Ringer's acetate

Ringer's acetate (RA) is a balanced cristalloid solution used as a fluid and electrolyte substitute in patients with normal acid-base balance or slight acidosis. Additionally, it serves as a short-term intravascular volume substitute in patients with isotonic or hypotonic dehydration (1). As with other balanced Ringer's solutions such as Ringer's lactated (RL), it is preferred over saline due to the electrolyte composition that is better adapted to the physiological electrolyte composition in the plasma (2, 3). Isotonic saline is markedly hyperchloremic, thus likely to induce metabolic acidosis in the human body (4). Additionally, the absence of bicarbonate may result in dilutional acidosis by lowering bicarbonate concentration in the plasma (5). In balanced crystalloid solution, the amount of chloride ions is reduced by the addition of organic anions such as acetate, malate, or lactate. Once in the body, these ions are metabolised to bicarbonate, which serves as a buffer. As such, it maintains blood pH at physiological levels and prevents dilutional acidosis (6). Potassium levels have been shown to remain stable with both lactated and acetated Ringer's infusions, even in patients with impaired kidney function (7, 8). Advantages and disadvantages of acetate versus lactate in balanced crystalloid solutions are widely discussed and part of ongoing research (9-11). Since lactate is mainly metabolised in the liver, it may accumulate in patients with impaired liver function and hinder the interpretation of lactate levels as a marker of tissue hypoxia (9). In such patients, RA may be the better option, as it is metabolised more widely, mostly in skeletal muscles, and only to a minor extent in the liver (12). On the other hand, the concern with RA lies in its potential cardiotoxic effects (13, 14). To date, the ideal balanced solution does not exist (11). In the KSB, RA is preferred.

2.2. Physico-chemical compatibility of drug products and carrier solutions

For hospital pharmacists, the main concern with both acetate- and lactate-buffered solutions is their compatibility with other intravenous drug products (DP). Incompatibility describes a physical or chemical reaction between two DPs, a DP and a solution or a DP and a material such as a polyvinyl infusion bag (15). Incompatibilities may visually be observed as precipitates, changes in colour, consistency, opalescence, or even as phase separation or gas development. Chemical reactions such as hydrolysis, oxidation, or reduction are usually not visible. Incompatibility often arises from alterations in the acidic or basic environment of the drug substances (DS) involved (16, 17). Nevertheless, also other reaction conditions necessitate consideration. For instance, dilution of a DP solution may lower the quantity of the co-solvent that may be required for stabilising a DS in aqueous solution, which may result in precipitation of the DS (18, 19). The stability of a dissolved DS may also depend on its concentration, as observed with trimethoprim/sulfamethoxazole (20). In the case of amoxicillin,

the stability within a solution is influenced by the carrier solution itself. Specifically, it shows higher stability in NaCl 0.9% than in dextrose 5% (D5). The underlying factor for this phenomenon has not been examined. One possible explanation may be the aforementioned difference in pH values, which lies between 3.5 and 5.5 in D5 and between 5 and 7 in NaCl 0.9% (21). Other antibiotics, such as tetracyclines, tend to precipitate in the presence of calcium and magnesium cations due to complex formation (18).

Calcium and magnesium are also present in RL and RA. An example of complex formation with calcium ions in RL has been observed with ceftriaxone sodium (22, 23). For this reason, the manufacturer of Rocephin® recommends avoiding the use of Ringer's solutions as a diluent (24). Ceftriaxon is not the only drug to be instable in Ringer's solutions. Other examples are remimazolam, ciprofloxacin, cyclosporine, diazepam, ketamine, lorazepam, phenytoin, and propofol (25, 26).

Data on compatible carrier solutions is typically available in the official product information provided by the manufacturer. It generally includes information on NaCl 0.9% and D5, and in some cases on RL and Ringer's solution. However, there is rarely specific information available concerning RA. This also applies to the two databases, Micromedex and Stabilis, which serve as essential references for hospital pharmacists when seeking compatibility and stability information (27, 28). Compatibility data of one type of Ringer's solution cannot not be extrapolated indiscriminately to another type, due to variations in electrolyte composition, electrolyte concentrations, and pH values, as detailed in the appendix (Table A1). In general, it is imperative to critically analyse the provided compatibility data, taking into account factors such as concentration, additives, composition, temperature, and the duration of contact (29). Apart from available hard data, good assumptions on compatibility may also be made from considering the chemical structure of the drugs involved. In particular, acid-base reactions and drug solubility may be predicted on the basis of the respective pKa values (determining the acid-base equilibrium) and the resulting pH of the solution (16). However, implementing such considerations within a daily clinical routine of a hospital pharmacist may prove difficult, as it is time-consuming and often unfeasible within the required timeframe. Nonetheless, a comparable analysis was conducted by the Kantonsspital Schaffhausen, as described in Chapter 2.5.

2.3. Consequences of incompatibilities

The consequences of an incompatibility can be hazardous for the patient. The occlusion or malfunction of venous catheters, reduced efficacy of drugs, embolism and inflammatory reactions are some of the consequences that have been reported (23, 30). The frequency of potential incompatibility reactions is poorly investigated. At the KSB, such incompatibilities have not been systematically documented to date. In an observational study conducted in a

German hospital, it was found that incompatible infusion regimens contributed to 25% of all errors that occurred during the preparation and administration of intravenous drugs (31). A similar study executed in a pediatric ICU revealed that 3.4% of drug-drug and drug-solvent combinations were incompatible, and for 10.3% of combinations no documented compatibility information was available (32). These findings are in line with a previous study, where 15% of medication errors in an ICU were attributed to the use of incompatible drug combinations (33). In none of those studies did the identified incompatibilities result in any discernible clinical consequences.

In cases where compatibility information is lacking, the patient's infusion regimen may be challenging, requiring additional and potentially more complex interventions. For example, a continuous infusion of a DP may need to be temporarily interrupted in order to rinse the infusion system with a neutral solution such as saline or dextrose, before initiating a short infusion of another DP. If this is not possible, a second venous access or even a multi-lumen central venous catheter may be required (34, 35). Such interventions inherently carry the risk of contamination with the human skin microbiome, which in turn can compromise the patient’s safety (36).

2.4. Analytical methods to examine compatibility

When investigating Y-site compatibilities between various DPs, solvents, and carrier solutions, established pharmacopoeial methods are commonly used. Such methods are designed to detect both visible changes, including turbidity, discoloration, and the release of gas, as well as non-visible changes such as that of pH. Specifically, the methods consist of visual examination (chapter 2.9.20 of the EP) (37-40), turbidimetry or nephelometry (chapter 2.2.1 of the EP) (41-43), light obscuration particle count (chapter 2.9.19 of the EP) (23, 25, 44, 45), pH assessment, and microscopy (37, 40, 46). In addition, RP-HPLC, in combination with UV/VIS detection, is frequently used for the assessment of chemical stability, in particular also to assess the compatibility of DS and additives, i.e., when mixing DPs or DPs with a carrier solution within the same infusion bag or syringe (39, 45, 47-49). General long-term stability tests are performed over hours or days, as most chemical reactions typically require prolonged contact time (36). According to the guidance provided by the EMA and outlined in the ICH Q1A document on long-term stability studies, the combination of DPs or a DP and carrier solution is considered unstable if the degradation of any of the components exceeds 5% (50).

Methods for studying compatibility of DPs vary considerably. To date, no standardized and validated methodology has been established for conducting compatibility studies within a clinical context, particularly regarding Y-site compatibility. Kanji et al. have proposed a list of quality indicators for guidance when studying compatibility (Table 1) (36).

Table 1. Quality indicators for pharmaceutical compatibility studies in hospital pharmacies, according to Kanji et al. (36), adapted.

• Description of study materials (drug substances, excipients, diluents; manufacturers, lot numbers) • Description of analytical methods (or reference to the methods) • Description of testing conditions (temperature, light, testing containers, duration of testing, number and frequency of observations, number of replicates)	
Assessing precipitation / insolubility	**Assessing chemical stability**
• Precipitate formation • Colour change • pH change over time • Gas development • Analysis at time zero and after at least one later time point	• Description or reference to validation of stability-indicating analytical method • Analysis at time zero and after at least one later time point

According to the ICH Q2 R1 guideline on validation of analytical procedures of the EMA, an analytical method to quantify an analyte in a given sample should be validated in terms of accuracy, precision, specificity, linearity and concentration range, for which the analyte shows a suitable level of precision, accuracy and linearity (51).

2.5. Presentation of compatibility data in a clinical setting

In hospital pharmacy settings, compatibility data is typically summarized in simplified charts that illustrate common drug-drug and drug-carrier combinations. Such combinations are categorized as either Y-site compatible, additive compatible, incompatible, or of unknown compatibility based on literature data (52). The selection of DPs for inclusion in such charts is based on their frequency of use within a particular hospital or hospital unit. However, a notable drawback of such an approach is the omission of critical specifications such as drug concentration, type of excipients, type of carrier solution, infusion duration, and infusion rate (29). Such factors might all have an influence on compatibility. An alternative system, which has been successfully implemented in the ICU of Kantonsspital Schaffhausen, involves a colour-coding system, where DSs are assigned different colours based on their physico-chemical properties. DSs of low pKa or with frequently reported incompatibilities with BLUE-coded DSs are coded with RED and must not be co-infused with BLUE-coded DSs. DSs of increased pKa or with frequently reported incompatibilities with RED-coded DSs are coded with BLUE and must not be co-infused with RED-coded DSs. YELLOW-coded DSs are stable

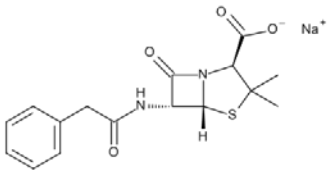
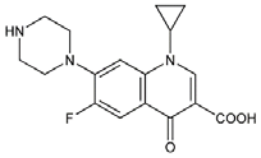
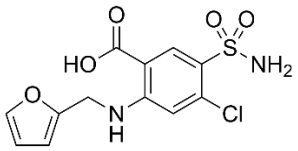
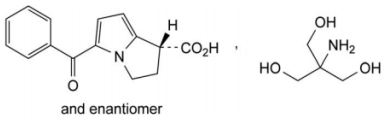
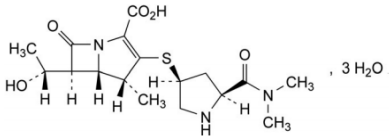
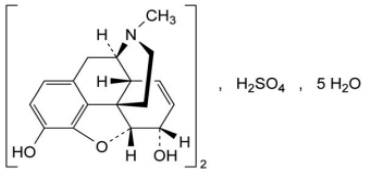
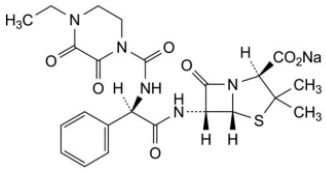
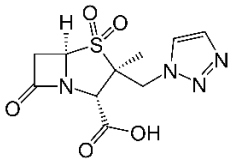
at neutral pH or are typically incompatible with each other. They can be co-administered with DSs from the red or blue group, but not with another YELLOW-coded DSs. Finally, BLACK-coded DSs are those with unknown and unpredictable compatibility and are not permitted to be co-infused with any other DS. This simplified yet comprehensible system has resulted in a sustained reduction of incompatibilities from 15% to 2% (53).

2.6. Goal and scope of the thesis

Appropriate postoperative fluid management is essential to maintain tissue perfusion and haemodynamic stability, particularly for patients undergoing major surgery. Although the superiority of balanced crystalloid solutions over NaCl 0.9% in terms of postoperative complications and in-hospital mortality has not been conclusively demonstrated, the surgical units at KSB exclusively use RA (54, 55). Post-surgery patients often receive intravenous analgesics and antibiotics (56, 57). The question of whether these classes of drugs are compatible with RA via the Y-site is therefore particularly common in surgical wards. Such uncertainties were confirmed in discussions with the head nurse of the surgical ward at KSB. The manufacturer of the RA solution used at KSB, Fresenius Kabi, has thoroughly tested the chemical stability and compatibility with various intravenous DPs (58, 59). The list is frequently accessed, as specified by an in-house examination of the click-through rates. However, for certain antibiotics and analgesics, there is a lack of corresponding studies, ambiguity in the findings, or the testing was performed with lower concentrations than those used at KSB.

Thus, the main goal of this thesis is to close some of these gaps by investigating the compatibility of certain antibiotics and analgesics when administered concurrently with RA via Y-site connections. From a broader perspective, it aims to facilitate safe and effective intravenous medication administration. The analysis will encompass the following DSs: morphine sulfate, piperacillin, tazobactam, ketorolac, meropenem, and benzylpenicillin (penicillin G). Table 2 provides an overview of the chemical structures, pKa and logP values, as well as the solubility of the DSs to be investigated. Their chemical stability in RA will be assessed using a validated quantitative HPLC analysis. The assessment of physical compatibility will involve an examination of particle formation through light obscuration particle counting, following the guidelines outlined in Chapter 2.9.19 of the EP. Finally, the data obtained will be integrated into the KSB hospital pharmacy's existing RA compatibility table.

Table 2. Overview of the drug substances under investigation and their physico-chemical properties.

Substance	Structure (60-68)	pKa (69)	Aqueous solubility (61-68)	logP (69)
Benzylpenicillin sodium		2.74 (60)	Very soluble	1.92
Ciprofloxacin		6.09 (70)	Practically insoluble	0.28
Furosemide		4.25	Practically insoluble	2.03
Ketorolac tromethamine		3.84	Freely soluble	2.1
Meropenem trihydrate		3.28	Sparingly soluble	- 0.6
Morphine sulfate		8.21	Soluble	0.87
Piperacillin sodium		3.49	Freely soluble	-0.26
Tazobactam		2.1	Soluble	-1.4

3. Materials and methods

3.1. Materials

Analytical reference standards of benzylpenicillin sodium, ciprofloxacin, furosemide, ketorolac tromethamine, meropenem trihydrate, morphine hydrochloride, piperacillin sodium and tazobactam for quantitative HPLC analysis were purchased from Sigma Aldrich (Buchs, Switzerland). Acetonitrile (ACN) gradient grade for liquid chromatography was obtained from Merck (Darmstadt, Germany) and formic acid (FA) 99-100 % analytical reagent grade from VWR Chemicals (Dietikon, Switzerland). Pure water for HPLC analysis was produced in-house at ETH Zurich with a water purification system (Labtec, Villmergen, Switzerland). DPs, carrier solutions and solvents used for preparation of the test and control samples (Table 3) were provided by the hospital pharmacy of KSB.

For dissolution and transfer of the drugs, Mini-Spikes® (for vials), Sterican® 18 G blunt needles with integrated 5 µm Filter (for ampoules) and sterile Omnifix® Luer Lock polypropylene syringes of different volumes were used (all from B. Braun Medical AG, Sempach, Switzerland). HPLC vials and inlets were obtained from BGB (Boeckten, Switzerland), screwcaps and septa from Infochroma AG (Goldau, Switzerland). Gilson pipettes and Gilson DIAMOND tips were used for all HPLC experiments (Mettmenstetten, Switzerland). Measurements of pH were carried out with a Lab 845 pH Meter connected to a BlueLine 13 pH electrode from SI Analytics (Mainz, Germany).

Table 3. Drug products and solvents used for the preparation of the test and control samples.

Trade name	Drug substance	Manufacturer, lot number
Tora-dol®	Ketorolac tromethamine	Bayer AG, F3016F01
Penicillin 10 Mega	Benzylpenicillin sodium	Grünenthal Pharma AG, KU3842
Meronem® 1 g	Meropenem trihydrate	Pfizer AG, 5A20G20
Morphin Sulfate Sintetica 500 mg/10 ml	Morphine sulfate	Sintetica SA, 205274
Piperacillin / Tazobactam Sandoz® 4.5 g	Piperacillin sodium, tazobactam sodium	Sandoz Pharmaceuticals AG, KY2753
Lasix® 20 mg/2 ml	Furosemide sodium	Sanofi Aventis AG, K0586
Ciproxin® 0.2 g/100 ml	Ciprofloxacin	Bayer AG, KP0EEET
Ringer-Acetat Fresenius	-	Fresenius Kabi AG, 14RD7315
Aqua ad iniectabilia Sintetica Injektionslösung 100 ml	-	Sintetica SA, 215018
NaCl 0,9% Bioren Infusionslösung	-	Sintetica SA, 202150
Glucose 5% B. Braun	-	B. Braun Medical AG, 211928131

3.2. HPLC assay

3.2.1. Method development

All HPLC assays were carried out at the Department of Chemistry and Applied Biosciences at ETH Zurich. The HPLC system was from VWR HITACHI Elite LaChrom and consisted of an autosampler (L-2200), a quaternary pump (L-2130), a column oven (L2350), and a diode array detector (L-2455). The mobile phase was composed of pure water with 0.1% FA (mobile phase A) and ACN with 0.1% FA (mobile phase D). Wavelength was set at λ = 210 nm for all substances. Three different columns were examined for their separation capacity: Zorbax SB C8, 4.6 x 150 mm, 5 μm (Agilent, Switzerland), Zorbax Eclipse Plus C18, 3.0 x 150 mm, 5 μm (Agilent, Switzerland), and Luna C18, 250 x 4.6 mm, 5 μm, 100 Å (Phenomenex, Switzerland). Two of them (Zorbax Eclipse Plus C18 and Luna C18) were tested in detail. Flow rate varied between 1.0 and 0.6 ml/min. Different column oven temperatures were applied (25 °C, 30 °C, 40 °C). Injection volume was 20 μl or 10 μl. Four different gradients were examined (see appendix Table A2).

3.2.2. Preparation of calibration and quality control samples

The reference substances were diluted separately in a solution of 50% ACN in pure water. From these stock solutions, two multianalyte groups were prepared and serially diluted with 50% ACN in pure water to acquire four different concentrations and two quality controls (QC high and QC low) within the calibration range of each analyte. One group consisted of meropenem trihydrate, piperacillin sodium, tazobactam and furosemide, the other of benzylpenicillin sodium and ketorolac tromethamine. The calibration range was set within the working range of the DP samples. QC high samples were chosen at 80% of the highest and QC low at 125% of the lowest calibrator. All samples were stored as aliquots of 250 µl in the freezer at - 26 °C.

Additionally, a single-point calibration was performed for ketorolac tromethamine and benzylpenicillin sodium. Calibration samples were 0.15 mg/ml ketorolac tromethamine and 0.2 mg/ml benzylpenicillin sodium in 50% ACN in pure water. QC samples were chosen at 90% of the calibrator and corresponded to 0.135 mg/ml ketorolac tromethamine and 0.18 mg/ml benzylpenicillin sodium.

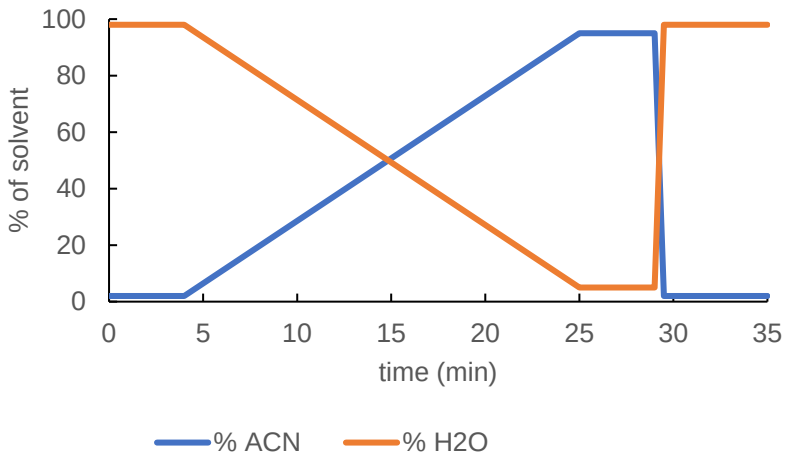
Table 4. Concentrations [mg/ml] of test samples (S), calibrator (K) and QC samples.

