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RESEARCH ARTICLE

Therapeutic Drug Monitoring of Protein Unbound Ciprofloxacin Concentrations to avoid inadequate Treatment of severe Bacterial Infections in Critically ill Patients

Nora J.D. Mabelis[1,*], Kimberly N. Shudofsky[1], Joost J. van Raaij[1], Sjoerd D. Meenks[1], Thomas Havenith[2], Sander Croes[2], Jos L.M.L. le Noble[3,4], Paddy K.C. Janssen[1,2]

[1] Department of Hospital Pharmacy, VieCuri Medical Center, Venlo, The Netherlands. [2]Department of Clinical Pharmacy and Toxicology, Maastricht University Medical Centre+, Maastricht, the Netherlands. [3]Department of Intensive Care, VieCuri Medical Center, Venlo, the Netherlands. [4]Department of Pharmacology and Toxicology, Maastricht University, the Netherlands.

***Correspondence:** N.J.D. Mabelis, Department of Hospital Pharmacy, VieCuri Medical Centre, Tegelseweg 210 5912 BL Venlo, The Netherlands. Phone +31 77320 5186; Fax: +31 77 320 6001. Email: nmabelis@viecuri.nl

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OBJECTIVES: To develop a reliable ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for therapeutic drug monitoring (TDM) of unbound ciprofloxacin concentrations in critically ill patients.

METHODS: Total and unbound ciprofloxacin concentrations of five randomly selected intensive care unit (ICU) patients were measured using UPLC-MS/MS. Method validation included accuracy, linearity, precision, repeatability, and limits of detection and quantification.

RESULTS: The median unbound ciprofloxacin fraction was 74.8%, with a median area under the curve from 0-24 h (AUC0-24) and maximum serum concentration (C_{max}) of 28.51 h mg/L and 4.45 mg/L respectively. Median free AUC0-24 (*f*AUC0-24) and free Cmax (fC_{max}) were 21.57 h mg/L and 3.53 mg/L respectively; 20% of patients reached the pharmacodynamic target. The UPLC-MS/MS method was validated using an intra-assay and inter-assay precision < 3%. Recoveries were between 90-110%

CONCLUSIONS: This UPLC-MS/MS method provided reliable unbound ciprofloxacin concentrations, allowing target attainment in critically ill patients and exploration of different dosing regimens.

KEYWORDS: ciprofloxacin, protein binding, mass spectrometry, UPLC-MS/MS, intensive care unit.

INTRODUCTION

Infections are common in critically ill patients and often contribute to the severity of their condition. Bacterial infection is the primary concern. Antibiotics are among the medications most frequently administered to the critically ill with high levels of intra and inter individual pharmacokinetic (PK) variability [1-3].

The fluoroquinolone ciprofloxacin has been a frequently used antibiotic in critically ill patients. Given the broad-spectrum bactericidal activity, the relative treatment safety and both parentally and orally administration options, fluoroquinolones are very important in treatment of ICU patients. Rapid identification and optimal treatment of bacterial infections, including minimizing the development of antibiotic resistance, is warranted in these critically ill patients [4,5].

The efficacy of ciprofloxacin is dependent on the maximum serum concentration (C_{max}) and the area under the curve (AUC). Both the free Cmax (fC_{max}) and free AUC from 0-24 h ($fAUC_{0-24}$) are pharmacodynamic predictors of the efficacy of ciprofloxacin [6]. Fluoroquinolones exhibit a mainly concentration-dependent killing, but show time-dependent effects as well. Moreover, the maximum dose to be administered is limited by dose-related toxicity of the central nervous system. Consequently, the time of ciprofloxacin concentrations above the MIC should be optimised. Therefore, the most commonly used and best predictor of ciprofloxacin efficacy is AUC/MIC, which combines the C_{max} and time above MIC [2,7-8].

A therapeutic target level of $fAUC_{0-24}$/minimum inhibitory concentration (MIC) ≥ 100 and fC_{max}/MIC ≥ 8 are predictive for effective antibiotic therapy in critically ill patients [6,9-10]. Unfortunately, PK/pharmacodynamic (PK/PD) targets are not always achieved in ICU patients. Approximately 70% of the patients did not reach the target AUC/MIC of 125 with a MIC of 0.5 mg/L [5-6,11].

