INTRODUCTION

β-lactam antibiotics are the cornerstone of antibacterial treatment and frequently prescribed drugs, especially in the intensive care units (ICU) of hospitals. Optimization of antibiotic dosing is a key intervention to improve clinical outcomes. Therapeutic drug monitoring (TDM) is a commonly used dosing strategy to optimize exposure and thereby minimize toxicity and maximize the efficacy. Currently, TDM of β-lactam antibiotics is rarely performed, due to poor availability in clinical practice.

The aim of this paper is to describe a newly developed and validated HPLC-MS method for the determination of ampicillin, amoxicillin, cefepime, ceftazidime, imipenem, meropenem and piperacillin in human plasma. The combination of antibiotics selected in this study is aimed at those commonly used in severely ill patients in the ICU of North Estonia Medical Centre.

EXPERIMENTAL

Chemicals, reagents and standards
Antibiotic standards (ACS grade) (Figure 1), internal standard (IS) fluoxacillin and acetonitrile (ACN) (HPLC grade) were purchased from Sigma-Aldrich. Milli-Q water was used for preparations of the solutions.

Calibrator and quality control solutions
Antibiotic stock solutions 1000 µg/mL were prepared in MilliQ water and stored at −80°C. Working standards and quality controls (QC) were prepared in three different matrices used in validation process: in MilliQ water (1), standards spiked into the pooled plasma before (3) and after (2) extraction in the range of 0.5-100 µg/mL, depending on the matrix. The QC solutions were prepared in a similar way from antibiotic standards with different LOT from those that were used for the calibrations.

Chromatographic conditions
Chromatographic separation was carried out on an Agilent 1200 Infinity system (Agilent Technologies, Santa Clara, CA) equipped with an Agilent Poroshell 120 EC-C18 (100 mm × 4.6 mm i.d.) with 2.7 µm particle size, operating at a temperature 40°C. A binary mobile phase with a gradient elution was used. Mobile phase A was MilliQ water/0.1% formic acid and mobile phase B was ACN/0.1% formic acid. The flow rate was set for 0.7 mL/min. The elution gradient was as follows: 0% B=30% B in 5 min, 95% B in 10 min and then 5 min at 95% B and afterwards the analytical column was re-equilibrated to initial gradient settings. The sample injection volume was 3 µL.

VALIDATION RESULTS

The developed procedure was validated according to the EMA Guidelines [1]. For selectivity assessment, ten independent blank and spiked blank plasmas fortified with IS were analysed by developed HPLC-MS method. Due to the selectivity of MS/MS-detection the absence of the endogenous signal contributions for any analyte of interest was confirmed according to EMA criteria: response was less than 20% of the LOQ for the analyte and 5% for the IS.

Table 3 depicts the results of the method validation regarding the linearity of the working range, instrumental quantitation limit (IQL) and limit of quantitation of the procedure (LOQ), extraction recoveries (R) and matrix effects (ME). The plots of the peak area ratios (analyte to IS) versus the concentrations of the standards in water (1), in prepared plasma (2) and in plasma with spiked standards before extraction (3) exhibited adequate linearity for the studied analytes, all with acceptable statistical parameters: the regression error (RE) were <10% and probability (P) associated with F statistic for a on-sided distribution with k-2 (MSp) and N-k (MSn) degrees of freedom: P>0.05 (lack-of-fit ANOVA), where k=6, N=18.

Matrix effects were assessed by the slope ratios of the calibration curves (2) (in the presence of matrix) and (1) (in the absence of matrix) according to: (ME) (%) = R1/R2 × 100

Significant ion enhancement effect (about 50%) was observed for IMP and ion suppression effect for CEF due to the presence of interfering components in the matrix. When quantifying β-lactam antibiotics the presence of matrix effects can be taken into account by using the calibration curves in the matrix.

The extraction recoveries from the sample matrix were assessed by the slope ratios of the calibration curves (3) and (2): R(3)/R(2) = b × 100

The intermediate precision and the accuracy were estimated by using six fortified matrix samples at three concentration levels – low (l), medium (M) and high (H) (Table 2). Precision and accuracy values were within the acceptance value (RSD ≤ 15%), with the exception of the IMP (16.1 and 18.3%), both for only the QC-L samples. The blank samples that were injected immediately after the analysis of high-level samples containing 100 µg/mL of each analyte showed no evidence of carry-over (the signals were below the LOD for all analytes).

CONCLUSIONS

We validated simple and rapid HPLC-MS/MS assay for the simultaneous determination of seven (potentially ten) frequently used β-lactam antibiotics. The method is accurate, reproducible and is successfully being used in TDM studies in the ICU of North Estonia Medical Centre.