	Test concentration S (dilution factor [a])		**K1**	**K2**	**K3**	**K4**	**QC high**	**QC low**
Meropenem trihydrate	0.1	(100)	0.3	0.15	0.03	0.01	0.25	0.0125
	0.05	(100)						
Piperacillin sodium	0.21	(500)	0.3	0.15	0.03	0.01	0.25	0.0125
	0.21	(200)						
Tazobactam	0.025	(500)	0.3	0.15	0.03	0.01	0.25	0.0125
	0.025	(200)						
Furosemide	0.1	(10)	0.2	0.1	0.02	0.007	0.16	0.008
Benzylpenicillin sodium	0.3	(100)	0.3	0.15	0.03	0.01	0.25	0.0125
	0.12	(100)		0.2 [b]			0.18 [b]	
Ketorolac tromethamine	0.15	(100)	0.3	0.15	0.03	0.01	0.25	0.0125
	0.06	(5)		0.15 [b]			0.135 [b]	
Ciprofloxacin	0.2	(10)	0.3	0.15	0.03	0.01	0.25	0.0125

[a] for HPLC analysis [b] for single-point calibration

3.2.3. Method validation

The gradient used for method validation is illustrated in Figure 1. Injection volume was 10 µl, flow 0.6 ml/min and oven temperature 30 °C. Detector wavelength was set at 210 nm. Peak integration was performed in Agilent EZChrom Elite (Version 3.2.0, 2007) software.



t (min)	% ACN + 0.1% FA	% H_2O + 0.1% FA
0.0	2	98
4.0	2	98
25.0	95	5
29.0	95	5
29.5	2	98
35.0	2	98

Figure 1. Gradient used for method validation and application. ACN: acetonitrile, FA: formic acid

For the multianalyte analysis, eight replicates of each calibrator were analysed on eight different days over four weeks. QC samples were equally measured in duplicates on each of the eight days. Regression lines were obtained by plotting the peak area (λ = 210 nm) of the calibrators against the corresponding concentrations. Weighted (1/x) least square linear regression analysis of the calibration data was computed using the equation y = mx + b. The weighted equations were then used to back-calculate the concentrations of the calibrators and QC samples. The acceptance criteria was ± 20% deviation from the nominal value. Accuracy, in terms of bias, of both QC high and QC low samples was calculated as the percent deviation of the mean from the nominal concentration. Intra- and inter-day precision were determined by calculating the relative standard deviation (RSD, or percent coefficient of variation) of the QC samples on the same day and on eight different days, respectively.

3.2.4. Preparation and quantitative analysis of the DP test and control samples

For each DP, two different concentrations were tested, corresponding to high and average concentrations used in the KSB (henceforth referred to as high- and low-dose DP samples). They were dissolved and diluted, if necessary, according to the manufacturer's specifications to the concentrations A shown in Table 5. Dilution with carrier solution was carried out in a borosilicate graduated cylindrical flask. To obtain the final test samples, henceforth referred to as concentration B, the DP solutions of concentration A were subsequently diluted, separately, in equal parts with RA (10 ml each), except for low-dose ketorolac tromethamine, for which concentration B was obtained by a 100-fold dilution. Equivalent control samples were prepared alongside the test samples. Here, final concentration B was obtained by dilution with a carrier solution recommended by the manufacturer, which was generally NaCl 0.9%, except for benzylpenicillin sodium, where D5 was used. Both DP test and control samples were mixed on a magnetic stirrer for a few seconds and transferred to a borosilicate beaker. They were stored at room temperature and under normal day light before and between measurements. The control solution of meropenem was stored in the refrigerator at 5 °C, as indicated by the manufacturer when storage time exceeds 3 h.

Ciprofloxacin and furosemide were used as "positive control" and mixed by adding 2 ml furosemide 10 mg/ml to 18 ml ciprofloxacin 2 mg/ml. The "positive control" was used to determine how an incompatibility visible to the naked eye affects drug concentration over time. The control solution of furosemide was obtained by diluting 2 ml furosemide 10 mg/ml with 18 ml NaCl 0.9%.

Generally, measurements of the DP test samples were taken immediately after preparation (t_0), after 1.35 h (t_1) and 2.55 (t_2) h, but low-dose ketorolac tromethamine (0.3 mg/ml) was measured immediately after preparation (t_0), and after 4 h (t_1) and 24 h (t_2). Measurements of pH were made at t_0 and after 2.55 h or, in the case of ketorolac tromethamine, after 24 h (t_2).

DP control samples were analysed at the end of the day of analysis (time not specified), or, in the case of ketorolac tromethamine, after 24 h. Their pH was measured at t_0 and at the end of the day of analysis (time not specified), or, as for ketorolac tromethamine, after 24 h.

HPLC analysis was performed using the gradient and conditions described in chapter 3.2.3. All samples were diluted with a solution of 50% ACN in pure water to the concentrations indicated in Table 4 of chapter 3.2.2. All measurements were done in triplicates.

A sample was considered chemically stable if its concentration at t_2 (generally 2.55 h, 24 h for ketorolac tromethamine) was equal to or greater than 95% of its initial concentration (t_0).

Table 5. Preparation of the drug product test and control samples for quantitative analysis.

Drug substance	solvent	Carrier solution	Concentration A [mg/ml]	concentration B [mg/ml]	
				in Ringer's acetate (= test sample)	in dextrose 5% or NaCl 0.9% (= control sample)
Ketorolac trometha-mine	-	-	30 15	15 0.3	15 0.3
Benzyl-penicillin sodium	WFI	dextrose 5%	60 24	30 12	30 12
Piperacillin	WFI	NaCl 0.9%	200 80	100 40	100 40
Tazobactam	WFI	NaCl 0.9%	25 10	12.5 5	12.5 5
Meropenem	WFI	NaCl 0.9%	20 10	10 5	10 5

Concentration A: concentration of the drug substance after dissolution with WFI (if necessary) and dilution in the carrier solution. Concentration A represents the drug infusion solution as it would be given to the patient.

Concentration B: concentration of the drug substance after mixing the sample of concentration A with Ringer's acetate (test sample) or with dextrose 5% or NaCl 0.9% (control sample).

For example: benzylpenicillin sodium was dissolved in WFI, then diluted with dextrose 5% to concentrations A, 60 mg/ml and 24 mg/ml, and then mixed 1:1 with Ringer's acetate to reach concentrations B, 30 mg/ml and 12 mg/ml, respectively.

WFI: water for injection

3.3. Measurement of particle concentration

3.3.1. Preparation of DP samples

Fresh DP test samples were prepared using the same solvents and carrier solutions presented in chapter 3.2.4., Table 5. DP control samples were not included. To minimize particle contamination during sample preparation, different equipment and preparation techniques were used than those described for HPLC analysis. To reach concentration A, benzylpenicillin sodium, piperacillin/tazobactam and meropenem were first dissolved in water for injection (WFI), before the necessary volume was withdrawn and transferred into the infusion bag containing the carrier solution. Then, 25 ml of the DP-solution were withdrawn and mixed with 25 ml RA within a polypropylene syringe. The syringe was closed with a Combi-Stopper (B. Braun Medical AG, Sempach, Switzerland). To prepare the ketorolac tromethamine 15 mg/ml and 0.3 mg/ml solutions, a total of 25 ml or 0.5 ml were directly withdrawn from the vials into a

polypropylene syringe using a sterile blunt 18G needle with an integrated 5 µm filter. 25 ml or 49.5 ml RA were filled into the same syringe. All samples were stored in the closed polypropylene syringes at room temperature and under normal day light. “Positive controls” with ciprofloxacin and furosemide were not performed, as particle formation was clearly visible.

With meropenem 10 mg/ml, the measurements were repeated using a modified technique: After diluting the sample in the NaCl 0.9% bag, as described above, 50 ml of the solution were transferred into a 100 ml volumetric flask and RA was added to a total volume of 100 ml. The flask was closed, the solution carefully rotated and then put into an ultrasonic bath for 5 min. The same procedure was done with a control solution, where NaCl 0.9% instead of RA was used in the final step. All samples were stored in closed borosilicate volumetric flasks at room temperature and under normal day light.

Particle concentrations were measured immediately after preparation of the samples (t_0), and after 2.55 h (t_2). Low-dose ketorolac tromethamine (0.3 mg/ml) was measured at t_0 and after 24 h. For meropenem, an additional measurement was performed after 5 h.

3.3.2. Instrumentation and method for particle measurement

Sub-visible particles were measured according to chapter 2.9.19 method 1B of the EP at the Institute of Hospital Pharmacy at Inselspital Bern. The system was a Syringe® particle counter equipped with a laser diode sensor LDS 30/30 usp and SW-CA software Version 3.01 (Klotz GmbH, Bad Liebenzell, Germany). It operates by passing the test liquid between a laser diode as the light source and a detector. When particles pass, they obfuscate the light and generate a shadow on the detector. Particle size is determined by the intensity of the light obscuration.

The instrument was rinsed with ethanol 70% and WFI before the first, and with WFI between each measurement. An environment test according to chapter 2.9.19 of the EP was performed before testing any samples, ensuring that test conditions were suitable. Glass ware was rinsed with WFI between each measurement.

For particle counting, the test solutions were carefully inverted three times before transferring 25 ml of them into a borosilicate beaker. Prior to starting the measurements, all samples stood for 2 min to release air bubbles. Four portions of 5 ml each were withdrawn. The first portion was used to rinse the system and not included in the particle counting. For the remaining three portions, the number of particles equal to or greater than 10 µm or 25 µm in diameter was determined. The mean particle count was calculated and extrapolated to the total volume of 25 ml.

Samples were considered physically compatible if they passed the acceptance criteria of chapter 2.9.19 of the EP method 1B for volumes less than 100 ml. The method allows a

maximum of 6000 particles equal to or greater than 10 μm in diameter, and a maximum of 600 particles equal to or greater than 25 μm in diameter per container.

3.4. Statistical analysis

Statistical analysis of the results was performed in excel, using a two-tailed, unpaired student's t-test. Additionally, deviation of the results from their nominal values were statistically analysed using a one-sample, two-tailed student's t-test. Results were considered significant at a threshold of $p < 0.05$.

4. Results

4.1. HPLC assay

4.1.1. Method development

To identify the most suitable method for multianalyte analysis, individual measurements of reference substances were conducted using different columns, varied gradients, flow rates (1.0 and 0.6 ml/min), and temperatures (25, 30, 40 °C). Optimal peak quality was achieved with the Zorbax Eclipse plus C18 and the Phenomenex Luna C18 columns (Figures 2-4). Four different gradients were tested on these columns (see appendix Table A2). Peak quality improved when altering the starting condition of the mobile phase from 95% to 98% solvent D (H_2O with 0.1% ACN). The temperature had no substantial impact on retention time (see appendix Tables A3-A4), and flow rate did not affect peak quality. Figure 2 illustrates the chromatogram of the multianalyte solution (each analyte with a concentration of 0.1 mg/ml) attained through the optimized gradient (refer to chapter 3.2.3), which was ultimately selected for method validation and application. Complete separation of benzylpenicillin, furosemide, and ketorolac was challenging. As a result, the analytes were divided into two groups for method validation. Group 1 comprised ciprofloxacin, benzylpenicillin, and ketorolac (Figure 3), whilst group 2 included piperacillin, tazobactam, meropenem, and furosemide (Figure 4). The analytes were separated with a baseline Rs > 1.5, which is necessary for quantitative analysis. In group 2, the flow rate was decreased to 0.6 ml/min, and the injection volume to 10 µl to improve the quality of the meropenem peak. Regarding morphine hydrochloride, its chromatograms persistently showed two peaks, and their assignment remained uncertain. Moreover, these peaks did not exhibit linear behaviour in the dilution series. Thus, morphine was not included in any subsequent experiments.

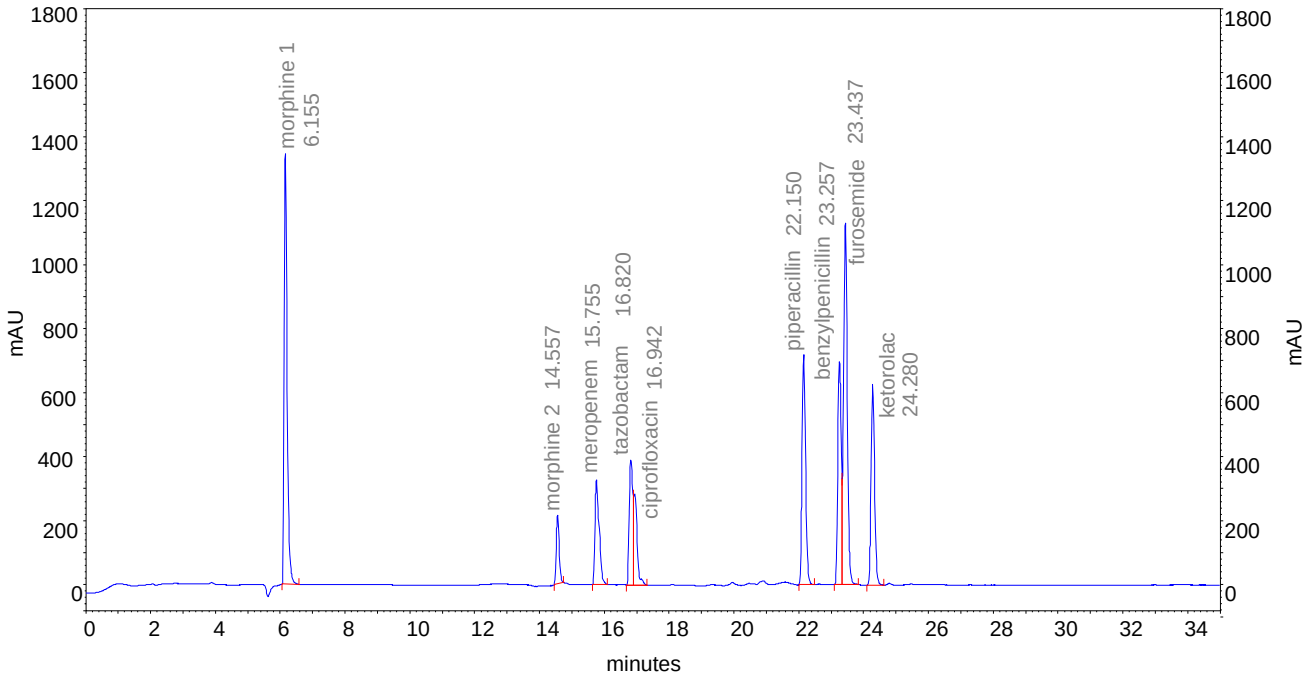


Figure 2. Separation of the mixed reference substances (each 0.1 mg/ml) on the Phenomenex Luna C18 column with gradient 8 at 30 °C. Flow rate 0.6 ml/min, injection volume 20 µl, detector wavelength 210 nm. From left to right: morphine (first two peaks, assignation uncertain), meropenem, tazobactam, ciprofloxacin, piperacillin, benzylpenicillin, furosemide, ketorolac. Ciprofloxacin elutes at the same time as tazobactam (double Peak at 16.8 min). The same can be observed with benzylpenicillin and furosemide (double peak at 23.3 min).

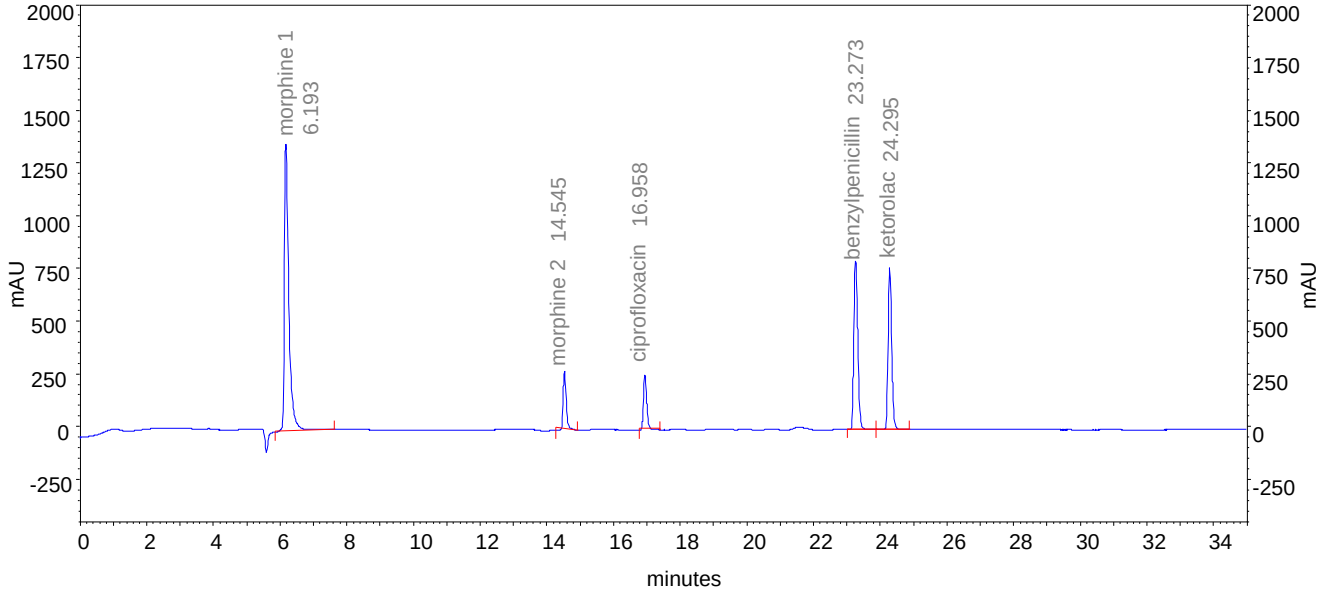


Figure 3. Separation of multianalyte group 1 (each 0.1 mg/ml) on the Phenomenex Luna C18 column with gradient 8 at 30 °C. Flow rate 0.6 ml/min, injection volume 20 µl, detector wavelength 210 nm. From left to right: morphine (first two peaks, assignation uncertain), ciprofloxacin, benzylpenicillin, ketorolac.