Approximately 70-80% of ciprofloxacin is present in the circulation as protein unbound drug, which accounts for the antibacterial activity [12,13]. However, in critically ill ICU patients, the amount of protein bound and unbound fractions is subject to change because of disease characteristics [14]. Fever, blood pH, elevated bilirubin, and decreased albumin levels can cause the ratio of bound and unbound ciprofloxacin to change [1,15-16]. Moreover, highly protein-bound co-medication could displace ciprofloxacin from proteins. Because of the divergent ratio of drug protein binding in ICU patients, therapeutic target levels are not always achieved [5-6,11]. This could lead to failure of antibiotic therapy and development of antibiotic resistance. Therapeutic drug monitoring (TDM) is used to optimise drug efficacy and minimise drug toxicity. Although TDM of ciprofloxacin is recommended [6], dose guidance based on plasma concentrations is not implemented in daily practice in hospitals.

To implement TDM of ciprofloxacin, an accurate analytical method is needed to measure plasma concentrations. Since unbound plasma levels reflect the antibacterial activity more closely than total plasma levels do, TDM of unbound ciprofloxacin concentrations is desirable when treating ICU patients with a high level of intra-individual variability in protein binding [1,15-16].

Several analytical methods for measuring total ciprofloxacin plasma concentrations have been described [17-21]. However, there is little information about methods for quantifying unbound ciprofloxacin plasma levels. Therefore, the aim of this observational study was to develop and implement a reliable ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method for the quantification of total and unbound ciprofloxacin plasma levels in hospital settings.

MATERIALS and METHODS

Setting and patients

The study was performed in a large teaching hospital. The ICU has 16 beds for medical and surgical patients.

Chemicals and standards

LC/MS grade ammonium acetate and methanol were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands) and VWR International (Amsterdam, The Netherlands), respectively while formic acid (98-100% purity) was also obtained from VWR Internation-

al. Purified water (MilliQ, Amsterdam, The Netherlands) was produced in our hospital. Pasteurised plasma protein solution (GPO) plasma and fresh frozen plasma (FFP) were purchased from Sanquin (Amsterdam, The Netherlands). Ciprofloxacin was purchased from Pharmachemie BV (Haarlem, The Netherlands) and cefazolin was purchased from Eurocept (Ankeveen, The Netherlands).

Plasma protein binding

In addition to quantifying total ciprofloxacin plasma concentrations, a method was developed to determine the plasma protein unbound ciprofloxacin concentrations. FFP was spiked 6 fold, to obtain concentrations of 0.2, 2.0 and 7.5 mg/L ciprofloxacin. First, 0.5 to 0.7 mL of the plasma samples was centrifuged at 1500 g for 25 min at 25 °C, using a Centrifree ultrafiltration device with a 30,000 molecular weight cut-off (Merck Millipore, Carrigtwohill, County Cork, Ireland). Then, 0.1 mL of the filtrate was prepared for UPLC/MS-MS analysis according the described method.

UPLC-MS/MS conditions

The chromatographic analyses were performed using an Acquity H-class UPLC system with a Xevo TQD detector, equipped with a BEH C18 50 × 2.1 mm 1.7 µm column (all obtained from Waters Corporation, Milford, USA). The data were processed using the MassLynx© software (Waters Corporation, Milford, USA). The mobile phase consisted of 2 mM ammonium acetate in water containing 0.1% formic acid (phase A) and 2 mM ammonium acetate in methanol containing 0.1% formic acid (phase B). The separation was run using a gradient of 90% phase A, followed by 100% phase B, and finally back to 90% phase A. The total run time was 5 min with a constant flow rate of 0.5 mL/min at a column temperature of 40 °C.

The analyses using electrospray ionization MS/MS were performed under the following conditions: desolvation gas flow, 1000 L/h at 600 °C; cone gas flow, 50 L/h; source temperature, 150 °C; cone voltage, 30 V; and capillary voltage, 1.0 kV. The transitions of precursor to product ions were detected at quantification and target trace of m/z 332→314.1, m/z 332→231.1 (retention time 2.00 min) and m/z 454.8→323.0, m/z 454.8→156.0 (retention time 2.04 min) for ciprofloxacin and cefazolin, respectively.

