J Antimicrob Chemother 2022; **77**: 2437–2440
https://doi.org/10.1093/jac/dkac195 Advance Access publication 23 June 2022

Journal of Antimicrobial Chemotherapy

New methods for quantification of amoxicillin and clindamycin in human plasma using HPLC with UV detection

Eva Greibe[1,2]*, Claus Ernst Moser[3,4], Niels Eske Bruun[5,6,7] and Elke Hoffmann-Lücke[1,2]

[1]Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark; [2]Institute for Clinical Medicine, Aarhus University, Aarhus, Denmark; [3]Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark; [4]Department for Immunology and Microbiology, Copenhagen University, Copenhagen, Denmark; [5]Department of Cardiology, Zealand University Hospital, Roskilde, Denmark; [6]Institutes of Clinical Medicine, Copenhagen University, Copenhagen, Denmark; [7]Institutes of Clinical Medicine, Aalborg University, Aalborg, Denmark

*Corresponding author. E-mail: evagreib@rm.dk

Received 5 April 2022; accepted 20 May 2022

Objectives: We aimed to develop simple and rapid HPLC methods for determination of amoxicillin and clindamycin in human plasma.

Methods: Plasma samples were pretreated by direct deproteinization with acetonitrile and the analytical separation took place on a reverse phase Poroshell 120 EC-C18 column (2.7 μm, 2.1 × 100 mm) with a gradient of acetonitrile. UV detection at 229 nm for amoxicillin and 204 nm for clindamycin was used for determination of the antibiotics in plasma.

Results: The calibration curves were linear over the concentration ranges of 1–100 mg/L for amoxicillin and 1–15 mg/L for clindamycin with a correlation coefficient of ≥0.98. Intra-assay precisions were all ≤15% and the accuracies were within ±15%. The limit of quantification (LOQ) was found to be 0.5 mg/L for amoxicillin and 1 mg/L for clindamycin with inter-assay imprecision coefficient of variances (CVs) of 18.7% and 15.6%, respectively. The present HPLC methods were successfully applied on spike-in samples and on plasma samples collected 4–6 and 3.5–5.5 h after oral antibiotic administration of 500 mg of amoxicillin and 600 mg of clindamycin, respectively.

Conclusions: We have developed HPLC methods with UV detection for quantification of amoxicillin and clindamycin in human plasma. The methods are fast, simple and suitable for use in routine settings and clinical studies.

Introduction

Amoxicillin and clindamycin are valuable antibiotic drugs that can be administered orally. Several reports have shown substantial inter-patient variations in antibiotic plasma concentrations.[1–3] Antibiotic effect is directly related to the concentration in relation to microbe susceptibility.[3,4] Several methods have been described in the literature for determination of amoxicillin and clindamycin in plasma, including radioimmunoassay, microbial assay, GC, LC-MS/MS and HPLC using different detection methods and columns.[5–9] Some of these methods have very complex designs, require expensive equipment and demand comprehensive pre-analytical handling.

The present paper reports on the development of simple, fast and sensitive methods using HPLC with UV detection for measurement of amoxicillin and clindamycin in human plasma. The strength of these methods is that they are quick and sensitive, and can easily be applied in other research and clinical laboratories. In order to demonstrate the usability of the methods and present proof of concept, we have analysed spike-in samples and human samples collected after antibiotic administration.

Materials and methods

Preparation of stock solutions, calibration standards and quality control (QC) samples

Amoxicillin (amoxicillin trihydrate; CAS number 61336-70-7; MW 419.45) and clindamycin (clindamycin hydrochloride, CAS number 58207-19-5; MW 479.46) were purchased from Merck (Soeborg, Denmark).

Stock solutions of amoxicillin and clindamycin were prepared by dissolving 25 mg in 25 mL of 0.9% NaCl. Standards were prepared for amoxicillin (blank, 1, 5, 12.5, 25, 50 and 100 mg/L) and clindamycin (blank, 1, 5, 10 and 15 mg/L) by adding stock solutions to EDTA plasma. The QCs were

prepared in plasma at concentrations levels of 2.5 and 50 mg/L for amoxicillin and 5 and 12 mg/L for clindamycin. The standard and QC samples were stored at −80°C until use.