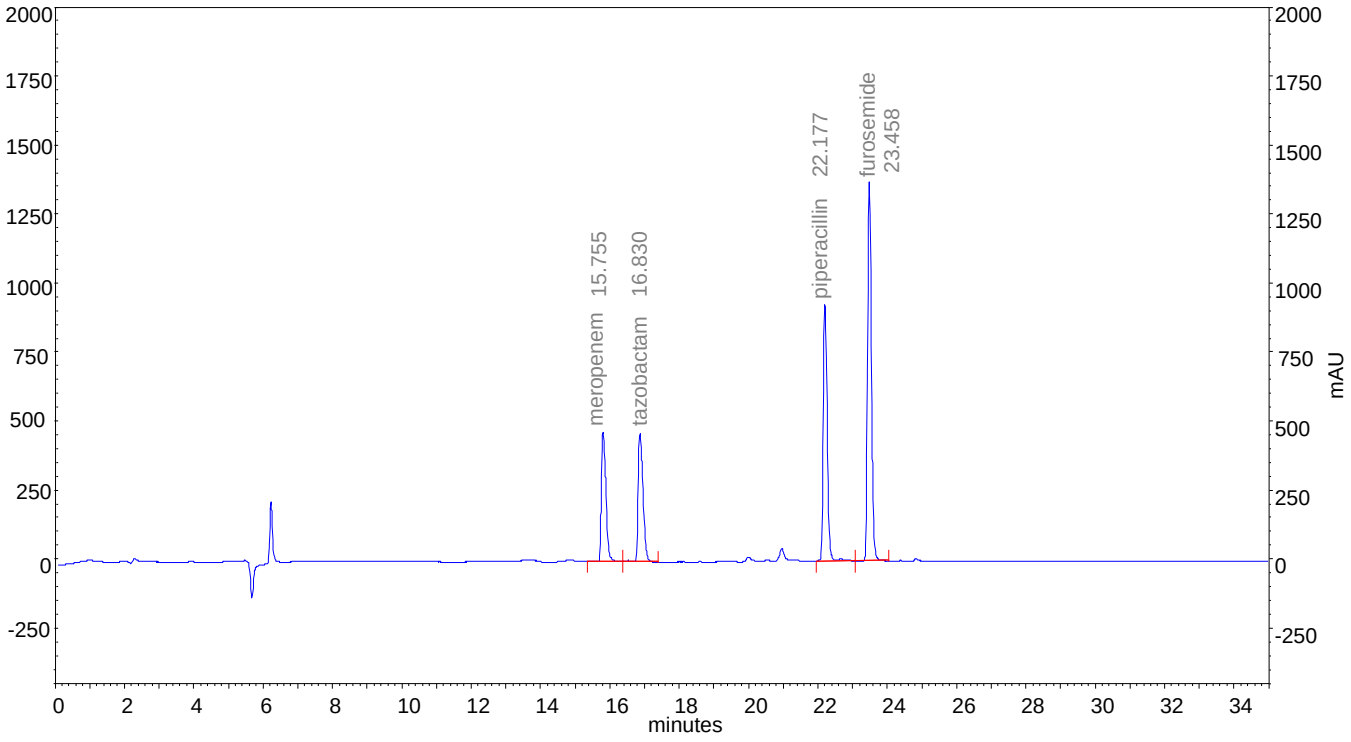


Figure 4. Separation of multianalyte group 2 (each 0.1 mg/ml) on the Phenomenex Luna C18 column with gradient 8 at 30 °C. Flow rate 0.6 ml/min, injection volume 20 µl, detector wavelength 210 nm. From left to right: meropenem, tazobactam, piperacillin, furosemide.

Linearity and calibration range were tested by performing a dilution series using the selected method (Figures 5 and 6). Group 1 exhibited an R^2-value of ≥ 0.97, whereas group 2 exhibited an R^2-value of ≥ 0.99. Peaks remained detectable at minimal concentrations of 5 µg/ml for ciprofloxacin, 3.75 µg/ml for meropenem trihydrate and tazobactam, and 2.5 µg/ml for benzylpenicillin sodium, ketorolac tromethamine, piperacillin sodium, and furosemide. However, the limit of detection was not investigated in detail as the concentration of the DP samples would be in the middle to high concentration range of the calibration curve. During method validation it was observed that the stability of benzylpenicillin in group 1 was affected in the presence of ciprofloxacin and ketorolac tromethamine, resulting in variable peak area and the appearance of additional peaks over time (data not shown). Consequently, ciprofloxacin was eliminated from further experiments, and a single-point calibration was employed during method application, replacing the multianalyte-analysis for group 1.

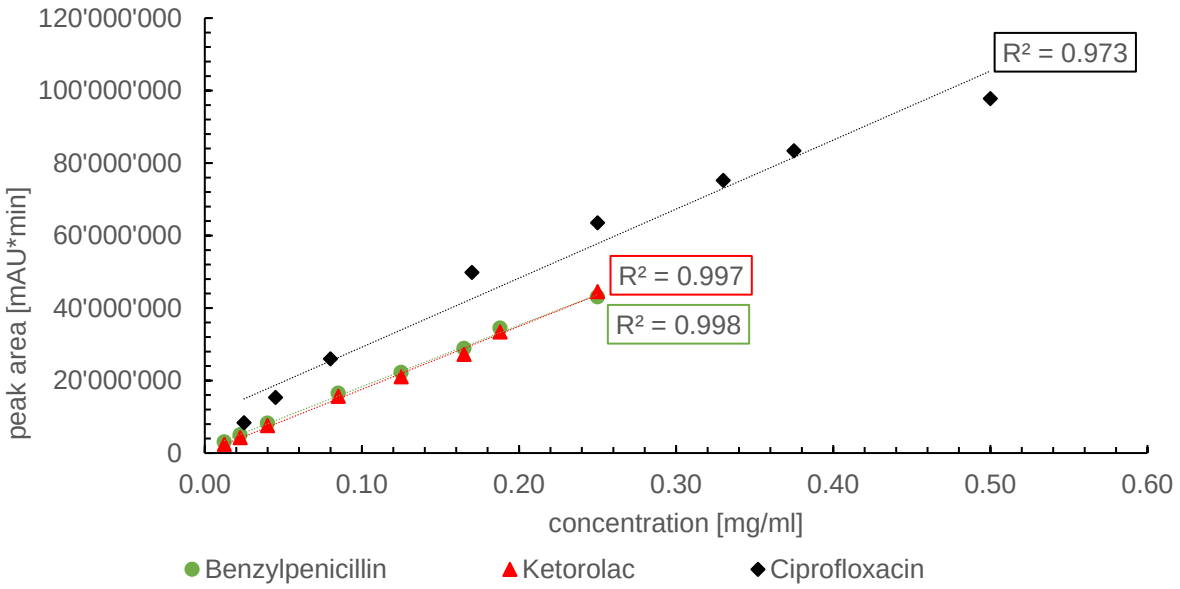


Figure 5. Linearity of the dilution series of group 1 with gradient 8 on the Phenomenex Luna C18 column, at 30 °C, flow rate 0.6 ml/min and injection volume 20 µl. Linear concentration range benzylpenicillin sodium and ketorolac tromethamine: 12.5 - 250 µg/ml. Linear concentration range ciprofloxacin: 25 - 500 µg/ml.

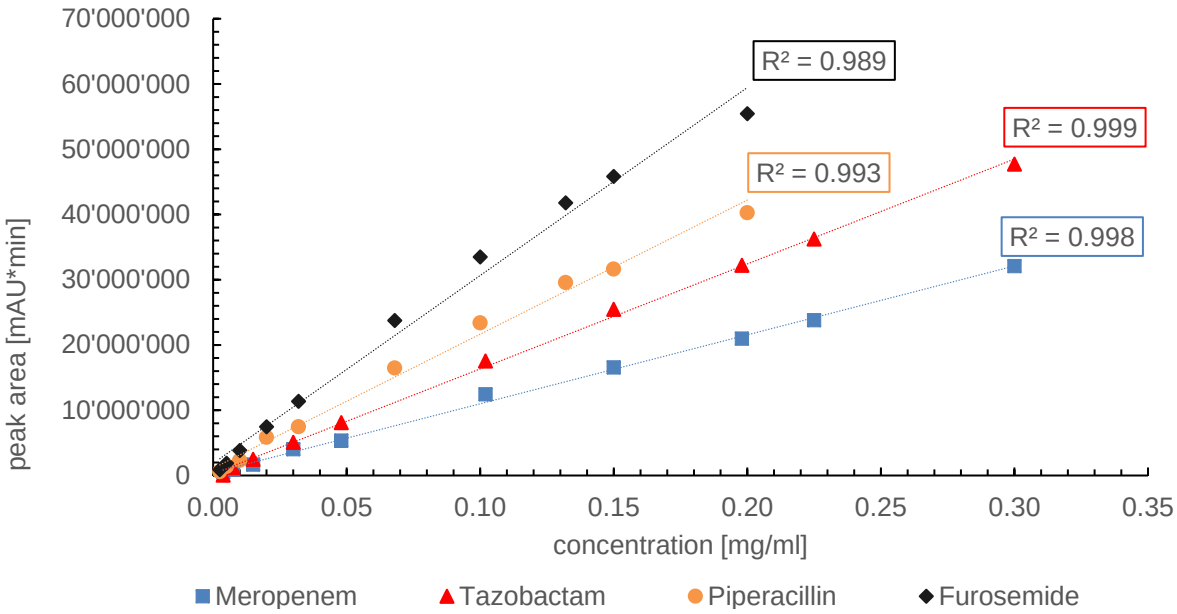


Figure 6. Linearity of the dilution series of group 2 with gradient 8 on the Phenomenex Luna C18 column, at 30 °C, flow rate 0.6 ml/min and injection volume 10 µl. Linear concentration range meropenem trihydrate and tazobactam: 3.75 - 300 µg/ml. Linear concentration range piperacillin sodium and furosemide: 2.5 - 200 µg/ml.

4.1.2. Method validation

Validation of the developed method for group 2 was conducted with regards to linearity, accuracy, precision and stability. The validation of eight days was carried out over a period of four weeks. Table 5 shows the calibration model, accuracy in terms of bias expressed as deviation from the expected value, and precision expressed as relative standard deviation (RSD, or coefficient of variation) for the analytes piperacillin sodium, tazobactam, meropenem trihydrate, and furosemide. To examine carry-over, blank runs were conducted subsequent to QC high samples. No carry-over was detected. The bias was found to be less than 10% for both QC high and low samples, meeting satisfactory criteria. The precision of QC high samples was acceptable with an RSD lower than 10%. However, the RSD for QC low samples was slightly higher across individual measurement days (RSD_T). To account for this discrepancy, concentrations of DP test and control samples were selected closer to the QC high samples rather than the QC low samples.

Table 5. Method validation data group 2.

	Range µg/ml	Cal. model	QC high			QC low		
			Bias [%]	RSD_R [%]	RSD_T [%]	Bias [%]	RSD_R [%]	RSD_T [%]
Piperacillin sodium	10.0 - 300.0	1/x	8.1	2.2	5.7	8.5	5.4	9.6
Tazobactam	10.0 - 300.0	1/x	5.6	2.3	5.8	4.6	5.0	10.3
Meropenem trihydrate	10.0 - 300.0	1/x	6.1	2.4	5.5	5.7	5.8	10.4
Furosemide	7.0 - 200.0	1/x	2.5	3.3	6.9	6.9	5.1	10.8

RSD: relative standard deviation (or coefficient of variation)

RSD_R: intra-day repeatability (RSD of the QC samples measured on the same day)

RSD_T: inter-day repeatability (RSD of the QC samples measured on different days)

The stability of the QC samples is demonstrated in Figures 7 to 10, which show the deviation of the QC samples from their nominal value. The deviation remained below 20% except for the QC low samples taken on day 8 (end of week 4) for tazobactam, piperacillin and furosemide.

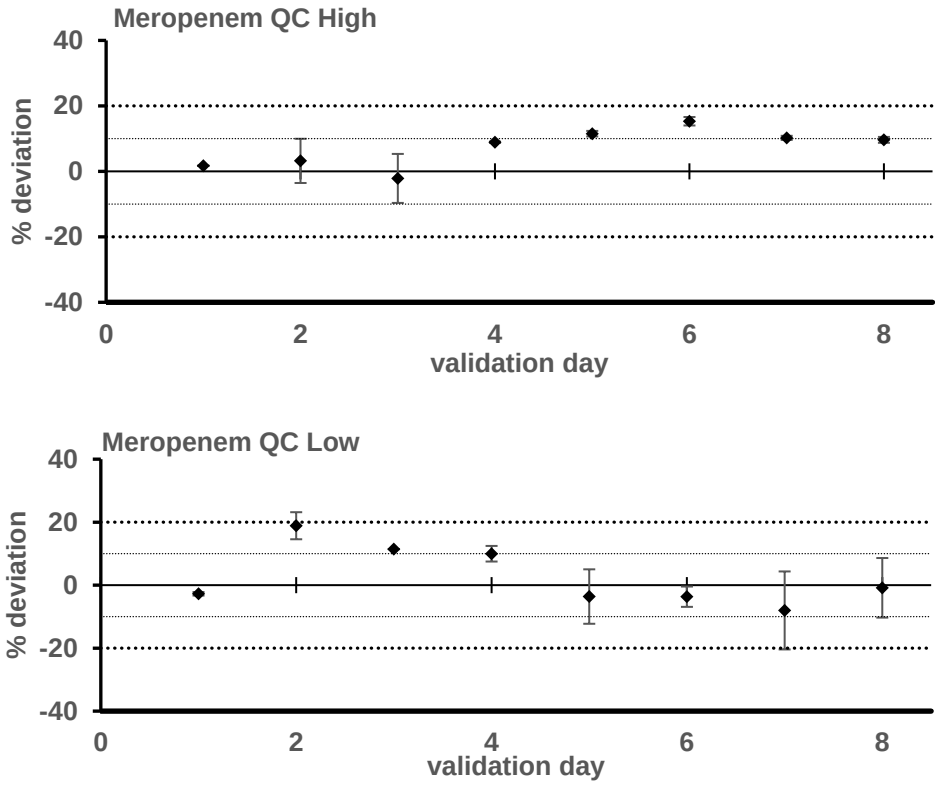


Figure 7. Stability of the QC samples of meropenem trihydrate over 8 days.

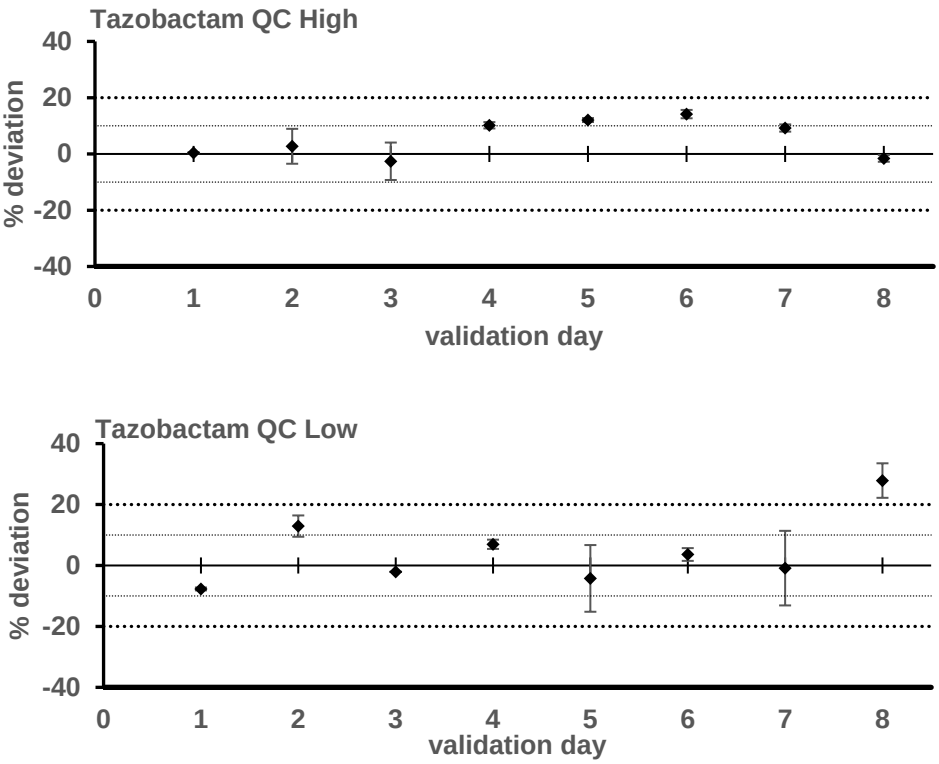


Figure 8. Stability of the QC samples of tazobactam over 8 days.

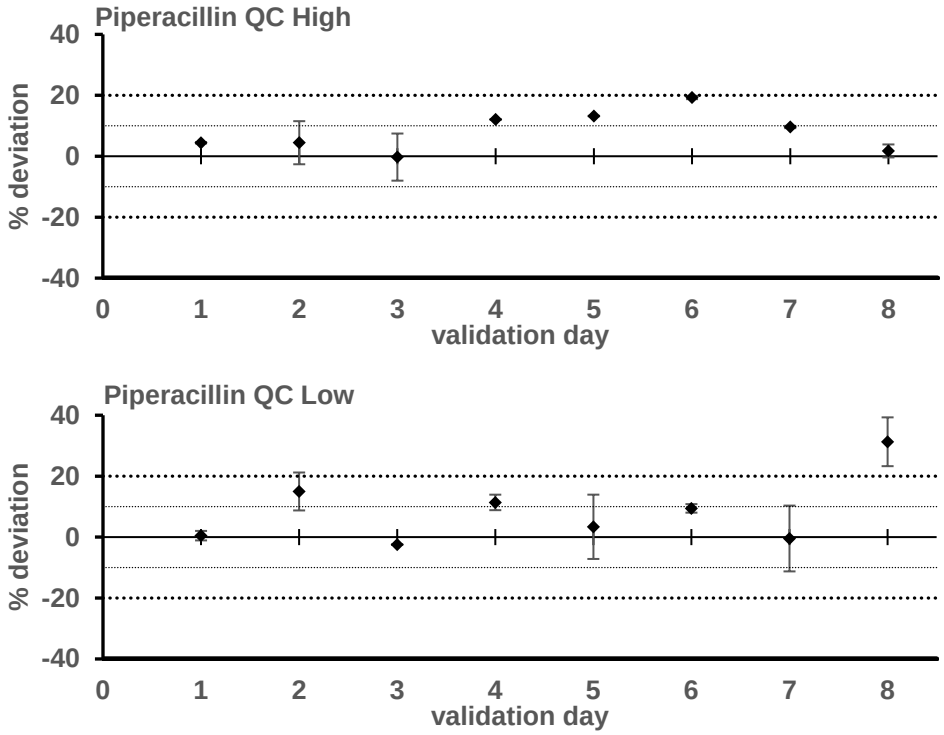


Figure 9. Stability of the QC samples of piperacillin sodium over 8 days.

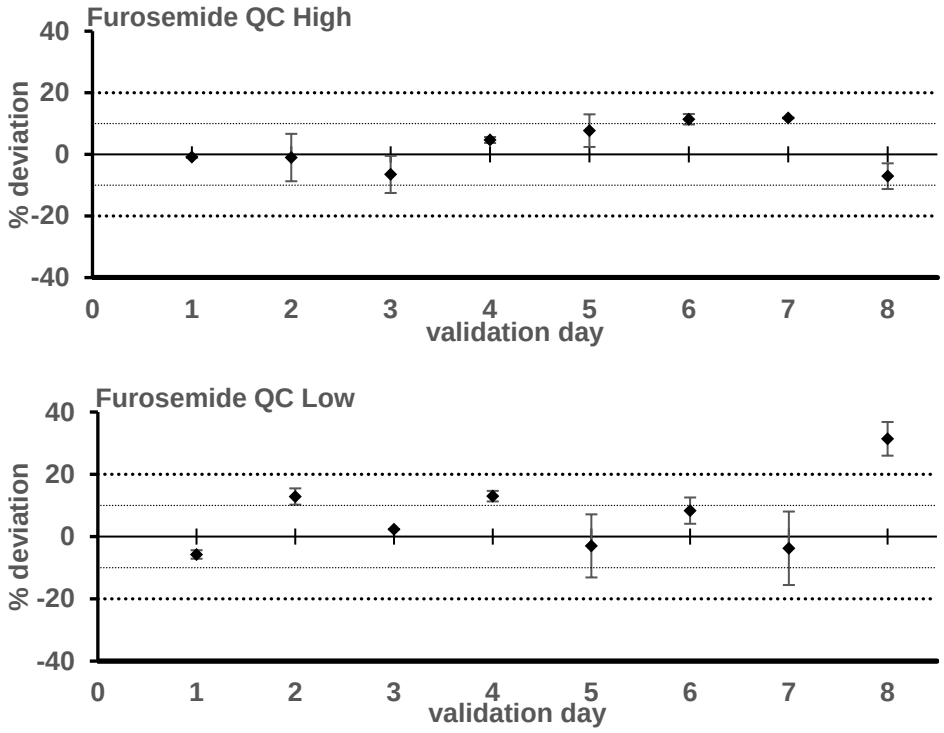


Figure 10. Stability of the QC samples of furosemide over 8 days.