UPLC-MS/MS method validation

The method was validated for specificity, linearity, repeatability, intermediate precision, recovery, and lower limit of quantitation (LOQ) based on the European guidelines [22,23]. To prove specificity of the method, GPO plasma samples were processed in ten-fold according to the UPLC-MS/MS method described. Multiple reaction monitoring (MRM) transitions of these samples were compared to those of a standard solution of 0.1 mg/L ciprofloxacin. The matrix effect was determined by measuring the multiple reaction monitoring (MRM) transitions of the blank matrix. These MRM transitions were compared to the MRM transitions of a 0.1 mg/L ciprofloxacin standard solution. The MRM transitions of the blank matrix should not exceed 5% compared to the MRM transitions of the standard solution.

The linearity of the method was determined at the concentration range of 0.1-10.0 mg/L (n = 3). The precision of the method was tested by demonstrating its repeatability (intra-assay precision) and intermediate precision (inter-assay precision) using blank plasma spiked with ciprofloxacin at concentrations of 0.5 and 5.0 mg/L (n = 10). To investigate the intermediate precision, the samples were analysed using two different laboratory methods on two different days (three or four runs each day). The accuracy was determined by analysing solutions spiked with 4.0, 40, and 100 mg/L ciprofloxacin (n = 10) using the UPLC-MS/MS method. Finally, the LOQ was defined as a two times dilution of the lowest concentration of the concentration range (0.1 mg/L) [22]. Data were processed using Regres 10.01 and Microsoft Office Excel (version 2010, Microsoft Inc, Radmond, WA, USA).

Patient samples

The study was approved by the medical ethical committee of the Academic Hospital Maastricht (METC 17-4-025). The residual blood samples from the Laboratory of Clinical Chemistry and Hematology were collected unless the patient objected to its use for clinical research. Blood samples were collected during the period June 2017 until February 2018. The blood samples were stored during an average of approximately three months. Five ICU patients treated with ciprofloxacin were randomly selected. Since the focus of this observational study was the development of a method to analyse unbound fractions of ciprofloxacin and its implementation in daily clinical practice, no sample size calculation was performed. Blood samples were analysed according to the described UPLC-MS/MS method to determine total and unbound ciprofloxacin plasma concentrations. To assess treatment efficacy, PK/PD targets of $fAUC_{0\text{-}24}$/MIC ≥ 100 and fC_{max}/MIC ≥ 8 were calculated using the non-species related MIC breakpoint of ciprofloxacin (0.5 mg/L) [24]. The $AUC_{0\text{-}24}$ and Cmax were estimated based on total and unbound ciprofloxacin concentrations of three blood samples using PK/PD software MwPharm© version 3.58 (Mediware, Groningen, The Netherlands). All analyses were performed in an ISO-15189 certified laboratory.

RESULTS

The UPLC-MS/MS method for quantitative determination of ciprofloxacin plasma levels was validated by examining six parameters: specificity, linearity, repeatability, intermediate precision, recovery, and LOQ. There were 0.0% MRM transitions detected in the blank matrix compared to the 0.1 mg/L standard solution of ciprofloxacin. The linearity of the method was confirmed using the calibration equation $y = 0.999x + 0.0008$ with correlation coefficient (r) = 0.9996 and regression coefficient (r^2) = 0.9992 in the range of 0.1–10.0 mg/L (**Figure 1A**). A corresponding Bland-Altman plot is shown in **Figure 1B**. The precision and accuracy of the method were within acceptable limits for clinical use. The intra-assay coefficients of variation (CVs) were 1.84–2.20% and inter-assay CVs were 1.50–1.88% The method was accurate, with a calculated accuracy of 91.1–109.4% and CV of 3.59–5.18%. Furthermore, the LOQ of the UPLC/MS-MS method was 0.05 mg/L (CV 2.84%). **Figure 2** shows a chromatogram of ciprofloxacin at the LOQ. The mean fraction of ciprofloxacin unbound plasma concentrations in the range of 0.2-7.5 mg/L was 60.5% ± 6.17% and the unbound fractions are shown in **Table 1**. The intraday variability in protein binding was 3.06% (1.29 – 5.93%).

Clinical application of UPLC-MS/MS method

Fifteen blood samples (three per patient) were collected from five critically ill patients admitted In the ICU and their characteristics are shown in **Table 2**. Patients were treated with 400 mg ciprofloxacin intravenously once or twice daily. Ciprofloxacin total and unbound plasma concentrations were measured. The inter patient variability in protein binding was 12.7% (67.6 – 90.4%); intra patient variability in protein binding was 12.1%

Table 1. Validation data: plasma protein unbound fractions of ciprofloxacin (n=6)

Total concentration measured (mg/L)	Unbound ciprofloxacin concentration (mg/L)	Plasma protein unbound fraction (%)	SD	% CV
0.21	0.132	62.2	3.68	5.93
2.11	1.327	63.0	0.81	1.29
8.02	4.517	56.3	1.11	1.97

SD = Standard Deviation; %CV = Coefficient of Variation.

