



Quantification of beta-lactam antibiotics cefuroxime and flucloxacillin in human synovial fluid, using ultra-performance convergence chromatography-tandem mass spectrometry

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ABSTRACT

Total hip- and knee arthroplasty generally result in successful outcomes. A small percentage of patients however suffer from periprosthetic joint infections (PJI) postoperatively, often with severe consequences. The standard treatment of chronic PJIs consists of a staged arthroplasty exchange during which antibiotic therapy plays a crucial role. For successful antibiotic treatment, adequate concentrations at the infection site are a prerequisite. Regarding the treatment of PJIs, knowledge is lacking with respect to the relationship between administered dosages and plasma- and infection site concentrations of the antibiotics. To gain insight into the antibiotic exposure at the infection site, validated analytical methods for analysis of the antibiotics in matrices at the site of the PJI are essential. We describe a validated ultra-performance convergence chromatography-tandem mass spectrometry (UPC²-MS/MS) method for quantification of the beta-lactam antibiotics cefuroxime and flucloxacillin in synovial fluid. This method was successfully validated for antibiotic quantification in synovial fluids according to the EMA guidelines and consists of a simple sample preparation. For both antibiotics, the accuracy and precision were within requirements (RSD < 15%). In addition, matrix effects and recovery were within the range of 80–120%. Carry over was less than 20% and stability in –80 °C was at least 2 months for standards and quality controls. The limits of quantification were adequate (1–100 mg/L) to cover potential cefuroxime and flucloxacillin concentrations in synovial fluid as described in literature ($r > 0.995$). The method has a run time of 4.5 min and 50 μ L synovial fluid is needed and the validated method will be applied during a PK/PD study to determine the exposure of the study antibiotics in synovial fluid at the site of PJIs.

1. Introduction

Total hip arthroplasty (THA) and total knee arthroplasty (TKA) are frequently performed joint replacement procedures with approximately 30.000 THAs and 29.000 TKAs conducted in the Netherlands in 2017 [1]. Successful joint replacement provides pain relief, restores joint function and improves the patient's quality of life [2,3]. However, in a number of patients (± 1 –2%) periprosthetic joint infections (PJI) occur as a postoperative complication, often leading to prosthesis failure [4].

The number of PJI diagnoses is expected to rise due to increment in the number of primary THAs and TKAs performed and the currently existing prostheses in patients [2]. In many cases, the standard treatment of chronic PJIs consists of the two-stage arthroplasty exchange, combining antimicrobial therapy with surgical interventions [2]. This staged exchange is considered to be the most effective strategy in terms of eradication of the bacteria underlying the infection and preservation of the joint function. The therapeutic success of an antimicrobial therapy is mainly determined by its degree of penetration into the infection site

Abbreviations: ESI, electrospray ionization; EMA, European Medicines Agency; HPLC, high-performance liquid chromatography; IS, internal standard; LLOQ, lower limit of quantification; LOD, limit of detection; LOQ, limits of quantification; MRM, multiple reaction monitoring; PJI, periprosthetic joint infection; QC, quality control; QCH, high quality control; QCL, low quality control; QCM, medium quality control; RSD, relative standard deviation; ULOQ, upper limit of quantification; UPC²-MS/MS, ultra-performance convergence chromatography-tandem mass spectrometry; THA, total hip arthroplasty; TKA, total knee arthroplasty.

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and susceptibility of the bacterial pathogen to the antibiotic. Therefore, adequate exposure of the antibiotics at the infection site is as important as plasma concentrations for an effective therapy. Research is lacking with respect to the relationship between administered dosages and plasma- and infection site concentrations of the administered antibiotics in PJI patients. In standard PK studies, only plasma concentrations are taken into account. Consequently, the selection and optimization of antibiotic dosing regimens for PJIs are not based on the extent of penetration into the infected periprosthetic synovial fluid, bone and joint tissues and therefore the antibiotic exposure at the infection site. Moreover, bone penetration studies have previously indicated differences in antibiotic concentrations at the infection site dependent on the location of the infected joint tissue in hip and knee [5]. The applied antibiotic dosing regimen may result in sub-inhibitory concentrations or concentrations greatly exceeding the therapeutic values at the site of PJIs, which can lead to treatment failure, antibiotic related side effects, and the development of antibiotic resistance. Insufficient treatment of PJIs can lead to persistence of local infection leading to disability of the joint for a longer period of time and multiple surgical interventions including amputation [6]. Additionally, several studies have suggested the importance of infection site concentrations of antibiotics and indicated that antibiotic plasma concentrations may not always be predictive of those at the target site [7–10]. Hence, more research is necessary to gain insight into the exposure of the antibiotics administered as part of the two-stage arthroplasty exchange at the site of PJI in synovial fluid, synovial tissue and bone, in relation to the dosage and the plasma concentration to assure optimal treatment.