Sample pretreatment

For analysis of total plasma amoxicillin, 75 μL of sample was transferred to a 96-well Captiva filter plate with 0.2 μm pore size (Agilent Technologies, Denmark) together with 450 μL of acetonitrile. After heat sealing at 170°C and 5 min of shaking, the plate was centrifuged for 10 min at 3000 rpm and the filtrate was evaporated under a gentle stream of nitrogen gas at 40°C. After approximately 40 min, dry extracts were obtained. The dry extracts were reconstituted with 150 μL of 0.1 M phosphate buffer with 5% acetonitrile, heat sealed and subjected to shaking for 5 min at 2000 rpm. A volume of 5 μL was injected into the HPLC system for analysis.

For analysis of total clindamycin, 75 μL of sample was transferred to a 96-well Captiva filter plate with 0.2 μm pore size (Agilent Technologies, Denmark) together with 360 μL of methanol and 90 μL of 10% zinc sulphate. After heat sealing and 5 min of shaking, the plate was centrifuged for 10 min at 3000 rpm and the filtrate was evaporated under a gentle stream of nitrogen gas at 40°C. After approximately 50 min, dry extracts were obtained. The dry extracts were reconstituted with 150 μL of 0.1 M phosphate buffer with 5% acetonitrile, heat sealed and subjected to shaking for 5 min at 2000 rpm. A volume of 5 μL was injected into the HPLC system for analysis.

HPLC

The plasma concentrations of amoxicillin and clindamycin were analysed using HPLC with UV detection. The HPLC-UV system consisted of an Agilent Series 1290 HPLC system with a diode array detector (DAD) (Agilent Technologies, Denmark). Analytical separation was performed on a Poroshell 120 EC-C18 column (2.7 μm, 2.1 × 100 mm) (Agilent Technologies, Denmark) at a temperature of 28°C (amoxicillin) or 40°C (clindamycin) controlled by a column heater.

The mobile phases had a flow rate of 0.5 mL/min and were composed of: (A) 5% acetonitrile in phosphate buffer, pH 3; and (B) acetonitrile. For analysis of amoxicillin, the gradient profile was: 0% B (0 min), 30% B (3 min), 30% B (3.5 min), 0% B (4 min). For analysis of clindamycin, the gradient profile was: 20% B (0 min), 20% B (0.5 min), 30% B (3 min), 20% B (4 min). The needle was cleaned with 50% methanol in water. The analytes were detected by UV detection (HPLC-UV) at 229 nm for amoxicillin and 204 nm for clindamycin.

Data analysis

All data used for qualification were collected and processed using OpenLAB software (Agilent Technologies, Denmark). The calibration settings were performed with a linear curve fit with equal weighing. As criteria of acceptance, all calibrator standards had to be within ±15% from the nominal concentration in each analytical run and the correlation coefficient (R^2) had to be ≥0.98.

Method validation and proof of concept

For validation of the developed methods, linearity, carry-over, lower limit of detection (LOD), limit of quantification (LOQ), precision [coefficient of variance (CV)], stability and accuracy were assessed.

The linearity of the developed methods was assessed over calibration ranges of 1–100 mg/L for amoxicillin and 1–15 mg/L for clindamycin. The acceptable criteria for the calibration curves are outlined above. The dilution integrity was assessed by making a dilution series (100%, 90%, 75%, 50%, 25%, 10%, 5%, 1%) from the highest standard (100 mg/L for amoxicillin and 15 mg/L for clindamycin) that was measured in three replicates for evaluation by linear regression. The validation criteria were that both precision and deviation from the nominal concentration for the means of the three replicates should be ≤15% from the nominal concentration and that the R^2 should be ≥0.98.