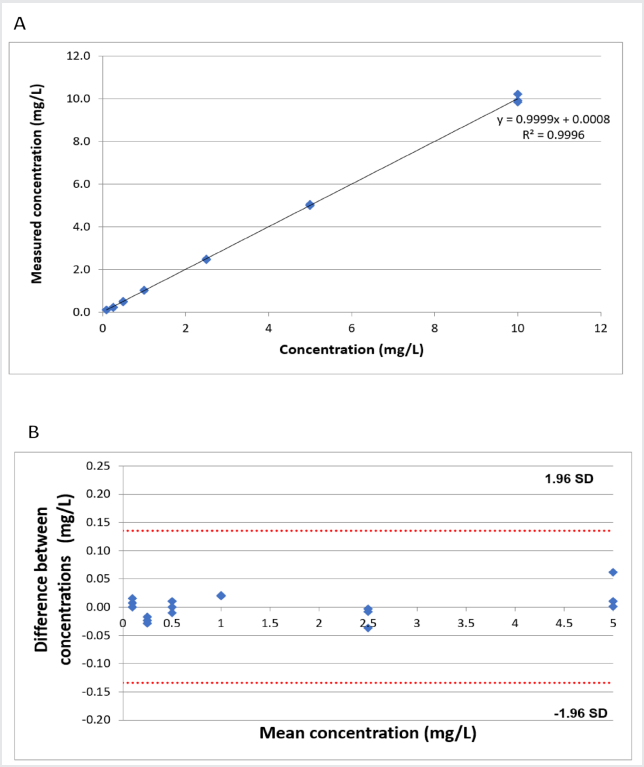

Figure 1. Linearity of developed method and Bland-Altman plot. (A) Linearity of ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis of ciprofloxacin; $y = 0.9999x + 0.0008$, $R^2 = 0.9992$ in the concentration range of 0.1–10 mg/L (n = 3). (B) Bland-Altman plot of the calculated ciprofloxacin concentrations versus the measured concentrations using the LC-MS/MS method; 95% limits are indicated with red dotted lines.

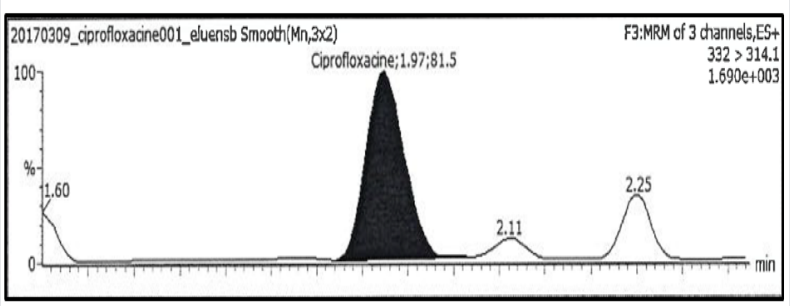

Figure 2. Chromatogram of ciprofloxacin plasma levels at LOQ (0.05 mg/L). The retention time of ciprofloxacin is shown at 1.97 minutes.

Table 2. Patient characteristics of five ICU patients treated with ciprofloxacin; SD = standard deviation.

	Mean ± SD	Range
Sex (male:female)	1:4	NA
Age (years)	74 (6.0)	67 – 83
Length (cm)	171 (6.0)	165 – 178
Weight (kg)	79 (14.4)	71 – 100
Creatinine (µmol/L)	127 (81.1)	37 – 281
Albumin (g/L)	31 (7.0)	21 – 37

SD = Standard Deviation.