Two of the commonly administered antimicrobial agents during the two-stage arthroplasty exchange are cefuroxime and flucloxacillin. To quantify the concentration of these antibiotics at the site of PJIs validated assays are fundamental to obtain robust and reliable analytical results. However, no specific guidelines have been published for the development and validation of assays for analysis of drug levels in synovial fluid as an alternative matrix. Several analytical methods have been applied in the past to quantify various types of beta-lactam antibiotics in synovial fluid by use of high-performance liquid chromatography (HPLC) [11–14]. To our knowledge, no studies have been published on validated methods for measurement of cefuroxime and flucloxacillin in synovial fluid by use of ultra-performance convergence chromatography-tandem mass spectrometry (UPC²-MS/MS). Ultra-performance convergence chromatography is based on supercritical fluid chromatography (SFC) and has many analytical advantages as it is easily operated and exhibits the ability to enhance separation selectivity. SFC is highly recommended for chromatographic separation of compounds with a high polarity, as beta-lactam antibiotics, to obtain optimal retention. The mobile phase consisting of primarily CO₂ exhibits limited toxicity and low costs and makes this method also economically advantageous.

When coupled with tandem mass spectrometry it results in an even higher sensitivity and specificity of the assay [15]. Bioanalysis with UPC²-MS/MS has many advantages due to properties as high efficiency, fast analytical run and high throughput [16,17].

We present a validated ultra-performance convergence chromatography-tandem mass spectrometry method for quantification of the beta-lactam antibiotics cefuroxime and flucloxacillin in synovial fluid. The validated method for analysis can be applied to determine the flucloxacillin and cefuroxime exposure at the site of PJIs with respect to blood concentrations.

2. Material and methods

2.1. Chemicals and reagents

Cefuroxime and meropenem-D₆ were purchased from Santa Cruz Biotechnology Inc (Huissen, The Netherlands); flucloxacillin sodium salt was bought from Toronto Research Chemicals Inc (Ontario, Canada).

Ammonium formate was obtained from Honeywell Research Chemicals (Landsmeer, The Netherlands). Methanol LCMS-grade was purchased from Biosolve BV (Valkenswaard, The Netherlands). Deionized water was prepared using a Milli-Q Advantage A10 System (Merck Millipore, Darmstadt, Germany).

2.2. Sampling of human synovial fluid

Blank, i.e. without cefuroxime or flucloxacillin, human synovial fluid was collected during surgical orthopedic interventions on the knee or hip and through arthrocentesis for diagnostic purposes. After collection, the samples were directly stored at –20 °C until analysis. Sampling was performed by two orthopedic surgeons (JvO and PB).

2.3. Instrumentation

Analysis was carried out on a Waters Acquity UPC²-MS/MS system (Waters Corp, Milford, MA), consisting of a Waters UPC² Torus Diol column (1.7 μm, 2.1x100 mm) connected to a Waters Xevo TQ-s micro mass spectrometer equipped with a triple quadrupole and electrospray ionization (ESI) probe. The UPC²-system consisted of a binary solvent manager, an isocratic solvent manager, a convergence manager, a sample manager and a column manager. Data was processed by Masslynx software 4.1 and TargetLynx 4.1 (Waters Corp).

2.3.1. Chromatographic conditions

A mobile phase consisting of carbon dioxide (eluent A) over 5% 10 mM ammonium formate in methanol (eluent B) was applied for chromatographic separation of the analytes at a column temperature of 55 °C. The retention time of cefuroxime and flucloxacillin were 1.73 min and 1.17 min, respectively. The flow rate was 0.75 mL/min and the make-up flow rate was 0.20 mL/min. A gradient was applied during the run as follows: starting gradient was 70% eluent A and 30% eluent B; eluent A decreased to 45% in 1 min, continued to be 45% during 3 min, and stabilized at 70% in 0.5 min; eluent B increased to 55% in 1 min, continued to be 55% during 3 min, and stabilized at 30% in 0.5 min. The auto sampler temperature was set at 5 °C. The volume of injection was 20 μL, with a run time of 4.5 min of each sample.