4.1.3. Method application

Table 6 presents the stability data of the DP test samples, which correspond to the DPs in RA, over time and in comparison to the control samples, which correspond to the DPs in either NaCl 0.9% (meropenem, piperacillin, tazobactam, ketorolac) or D5 (benzylpenicillin). The single-point calibration approach for benzylpenicillin and ketorolac was deemed valuable as the primary objective was to ascertain whether a specific threshold (95% of the time zero concentration) was met. The accuracy of the quality control sample (QC_{90}, see appendix Figure A4) was considered acceptable in this context.

All the samples met the acceptance criteria of a final drug concentration of at least 95% relative to the initial concentration. Differences regarding the concentrations of the DP test samples between the initial (t_0 = immediately after preparation) and the final (t_2 = 2.55 hours, or 24 hours for ketorolac tromethamine) measurement were not significant ($p > 0.05$, Table 6). Final concentrations of both the test and control solutions of ketorolac tromethamine, tazobactam and high-dose piperacillin sodium (104.25 mg/ml) were also within 90% and 110% of the nominal value. Both test and control solutions of low-dose benzylpenicillin sodium (12 mg/ml) were below 90%, those of high-dose benzylpenicillin sodium (30 mg/ml) above 110% of the nominal value at ($p < 0.05$). This significant difference was consistent between the three time points. Low-dose meropenem (5 mg/ml) test and control solution, as well as high-dose meropenem (10 mg/ml) control solution exceeded their nominal values by more than 80% and more than 30%, respectively. This deviation is statistically significant for the test solution of low-dose meropenem, and for the control solutions of both high- and low-dose meropenem ($p < 0.05$). The same was observed when the experiment was repeated by a laboratory assistant three months later, with both the high- and low-dose samples exceeding their nominal concentrations by more than 50% (see appendix Figure A6).

Generally, no significant differences in the DS concentrations between the DP test and control samples could be observed ($p > 0.05$). Only for high-dose benzylpenicillin sodium, the concentrations of the test samples were significantly higher than those of the control samples.

The “positive control” with furosemide and ciprofloxacin hydrochloride (see appendix Figure A5) showed a significant decline in furosemide concentration to 25% of the initial concentration at t_2 ($p < 0.05$).

Table 6. Stability data of the test solutions (drug products diluted in Ringer's acetate) and control solutions (drug products diluted in NaCl 0.9 % or dextrose 5 %).

	Ringer's acetate			NaCl 0.9% or dextrose 5% [c, d]
	Time [h]	Content relative to nominal [%] [a]	Content relative to initial [%] [b]	Content relative to nominal [%] [a]
Meropenem 10 mg/ml	0 (t_0)	108.6 ± 10.7		
	1.35 (t_1)	109.1 ± 12.6		
	2.55 (t_2)	112.4 ± 12.4	**103.4 ± 2.5**	138.7 ± 4.1 **
Meropenem 5 mg/ml	0 (t_0)	188.1 ± 2.5		
	1.35 (t_1)	185.6 ± 1.0		
	2.55 (t_2)	184.8 ± 2.1 **	**98.2 ± 0.8**	182.3 ± 0.8 **
Piperacillin sodium 104.25 mg/ml	0 (t_0)	104.0 ± 2.5		
	1.35 (t_1)	101.7 ± 1.1		
	2.55 (t_2)	101.6 ± 1.7	**97.8 ± 2.5**	104.4 ± 3.1
Piperacillin sodium 41.7 mg/ml	0 (t_0)	111.5 ± 2.6		
	1.35 (t_1)	111.6 ± 2.9		
	2.55 (t_2)	113.9 ± 0.2 **	**102.2 ± 2.4**	115.6 ± 2.6 **
Tazobactam 12.5 mg/ml	0 (t_0)	99.1 ± 2.5		
	1.35 (t_1)	95.7 ± 0.8		
	2.55 (t_2)	97.2 ± 1.3	**98.1 ± 3.8**	98.6 ± 3.1
Tazobactam 5 mg/ml	0 (t_0)	105.2 ± 2.9		
	1.35 (t_1)	104.2 ± 1.1		
	2.55 (t_2)	106.8 ± 1.3	**101.7 ± 1.7**	110.1 ± 3.8 **
Benzylpenicillin sodium 30 mg/ml	0 (t_0)	85.4 ± 0.8		
	1.35 (t_1)	85.1 ± 1.0		
	2.55 (t_2)	85.4 ± 0.8 **	**100.0 ± 0.7**	83.2 ± 0.4 **
Benzylpenicillin sodium 12 mg/ml	0 (t_0)	112.1 ± 0.1		
	1.35 (t_1)	113.2 ± 0.3		
	2.55 (t_2)	114.7 ± 0.7 **	**102.3 ± 0.7**	117.9 ± 1.2 **
Ketorolac tromethamine 15 mg/ml	0 (t_0)	102.7 ± 3.3		
	1.35 (t_1)	104.4 ± 4.9		
	2.55 (t_2)	102.0 ± 2.8	**99.3 ± 1.0**	102.2 ± 1.3
Ketorolac tromethamine 0.3 mg/ml	0 (t_0)	107.2 ± 0.7		
	4 (t_1)	106.7 ± 0.6		
	24 (t_2)	106.5 ± 0.3	**99.4 ± 0.9**	105.6 ± 0.2

Note: Significant differences between measured and nominal values are labelled with stars (** $p < 0.05$). Differences regarding the concentrations of the test solutions between t_0 and t_2 were not significant ($p > 0.05$).

[a] relative to nominal concentration (mean ± SD, n = 3)

[b] relative to initial concentration at t_0 = 0 h (mean ± SD, n = 3)

[c] NaCl 0.9% for meropenem, piperacillin, tazobactam and ketorolac; dextrose 5% for benzylpenicillin

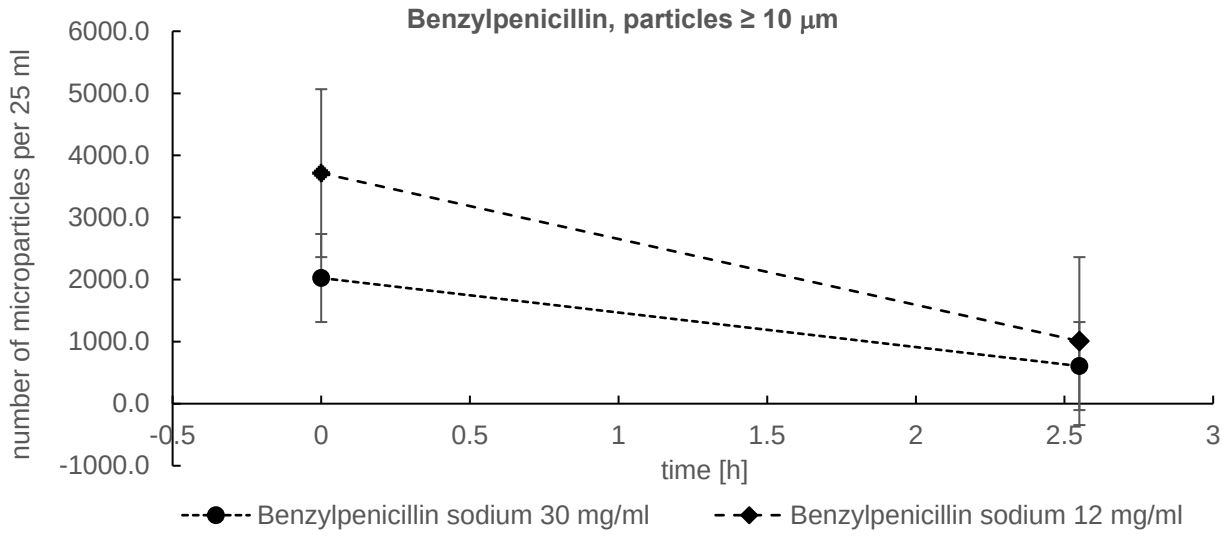
[d] the control solutions were measured at the end of the day of analysis

4.2. Measurement of particle counts

Figures 11 to 14 illustrate the mean change in number of particles equal to or greater than 10 or 25 μm in diameter (n = 3) in the DP test solutions, between t_0 and t_2. Figure 13 illustrates the repeat measurement of meropenem, where sample preparation was slightly modified, i.e., samples were put into an ultrasonic bath prior to testing, and an additional measurement was performed after 5 h. The first modification was intended to reduce the high number of air bubbles that were observed during the first measurement, and the second to test whether the number of particles remained low even at a prolonged time of contact. No DP test sample exceeded the threshold of 6000 particles ≥ 10 μm in diameter, and of 600 particles ≥ 25 μm in diameter. Particle counts of benzylpenicillin sodium (Figure 11) and meropenem trihydrate (Figure 12) diluted in RA (= test solutions), tended to decline between t_0 and t_2. The control solution of meropenem 10 mg/ml, i.e. meropenem diluted in pure NaCl 0.9%, showed the same pattern. An interesting observation is that the apparent decline in particle count for both the test and control solution of meropenem plateaus after 2.5 h. Moreover, the particle count seems to be lower in the test than in the control sample, and lower in the high- than in the low-dose samples. Similar results were obtained during the first measurements of meropenem, where no ultrasonic bath was used (see appendix Figure A7).

For piperacillin/tazobactam (Figure 12), the number of the ≥ 10 μm particles in the high-dose test sample (112.5 mg/ml) tended to decline between t_0 and t_2, while the number of the ≥ 25 μm particles appeared to slightly increase. For high-dose ketorolac tromethamine (15 mg/ml, Figure 14), the particle count appears to be lower at t_2 compared to t_0. Conversely, the particle counts of the low-dose test sample (0.3 mg/ml) tended to increase between time 0 and 24 h. The particle count also appeared higher in high-dose than in low-dose samples for both piperacillin/tazobactam and ketorolac tromethamine, with the exception of piperacillin/tazobactam at t_2 with regard to the ≥ 25 μm particles.

Despite the presented tendencies, the changes in particle count between t_0 and t_2 of the DP test samples are statistically insignificant ($p > 0.05$).



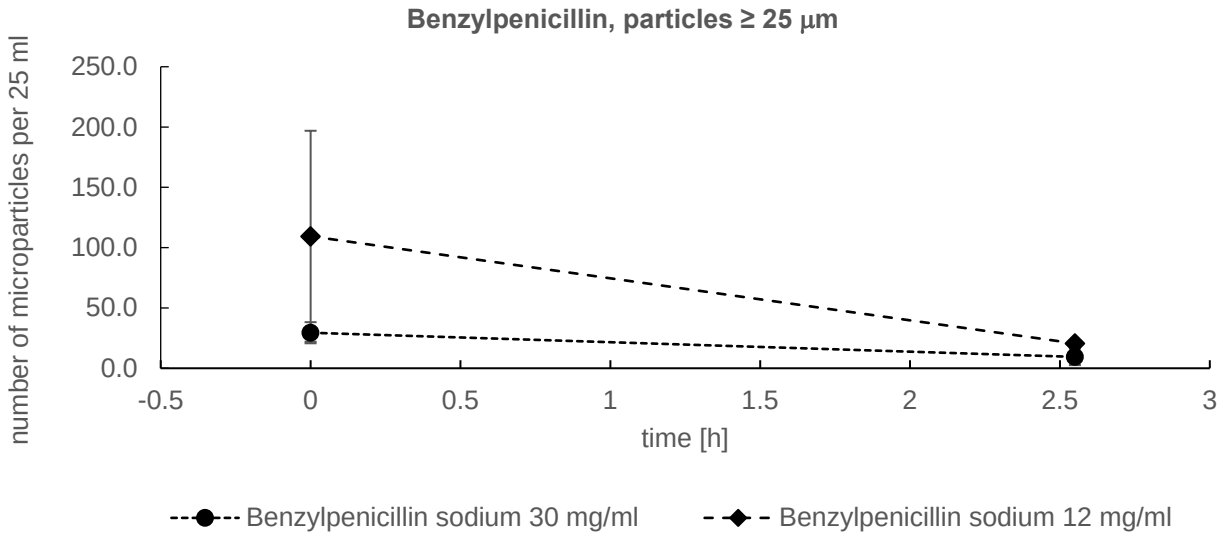
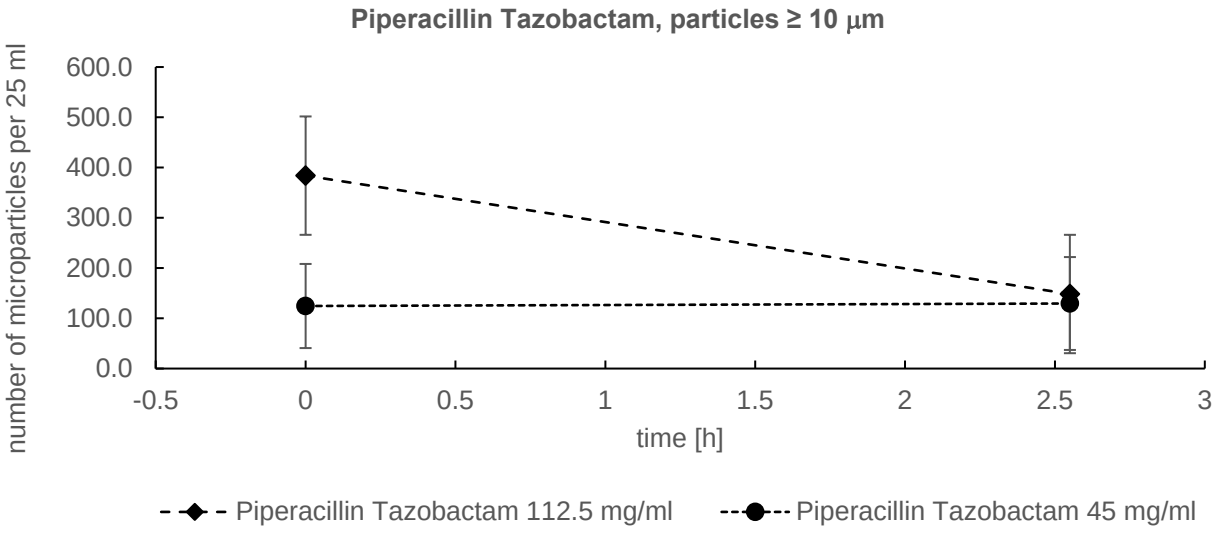


Figure 11. Change in number of particles ≥ 10 and ≥ 25 µm in diameter between t_0 (immediately after preparation) and t_2 (2.55 h) of high- and low-dose benzylpenicillin sodium in RA ($n = 3$, $p > 0.05$).



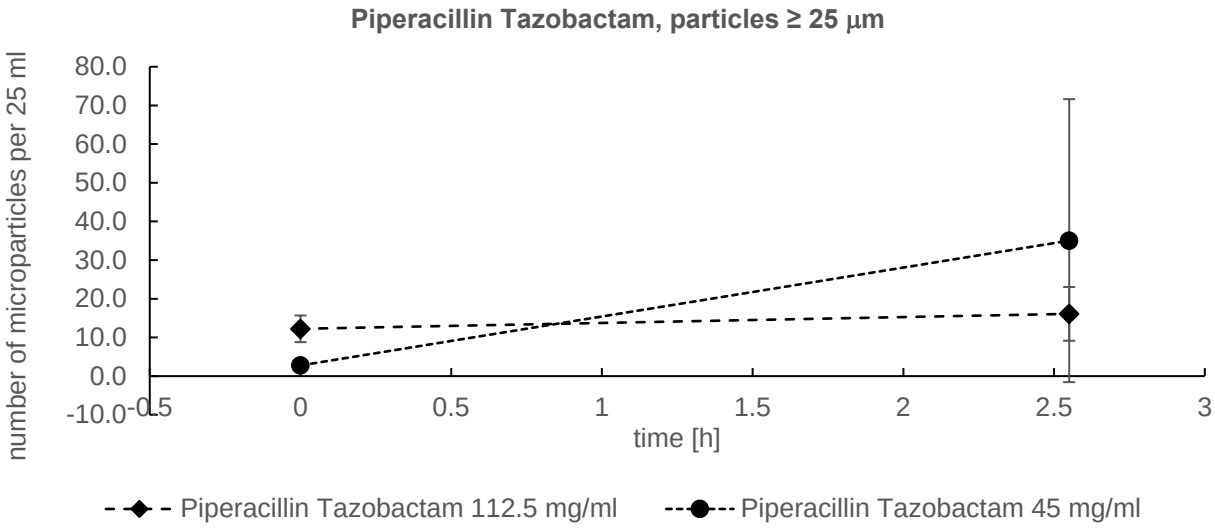


Figure 12. Change in number of particles ≥ 10 and ≥ 25 µm in diameter between t_0 (immediately after preparation) and t_2 (2.55 h) of high- and low-dose piperacillin/tazobactam in RA ($n = 3$, $p > 0.05$).

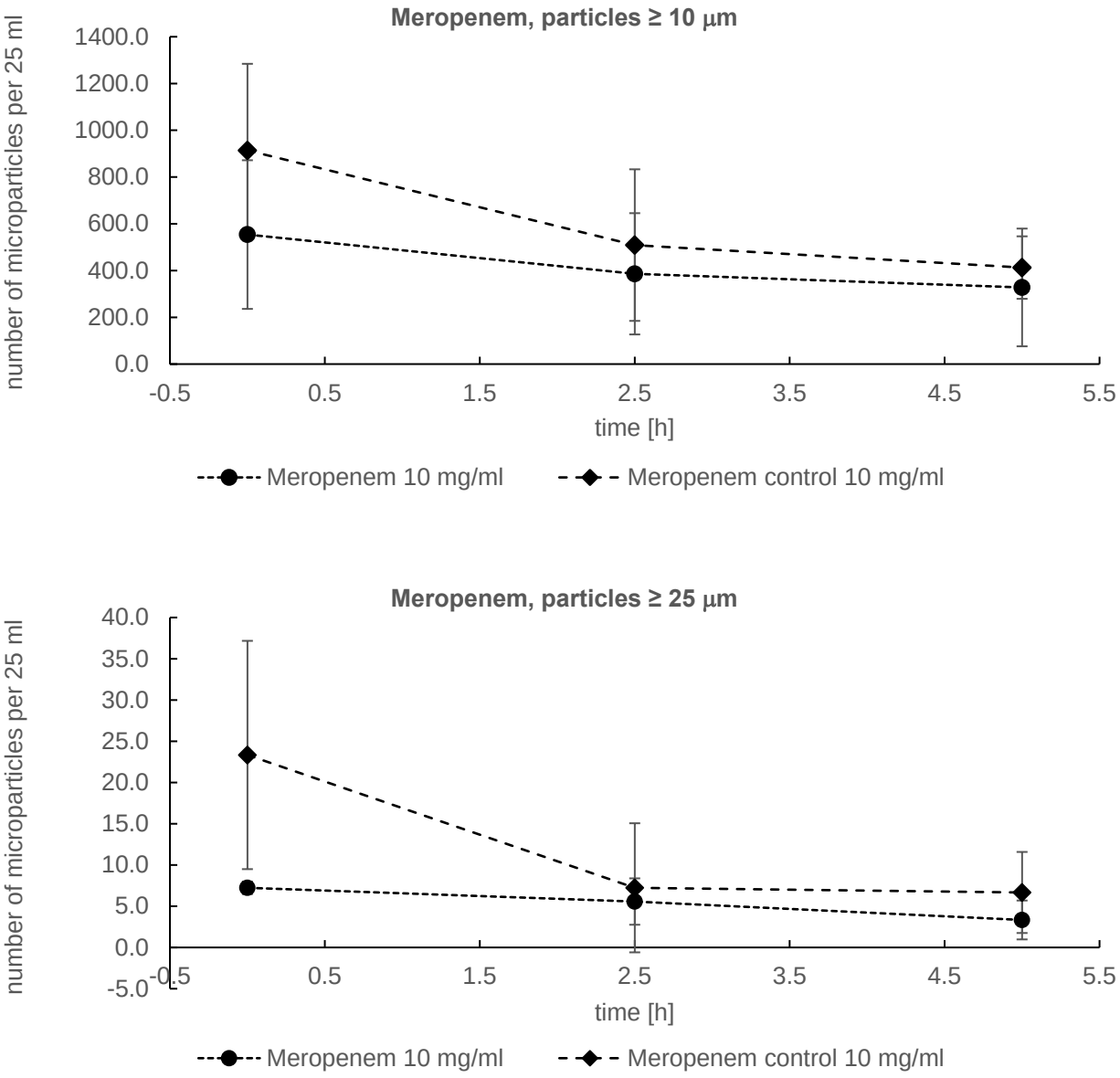


Figure 13. Change in number of particles ≥ 10 and ≥ 25 µm in diameter between t_0 (immediately after preparation) and t_2 (5 h) of meropenem 10 mg/ml in RA (test solution) and in NaCl 0.9 % (control solution) (n = 3, p > 0.05).

