Bioanalytical UPLC-MS/MS method development and validation for measuring penicillins in human blood plasma– analyte stability issues

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Antimicrobials are the medicines most commonly prescribed to children, so the correct dose is of key importance to improve outcome and reduce toxicity [1]. This is particularly true for patients in intensive care, where effective antimicrobial therapy is absolutely crucial, and influences therapeutic outcomes in both children and adults [2]. One key factor in improving treatment outcomes is the optimization of antibiotic dosing [3,4,5,6]. On average, each child in Europe receives one course of antibiotics per year, but there is considerable variation in the rate of antimicrobial prescribing in different countries and also the doses used. Even for very common antibiotics, there remains a marked lack of information about the optimal dosing in the context of critical illness. Although antibiotics are extensively used for patients in intensive care (ICU), very few pharmacokinetic (PK) studies have been conducted in this patient population [7], despite numerous studies on toxicity.

Penicillins are the important group of antimicrobials widely used in children and adults for over 50 years. Penicillins are β-lactam antimicrobials and therefore especially intolerant to the stress conditions since the degradation of penicillins occur in different ways in different conditions. The stability of penicillins have been evaluated in stress conditions previously [8]. The instability of penicillins is reported through the β-lactam ring opening (Fig 1) in acidic and basic conditions, enzymatic (hydrolysis and aminolysis) degradation, degradation by the presence of metal ions and by temperature changes.

Figure 1. β-lactam ring opening.
The list of penicillins studied consisted ampicillin, amoxicillin, penicillin G, piperacillin and flucloxacillin as the most commonly used penicillins in the United Kingdom for children intensive care (Fig 2).

Figure 2. Chemical structures of penicillins.

The UPLC-MS/MS method (using Waters Acquity UPLC coupled with TQ detector) was using protein precipitation with acetonitrile (penicillin G-D7 as internal standard (IS)) for sample preparation. For the separation of compounds, 0.1 % formic acid in water and methanol with gradient elution was used in reversed phase (analytical column: 50mm x 2.1mm; 1.7 µm Acquity UPLC BEH C18). Multiple reaction monitoring (MRM) mode was used for detection of penicillins. With 3Q detector transitions m/z 335 [M+1] -> m/z 160; 176 (for penicillin G); m/z 350 [M+1] -> m/z 106; 160 (for ampicillin); m/z 366 [M+1] -> m/z 114; 208 (for amoxicillin); m/z 518 [M+1] -> m/z 143; 160 (for piperacillin); m/z 454 [M+1] -> m/z 160;
295 (for flucloxacillin) and m/z 342 \([M+1]\) -> m/z 160 (for penicillin G-D7, IS) were used for quantification and qualification.

The aim of developing and validating the bioanalytical method for measuring penicillins in blood plasma was to use it for the measurement of ICU patients’ plasma samples, in order to use the data for the population PK modelling and dose optimization of the drugs.

In the method validation step the stability of the analyte must be carefully examined. Through the validation of the bioanalytical method according EMA guideline [9], the stability of penicillins was carefully studied for freeze-thaw stability, long-term freezer stability, bench-top stability, stock solution stability, processed sample stability in different conditions.

The rapid degradation of ampicillin occurred for the samples kept in the cooled (+10 °C) autosampler for 24 h, only 35-57% of drugs original concentrations remained to the samples. However, all other penicillins maintained 85-99% of their original content. The freeze-thaw stability of ampicillin indicated also quicker degradation of the compound in plasma samples, since 82-99% of the original content remained to the plasma samples after 3 freeze-thaw cycles, while rest of the penicillins maintained approximately 98-100% of their original content in the plasma samples.

Bench-top stability of the penicillins at the room temperature (23 ± 2°C) for 24 h indicated the degradation of the flucloxacillin, piperacillin and penicillin G in the plasma samples, since only 40-63%, 52-64% and 66-70%, respectively, of the drug was detectable after applying the room temperature as a stress condition. Ampicillin and amoxicillin however had a slightly better stability in the bench-top, 89-96% and 71-89%, respectively, of the drugs were detectable after 24 h in the room temperature.

The method was fully validated and matrix effects, accuracy, precision, linearity, limits of quantification, limit of detection and method’s uncertainty estimation was evaluated.

The aim of the clinical study is to characterise the pharmacokinetic profiles of these penicillins in intensive care patients when used in hospital in the routine clinical context, in order to further understanding of the extent of interindividual pharmacokinetic variability within a critically ill patient population.
References