2.3.2. Mass-spectrometry conditions

The antibiotics and internal standard (IS) meropenem-D₆ were detected with a quadrupole mass spectrometer and positive ESI. The applied conditions were as follows: capillary voltage 3.50 kV, cone gas flow 1 L/h, source block temperature 150 °C, and desolvation temperature 350 °C at a desolvation gas flow of 650 L/h. The collision gas was argon with a gas flow of 5 μL/min. Multiple reaction monitoring (MRM) was used for data acquisition; the MS/MS-parameters are listed in Table 1.

2.4. Stock solutions

Stock solutions of cefuroxime (1000 mg/L), flucloxacillin (10,000 mg/L), and IS meropenem-D₆ (2 mg/L) were prepared in methanol LCMS-grade and stored at –20 °C. The stock solutions were prepared separately for the calibration standards and quality control (QC)

Table 1
MS/MS-parameters of the study antibiotics and internal standard.

Analyte	Parent ion (m/z)	Daughter ion (m/z)	CV (V)	CE (V)	Dwell time (s)	ESI mode
Cefuroxime	441.98	364.01	16	8	0.05	+
Flucloxacillin	453.88	160.02	12	16	0.05	+
Meropenem-D ₆	390.02	147.09	12	32	0.05	+

CV, cone voltage; CE, collision energy.

samples.

2.5. Calibration standards and quality controls

Batches of blank synovial fluid were pooled to obtain a larger amount of matrix and centrifuged (Eppendorf Centrifuge 5810R) for 6 min at 3000 rpm to withdraw any diffuse solid material. Calibration standards at eight concentration levels (within the range of 1–100 mg/L) and low, medium and high QCs (respectively, QCL, QCM, and QCH) (at concentration levels 15, 50 and 80 mg/L) were prepared by spiking the antibiotic stock solutions into blank synovial fluid. The highest calibration standard was prepared by first adding the cefuroxime stock solution and evaporating the methanol solvent to dryness under a nitrogen flow at 40 °C, before adding the flucloxacillin stock solution. This resulted in a spiked volume of stock solutions that contributed for less than 5% of the total volume of the highest calibration standard. The remaining standards and QCs were prepared by diluting the highest concentration standard and QC level. Aliquots of 50 µL of each standard and QC were stored at –80 °C, until analysis.

2.6. Sample preparation

The extraction procedure of beta-lactam antibiotics validated for plasma was adapted for the sample preparation of synovial fluid. Briefly, frozen standards and QCs were thawed and mixed with 800 µL meropenem-D₆ in methanol for protein precipitation. After shaking for 10 s (Scientific Industries, Vortex Genie 2), the samples were centrifuged (Eppendorf centrifuge) at 14680 rpm for 5 min. A volume of 50 µL supernatant was mixed with 950 µL of 5% 10 mM ammonium formate in methanol, followed by shaking for 5 s. The samples were then injected, with a volume of 20 µL, into the UPC²-MS/MS apparatus.

2.7. Method validation

Method validation was carried out in accordance with the European Medicines Agency (EMA) guidelines on bioanalytical method validation [18]. The ensuing parameters were validated as described in the paragraphs below: linearity, limits of quantification, accuracy and precision, carry-over, auto sampler- and long-term stability, recovery and matrix effects.

2.7.1. Linearity

A calibration curve with eight concentration levels was established. Each standard was measured in duplicate, along with a blank sample without internal standard and zero-sample with internal standard. The regression line was analyzed by conducting a linear least-square-regression; the origin of the curve was included and a weighting factor of 1/x was applied. The peak area ratios (response of analyte versus internal standard) were calculated and plotted against the theoretical concentrations of the calibration standards. The correlation coefficient *r* has to be at least 0.995 for each analyte of interest.