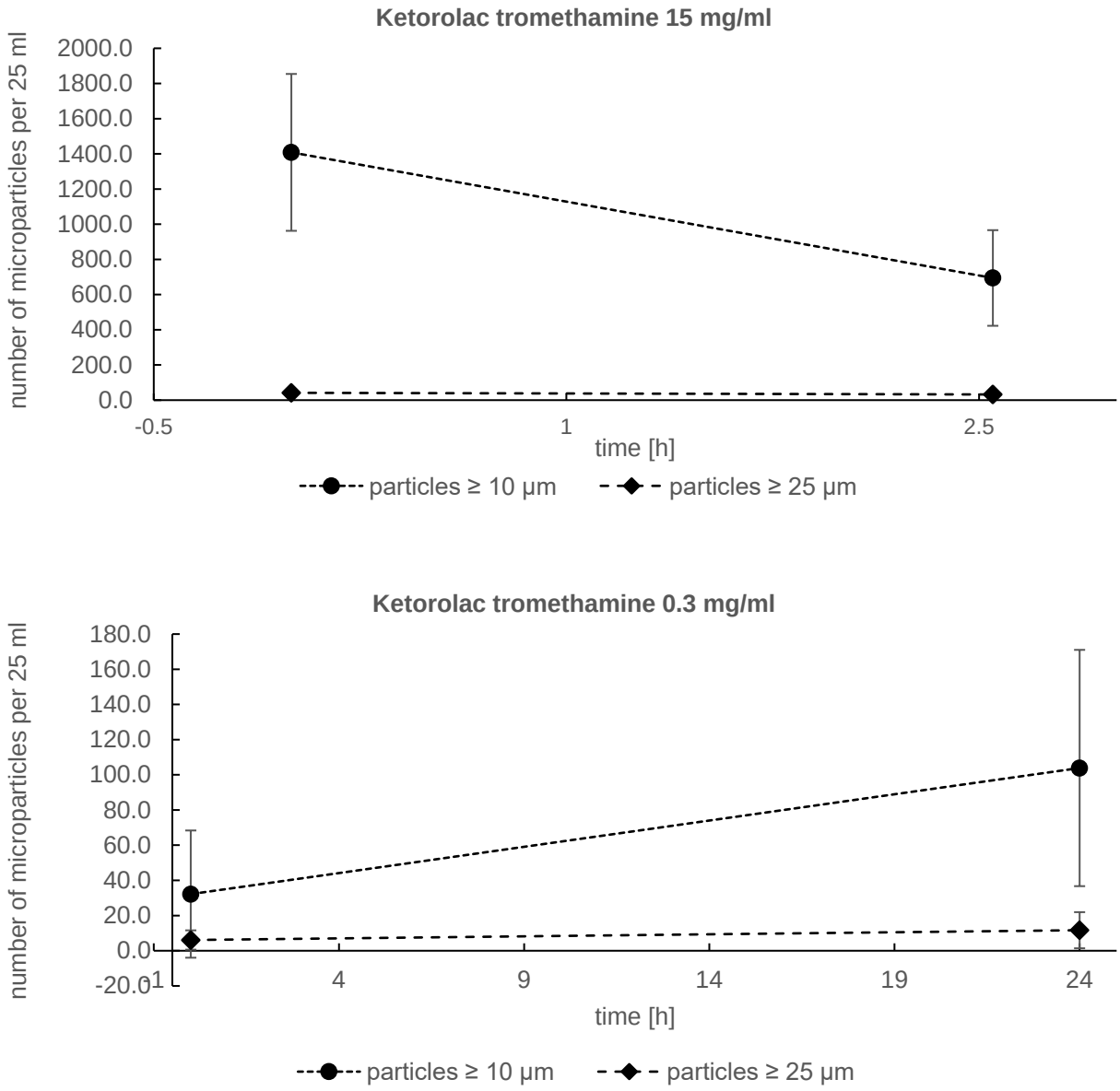


Figure 14. Change in number of particles ≥ 10 and ≥ 25 µm in diameter of ketorolac tromethamine in RA. High-dose samples (15 mg/ml) were tested at t_0 (immediately after preparation) and after 2.55 h, low-dose samples (0.3 mg/ml) at t_0 and after 24 h ($n = 3$, $p > 0.05$).

5. Discussion

In this study, the Y-site compatibility of five different DSs with RA was assessed by using RP-HPLC-UV/VIS for the analysis of chemical stability, and light obscuration particle counting for the examination of sub-visible particle formation, resulting from compound precipitation. The DSs - piperacillin sodium, tazobactam, benzylpenicillin sodium, meropenem trihydrate and ketorolac tromethamine - were selected because of their frequent use in the surgical ward of the KSB. In the surgical ward, RA is extensively used as basic infusion or volume replacement over prolonged durations. All DSs are typically administered as infusions lasting between 15 to 30 minutes, resulting in relatively brief contact times with RA in the joint infusion line. Given the continuous flow, the estimated contact time during Y-site co-administration ranges between 15 and maximum 60 minutes, even at low infusion rate (15, 36, 71). Consequently, stability was assessed for a maximum of 2.5 hours. Only low-dose ketorolac tromethamine (0.3 mg/ml) was tested over 24 hours to simulate prolonged infusion time with RA as the carrier solution.

To assess chemical compatibility, a multianalyte RP-HPLC-UV/VIS method was developed and validated in terms of linearity, accuracy, precision, and stability in accordance with the ICH Q2 R1 guideline of the EMA. Due to inadequate resolution ($R_s < 1.5$) between benzylpenicillin, furosemide and ketorolac, as well as stability issues observed between benzylpenicillin, ciprofloxacin, and ketorolac, the multianalyte method was subsequently restricted to meropenem, piperacillin, tazobactam, and furosemide. Benzylpenicillin and ketorolac were quantified separately using a single-point calibration. DPs were considered chemically stable if their final DS concentrations remained above or equal to 95% of the initial concentration. This limit is in accordance with the EMA ICH Q1A guideline on long-term stability studies of authorized drug products. None of the tested DP-solutions fell below this threshold. To enhance plausibility, the results were further assessed in relation to the content specifications for pharmaceutical preparations outlined in the Pharmacopoea Helvetica, where a minimum of 90% and a maximum of 110% of the declared DS content is acceptable. Most samples fell within or in close proximity to these limits. However, challenges were encountered with low-dose benzylpenicillin, as complete removal of the dissolved drug from the original vial was technically unfeasible, resulting in a relative content of less than 90%. The issue with meropenem is more complex. Its concentration relative to the nominal value was exceptionally high in both the test and control solutions. Although the peak area of the reference standard meropenem did not decrease over time and no additional peaks were observed during method validation, it is plausible that the analyte's stability was compromised when the DS assay measurements commenced. In such a scenario, the regression analysis equation calculated during method validation would have been based on a lower reference standard concentration than assumed for the stability evaluation. This would also explain the larger deviation from the

nominal concentration when measurements were repeated three months later by a laboratory assistant, presumably due to further degradation of the reference standard.

According to the general EP monograph on parenteralia, formulations must conform with the test on sub-visible particles (72). Consequently, hospital pharmacies engaged in small-scale industrial production of parenteral preparations are usually equipped with a light-obscuration particle counter. The method is characterized by simplicity and speed, demanding minimal space for instrumentation, and the quality of the results is independent of the analyst's visual acuity. Therefore, it stands as a reasonable analytical method for assessing compatibility in terms of precipitation, although other methods such as turbidimetry or nephelometry are more frequently used (37, 39-43). Pharmaceutical samples were considered physically compatible if they met the acceptance criteria outlined in chapter 2.9.19 of the EP method 1 B for volumes less than 100 ml. This method allows a maximum of 6000 particles equal to or greater than 10 μm in diameter, and a maximum of 600 particles equal to or greater than 25 μm in diameter per container. All test samples met these criteria for the used DS concentrations, environmental conditions (20 - 25 °C, day light), and the duration of 2.5 hours (24 hours for ketorolac tromethamine 0.3 mg/ml). Surprisingly, particle counts were higher immediately after sample preparation than after 2.5 hours, possibly due to the presence of air bubbles resulting from the various manipulations for the preparation of the DP solutions. The sub-visible particle test is not typically applied to samples that have undergone such diverse manipulations just prior to testing. The use of an ultrasonic bath did not significantly reduce the presence of air bubbles.

Based on the results of both the HPLC and particle counting tests, it is inferred that Piperacillin/Tazobactam Sandoz®, Penicillin "Grünenthal"®, and Tora-dol® can be concurrently administered as infusions through a Y-site with RA, adhering to the specified concentrations, carrier solutions, and environmental conditions, as outlined in Table 7. Tora-dol® 0.3 mg/ml may even be administered as a continuous infusion using RA as the carrier solution (i.e., admixture). No precipitation or changes in the DS-content should be expected. Furthermore, no discernible alterations in pH over time were detected in any of the DP-samples when compared to the control solutions. This eliminates concerns about instabilities caused by pH fluctuations. Although Meronem® may also be deemed compatible under the given conditions, the observed deviation from its nominal concentration renders the data inconclusive. Therefore, it is advisable to replicate the experiments using an alternative technique with confirmed stability of the reference substance during the entire measurement period.

Table 7. Summary of the compatibility of the tested drug substances in Ringer's acetate.

Drug product (drug substance)	**Concentration of drug substance [mg/ml]**	**Chemically stable in RA**	**Physically compatible with RA**	**Y-site co-administration possible**
Meronem® (meropenem) in NaCl 0.9%	10	(✓)	✓	(×)
	20	(✓)	✓	(×)
Piperacillin/ Tazobactam Sandoz® (piperacillin, tazobactam) in NaCl 0.9%	225	✓	✓	✓
	90	✓	✓	✓
Penicillin "Grünenthal"® (benzylpenicillin sodium) in dextrose 5%	60	✓	✓	✓
	24	✓	✓	✓
Tora-dol® (ketorolac tromethamine) in NaCl 0.9%	30	✓	✓	✓
	0.3	✓	✓	✓✓

Note: study period was 2.5 hours (24 hours in the case of Tora-dol® 0.3 mg/ml). Samples were stored at room temperature (20 - 25 °C) and kept under normal day light.

Chemically stable: no significant decline in drug concentration of more than 5% was observed.

Physically compatible: maximum sub-visible particle counts according to chapter 2.9.19 of the European Pharmacopeia (method 1B) were met.

RA: Ringer's acetate

✓ yes
(✓) no (data inconclusive)
(×) not recommended, further studies required
✓✓ both Y-site co-administration and continuous infusion as admixture possible

The Y-site compatibility of morphine sulphate with RA could not be tested due to unsuccessful separation of the reference substance, morphine hydrochloride, using the selected reversed-phase columns and mobile phases. Since morphine hydrochloride is a markedly polar substance, it is hypothesized that it immediately escaped from the stationary phase following injection. Compatibility data with RA is still of interest due to the high demand for patient-controlled analgesia (PCA) with morphine sulphate at the KSB. As both RA and morphine sulphate are continuously infused for 24 hours, simultaneous infusion at the Y-site using a single venous access would enhance patient's comfort and safety. RP-HPLC assays have been successfully reported and detailed in both the EP and United States Pharmacopeia (USP) (73-75). Therefore, by using a different column, mobile phase, or optimized gradient, it should be possible to quantify the DS. A multi-analysis, preferably including other structurally similar DSs found in PCA devices, such as pethidine, hydromorphone, and methadone, would be preferable from an economic standpoint.

The findings of this study are considered reliable in comparison to similar investigations, despite certain discrepancies. Notably, according to the manufacturer's specifications for RA (Fresenius), piperacillin/tazobactam should be stable in RA solely at low concentrations. At elevated concentrations, either a physical incompatibility or a chemical instability may arise after four hours at room temperature (58). The available data do not conclusively identify whether the reported instability is of physical or chemical nature. Furthermore, all generic manufacturers of piperacillin/tazobactam, including Sandoz, specify in their product documentation that the DP is incompatible with RL. Conversely, the Tazobac® formulation from Pfizer, which contains edetate disodium dehydrate (EDTA) as chelating agent and sodium citrate as a buffer agent appear to be stable in various Ringer's solutions, including lactated and acetated Ringer's (76). It has been shown that piperacillin forms a less soluble dimer in the presence of metal ions, such as Ca^{2+} in Ringer's solutions. Although the reaction rate is unknown, it appears to be enhanced in acidic solutions (76). It is possible that the reaction rate is sufficiently slow to permit Y-site short infusions with RA without showing any relevant instability.

Meropenem solutions appear to be generally unstable, at least in NaCl 0.9%, D5, and Ringer's (77-79). Patel et al. tested the stability of meropenem 1 mg/ml and 20 mg/ml in Ringer's solution and lactated Ringer's solution using HPLC analysis (77). The decrease in concentration was less than 5% after four hours at room temperature but raised to 7% (in Ringer's solution) and 8.5% (in lactated Ringer's solution) of the initial concentration after eight hours. No data are available for RA. The Meronem® product data sheet recommends NaCl 0.9% or D5 as carrier solutions and does not mention any specific incompatibilities with other solutions or drugs (80). The combination has also been recently tested by Fresenius. It appears to be chemically stable at concentrations between 0.5 and 5 mg/ml for 24 h at room temperature (59). Additionally, the pH of RA should not pose a problem, since meropenem has its pH optimum at approx. 6 (79, 81) In conclusion, although further experiments are needed for high concentrated meropenem, it is likely that it can be co-administered with RA.

No compatibility data for Ringer's solution and benzylpenicillin is available on the Micromedex database (82). Fresenius reports that Penicillin "Grünenthal"® is both chemically and physically stable in RA at low concentrations for 24 hours at room temperature (59). The Swissmedic data sheet for the same product does not recommend further dilution with infusion solutions other than D5 (83). Benzylpenicillin solutions demonstrate the highest stability in the pH range of 6 to 7, exhibiting instability below pH 5 or above pH 8 (83). Thus, the stability of Penicillin "Grünenthal"® in RA was anticipated and confirmed by our data.

According to Floy B. et al., ketorolac tromethamine is chemically stable and physically compatible with Ringer's solution and lactated Ringer's solution for 48 hours at room

temperature, at test concentrations of 0.06 and 0.6 mg/ml (84). Fresenius conducted stability testing in RA at a similar concentration for 24 h at room temperature. According to their findings, ketorolac tromethamine is both physically and chemically stable (59). Our data further support its stability even at higher concentrations. The pH range of the ketorolac tromethamine solution is 5.7 to 6.7, with the pH optimum specified in market products lying between 6.9 and 7.9, when adjusted with sodium hydroxide and hydrochloric acid (85, 86). Therefore, the pH of RA should not present a concern.

While this study provides important information on the combined use of DPs in RA, the reported findings have some limitations. First of all, the degree of coloration of the samples was not investigated, although a change in coloration over time might also indicate an incompatibility reaction (87). Both the EP and USP describe an instrumental and visual assessment in chapters 2.2.2 and 631, respectively. In addition, the statistical significance is weakened by the fact that only three replicates per DP and concentration were measured. The reliability of the data should be strengthened through multiple assessments involving other laboratories and the blinding of the samples. Moreover, there were problems regarding the stability of certain reference standards in the multianalyte solution. These could have been avoided by conducting a more comprehensive stability study either prior to or concurrently with method validation. Multianalyte analysis is a reasonable approach, as it saves costs and other resources. It has already been successfully applied to some of the DSs tested in this thesis, namely the combination of piperacillin and tazobactam (88, 89), of piperacillin and meropenem (90), of piperacillin, tazobactam and meropenem (91), and of benzylpenicillin, piperacillin and meropenem (92). These molecules share a similar structure, thereby simplifying method development. In this regard, the instability of benzylpenicillin in the presence of ciprofloxacin could have been anticipated considering their distant pH optima (3.5 - 4.6 versus 6.8) (83, 93). The instability of benzylpenicillin in the presence of ketorolac tromethamine, on the other hand, was less predictable. It is assumed that tromethamine accelerates the hydrolysis of benzylpenicillin (94). Additionally, it has not been demonstrated whether or not the used HPLC method is stability-indicating, i.e., that it can effectively differentiate and separate the intact drug molecule from its degradation products. This should be tested by either spiking the drug reference standard with impurities or by inducing degradation through exposure to extreme pH values or heating (36, 51, 95). Moreover, the HPLC run time of 35 minutes is relatively long, consuming a significant amount of time for the analysis. A shorter run time of up to 10 minutes would be preferable, which could potentially be achieved with the use of ultra-high-performance liquid chromatography (UHPLC) (96). Finally, the compatibility data apply only to the commercial products used in this study. Extrapolation to products from other manufacturers should be feasible provided that the compositions are identical; otherwise such extrapolation is not recommended, because the presence of different salts, excipients, or solvents may

greatly affect the DS's stability. This also applies to the use of other carrier solutions. In this study, only one carrier solution (either NaCl 0.9% or D5) was used per product to determine compatibility. An extension to other carrier solutions would be beneficial.

6. Conclusions and outlook

The present thesis addressed missing or inconclusive Y-site compatibility data of different DP-solutions with RA infusion from Fresenius, focusing on selected antibiotics and analgetics. Chemical stability of Meronem®, Penicillin "Grünenthal"®, Tora-dol®, and Piperacillin/Tazobactam Sandoz® was assessed by quantitative analysis using a multianalyte HPLC method, which was validated in accordance with the ICH guidelines on validation of analytical procedures. Compatibility was additionally investigated in terms of formation of precipitates by detection of sub-visible particles through light obscuration. The results of both test methods indicate that the following DP-solutions can be safely co-administered via Y-site with RA for up to 2.5 h: Penicillin "Grünenthal"® at maximum concentration of 60 mg/ml, in D5, Piperacillin/Tazobactam Sandoz® at maximum concentration of 225 mg/ml, in NaCl 0.9%, and Tora-dol® 30 mg/ml, undiluted. Additionally, Tora-dol® proved to be chemically and physically stable in RA for up to 24 h at maximum concentration of 0.3 mg/ml, hence confirming prolonged compatibility enabling continuous infusion within the RA infusion bag (i.e., admixture). All results were obtained at ambient temperature (20 - 25 °C) under daylight. For Meronem®, the data is inconclusive and further investigations are necessary to justify co-administration. The data obtained from this project has been integrated into the KSB pharmacy's list of RA compatibility, a document frequently consulted by nurses. It assists both doctors and nurses in planning infusion regimes and contributes to a safe and effective intravenous drug administration.

