Evaluation of the Mitra microsampling device for dry sample processing in a pharmacokinetic/pharmacodynamic study of beta-lactams

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Introduction

Antimicrobials form an essential part of the treatment of critically ill patients, yet there is often very limited evidence to support the dose regimens used routinely in intensive care (ICU). Typically, the pharmacokinetic data used to derive current ICU antibiotic dosing recommendations were based on studies in healthy volunteers and non-critically ill patients, with little consideration for the significant pharmacokinetic changes that occur in sepsis. Beta-lactams are an important group of antimicrobials, commonly used in ICU settings, which lack chemical stability, therefore the sample processing and analysis should be carefully controlled. Moreover, paediatric patient blood sampling requires careful considerations of the blood volume and therefore the microsampling techniques are much preferred. Novel microsampling device called Mitra was introduced by Phenomenex last year.

Objectives

Penicillins are β-lactam antimicrobials and therefore especially intolerant to stress conditions since the degradation of penicillins occurs in several ways under different conditions. The instability of penicillins can result from the β-lactam ring opening in acidic and basic conditions, enzymatic (hydrolysis and aminolysis) degradation, degradation by the presence of metal ions and from temperature changes.
Therefore, stability (ST%) studies during bioanalytical microsampling assay validation are crucial. 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 (Fig1). We aimed to investigate a dry blood and blood plasma sampling device, Mitra (Fig 2), which could potentially improve beta-lactam stability in the sampling and storage procedures.

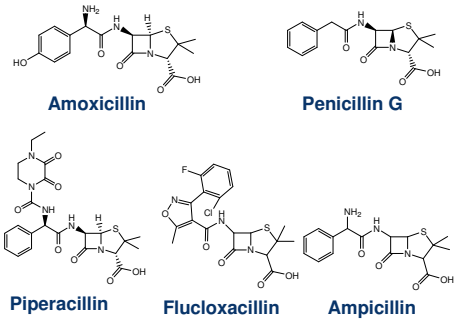

Figure 1. Chemical structures of penicillins.

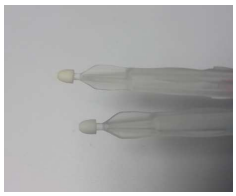

Figure 2. Mitra microsampling device.

Materials and methods

Sampling and sample preparation

10 µL of blood plasma sampled with Mitra

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Add 50 µL de-ionised water and 100 µL internal standard (Penicillin G-D7) in acetonitrile

↓

Mix for 9 min and centrifuge 13500 x g for 5 min

↓

Add 500 µL of de-ionised water to 100µL of supernatant, vortex for 10 seconds

↓

Inject 10µL of extract into the analytical column

UPLC-MS/MS conditions

The UPLC-MS/MS method used Waters Acquity UPLC coupled with tandem quadrupole detector.

UPLC conditions:
Mobile phase:
0.1% Formic acid in water
0.1% Formic acid in methanol
Gradient elution with starting at 95/5.
Column: 50mm x 2.1mm Acquity UPLC BEH C18
Flow rate: 250µL/min

Sample ID	Initial ion (m/z)	Product ion (m/z)
PenicillinG	335	159.9 (±0.5) 176.02 (±0.5)
Ampicillin	350	105.99 (±0.5) 159.94 (±0.5)
Amoxicillin	366	113.91 (±0.5) 208.02 (±0.5)
Piperacillin	518	143.03 (±0.5) 159.93 (±0.5)
Flucloxacillin	454	159.94 (±0.5) 294.92 (±0.5)
Penicillin G-D7	342	159.93 (±0.5)

Results and discussion

Through the validation of this bioanalytical method according to the European Medicines Agency (2011) Guideline on bioanalytical method validation, the accuracy and precision using Mitra microsampling was carefully studied. The stability of penicillins analysed with dried blood plasma sampling using Mitra was compared with regular liquid sampling.

Accuracy and precision using Mitra microsampling

Penicillins have a linear (R2>0.995) range from 0.15 µg/mL-200 µg/mL in plasma using Mitra devices. Accuracy and precision were tested (n=5) in low (0.15 µg/mL), medium (20 µg/mL) and high (80 µg/mL) concentrations.

Compound	Within-day accuracy		Within-day precision (CV%)	
	Mitra sampling	10 µL blood plasma pipetting	Mitra sampling	10 µL blood plasma pipetting
Amoxicillin	2-5%	4-9%	2-4%	5-12%
Ampicillin	3-6%	4-8%	1-3%	4-7%
PenicillinG	1-5%	6-11%	2-6%	5-10%
Piperacillin	2-6%	4-10%	2-5%	5-11%
Flucloxacillin	2-7%	5-9%	3-6%	5-9%

Penicillins stability using Mitra microsampling

Penicillins have shown demonstrated poor stability at room temperature, +4 ˚C and even storage at -20 ˚C.
Storage at -20 ˚C indicated degradation of all penicillins in liquid sampling. Ampicillin was most affected and only 53-58% of the drug original content remained in the sample after 30 days storage. Mitra microsampling and storage in dry form, however, caused even more rapid degradation, only 8-16% of the drug was detected after 30 days storage at -20 ˚C (Fig. 3).
Testing at room temperature (23 ± 2˚C) for 24 h showed degradation of flucloxacillin, piperacillin and penicillin G in the liquid plasma samples, since only 40-63%, 52-64% and 66-70%, respectively, of the drug was detectable after applying room temperature as a stress condition. Ampicillin and amoxicillin had slightly better stabilities on the bench-top, 89-96% and 71-89%, respectively, of the drug was detectable after 24 h at room temperature.
Interestingly, rapid degradation of penicillins occurred using Mitra microsampling (Fig 4).

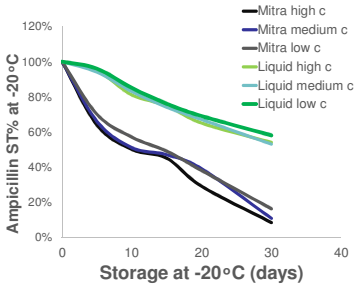

Figure 3. Ampicillin long-term storage stability at -20 ˚C for 30 days.

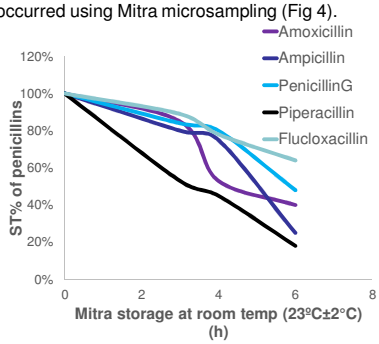

Figure 4. Short-term storage stability using Mitra at room temperature for 6 h.

Storage at +4 ˚C indicated a similar trend for Mitra and additional drying using desiccator did not improve the penicillins' stability. Storage at -80 ˚C was comparable to stability in the liquid samples.

Conclusion

- ✓ As a promising technique, Mitra microsampling device sampling accuracy and precision is better than 10 µL blood plasma pipetting.
- ✓ The analysis of penicillins is limited by the instability of these analytes.
- ✓ Surprisingly, penicillins' stability at room temperature, +4 ˚C and -20 ˚C was better in liquid form.
- ✓ Mitra storage with a desiccator did not improve penicillins stability.
- ✓ Penicillins are stable at -80 ˚C in Mitra devices and in regular blood plasma.

Acknowledgments

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