2.7.2. Limits of quantification

The upper limit of quantification (ULOQ) was set at the highest calibration standard of both analytes. The lower limit of quantification (LLOQ) was assessed by analyzing six replicates of the LLOQ-standard in duplicate, measured over six different days. The LLOQ-standard corresponded with the lowest calibration standard of both analytes. The limit of detection (LOD) was determined by analyzing five blank samples with IS, over five different days. The relative standard deviation (RSD) for the precision and accuracy should be ≤ 20%.

2.7.3. Accuracy and precision

The accuracy and within-run precision of the method were evaluated by measuring six replicates of QCL, QCM and QCH in a single run. The between-run precision was determined by analyzing each QC level in

duplicate on six different days. The calculated RSD for accuracy and precision should be ≤ 15%.

2.7.4. Matrix effect and recovery

Matrix effects and recovery were investigated according to the method of Matuszewski [19]. Three sets of QCL, QCH and blank samples, were prepared in duplicate. In set A, the samples were prepared in Milli-Q water (total of 6 samples). Blank synovial fluid was collected from different sources and separated in five batches of blank matrix. In set B, from each batch of blank matrix samples were prepared and spiked with cefuroxime and flucloxacillin after extraction (6 samples per batch, total 30 samples). In set C, each batch of blank matrix was spiked before extraction, according to the standard sample preparation method as described in this study (6 samples per batch, total 30 samples). Matrix effects were calculated as a ratio of the sample response of set B and set A (B/A*100%). Recovery was defined as a ratio of the sample response of set C and set B (C/B*100%). Matrix effects and recovery should be between 80% and 120%.

2.7.5. Stability

The auto sampler stability was determined by storing QCs of each level after sample preparation in the auto sampler (at 5 °C) during 24 h. The QCs were measured at this time-point along with freshly prepared calibration standards. The recovery of the study antibiotics at each time-point was calculated and should be between the 90% and 110% of the initial injection (at 0 h). The long-term stability of the samples stored at –80 °C was investigated after storage for up to two months. QC samples of each level and freshly spiked calibration standards were measured (the recovery should be between the 90% and 110%).

2.7.6. Carry-over

To investigate carry-over effect, a blank sample with internal standard was injected directly following the injection of the ULOQ-standard. Carry-over of the antibiotics in the blank sample should not exceed 20% of the LLOQ-concentration.

3. Results and discussion

We validated an UPC²-MS/MS based method for quantification of cefuroxime and flucloxacillin in synovial fluid. The sample preparation method and chromatographic and mass-spectrometric conditions for beta-lactam antibiotics in plasma proved to be applicable for processing synovial fluid. A simple and rapid extraction procedure, including protein precipitation with methanol, was performed. A low sample volume of 50 µL synovial fluid is sufficient for analysis of cefuroxime and flucloxacillin.

3.1. Linearity

The linearity of the method was accomplished. The calibration curve was obtained by use of eight calibration levels in duplicate as no measurements were excluded. Concentrations of the calibration standards of each analyte, and the linearity data are presented in Table 2. The linearity of the method was validated by a linear least-square-regression. Due to the wide calibration range, the application of a weighting factor (1/x) was necessary to reliably quantify the lower calibration levels. Cefuroxime and flucloxacillin were successfully quantified within the calibration range of 1 mg/L to 100 mg/L and the fitted curve was linear within the analyzed range as the correlation coefficient (*r*) values were > 0.995 for both analytes.

3.2. Limits of quantification

The limits of quantification (LOQ) for cefuroxime and flucloxacillin are shown in Table 2. The lowest calibration standard, with a concentration of 1 mg/L of both study antibiotics, was set as the LLOQ standard.

Table 2

Quantification data of the calibration standards and limits of detection. The calibration curve was constructed based on the complete run consisting of eight calibration standards in duplicate.

Analyte	Calibration standards (mg/L)								<i>r</i>	<i>r</i> ²	LLOQ (mg/L)	ULOQ (mg/L)
	S1	S2	S3	S4	S5	S6	S7	S8				
Cefuroxime	1	10	20	40	60	70	90	100	0.9999	0.9998	1	100
Flucloxacillin	1	10	20	40	60	70	90	100	0.9998	0.9995	1	100

S, standard; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification; *r*, correlation coefficient; *r*², determination coefficient.