The analytical methods presented here may provide other hospital pharmacies a foundation for future compatibility studies. Both the HPLC analysis and measurement of sub-visible particles through light obscuration are methods and equipment commonly used in larger hospital pharmacies, as they are required for the release of substances and of pharmaceutical preparations. Hence, these methods provide a reasonable approach to study compatibility issues. Multianalyte HPLC analysis is a particularly interesting practice that should be pursued further, as it may safe time, cost, and resources. Currently, there is a lack of guidelines on how to conduct Y-site compatibility studies, which is reflected in the heterogeneity of published data. The adoption of a standardised approach and presentation of data would be highly desirable. In addition, nationwide cooperation and coordination among hospital pharmacies in data acquisition and presentation would enhance both the quality and quantity of the data,

thereby further improving medication safety. Ultimately, proficiency in this area is an indispensable skill for all pharmacists.

In addition, it would be interesting to analyse the incidence of medication errors in Swiss healthcare institutions due to the simultaneous infusion or mixing of incompatible or potentially incompatible drug solutions. An examination of the clinical consequences of these medication errors, such as the need to stop an infusion due to precipitation in the catheter, would provide valuable insights. Furthermore, investigating whether healthcare facilities using the services of hospital pharmacists have a lower incidence of such errors would contribute to our awareness of the role of hospital pharmacists in reducing such errors.

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8. Appendix

Table A1. Overview of the different ringer's solutions on the Swiss market.

Name	pH	Theoretical osmolality [mosmol/l]	Na+ [mmol/l]	K+ [mmol/l]	Ca++ [mmol/l]	Mg++ [mmol/l]	Cl- [mmol/l]	Actetat- [mmol/l]	Lactat- [mmol/l]	Carbonat- [mmol/l]	Malat 2- [mmol/l]	Gluconat- [mmol/l]
Blood plasma	7.4	291	142.00	4.50	2.50	1.25	103.00	-	1.5	24.00	-	-
Plasma Lyte A Baxter	6.5 – 8.0	295	140.10	4.96	0.00	1.48	97.93	27.05	0.00	0.00	0.00	23.01
Ringeracetat Bichsel	5.0 - 7.0	291	137.00	4.00	1.65	1.25	110.00	36.80	0.00	0.00	0.00	0.00
Ringeracetat Fresenius	5.0 - 7.0	291	137.00	4.00	1.65	1.25	110.00	36.80	0.00	0.00	0.00	0.00

Name	pH	Theoretical osmolality [mosmol/l]	Na+ [mmol/l]	K+ [mmol/l]	Ca++ [mmol/l]	Mg++ [mmol/l]	Cl- [mmol/l]	Actetat- [mmol/l]	Lactat- [mmol/l]	Carbonat- [mmol/l]	Malat 2- [mmol/l]	Gluconat- [mmol/l]
Ringerfundin B. Braun	5.1 - 5.9	309	145.00	4.00	2.50	1.00	127.00	24.00	0.00	0.00	5.00	0.00
Ringerlactat Bichsel	k. A.	276	131.00	4.00	1.50	0.00	110.00	0.00	28.00	0.00	0.00	0.00
Ringerlactat Bichsel mit Dextrose 1 % / 2 % / 5 %	k. A.	330 / 384 / 550	131.00 / 130.00 / 130.00	4.00	1.50	0.00	110.00 / 109.00 / 109.00	0.00	28.00	0.00	0.00	0.00
Ringerlactat Bioren	k. A.	278	131.00	5.40	1.80	0.00	111.60	0.00	28.40	0.00	0.00	0.00
Ringerlactat kaliumfrei Bioren	k. A.	288	140.00	0.00	1.875	0.75	104.25	0.00	41.00	0.00	0.00	0.00
Ringerlactat Fresenius	5.0 - 7.0	278	130.90	5.40	1.84	0.00	111.70	0.00	28.30	0.00	0.00	0.00

Name	pH	Theoretical osmolality [mosmol/l]	Na+ [mmol/l]	K+ [mmol/l]	Ca++ [mmol/l]	Mg++ [mmol/l]	Cl- [mmol/l]	Actetat- [mmol/l]	Lactat- [mmol/l]	Carbonat- [mmol/l]	Malat 2- [mmol/l]	Gluconat- [mmol/l]
Ringerlactat B. Braun (nach Hartmann)	5.5 - 7.0	277	131.00	5.40	1.80	0.00	112.00	0.00	28.00	0.00	0.00	0.00
Ringerlactat kaliumfrei B. Braun	4.5 - 7.0	288	140.00	0.00	1.75	0.75	105.00	0.00	40.00	0.00	0.00	0.00
Ringer B. Braun (ohne Carbonat)	5.0 - 7.0	309	147.20	4.00	2.20	0.00	155.70	0.00	0.00	0.00	0.00	0.00
Ringer-Lösung Bichsel	k. A.	327	155.20	4.00	2.70	0.00	163.40	0.00	0.00	1.20	0.00	0.00

Table A2. Overview of applied gradients during method development for HPLC analysis.

Gradient 5			Gradient 6			Gradient 7			Gradient 8		
t (min)	%ACN	%H_2O	t (min)	%ACN	%H_2O	t (min)	%ACN	%H_2O	t (min)	%ACN	%H_2O
0.0	5	95	0.0	5	95	0.0	2	98	0.0	2	98
2.0	5	95	2.0	5	95	2.0	2	98	4.0	2	98
20.0	95	5	20.0	95	5	20.0	95	5	25.0	95	5
23.0	95	5	24.4	95	5	23.0	95	5	29.0	95	5
23.1	5	95	25.0	5	95	23.1	2	98	29.5	2	98
26.0	5	95	30.0	5	95	26.0	2	98	35.0	2	98

Note: both ACN and H_2O contained 0.1% formic acid.

ACN: acetonitrile

Table A3. Retention times [min] of the reference substances at different gradients, flow rates and temperatures on the column Zorbax Eclipse plus C18.

Zorbax Eclipse plus C18						
	Gradient 5		Gradient 7			
	1.0 ml/min 30°C	1.0 ml/min 40°C	1.0 ml/min 25°C	1.0 ml/min 30°C	1.0 ml/min 40°C	0.6 ml/min 40°C
Benzylpenicillin sodium	11.0	10.6	11.3	11.2	11.0	13.2
Ciprofloxacin	7.2	6.8	7.8	7.7	7.5	1.4 (?)
Furosemide	11.3	10.7	11.7	11.5	11.2	13.3
Ketorolac tromethamine	11.8	11.4	12.1	12.0	11.8	14.1
Meropenem	6.1	5.1	6.8	6.7	6.6	1.4 (?)
Morphine hydrochloride	1.5	1.2	2.5	2.2	1.8	1.4 (?)
Piperacillin sodium	Not tested	Not tested	Not tested	Not tested	Not tested	12.8
Tazobactam	2.5	1.9	4.5	3.9	3.1	1.5 (?)

Note: As piperacillin was not available from the beginning, some data is missing. For some peaks (marked with a question mark), the signal could not be clearly assigned, irrespective of the wavelength of the detector.

Table A4. Retention times [min] of the reference substances at different gradients, flow rates and temperatures on the column Phenomex Luna C18.

Phenomenex Luna C18				
	Gradient 6	Gradient 7		Gradient 8
	1.0 ml/min 30°C	0.6 ml/min 30°C	0.6 ml/min 40°C	0.6 ml/min 30°C
Benzylpenicillin sodium	15.5	19.8	19.6	23.3
Ciprofloxacin	10.4	5.8 (?)	5.9 (?)	16.9
Furosemide	15.7	20.0	19.5	23.4
Ketorolac tromethamine	16.3	20.7	20.5	24.3
Meropenem	9.3	5.9 (?)	5.7 (?)	15.7
Morphine hydrochloride	3.5	5.9 (?)	5.5 (?)	6.2 or 14.5 (two signals)
Piperacillin sodium	Not tested	18.8	18.7	22.1
Tazobactam	9.3	14.0	13.7	16.9

Note: As piperacillin was not available from the beginning, some data is missing. For some peaks (marked with a question mark), the signal could not be clearly assigned, irrespective of the wavelength of the detector.

Table A5. Comparison of the retention times and peak areas of the drug reference standards in the mix and as single reference standards on the column Phenomex Luna C18 with gradient 8.

	Single substance		Mix	
	Retention time [min]	**Peak area [mAU*min]**	**Retention time [min]**	**Peak area [mAU*min]**
Benzylpenicillin sodium	23.3	23'065'313	23.3	n/d [a]
Ciprofloxacin	16.9	15'620'901	16.8 [b]	n/d [b]
Furosemide	23.4	42'639'113	23.4	n/d [c]
Ketorolac tromethamine	24.3	19'487'153	24.3	19'130'633
Meropenem	15.8	11'692'168	15.8	12'669'122
Morphine hydrochloride	6.2 and 14.5	35'244'008 4'982'046	6.2 and 14.6	36'018'671 6'283'431
Piperacillin sodium	22.1	22'892'383	22.1	22'053'448
Tazobactam	16.9	14'737'000	16.8[b]	n/d [b]

Note: Oven temperature was set to 30 °C. The concentration was 0.1 mg/ml for all analytes.

n/d = not determinable

[a] double peak with furosemide

[b] peak of ciprofloxacin is below peak of tazobactam

[c] double peak with benzylpenicillin

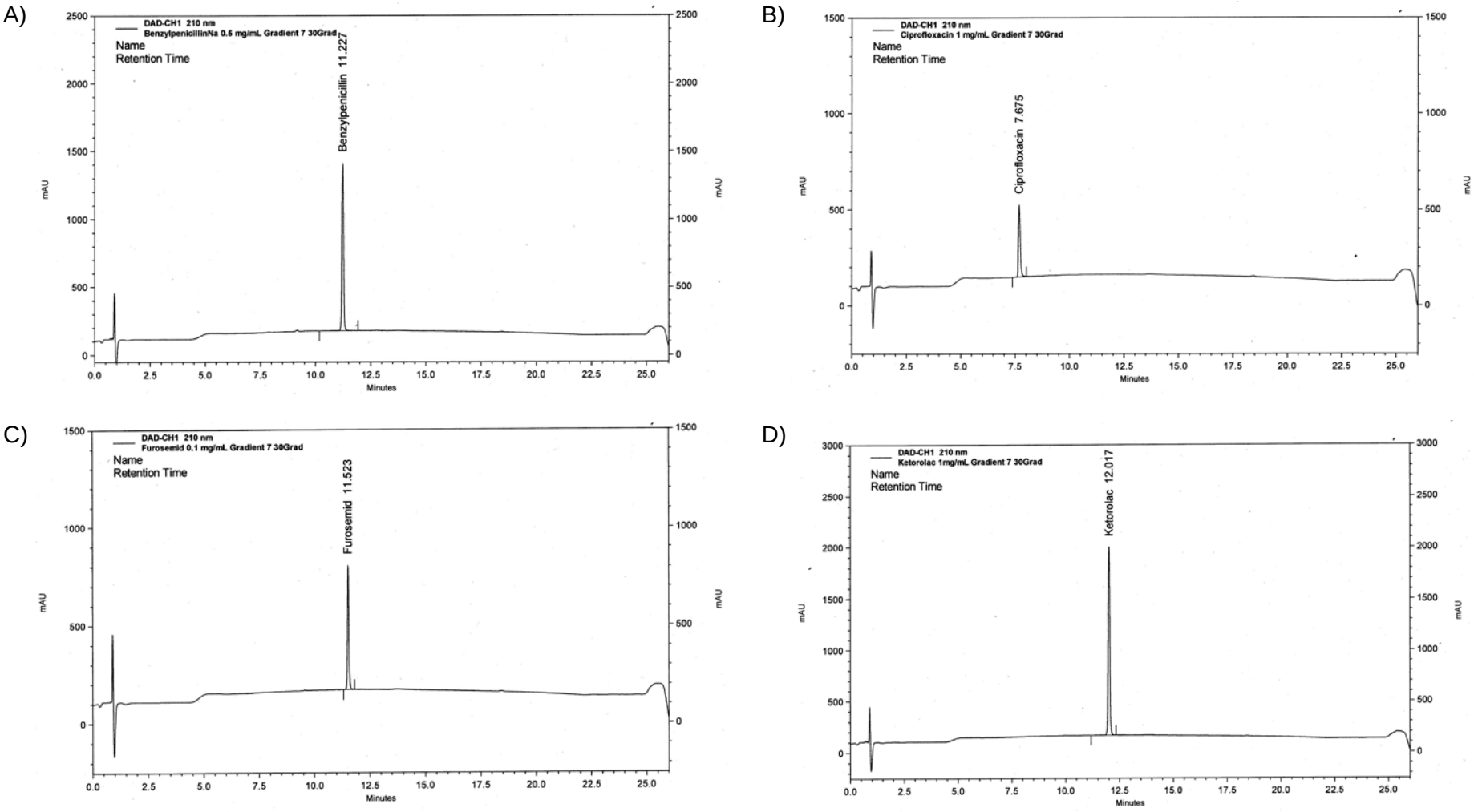


Figure A1. Chromatograms at 210 nm of A) benzylpenicillin sodium (0.5 mg/ml), B) ciprofloxacin (1 mg/ml), C) furosemide (0.1 mg/ml) and D) ketorolac tromethamine (1 mg/ml) on the Zorbax Eclipse Plus C18 with gradient 7. Flow rate 1.0 ml/min, oven temperature 30 °C, injection volume 10 µl.

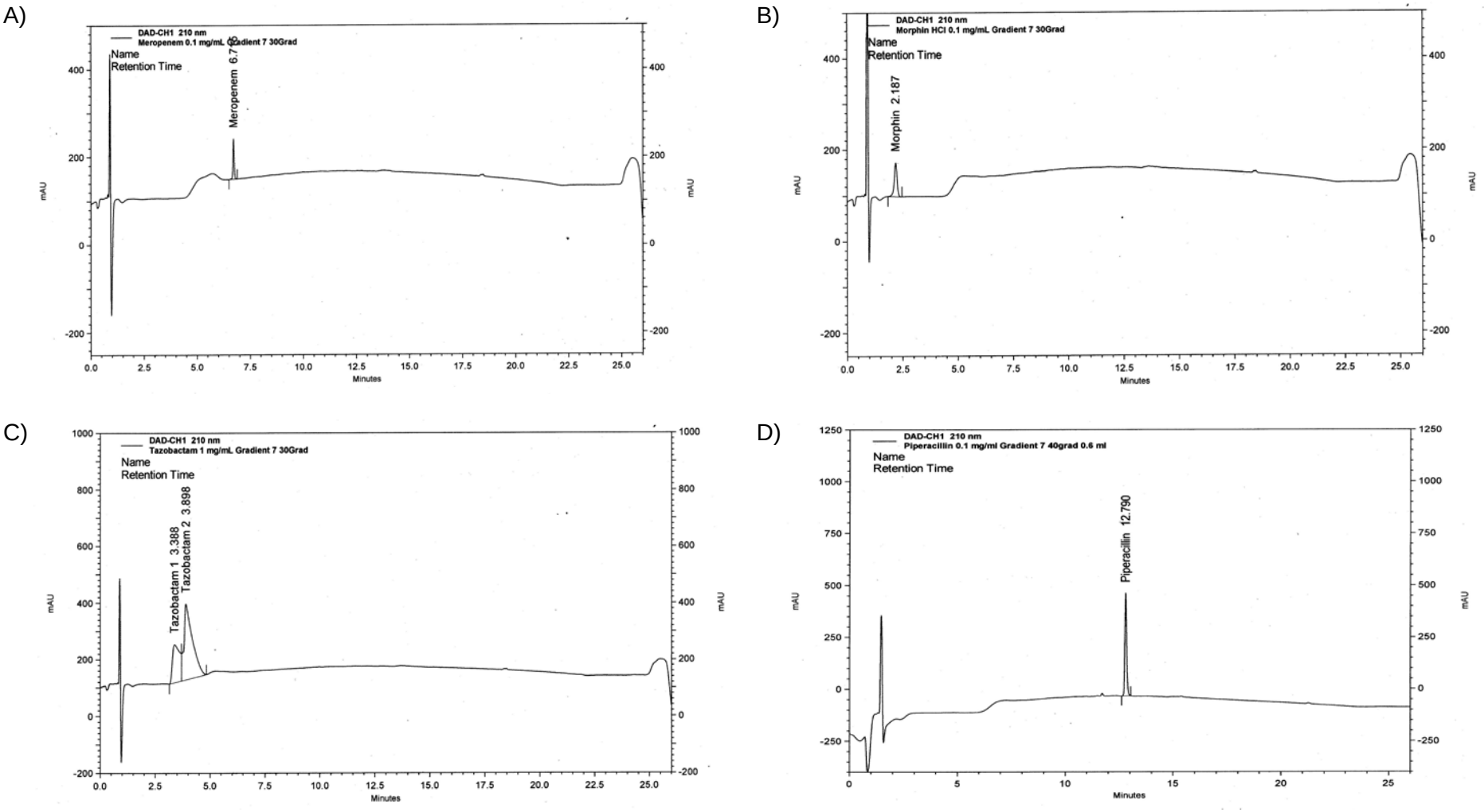


Figure A2. Chromatograms at 210 nm of A) meropenem trihydrate (0.1 mg/ml), B) morphine HCl (0.1 mg/ml), C) tazobactam (1 mg/ml) and D) piperacillin sodium (0.1 mg/ml) on the Zorbax Eclipse Plus C18 with gradient 7. Flow rate 1.0 ml/min (0.6 ml/min for piperacillin sodium), oven temperature 30 °C (40 °C for piperacillin sodium), injection volume 10 µl.

Table A6.1. Back-calculated concentrations of the calibration standards and quality control samples of **meropenem trihydrate** at different days, using 1/x-weighted least square linear regression analysis.

		Calibration standards				Quality control samples			
		K1	**K2**	**K3**	**K4**	**QC high, sample 1**	**QC high, sample 2**	**QC low, sample 1**	**QC low, sample 2**
	nominal concentration [mg/ml]:	**0.3**	**0.15**	**0.03**	**0.01**	**0.25**	**0.25**	**0.0125**	**0.0125**
back-calculated concentration [mg/ml]	day 1	0.2995	0.1511	0.0292	0.0102	0.2542	0.2545	0.0121	0.0122
	day 2	0.3171	0.1361	0.0246	0.0121	0.2411	0.2749	0.0143	0.0154
	day 3	0.3084	0.1432	0.0274	0.0110	0.2633	0.2259	0.0139	n/d [a]
	day 4	0.3158	0.1390	0.0224	0.0127	0.2727	0.2718	0.0141	0.0134
	day 5	0.3229	0.1279	0.0278	0.0115	0.2809	0.2766	0.0131	0.0110
	day 6	0.3029	0.1489	0.0274	0.0109	0.2850	0.2915	0.0124	0.0116
	day 7	0.3235	0.1234	0.0331	0.0100	0.2740	0.2771	0.0099	0.0130
	day 8	0.3304	0.1241	0.0223	0.0133	0.2446	0.2558	0.0141	0.0167
	mean ± SD [mg/ml]	0.3151 ± 0.0100	0.1367 ± 0.0101	0.0268 ± 0.0034	0.0115 ± 0.0011	0.2653 ± 0.0146 [b]		0.0132 ± 0.0014 [b]	

[a] not detectable due to missing vial

[b] interday precision

Table A6.2. Weighted (1/x) least square linear regression analysis (y = mx + b) of **meropenem trihydrate** calibration standards.

validation day:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
y intercept (b)	-41498	-219806	-146071	-211458	-219955	-102197	-71917	-285484
slope (m)	51594930	48607144	50819557	49350514	48705929	45590779	48393275	46972519
R^2	1.00	0.99	1.00	1.00	0.99	1.00	0.98	0.98

Table A6.3. Back-calculated content relative to the nominal value of the calibration standards and quality control samples of **meropenem trihydrate** at different days, using 1/x-weighted linear regression analysis.