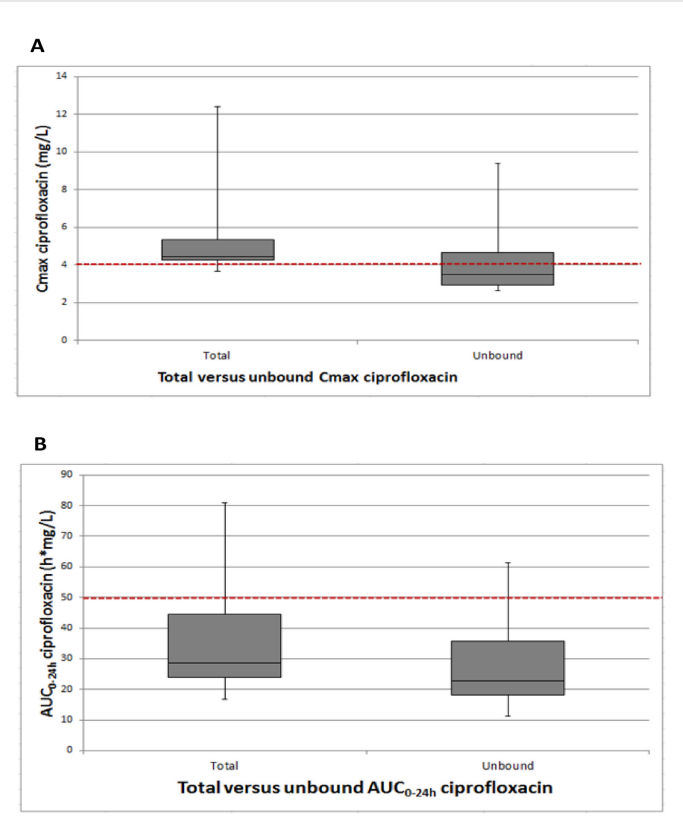

Figure 3. Boxplot of total and free drug concentrations and their calculated pharmacokinetic parameters.
(A) Boxplot span inter quartile ranges with median values. T-bars are set at minimum and maximum ciprofloxacin concentrations of analysed blood samples. The pharmacodynamic (PD) target of the maximum serum concentration (C_{max}) 4 mg/L is shown as a red dashed axis.(B) Boxplot of $AUC_{0\text{-}24h}$ calculated using both total and free drug. Boxplot span inter quartile ranges with median values. T-bars are set at minimum and maximum ciprofloxacin $AUC_{0\text{-}24h}$ of analysed blood samples. The PD target $AUC_{0\text{-}24h}$ of 50 h·mg/L is shown as a red dashed axis.

(±6.0). Subsequently, the AUC_{0-24} and C_{max} were estimated (**Figure 3**). The median unbound fraction was 74.8% (60.8–107.9%). The median C_{max} of the total ciprofloxacin concentrations was 4.45 mg/L (3.66–12.40) with a corresponding median AUC_{0-24} of 28.51 h·mg/L (16.80–80.94). The median unbound ciprofloxacin fractions were estimated at fC_{max} = 3.53 mg/L (2.73–9.41) and $fAUC_{0-24}$ = 21.57 h·mg/L (11.20–61.46).

Discussion

This observational study showed that our UPLC-MS/MS analysis was a reliable method for measuring total and protein unbound ciprofloxacin plasma concentrations within the therapeutic range of 0.1–10 mg/L and it had acceptable limits for clinical use [24,25]. In addition, we showed that by using the UPLC-MS/MS method in everyday clinical practice of the ICU, ciprofloxacin target attainment differed widely between calculations based on bound and unbound ciprofloxacin levels. Moreover, most patients did not achieve the therapeutic target based on ciprofloxacin unbound plasma concentration, which is known to be a more accurate parameter to predict efficacy than total plasma concentrations.

Our results indicate that in daily clinical practice, ICU patients benefit from TDM of unbound ciprofloxacin levels. Routine TDM of quinolones is practiced in a few hospitals and there are very few reports in the literature. A positive outcome correlates with PD targets of an $fAUC_{0-24}$/MIC ≥ 100 and fC_{max}/MIC ≥ 8, which have been proposed as targets to reach clinical improvement in critically ill patients [6,9,10]. With a clinical breakpoint of 0.5 mg/L, the $fAUC_{0-24}$ should be at least 50 h·mg/L and the fCmax at least 4 mg/L. In our study, one out of five ICU patients reached the target $fAUC_{0-24}$ of 50 h·mg/L.

The results showed that all five patients reached the target C_{max} of 4 mg/L. However, the unbound ciprofloxacin levels indicate that only two patients reached the fC_{max} levels of ≥ 4 mg/L. The results show that the $fAUC_{0-24}$ and fC_{max} based on total ciprofloxacin levels overestimated the clinically relevant PK/PD parameters of the unbound drug.