In Fig. 1 representative chromatograms of the study antibiotics at LLOQ-level and the internal standard in synovial fluid are presented along with the corresponding retention times. The RSDs for precision and accuracy of the LLOQ standard were within the acceptance criteria (RSD ≤ 20%: 4.4% and 2.5% for cefuroxime and flucloxacillin, respectively), indicating a reliable accuracy and precision of the method for quantification of lower concentrations of cefuroxime and flucloxacillin in synovial fluid. The ULOQ was quantified to be similar to the highest concentration standard. The LOD was 0.1 mg/L for cefuroxime and 0.02 mg/L for flucloxacillin.

Contrary to plasma, no therapeutic range has been defined for cefuroxime and flucloxacillin in synovial fluid. The type of antimicrobial treatment applied during the two-stage arthroplasty exchange is dependent on the bacteria causing the PJI. The validated concentration range and the limits of quantification are sufficient to measure cefuroxime and flucloxacillin in the potential clinically relevant concentrations in synovial fluid, based on the minimal inhibitory concentration (MIC) of the potential pathogens. Cefuroxime is part of the eradication therapy of PJIs caused by *Enterobacter* for which it has a MIC of 8 mg/L, while flucloxacillin is commonly applied in case of a *Staphylococcus aureus* infection with a MIC of 1 mg/L [20]. For adequate inhibition of these bacteria, the concentrations at the site of the PJI should exceed the abovementioned MIC values. The LLOQ concentration of our presented assay is 1 mg/L for both antibiotics; since the assay should have a sufficient sensitivity to measure at least the MIC-values of the concerned pathogens this LLOQ level is justifiable for our study antibiotics. Nevertheless, this might be a limitation when these beta-lactam

antibiotics are administered for eradication of bacteria with a MIC lower than 1 mg/L.

A previous study determined a cefuroxime maximum concentration in synovial fluid of 57.3(±27.7) mg/L and a minimum concentration of approximately 10 mg/L after intravenous (IV) administration, whereas in another study a mean flucloxacillin concentration of 12.9(±5.25) mg/L was reached several hours after IV administration [8,21]. Despite that target site concentrations largely depend on the dosage and route of administration, the wide calibration range of our linearity model, is expected to cover the potential cefuroxime and flucloxacillin concentrations in synovial fluid as described in literature.

3.3. Accuracy and precision

Accuracy and precision data for the quality controls are summarized in Table 3. Each quality control level was spiked with cefuroxime and flucloxacillin in synovial fluid at a concentration different from those of the calibration standards. The accuracy of the three quality control levels was in accordance with the acceptance value (RSD ≤ 15%). The within-run and between-run precision of the assay were within the validation norm (RSD ≤ 15%).

3.4. Matrix effect and recovery

Matrix effect and extraction recovery data are presented in Table 4. No significant ion suppression was observed for the analysis of flucloxacillin in synovial fluid due to the matrix composition. Analysis of