Carry-over was assessed in combination with the linearity studies by running two subsequent blank samples after the 100%, 90% and 75% stock solutions. The concentration of analyte in the blank samples should be below the LOD to rule out carry-over. The LOD was assessed as the mean value of 25 replicates of blank samples plus five times SD value of the blanks. The LOQ was determined as the concentration with a signal-to-noise ratio of 10.

The precision of the assays was determined at three concentration levels (LOQ, QC1 and QC2) by analysing five replicates in three runs ($n = 15$, LOQ) or five runs ($n = 25$, QC1 and QC2). The validation criteria were that the inter-assay imprecision should be ≤15% (or ≤25% at LOQ).

The two levels of QC samples were used to evaluate the freeze–thaw stability and the long-term stability of the antibiotics in plasma after 2 months at −80°C.

To test the accuracy, we analysed plasma samples spiked with seven different concentrations of amoxicillin [100, 80, 60, 30, 10, 5 and 0 (blank) mg/L] and clindamycin (18, 13, 10, 5, 2, 1 and 0 mg/L). The stocks were analysed in triplicates and the mean concentrations were used to evaluate accuracy/recovery. The acceptance criterion was that the recovery should be within ±15% of the nominal spike-in concentration and that the inter-assay precision should be ≤15%.

To explore the usability of the methods and as proof of concept, we analysed plasma samples from patients undergoing haemodialysis ($n = 5$) collected 4–6 and 3.5–5.5 h after oral administration of 500 mg of amoxicillin and 600 mg of clindamycin, respectively. The plasma was collected in relation to another ongoing study (ClinTrials.gov, NCT05248620) approved by the Danish Ethical Committee (approval H-20026735). For this study, non-fasting whole blood samples were collected in EDTA tubes and centrifuged (10 min at 2300 **g**) within 2 h of being drawn at Herlev and Gentofte Hospital, Denmark, and at Roskilde hospital, Denmark. Plasma was stored at −80°C and shipped on dry ice to Aarhus University Hospital, Denmark, for analysis of amoxicillin and

Table 1. Recoveries of amoxicillin and clindamycin in human plasma

Spiked concentration (mg/L)	Measured concentration (mg/L), mean[a]	Recovery (%)[b]	CV (%)[c]
Amoxicillin			
100	105.0	105.0	0.3
80	80.4	100.4	0.3
60	60.5	100.9	0.7
30	30.9	100.9	0.1
10	12.2	102.2	1.5
5	5.2	100.3	1.1
Clindamycin			
18	17.8	99.8	0.6
13	13.7	100.7	0.3
10	10.1	100.1	0.1
5	4.9	99.9	0.4
2	1.8	99.8	7.4
1	0.9	99.9	2.2

[a]Results are given as means of triplicates.
[b]Recovery (%) is expressed as [(mean measured concentration−nominal concentration)/(nominal concentration)] × 100 (accept criterion, ±15%).
[c]CV (%) is expressed as [(SD/mean) × 100] (accept criterion, ≤15%) of three replicates.