Our results are in line with those of previous studies addressing the PK of ciprofloxacin in critically ill patients [5,11,18,19]. The precision and accuracy of our UPLC/MS-MS method were comparable with those of previously reported methods for analysing total ciprofloxacin plasma concentrations [18,19]. In a prospective study, Van Zanten et al. [5] showed that administration of ciprofloxacin 400 mg intravenously to critically ill patients did lead to inadequate AUC_{0-24}/MIC and C_{max}/MIC ratios. Haeseker et al. [11] showed that even a substantial proportion of the general hospitalized patients was treated suboptimally with the recommended doses.

In contrast to previously described methods, our UPLC/MS-MS method could be used to measure ciprofloxacin unbound fractions. There is a paucity of data on analysis of unbound ciprofloxacin levels using MS/MS, making a comparison of our results with those of previous studies cumbersome. Both total and unbound ciprofloxacin fractions showed a high inter- patient variability as demonstrated by the altered PK parameters in our cohort of critically ill patients. Our patients showed low albumin levels and were treated for sepsis, had elevated liver enzymes and were treated with fluid resuscitation. This could explain the high inter individual variability due to a reduced availability of albumin by loss of liver function or a higher volume of distribution caused by oedema and excessive fluid administration.

Our data suggest that measuring the unbound fractions of ciprofloxacin, could achieve accurate and personalized dosing strategies to optimise plasma concentrations. These measurements could improve patient clinical outcomes in future patients. TDM of quinolones, including protein unbound fractions, is likely warranted in ICU patients since protein plasma binding in these patients is unpredictable due to altered PK [15]. Therefore, TDM of unbound ciprofloxacin plasma concentrations should be considered to generate relevant information for the development of accurate and personalized dosing strategies.

Furthermore, this would facilitate the optimisation of ciprofloxacin plasma concentrations, and thereby possibly improving patient clinical outcomes.
To the best of our knowledge, this study is the first report a UPLC/MS-MS method for analysing protein unbound plasma concentrations of ciprofloxacin. We randomly selected the patient blood samples to avoid selection bias. In this way, we demonstrated the clinical use of the method in an average ICU population of a general teaching hospital.
Our study has some limitations. It was a single centre study and the sample size was small. We did not perform sample size calculations because the study was focused on the technical aspects of the UPLC/MS-MS method. Patient samples were analysed to elucidate the clinical feasibility of the method application. Moreover, the clinical breakpoint of ciprofloxacin was used to interpret the PK-PD parameters. This could overestimate the percentage of ICU patients with suboptimal treatment in our hospital.

CONCLUSION

In conclusion, our UPLC-MS/MS method provides reliable, making target attainment in critically ill patients and exploration of different dosing regimens possible. TDM of antibiotic drugs based on the calculation of unbound drug fractions could become a daily practice in general hospitals. Future research in our hospital will be focused on TDM of antibiotics in ICU patients.

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REFERENCES

1. Tsai D, Lipman J, Roberts JA. Pharmacokinetic/pharmacodynamic considerations for the optimization of antimicrobial delivery in the critically ill. Curr Opin Crit Care 21(5), 412-20 (2015).
2. Forrest A, Nix DE, Ballow CH, Goss TF, Birmingham MC, Schentag JJ. Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. Antimicrob Agents Chemother 137(5), 1073-81 (1993).
3. Pea F, Poz D, Viale P, Pavan F, Furlanut M. Which reliable pharmacodynamic breakpoint should be advised for ciprofloxacin monotherapy in the hospital setting? A TDM-based retrospective perspective. J Antimicrob Chemother 58(2), 380-6 (2006).
4. Luyt CE, Bréchot N, Trouillet JL, Chastre J. Antibiotic stewardship in the intensive care unit. Crit Care 18(5), 480 (2014).
5. van Zanten AR, Polderman KH, van Geijlswijk IM, van der Meer GY, Schouten MA, Girbes AR. Ciprofloxacin pharmacokinetics in critically ill patients: a prospective cohort study. J Crit Care 23(3), 422–430 (2008).
6. Conil JM, Georges B, de Lussy A, Khachman D, Seguin T, Ruiz S, Cougot P, Fourcade O, Houin G, Saivin S. Ciprofloxacin use in critically ill patients: pharmacokinetic and pharmacodynamic approaches. Int J Antimicrob Agents 32(6), 505–510 (2008).
7. Kuti JL. Optimizing antimicrobial pharmacodynamics: a guide for your stewardship program. Revista Médica Clínica Las Condes 27(5), 615-624 (2016).
8. Roberts, J.A. & Lipman. Antibacterial Dosing in Intensive Care. J Clin Pharmacokinet 45, 755 (2006).
9. Thomas JK, Forrest A, Bhavnani SM, Hyatt JM, Cheng A, Ballow CH, Schentag JJ. Pharmacodynamic evaluation of factors associated with the development of bacterial resistance in acutely ill patients during therapy. Antimicrob Agents Chemother 42(3), 521–527 (1998).
10. Madaras-Kelly KJ, Ostergaard BE, Hovde LB, Rotschafer JC. Twenty-four-hour area under the concentration-time curve/MIC ratio as a generic predictor of fluoroquinolone antimicrobial effect by using three strains of Pseudomonas aeruginosa and