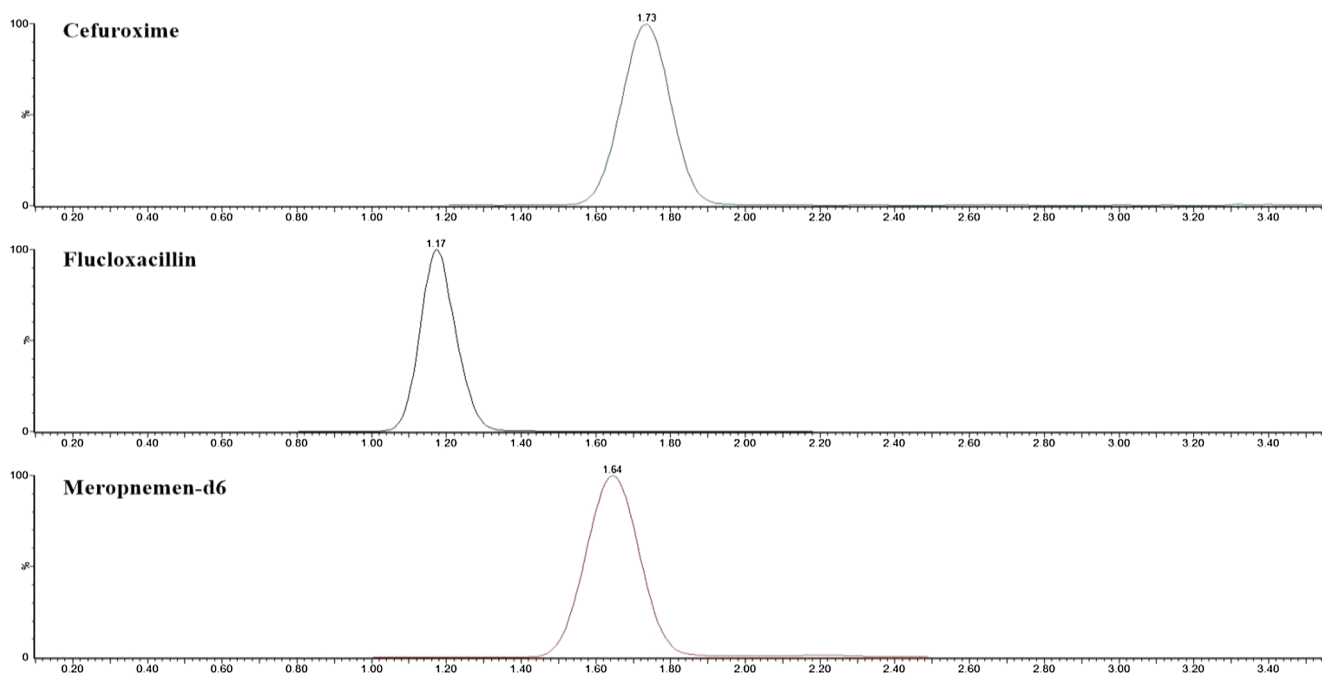


Fig. 1. Chromatograms of cefuroxime, flucloxacillin and the internal standard (meropenem-d₆) at LLOQ-level. Plots represent the signal (abundance %) as a function of retention time (min). Retention times: cefuroxime 1.73 min, flucloxacillin 1.17 min, and meropenem-d₆ 1.64 min (shown above the corresponding peak). Peak areas: cefuroxime 2219, flucloxacillin 4338, meropenem-d₆ 66265.

Table 3

Accuracy, within-run- and between-run precision data. The within-run precision and accuracy were based on the data of six replicates of each QC level in a single run. The between-run precision was determined by analysing each QC level in duplicate during six runs on separate days.

Analyte	Quality control levels	Quality control concentration (mg/L)	Accuracy RSD (%)	Within-run precision RSD (%)	Between-run precision RSD (%)
Cefuroxime	QCL	15	-10.9	0.6	2.1
	QCM	50	-13.8	1.4	2.0
	QCH	80	-8.7	2.9	2.2
Flucloxacillin	QCL	15	-3.7	2.3	3.7
	QCM	50	-3.1	2.8	3.9
	QCH	80	-2.0	5.7	2.4

QCL, low quality control; QCM, medium quality control; QCH, high quality control; RSD, relative standard deviation.

Table 4

Matrix effect and extraction recovery of cefuroxime and flucloxacillin in synovial fluid. Parameter values are obtained by using five different batches of blank matrix and adding the analytes before and after extraction.

Analyte	Matrix effect (%)				Recovery (%)			
	QCL	QCH	Mean	RSD	QCL	QCH	Mean	RSD
Cefuroxime	71	75	73	3.5	91	91	90.8	0.3
Flucloxacillin	86	94	90	7.0	85	86	85.3	0.4

QCL, low quality control; QCH, high quality control; RSD, relative standard deviation.

cefuroxime in synovial fluid, however, indicated a slightly larger ion suppression. In order to determine the matrix effects, analyte response in samples prepared in Milli-Q water (set A) were compared to the response in synovial fluid. For evaluation of the matrix effects, it should be considered that Milli-Q water and synovial fluid exhibit differences in composition but also in characteristics as pH. This might have influenced the extent to which ion suppression is observed, since it can affect the state of compounds in the matrix and therefore result in different analyte responses in matrix and Milli-Q water [22]. Additionally, small differences in the responses of the analytes spiked into synovial fluid extracts from five different batches were observed for both low and high concentrations (cefuroxime RSD QCL 2.2% and QCH 1.8%; flucloxacillin RSD QCL 3.4% and QCH 4.3%), suggesting that differences in synovial fluid samples between patients, with respect to factors as co-eluting components and pH, will have a negligible effect on the precision and reliability of the analyte quantification. Furthermore, adequate recoveries were achieved for both concentration levels of the analytes in the matrix, indicating, along with the observed matrix effects, that the overall method has an adequate precision and accuracy.