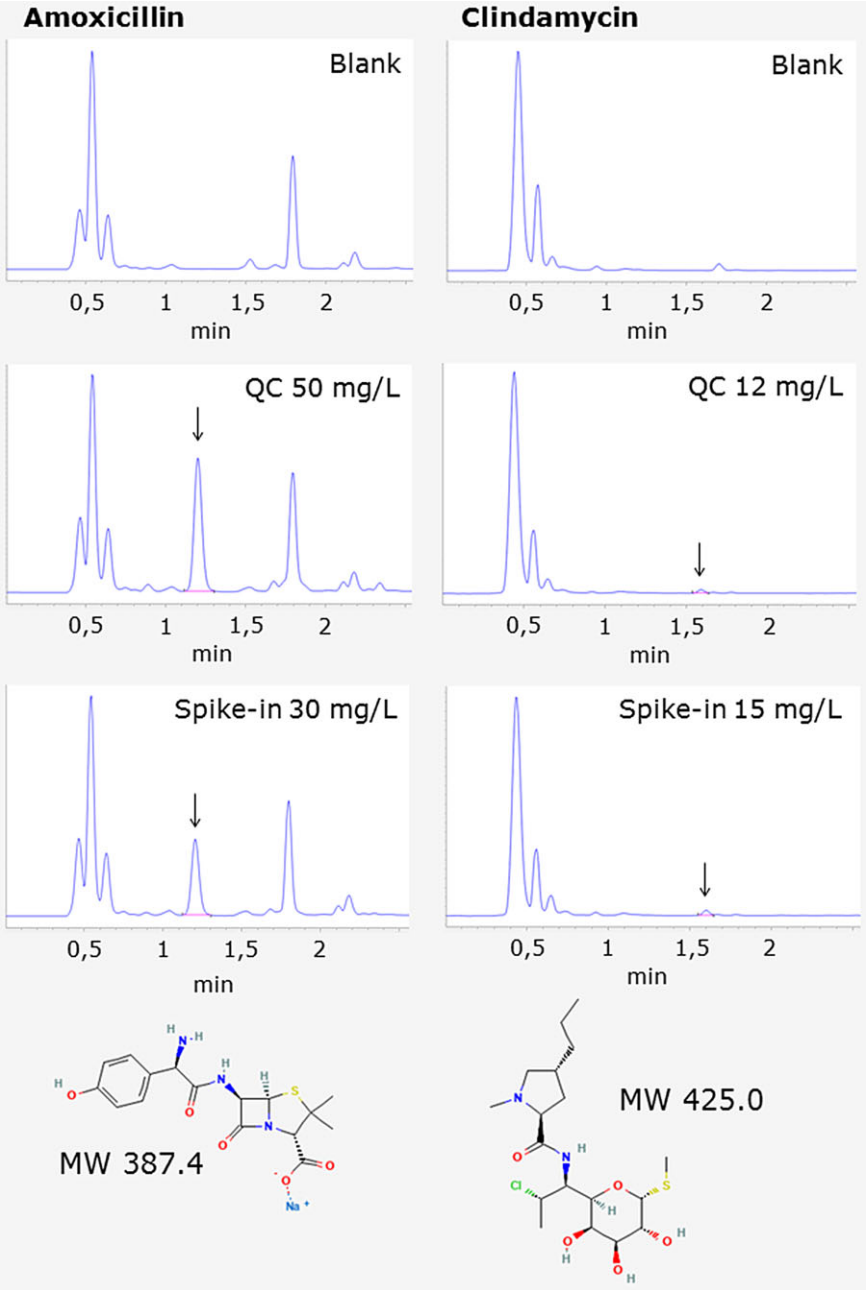

Figure 1. Typical chromatograms for amoxicillin and clindamycin in human plasma shown for blank samples, QCs and spike-in samples. The structures are from PubChem. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

clindamycin. The anonymized plasma samples were analysed in duplicates.

Results and discussion

Method validation and application

In each validation batch, the calibration standards were analysed and the calibration curves showed a linear response in the concentration range of 1–100 mg/L for amoxicillin and 1–15 mg/L for clindamycin. The R^2 values of all calibration curves were ≥0.98, which demonstrated a good linearity within the concentration range.

No carry-over was found at 100 mg/L for amoxicillin and 15 mg/L for clindamycin. The LOQ was 0.5 mg/L for amoxicillin and 1 mg/L for clindamycin, and the total imprecisions (CV) were within the acceptance criteria (≤15%; ≤25% at LOQ). For amoxicillin, the mean precisions were found to be 14.3%

(2.5 mg/L) and 10.8% (50 mg/L) and 18.7% (0.5 mg/L, LOQ). For clindamycin, the figures were 5.2% (5 mg/L) and 4.5% (12 mg/L) and 15.6% (1 mg/L, LOQ).

Long-term stability of plasma samples spiked with amoxicillin and clindamycin showed no loss of analytes when they were stored at −80°C for 2 months or subjected to three freeze–thaw cycles (data not shown).