		Calibration standards				Quality control samples			
		K1	**K2**	**K3**	**K4**	**QC high, sample 1**	**QC high, sample 2**	**QC low, sample 1**	**QC low, sample 2**
	Nominal concentration [mg/ml] :	**0.3**	**0.15**	**0.03**	**0.01**	**0.25**	**0.25**	**0.0125**	**0.0125**
Content relative to nominal value [%]	day 1	99.8	100.7	97.5	102.0	101.7	101.8	96.7	97.8
	day 2	105.7	90.8	82.1	121.4	96.4	110.0	114.6	123.2
	day 3	102.8	95.4	91.3	110.5	105.3	90.4	111.4	n/d [a]
	day 4	105.3	92.7	74.7	127.4	109.1	108.7	112.5	107.5
	day 5	107.6	85.3	92.5	114.6	112.4	110.7	105.0	87.7
	day 6	101.0	99.2	91.2	108.6	114.0	116.6	99.6	93.1
	day 7	107.8	82.3	110.4	99.5	109.6	110.9	79.6	104.4
	day 8	110.1	82.7	74.2	133.0	110.5	108.7	108.6	89.7
	mean ± SD [mg/ml]	105.0 ± 3.3	91.1 ± 6.7	89.2 ± 11.3	114.6 ± 11.1	107.3 ± 5.4 [b]		102.7 ± 8.4 [b]	

[a] not detectable due to missing vial

[b] interday precision

Table A7.1. Back-calculated concentrations of the calibration standards and quality control samples of **tazobactam** at different days, using 1/x-weighted least square linear regression analysis.

		Calibration standards				Quality control samples			
		K1	**K2**	**K3**	**K4**	**QC high, sample 1**	**QC high, sample 2**	**QC low, sample 1**	**QC low, sample 2**
	nominal concentration [mg/ml]:	**0.3**	**0.15**	**0.03**	**0.01**	**0.25**	**0.25**	**0.0125**	**0.0125**
back-calculated concentration [mg/ml]	day 1	0.2986	0.1508	0.0308	0.0097	0.2516	0.2505	0.0115	0.0116
	day 2	0.3127	0.1391	0.0268	0.0114	0.2413	0.2723	0.0137	0.0146
	day 3	0.3086	0.1421	0.0285	0.0107	0.2601	0.2269	0.0122	n/d [a]
	day 4	0.3155	0.1377	0.0246	0.0121	0.2727	0.2783	0.0136	0.0132
	day 5	0.3226	0.1279	0.0282	0.0113	0.2819	0.2784	0.0133	0.0106
	day 6	0.3050	0.1465	0.0277	0.0108	0.2817	0.2891	0.0132	0.0127
	day 7	0.3236	0.1233	0.0331	0.0099	0.2698	0.2763	0.0109	0.0139
	day 8	0.3280	0.1260	0.0231	0.0130	0.2431	0.2490	0.0153	0.0167
	mean ± SD [mg/ml]	0.3143 ± 0.0094	0.1367 ± 0.0094	0.0279 ± 0.0030	0.0111 ± 0.0010	0.2639 ± 0.0154 [b]		0.0131 ± 0.0013 [b]	

[a] not detectable due to missing vial

[b] interday precision

Table A7.2. Weighted (1/x) least square linear regression analysis (y = mx + b) of **tazobactam** calibration standards.

validation day:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
y intercept (b)	-2987	-189601	-133700	-184797	-258593	-126885	-53524	-306246
slope (m)	69162594	65755016	68128912	65979551	64252590	61485238	66267332	65588758
R^2	1.00	1.00	1.00	1.00	0.99	1.00	0.98	0.98

Table A7.3. Back-calculated content relative to the nominal value of the calibration standards and quality control samples of **tazobactam** at different days, using 1/x-weighted least square linear regression analysis.

		Calibration standards				Quality control samples			
		K1	**K2**	**K3**	**K4**	**QC high, sample 1**	**QC high, sample 2**	**QC low, sample 1**	**QC low, sample 2**
	Nominal concentration [mg/ml] :	**0.3**	**0.15**	**0.03**	**0.01**	**0.25**	**0.25**	**0.0125**	**0.0125**
Content relative to nominal value [%]	day 1	99.5	100.6	102.8	97.1	100.6	100.2	91.8	92.8
	day 2	104.2	92.8	89.4	113.6	96.5	108.9	109.4	116.4
	day 3	102.9	94.8	95.0	107.3	104.1	90.7	97.9	n/d [a]
	day 4	105.2	91.8	82.1	120.9	109.1	111.3	108.5	105.4
	day 5	107.5	85.3	93.9	113.3	112.8	111.4	106.7	84.8
	day 6	101.7	97.7	92.2	108.5	112.7	115.6	105.7	101.5
	day 7	107.9	82.2	110.5	99.5	107.9	110.5	86.9	111.4
	day 8	109.3	84.0	76.9	129.8	97.2	99.6	122.2	133.5
	mean ± SD [mg/ml]	104.8 ± 3.1	91.1 ± 6.3	92.9 ± 10.0	111.2 ± 10.1	105.6 ± 6.2 [b]		104.6 ± 10.7 [b]	

[a] not detectable due to missing vial

[b] interday precision

Table A8.1. Back-calculated concentrations of the calibration standards and quality control samples of **piperacillin sodium** at different days, using 1/x-weighted least square linear regression analysis.

		Calibration standards				Quality control samples			
		K1	**K2**	**K3**	**K4**	**QC high, sample 1**	**QC high, sample 2**	**QC low, sample 1**	**QC low, sample 2**
	nominal concentration [mg/ml]:	**0.3**	**0.15**	**0.03**	**0.01**	**0.25**	**0.25**	**0.0125**	**0.0125**
back-calculated concentration [mg/ml]	day 1	0.2946	0.1545	0.0315	0.0094	0.2605	0.2616	0.0128	0.0124
	day 2	0.3122	0.1398	0.0265	0.0114	0.2434	0.2788	0.0136	0.0152
	day 3	0.3048	0.1459	0.0288	0.0105	0.2686	0.2300	0.0122	n/d [a]
	day 4	0.3128	0.1415	0.0234	0.0123	0.2806	0.2798	0.0142	0.0136
	day 5	0.3203	0.1307	0.0275	0.0114	0.2832	0.2828	0.0142	0.0116
	day 6	0.3015	0.1504	0.0272	0.0108	0.2969	0.2996	0.0139	0.0135
	day 7	0.3192	0.1262	0.0354	0.0091	0.2731	0.2749	0.0111	0.0138
	day 8	0.3286	0.1255	0.0229	0.0131	0.2490	0.2597	0.0154	0.0174
	mean ± SD [mg/ml]	0.3118 ± 0.0104	0.1393 ± 0.0102	0.0279 ± 0.0039	0.0110 ± 0.0013	0.2702 ±0.0155 [b]		0.0136 ±0.0013 [b]	

[a] not detectable due to missing vial

[b] interday precision

Table A8.2. Weighted (1/x) least square linear regression analysis (y = mx + b) of **piperacillin sodium** calibration standards.

validation day:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
y intercept (b)	50727	-257756	-149386	-300251	-413753	-192098	-60341	-464158
slope (m)	96554894	92191633	96523507	93720949	91898158	85633721	94622833	90562030
R^2	1.00	1.00	1.00	1.00	0.99	1.00	0.99	0.98

Table A8.3. Back-calculated content relative to the nominal value of the calibration standards and quality control samples of **piperacillin sodium** at different days, using 1/x-weighted linear regression analysis.

		Calibration standards				Quality control samples			
		K1	**K2**	**K3**	**K4**	**QC high, sample 1**	**QC high, sample 2**	**QC low, sample 1**	**QC low, sample 2**
	Nominal concentration [mg/ml] :	**0.3**	**0.15**	**0.03**	**0.01**	**0.25**	**0.25**	**0.0125**	**0.0125**
Content relative to nominal value [%]	day 1	98.2	103.0	105.0	93.7	104.2	104.7	102.0	98.9
	day 2	104.1	93.2	88.5	114.2	97.4	111.5	108.8	121.2
	day 3	101.6	97.3	96.0	105.1	107.5	92.0	97.5	n/d [a]
	day 4	104.3	94.3	78.0	123.5	112.2	111.9	113.9	108.8
	day 5	106.8	87.1	91.8	114.3	113.3	113.1	113.9	92.8
	day 6	100.5	100.3	90.8	108.4	118.7	119.8	110.8	108.0
	day 7	106.4	84.2	118.2	91.3	109.2	110.0	88.7	110.3
	day 8	109.5	83.7	76.3	130.5	99.6	103.9	123.3	139.3
	mean ± SD [mg/ml]	103.9 ± 3.5	92.9 ± 6.8	93.1 ± 12.8	110.1 ± 12.6	108.1 ± 6.2 [b]		108.5 ± 10.4 [b]	

[a] not detectable due to missing vial

[b] interday precision

Table A9.1. Back-calculated concentrations of the calibration standards and quality control samples of **furosemide** at different days, using 1/x-weighted least square linear regression analysis.

		Calibration standards				Quality control samples			
		K1	**K2**	**K3**	**K4**	**QC high, sample 1**	**QC high, sample 2**	**QC low, sample 1**	**QC low, sample 2**
	nominal concentration [mg/ml]:	**0.2**	**0.1**	**0.02**	**0.007**	**0.16**	**0.16**	**0.008**	**0.008**
back-calculated concentration [mg/ml]	day 1	0.1972	0.1023	0.0209	0.0066	0.1589	0.1583	0.0076	0.0074
	day 2	0.2087	0.0921	0.0184	0.0078	0.1460	0.1707	0.0088	0.0092
	day 3	0.2048	0.0957	0.0191	0.0074	0.1592	0.1399	0.0082	n/d [a]
	day 4	0.2085	0.0938	0.0162	0.0085	0.1690	0.1659	0.0092	0.0089
	day 5	0.2146	0.0856	0.0190	0.0079	0.1808	0.1639	0.0086	0.0069
	day 6	0.2028	0.0987	0.0178	0.0078	0.1755	0.1810	0.0090	0.0083
	day 7	0.2134	0.0838	0.0233	0.0065	0.1787	0.1791	0.0068	0.0086
	day 8	0.2175	0.0849	0.0156	0.0090	0.1554	0.1420	0.0101	0.0109
	mean ± SD [mg/ml]	0.2084 ± 0.0063	0.0921 ± 0.0064	0.0188 ± 0.0023	0.0077 ± 0.0008	0.1640 ± 0.0112 [b]		0.0086 ± 0.0009 [b]	

[a] not detectable due to missing vial

[b] interday precision

Table A9.2. Weighted (1/x) least square linear regression analysis (y = mx + b) of **furosemide** calibration standards.

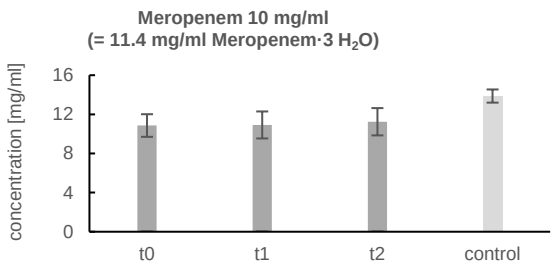
validation day:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
y intercept (b)	44014	-337027	-195945	-369069	-428837	-185987	-77432	-550384
slope (m)	174190478	167349224	170632452	167879105	164390750	147596796	170354236	161195829
R^2	1.00	1.00	1.00	1.00	0.99	1.00	0.98	0.99

Table A9.3. Back-calculated content relative to the nominal value of the calibration standards and quality control samples of **furosemide** at different days, using 1/x-weighted least square linear regression analysis.

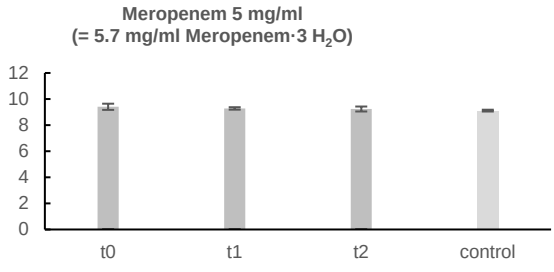
		Calibration standards				Quality control samples			
		K1	**K2**	**K3**	**K4**	**QC high, sample 1**	**QC high, sample 2**	**QC low, sample 1**	**QC low, sample 2**
	Nominal concentration [mg/ml] :	**0.2**	**0.1**	**0.02**	**0.007**	**0.16**	**0.16**	**0.008**	**0.008**
Content relative to nominal value [%]	day 1	98.6	102.3	104.5	94.6	99.3	98.9	95.6	92.9
	day 2	104.4	92.1	91.8	111.7	91.3	106.7	110.2	115.5
	day 3	102.4	95.7	95.6	106.4	99.5	87.5	102.4	n/d [a]
	day 4	104.3	93.8	81.0	120.9	105.6	103.7	114.7	111.3
	day 5	107.3	85.6	94.8	112.3	113.0	102.4	107.1	86.9
	day 6	101.4	98.7	88.8	111.1	109.7	113.1	112.6	104.1
	day 7	106.7	83.8	116.5	92.9	111.7	112.0	84.4	108.0
	day 8	108.8	84.9	78.0	128.4	97.1	88.8	126.0	136.8
	mean ± SD [mg/ml]	104.2 ± 3.1	92.1 ± 6.4	93.9 ± 11.6	109.8 ± 11.2	102.5 ± 7.0 [b]		106.9 ± 11.5 [b]	

[a] not detectable due to missing vial

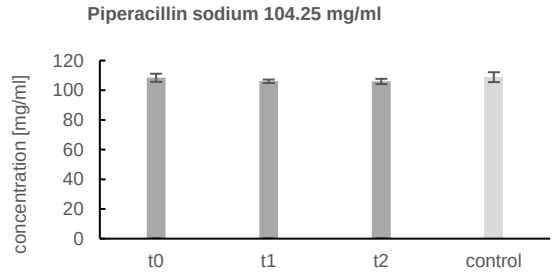
[b] interday precision



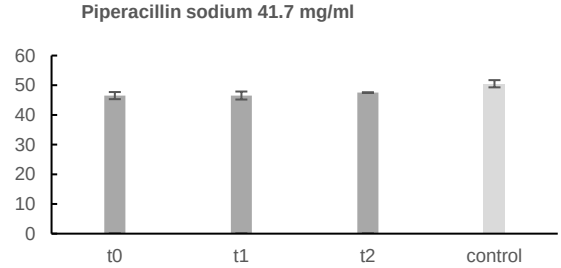
	concentration [mg/ml]
t_0	10.9 ± 1.2
t_1	10.9 ± 1.4
t_2	11.2 ± 1.4
control :	13.9 ± 0.6



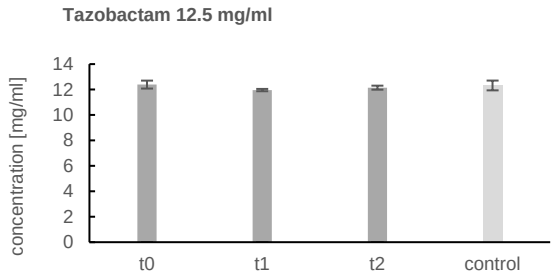
	concentration [mg/ml]
t_0	9.4 ± 0.2
t_1	9.3 ± 0.1
t_2	9.2 ± 0.2
control :	9.1 ± 0.1



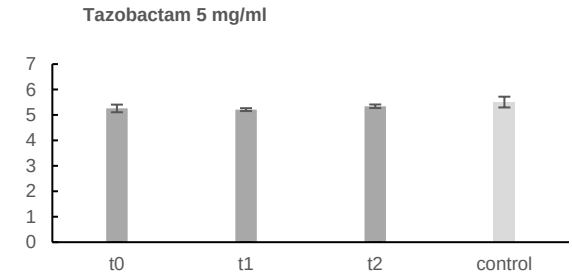
	concentration [mg/ml]
t_0	108.4 ± 2.7
t_1	106.0 ± 1.2
t_2	105.9 ± 1.8
control :	108.8 ± 3.3



	concentration [mg/ml]
t_0	46.5 ± 1.2
t_1	46.5 ± 1.3
t_2	47.5 ± 0.1
control :	48.2 ± 1.2

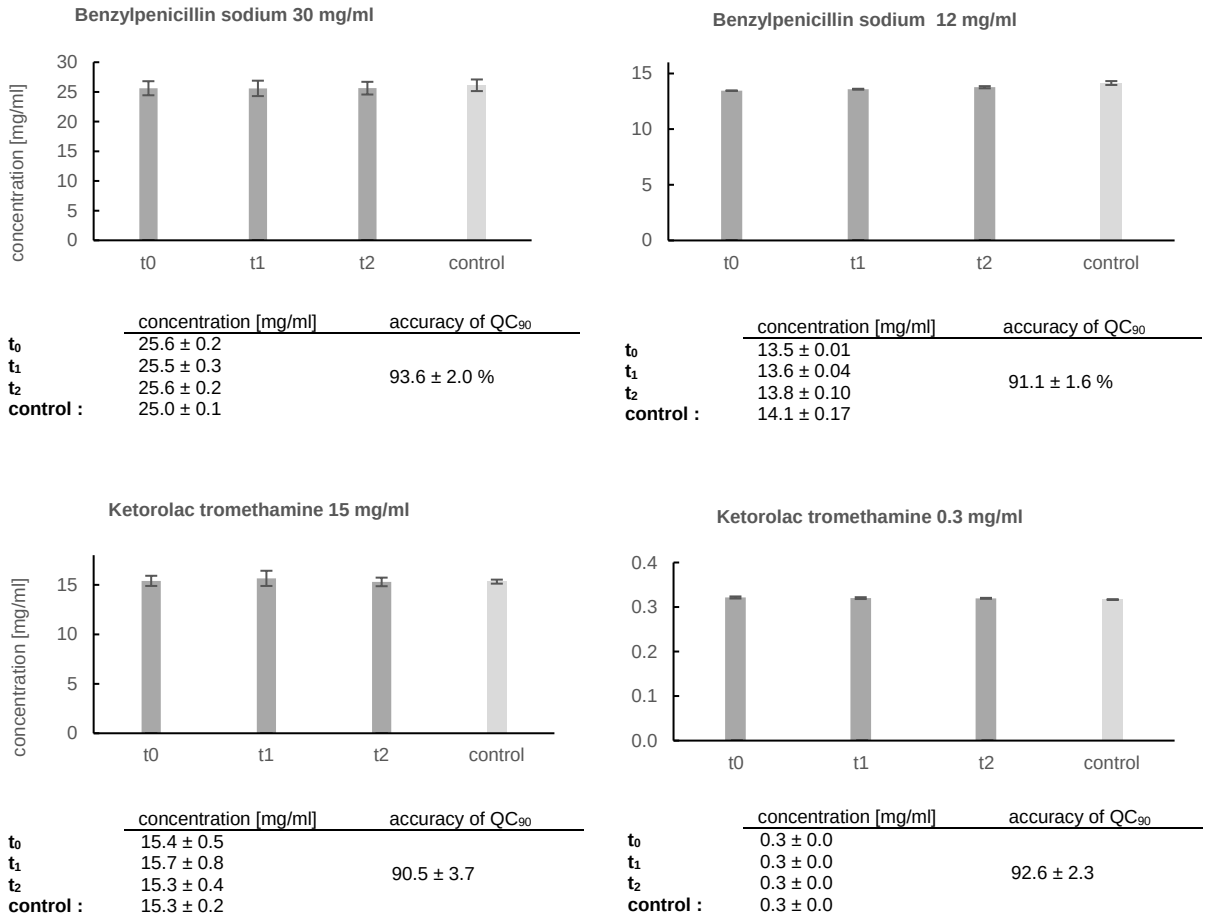


	concentration [mg/ml]
t_0	12.4 ± 0.3
t_1	12.0 ± 0.1
t_2	12.1 ± 0.2
control :	12.3 ± 0.4



	concentration [mg/ml]
t_0	5.3 ± 0.2
t_1	5.2 ± 0.1
t_2	5.3 ± 0.1
control :	5.5 ± 0.2

Figure A3. Stability of high and low concentrations of meropenem trihydrate, piperacillin sodium and tazobactam in Ringer's acetate. T_1 corresponds to 1.35 hours, t_2 to 2.55 hours. The control solutions were diluted in NaCl 0.9 % (n = 3).



	concentration [mg/ml]	accuracy of QC_{90}
t_0	25.6 ± 0.2	93.6 ± 2.0 %
t_1	25.5 ± 0.3	
t_2	25.6 ± 0.2	
control :	25.0 ± 0.1	

	concentration [mg/ml]	accuracy of QC_{90}
t_0	13.5 ± 0.01	91.1 ± 1.6 %
t_1	13.6 ± 0.04	
t_2	13.8 ± 0.10	
control :	14.1 ± 0.17	

	concentration [mg/ml]	accuracy of QC_{90}
t_0	15.4 ± 0.5	90.5 ± 3.7
t_1	15.7 ± 0.8	
t_2	15.3 ± 0.4	
control :	15.3 ± 0.2	

	concentration [mg/ml]	accuracy of QC_{90}
t_0	0.3 ± 0.0	92.6 ± 2.3
t_1	0.3 ± 0.0	
t_2	0.3 ± 0.0	
control :	0.3 ± 0.0	

Figure A4. Stability of high and low concentrations of benzylpenicillin sodium and ketorolac tromethamine in Ringer's acetate (n = 3). T_1 corresponds to 1.35 h (4 h for ketorolac tromethamine 0.3 mg/ml), t_2 to 2.55 h (24 h for ketorolac tromethamine 0.3 mg/ml). The control solution of benzylpenicillin sodium was diluted in D5, that of ketorolac tromethamine in NaCl 0.9 %.