3.5. Stability

The auto sampler stability was evaluated by storing quality control sample extracts at 5 °C in the auto sampler during 24 h. The recovery of each quality control level of cefuroxime and flucloxacillin after 24 h did not meet the criteria for stability: cefuroxime QCL 116.4%, QCM 115.5%, QCH 114.9%; flucloxacillin QCL 129.7%, QCM 123.9%, QCH 123.9%. Both study antibiotics in synovial fluid have a limited auto sampler stability. The synovial fluid extracts should therefore be measured shortly after sample preparation when containing cefuroxime and flucloxacillin. The stability of the samples after storage for up to two months was adequate: recoveries cefuroxime QCL 106.8%, QCM 103.2%, QCH 100.8%; flucloxacillin QCL 103.2%, QCM 95.5%, QCH 94.6%. The calibration standards and QC samples are stable during storage for a period of at least two months at -80 °C.

3.6. Carry-over

Carry-over of the study antibiotics in the blank samples following injection of the ULOQ-standard was not observed for this assay, which is pertinent due to the large concentration range used.

4. Conclusions

We successfully validated an UPC²-MS/MS based assay for quantification of cefuroxime and flucloxacillin in synovial fluid. This method can be applied to accurately and reliably quantify cefuroxime and flucloxacillin concentrations in synovial fluid within the range of 1–100 mg/L and presents validation parameters within the acceptance criteria (Tables 2–4). It will be applied during a clinical study to measure the exposure of the administered antibiotics at the site of the PJI during the two-stage arthroplasty exchange. Since PJIs occur in bone and joint tissue, antibiotic concentrations in synovial fluid will provide knowledge into the exposure near the infection site. In order to determine the exposure at the actual infection site to a more accurate extent we will also develop and validate an analytical method for quantification of cefuroxime and flucloxacillin in bone and synovial tissue.

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Z. Demir: Methodology, Investigation, Writing - original draft, Writing - review & editing. **S. Bahmany:** Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **C. Bethlehem:** Conceptualization. **J. van Oldenrijk:** Conceptualization, Writing - original draft. **P.K. Bos:** Conceptualization, Supervision. **B.C. P. Koch:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] Dutch Arthroplasty Register, Online LROI annual report 2018, (2018) 1–213. http://www.lroi-rapportage.nl/media/pdf/PDF_Online_LROI_annual_report_2018.pdf.
- [2] A.J. Tande, R. Patel, Prosthetic joint infection, Clin. Microbiol. Rev. 27 (2) (2014) 302–345, <https://doi.org/10.1128/CMR.00111-13>.