To demonstrate the usability of the methods in human plasma, we analysed plasma samples spiked with different concentrations of amoxicillin and clindamycin. The results are shown in Table 1. All the blank samples provided an analyte concentration below the detection limit and the spike-in samples had recoveries (%) within ±15% and CV (%) ≤15%. Chromatograms from the analysis of a blank sample, a QC sample and a spike-in sample are shown in Figure 1.

The analysed plasma collected 4–6 and 3.5–5.5 h after oral administration of 500 mg of amoxicillin and 600 mg of clindamycin, respectively, were found to have a mean ± SD concentration of 3.5 ± 1.2 mg/L ($n=5$) and 5.5 ± 1.9 mg/L ($n=5$) of amoxicillin and clindamycin, respectively.

Conclusions

In conclusion, we have developed HPLC methods with UV detection for quantification of amoxicillin and clindamycin in human plasma. The methods were successfully validated with the analysis of spiked human samples and samples collected after oral antibiotic administration. The methods provide simple, fast and accurate ways of quantitatively analysing amoxicillin and clindamycin, and are considered to be easy to implement in laboratories and suitable for use in routine settings and in clinical studies for the antibiotics used alone or in combination.

Acknowledgements

We thank Margrethe Sønderskov Christensen and Katrine Agerbo Bjerring, Department of Clinical Biochemistry, Aarhus University Hospital, Denmark, for excellent technical assistance.

Funding

This work was supported by an unrestricted grant from the Novo Nordisk Foundation and by the Department of Clinical Biochemistry, Aarhus University Hospital, Denmark.

Transparency declarations

N.E.B has received investigator-initiated grants from the Novo Nordisk Foundation, the Augustinus Foundation, the Kaj Hansen Foundation and Health Insurance Denmark. All other authors: none to declare.

Author contributions

E.G.: main author of this manuscript; principal investigator; designed the study; and analysed and interpreted data. C.E.M.: designed the study; critical revision; final approval of the version to publish; and acquired funding. N.E.B.: designed the study; critical revision; final approval of the version to publish; and acquired funding. E.H.-L.: designed the study; critical revision; final approval of the version to publish; acquired funding; and supervision of research.

References

1 Roberts JA, Paul SK, Akova M *et al.* DALI: defining antibiotic levels in intensive care unit patients: are current β-lactam antibiotic doses sufficient for critically ill patients. *Clin Infect Dis* 2014; **58**: 1072–83.

2 Høiby N, Pers C, Johansen HK *et al.* Excretion of β-lactam antibiotics in sweat—a neglected mechanism for development of antibiotic resistance? *Antimicrob Agents Chemother* 2000; **44**: 2855–7.

3 Moser C, Lerche C, Thomsen K *et al.* Antibiotic therapy as personalized medicine – general considerations and complicating factors. *APMIS* 2019; **127**: 361–71.

4 Mouton JW, Brown DFJ, Apfalter P *et al.* The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach. *Clin Microbiol Infect* 2012; **18**: E37–45.

5 Ball AP, Davey PG, Geddes AM *et al.* Clavulanic acid and amoxycillin: a clinical, bacteriological, and pharmacological study. *Lancet* 1980; **315**: 620–3.

6 Metzler CM, Dehaan R, Schellenberg D *et al.* Clindamycin dose-bioavailability relationships. *J Pharm Sci* 1973; **62**: 591–8.

7 Fieger-Büschges H, Schussler G, Larsimont V *et al.* Determination of clindamycin in human plasma by high-performance liquid chromatography using coupled columns. *J Chromatogr Biomed Sci Appl* 1999; **724**: 281–6.

8 Yu L-L, Chao C-K, Liao W-J *et al.* Determination of clindamycin in human plasma by liquid chromatography-electrospray tandem mass spectrometry: application to the bioequivalence study of clindamycin. *J Chromatogr B Biomed Sci Appl* 1999; **724**: 287–94.

9 Yoon K-H, Lee S-Y, Kim W *et al.* Simultaneous determination of amoxicillin and clavulanic acid in human plasma by HPLC-ESI mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2004; **813**: 121–7.