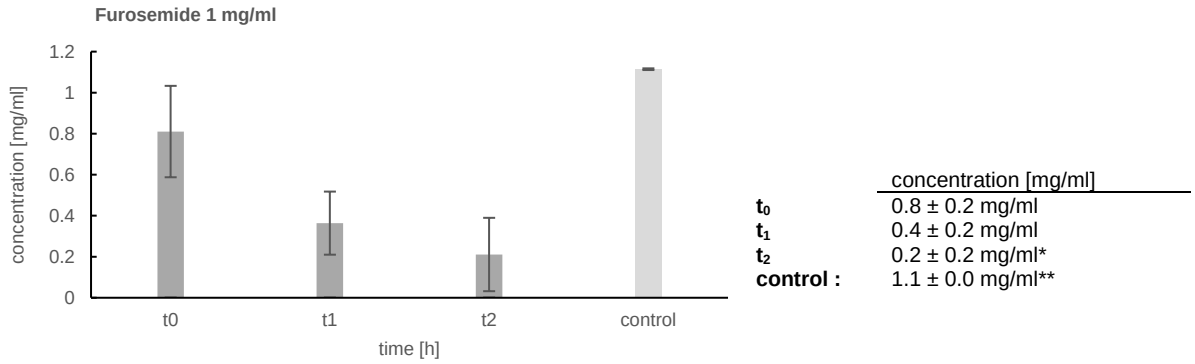


Figure A5. Instability of the "positive control" test solution of furosemide 1.0 mg/ml in ciprofloxacin hydrochloride 1.8 mg/ml /ml (n = 3). T_1 corresponds to 1.35 h, t_2 to 2.55 h. Furosemide control solution was diluted in NaCl 0.9 % (n = 3). Deviation of the furosemide concentration at t_2 from both the initial concentration and the nominal concentration was statistically significant (* $p < 0.05$). Deviation of the concentration of the furosemide test solution from the control solution at t_2 was statistically significant (** $p < 0.05$).

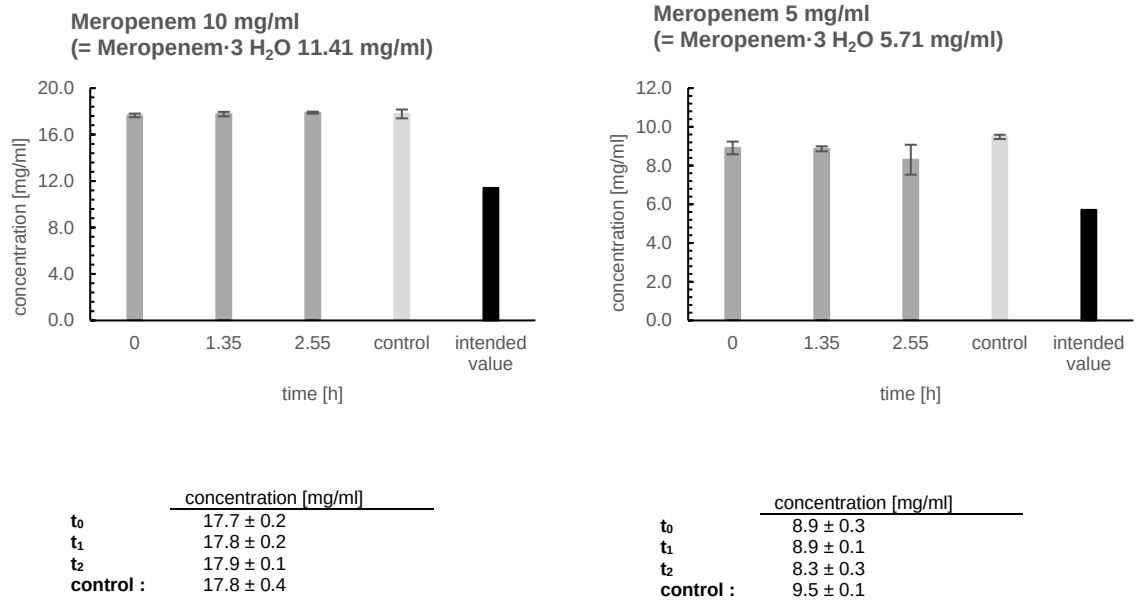


Figure A6. Repetition of meropenem measurements by a laboratory assistant, testing compatibility in RA (10 mg/ml and 5 mg/ml) at different time intervals (t_0, t_1 = 1.35 h, t_2 = 2.55 h, n = 3). The control solution corresponds to meropenem diluted in pure NaCl 0.9 % (n = 3).

Table A10. Results of the pH measurements of the drug product test samples (drug products diluted in Ringer's acetate), control samples (drug products diluted in NaCl 0.9% or dextrose 5%), and the "positive control" (furosemide diluted in ciprofloxacin HCl solution)

	pH at t_0	pH at t_2
Meropenem (Meronem®)		
10 mg/ml		
in Ringer's acetate	7.85 ± 0.00	7.94 ± 0.01
in NaCl 0.9%	7.94 ± 0.02	not measured
5 mg/ml		
in Ringer's acetate	7.88 ± 0.05	7.95 ± 0.05
in NaCl 0.9%	7.94 ± 0.03	not measured
Piperacillin/tazobactam (Piperacillin/Tazobactam Sandoz®)		
104.25/12.5 mg/ml		
in Ringer's acetate	6.12 ± 0.01	6.06 ± 0.01
in NaCl 0.9%	5.85 ± 0.02	5.63 ± 0.01
41.7/5 mg/ml		
in Ringer's acetate	6.16 ± 0.00	6.13 ± 0.01
in NaCl 0.9%	5.83 ± 0.01	5.58 ± 0.08
Furosemide (Lasix®)		
1 mg/ml		
in Ciproxin® 2 mg/ml	4.55 ± 0.14	4.40 ± 0.03
in NaCl 0.9%	8.90 ± 0.00	8.64 ± 0.15
Benzylpenicillin sodium (Penicillin «Grünenthal»®)		
30 mg/ml		
in Ringer's acetate	6.20 ± 0.03	6.16 ± 0.01
in dextrose 5%	6.06 ± 0.05	5.63 ± 0.09
12 mg/ml		
in Ringer's acetat	6.19 ± 0.00	6.18 ± 0.03
in dextrose 5%	5.89 ± 0.03	5.73 ± 0.11
Ketorolac tromethamine (Tora-dol®)		
15 mg/ml		
in Ringer's acetate	7.29 ± 0.00	7.29 ± 0.03
in NaCl 0.9%	7.34 ± 0.00	7.37 ± 0.01
0.3 mg/ml		
in Ringer's acetate	6.25 ± 0.00	6.26 ± 0.01
in NaCl 0.9%	7.24 ± 0.00	6.93 ± 0.02

mean ± SD, n = 3

Table A11.1. Peak areas of the drug product test samples (drug product in Ringer's acetate) and control samples (drug product in NaCl 0.9%) and results of the 1/x-weighted least square linear regression analysis (y = mx + b, b corresponding to y intercept, m to slope, R^2 to coefficient of determination) of the calibration standards used to back-calculate the concentrations of meropenem, piperacillin sodium, tazobactam and furosemide.

	A at t_0 [mAU*min]	**A at t_1 [mAU*min]**	**A at t_2 [mAU*min]**	**y intercept**	**slope**	**R^2**
Meropenem (Meronem®)						
10 mg/ml						
in RA	5204270 ± 579843	5233542 ± 692182	5398377 ± 699282			
in NaCl 0.9%	-	-	6717504 ± 287714	-239568	50145274	0.99
5 mg/ml						
in RA	3386774 ± 762735	3603038 ± 1020567	3320372 ± 737525			
in NaCl 0.9%	-	-	4472104 ± 529674			
Piperacillin sodium (Piperacillin/Tazobactam Sandoz®)						
104.25 mg/ml						
in RA	21293186 ± 543944	21080184 ± 229924	21055684 ± 357716			
in NaCl 0.9%	-	-	21637384 ± 664981	-118608	99959590	0.98
41.7 mg/ml						
in RA	23126822 ± 598961	23139898 ± 674241	23629661 ± 55377			
in NaCl 0.9%	-	-	23980047 ± 577411			
Tazobactam (Piperacillin/Tazobactam Sandoz®)						
12.5 mg/ml						
in RA	1642410 ± 44835	1580366 ± 13042	1607614 ± 22592	-136328	71781368	0.98
in NaCl 0.9%	-	-	1632412 ± 55224			

	A at t_0 [mAU*min]	A at t_1 [mAU*min]	A at t_2 [mAU*min]	y intercept	slope	R^2
5 mg/ml						
in RA	1750636 ± 53910	1733888 ± 20222	1781104 ± 25220			
in NaCl 0.9%			1840337 ± 75975			
Furosemide (Lasix®)						
1 mg/ml						
in Ciproxin® 2 mg/ml	14057837 ± 3915530	6213159 ± 2702641	3525412 ± 3142750	-178909	175701839	0.98
in NaCl 0.9%	-	-	19400858 ± 71556			

mean ± SD, n = 3

RA = Ringer's acetate

A = peak area (mean ± SD, n = 3)

t_0 = immediately after preparation, t_1 = 1.35 h, t_2 = 2.55 h

Table A11.2. Peak areas of the drug product test samples (drug products in Ringer's acetate) and control samples (drug products in NaCl 0.9% or dextrose 5%) of benzylpenicillin sodium and ketorolac tromethamine.

	A at t_0 [mAU*min]	A at t_1 [mAU*min]	A at t_2 [mAU*min]	c_{cal} [mg/ml]	A_{cal} [mAU*min]
Benzylpenicillin sodium (Penicillin « Grünenthal »)					
30 mg/ml (0.3 mg/ml [a])					
in RA	46857184 ± 463769	47133302 ± 473662	47550183 ± 349162		38062907 ± 34996
in D5	-	-	48463415 ± 467991	0.2	
12 mg/ml (0.12 mg/ml [a])					
in RA	25595598 ± 17345	25845143 ± 70050	26186911 ± 184700		38036027 ± 100326
in D5	-	-	26906853 ± 339739		
Ketorolac tromethamine (Tora-dol®)					
15 mg/ml (0.15 mg/ml [a])					
in RA	27621013 ± 684298	28070711 ± 1105493	27430485 ± 515030		26814550 ± 444290
in NaCl 0.9%	-	-	27726947 ± 426398	0.15	
0.3 mg/ml (0.06 mg/ml [b])					
in RA	11674595 ± 84473	11622335 ± 74810	11603416 ± 38768		27282683 ± 1044458
in NaCl 0.9%	-	-	11502524 ± 6044		

Note: the concentration of the drug substance was calculated using the equation $c_{sample} = \frac{c_{cal} \times A_{sample}}{A_{cal}}$, where c_{sample} = concentration of the drug substance in the drug product, c_{cal} = theoretical concentration of the calibration standard, A_{sample} = peak area of the drug substance in the drug product (mean ± SD, n = 3), A_{cal} = peak area of the calibration standard (mean ± SD, n = 3)

[a] samples were diluted 1:100 for HPLC analysis, [b] samples were diluted 1:5 for HPLC analysis

A = peak area (mean ± SD, n = 3), t_0 = immediately after preparation, t_1 = 1.35 h (4 h for ketorolac tromethamine), t_1 = 2.55 h (24 h for ketorolac tromethamine)

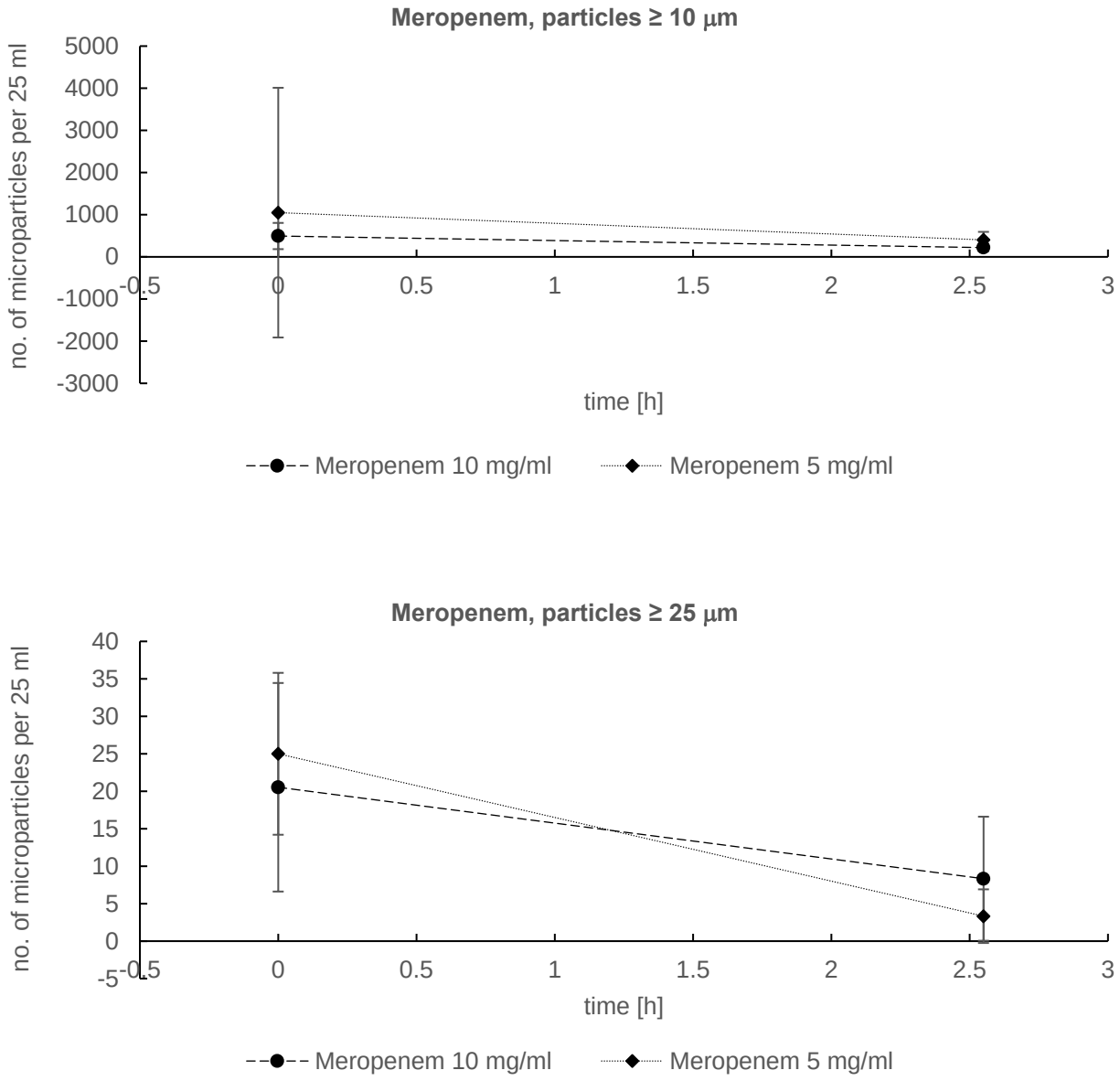


Figure A7. First round of particle counting of meropenem 5 mg/ml and 10 mg/ml in Ringer's acetate. The diagrams show the change in 10 μm and 25 μm particle counts from t_0 (immediately after preparation) to t_2 (2.55 h) of high and low-dose meropenem in Ringer's acetate. Control solutions were not included.

Table A12. Number of particles ≥ 10 μm and ≥ 25 μm in diameter per 25 ml in the drug product test samples at different time points.

sample	time [h]	number of particles per 25 ml	
		≥ 10 μm	**≥ 25 μm**
Benzylpenicillin sodium (Penicillin "Grünenthal"®)			
30 mg/ml	0	2024 ± 807	30 ± 9
	2.5	606 ± 294	9 ± 7
12 mg/ml	0	3714 ± 2491	109 ± 88
	2.5	1008 ± 637	21 ± 1
Piperacillin/Tazobactam (Piperacillin/Tazobactam® Sandoz)			
112.5 mg/ml	0	384 ± 140	12 ± 3
	2.5	148 ± 18	16 ± 7
45 mg/ml	0	125 ± 84	3 ± 1
	2.5	129 ± 92	35 ± 37
Meropenem (Meronem®)			
10 mg/ml	0	554 ± 318	7 ± 1
	2.5	386 ± 259	6 ± 3
	5.0	327 ± 252	3 ± 2
5 mg/ml	0	1050 ± 2965	25 ± 11
	2.5	403 ± 190	3 ± 4
Meropenem *control* [a] (Meronem®)			
10 mg/ml	0	914 ± 370	23 ± 14
	2.5	509 ± 324	7 ± 8
	5.0	413 ± 134	7 ± 5
Ketorolac tromethamine (Tora-dol®)			
15 mg/ml	0	1408 ± 446	42 ± 19
	2.5	694 ± 272	32 ± 21
0.3 mg/ml	0	32 ± 36	6 ± 6
	24	104 ± 67	12 ± 10

mean ± SD, n = 3

[a] the control solution was diluted in NaCl 0.9 % instead of Ringer's acetate