- [3] T. Karachalios, G. Komnos, A. Koutalos, Total hip arthroplasty: Survival and modes of failure, *EFORT Open Rev.* 3 (5) (2018) 232–239, <https://doi.org/10.1302/2058-5241.3.170068>.
- [4] H. Scheper, M. Wouthuyzen-bakker, K.E. Veldkamp, R.J.P. Van Der Wal, H.C. Vogely, Geïnfecteerde gewrichtsprothese, *Ned Tijdsch Geneesk.* (2019) 1–8.
- [5] Fay R K Sanders, J Carel Goslings, Ron A A Mathôt, Tim Schepers, Target site antibiotic concentrations in orthopedic/trauma extremity surgery: is prophylactic cefazolin adequately dosed? A systematic review and meta-analysis, *Acta Orthop.* 90 (2) (2019) 97–104, <https://doi.org/10.1080/17453674.2019.1577014>.
- [6] L. Pulido, E. Ghanem, A. Joshi, J.J. Purtill, J. Parvizi, Periprosthetic joint infection: The incidence, timing, and predisposing factors, *Clin. Orthop. Relat. Res.* 466 (2008) 1710–1715, <https://doi.org/10.1007/s11999-008-0209-4>.
- [7] A. Hosmann, L.C. Ritscher, H. Burgmann, Z. Oesterreicher, W. Jäger, S. Poschner, E. Knosp, A. Reinprecht, A. Gruber, M. Zeitlinger, Concentrations of Cefuroxime in Brain Tissue of Neurointensive Care Patients, *Antimicrob. Agents Chemother.* 62 (2018) 1–10.
- [8] R. Schwameis, S. Syré, D. Marhofer, A. Appelt, D. Burau, K. Sarahrudi, C. Kloft, M. Zeitlinger, Pharmacokinetics of cefuroxime in synovial fluid, *Antimicrob. Agents Chemother.* 61 (2017) 1–7, <https://doi.org/10.1128/AAC.00992-17>.
- [9] Christian Joukhadar, Martin Frossard, Bernhard X. Mayer, Martin Brunner, Nikolas Klein, Peter Siostrzonek, Hans Georg Eichler, Markus Müller, Impaired target site penetration of β -lactams may account for therapeutic failure in patients with septic shock, *Crit. Care Med.* 29 (2) (2001) 385–391, <https://doi.org/10.1097/00003246-200102000-00030>.
- [10] Mario Cazzola, Maria Gabriella Matera, Paolo Noschese, Parenteral antibiotic therapy in the treatment of lower respiratory tract infections. Strategies to minimize the development of antibiotic resistance, *Pulm. Pharmacol. Ther.* 13 (6) (2000) 249–256, <https://doi.org/10.1006/pupt.2000.0253>.
- [11] J. Renneberg, O.M. Christensen, N.O.B. Thomsen, C. Tørholm, Cefuroxime concentrations in serum, joint fluid and bone in elderly patients undergoing arthroplasty after administration of cefuroxime axetil, *J. Antimicrob. Chemother.* 32 (1993) 751–755. <https://doi.org/10.1093/jac/32.5.751>.
- [12] Sven Åke Hedström, Lars Lidgren, Ingrid Nilsson-Ehle, Cefuroxime in acute septic arthritis, *Scand. J. Infect. Dis.* 16 (1) (1984) 79–82, <https://doi.org/10.3109/00365548409068412>.
- [13] S.W.N. Ueng, P.H. Hsieh, H.N. Shih, Y.S. Chan, M.S. Lee, Y. Chang, Antibacterial activity of joint fluid in cemented total-knee arthroplasty: An in vivo comparative study of polymethylmethacrylate with and without antibiotic loading, *Antimicrob. Agents Chemother.* 56 (2012) 5541–5546. <https://doi.org/10.1128/AAC.01067-12>.
- [14] E Somekh, L Heifetz, M Dan, F Poch, H Hafeli, A Tanai, Penetration and bactericidal activity of cefixime in synovial fluid, *Antimicrob. Agents Chemother.* 40 (5) (1996) 1198–1200, <https://doi.org/10.1128/AAC.40.5.1198>.
- [15] H. Kirchherr, The Pros and Cons of Mass Spectrometry in the TDM Laboratory, *Pharmacopsychiatry.* 41 (2008) A20, <https://doi.org/10.1055/s-0028-1088258>.
- [16] Amandine Dispas, Hugues Jambo, Sébastien André, Eva Tyteca, Philippe Hubert, Supercritical fluid chromatography: A promising alternative to current bioanalytical techniques, *Bioanalysis.* 10 (2) (2018) 107–124, <https://doi.org/10.4155/bio-2017-0211>.
- [17] Caroline West, Current trends in supercritical fluid chromatography, *Anal. Bioanal. Chem.* 410 (25) (2018) 6441–6457, <https://doi.org/10.1007/s00216-018-1267-4>.
- [18] European Medicines Agency, Guideline on bioanalytical method validation, (2011).
- [19] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS, *Anal. Chem.* 75 (2003) 3019–3030. <https://doi.org/10.1021/ac020361s>.
- [20] European Committee on Antimicrobial Susceptibility Testing, EUCAST: Clinical breakpoints and dosing of antibiotics, (n.d.). https://eucast.org/clinical_breakpoints/ (accessed October 3, 2019).
- [21] S. Holm, S.E. Larsson, The penetration of flucloxacillin into cortical and cancellous bone during arthroplasty of the knee, *Int. Orthop.* 6 (1983) 243–247, <https://doi.org/10.1007/BF00267145>.
- [22] Alan Abdulla, Soma Bahmany, Rixt A. Wijma, Bart C.H. van der Nagel, Birgit C. P. Koch, Simultaneous determination of nine β -lactam antibiotics in human plasma by an ultrafast hydrophilic-interaction chromatography–tandem mass spectrometry, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 1060 (2017) 138–143, <https://doi.org/10.1016/j.jchromb.2017.06.014>.