an in vitro pharmacodynamic model. Antimicrob Agents Chemother 40(3), 627–632 (1996).
11. Haeseker M, Stolk L, Nieman F, Hoebe C, Neef C, Bruggeman C, Verbon A. The ciprofloxacin target AUC:MIC ratio is not reached in hospitalized patients with the recommended dosing regimens. Br J Clin Pharmacol 75(1), 180–185 (2013).
12. Summary of product characteristics. Ciprofloxacine 2 mg/ml PCH, solution for infusion. Pharmachemie B.V. Last update 24th May 2018; accessed 03-08-2018.
13. The Royal Dutch Pharmacists Association. Monograph ciprofloxacin of the Informatorium Medicamentorum. https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/S2078.html [accessed 28-12-2017].
14. Pea F, Viale P, Furlanut M. Antimicrobial therapy in critically ill patients: a review of pathophysiological conditions responsible for altered disposition and pharmacokinetic variability. Clin Pharmacokinet 44(10),1009–1034 (2005).
15. Ulldemolins M, Roberts JA, Rello J, Paterson DL, Lipman J. The effects of hypoalbuminaemia on optimizing antibacterial dosing in critically ill patients. Clin Pharmacokinet 50(2), 99–110 (2011).
16. Zeitlinger MA, Derendorf H, Mouton JW, Cars O, Craig WA, Andes D, Theuretzbacher U. Protein binding: do we ever learn? Antimicrob Agents Chemother 2011 55(7), 3067-74 (2011).
17. van Geijlswijk IM, van Zanten ARH, van der Meer YG. Reliable new high-performance liquid chromatographic method for the determination of ciprofloxacin in human serum. Ther Drug Monit 28(2), 278–281 (2006).
18. Zander J, Maier B, Suhr A, Zoller M, Frey L, Teupser D, Vogeser M. Quantification of piperacillin, tazobactam, cefepime, meropenem, ciprofloxacin and linezolid in serum using an isotope dilution UHPLC-MS/MS method with semi-automated sample preparation. Clin Chem Lab Med 53(5), 781–791 (2015).
19. Choudhury H, Gorain B, Paul A, Sarkar P, Dan S, Chakraborty P, Pal TK. Development and validation of an LC-MS/MS-ESI method for comparative pharmacokinetic study of ciprofloxacin in healthy male subjects. Drug Res 67(2), 94–102 (2017).
20. Lefeuvre S, Bois-Maublanc J, Hocqueloux L, Bret L, Francia T, Eleout-Da Violante C, Billaud EM, Barbier F, Got L. A simple ultra-high-performance liquid chromatography-high resolution mass spectrometry assay for the simultaneous quantification of 15 antibiotics in plasma. J Chromatogr B Analyt Technol Biomed Life Sci 15, (1065–1066), 50–58 (2017).
21. Mack G; Improved high-performance liquid chromatographic determination of ciprofloxacin and its metabolites in human specimens. J Chromatogr 582(1-2), 263–267 (1992).
22. ICH guidelines “Validation of Analytical Procedures: Tekst and Methodology Q2(R1)”, November 2005.
23. EDQM guideline “Validation of Analytical Procedures: PA/PH/OMCL (05) 47 DEF, Juni 2005”.
24. EUCAST; European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.1, (2018). http://www.eucast.org/ [accessed 03-08-2018].
25. Academic Medical Hospital Groningen. Monograph ciprofloxacin. https://www.umcg.nl/NL/Zorg/Professionals/dienstverlening/Laboratoriumgeneeskunde/bepalingenwijzer/Paginas/default.aspx [accessed 28-12-2017].