

Letter to the Editor

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Exploration of the pre-analytical stability of β -lactam antibiotics in plasma and blood – implications for therapeutic drug monitoring and pharmacokinetic studies

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To the Editor,

Severe infection and sepsis are the most common causes of morbidity and mortality in non-cardiac intensive care units worldwide [1]. Research has shown that initiating appropriate and timely antibiotic therapy is crucial for survival [2]. However, reaching adequate antibiotic concentrations may also be important, as recent data suggested a correlation between serum concentrations of β -lactam antibiotics and clinical outcomes in the critically ill [3]. However, because of pathophysiological changes and treatment interventions, dose optimization in these patients remains difficult for the treating physician [4]. There is a growing interest in therapeutic drug monitoring (TDM) of plasma concentrations of β -lactam antibiotics, as this may maximize efficacy and minimize toxicity [5, 6]. However, little is known about the pre-analytical stability

of these antibiotics, which are generally considered to be very unstable. Therefore, labor intensive measures are currently used, such as stabilization of carbapenems using non-nucleophilic buffers, transportation of the blood sample on ice and immediate centrifugation and subsequent storage of the plasma at -80°C , which makes routine therapeutic monitoring of these drugs more challenging [7–10].

The objective of this study was to evaluate the pre-analytical stability of three commonly used β -lactam antibiotics, both in whole blood and plasma. This study was conducted at the intensive care unit of Ghent University Hospital, Belgium between February and April 2014. The trial was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of Ghent University Hospital (registration number 2012/229). Patients were invited to participate but, as no patient data were used and no extra blood was taken, the need to obtain written informed consent was waived.

To evaluate the pre-analytical stability of these antibiotics, two blood tubes were drawn at the same time from patients treated with amoxicillin ($n=8$, range 4–45 mg/L), meropenem ($n=7$, range 9–36 mg/L) or piperacillin ($n=10$, range 34–263 mg/L): one Li-heparinized tube with (Venosafe VF-052SAHL, tube A) and one without (Venosafe VF-052SHL, tube B) a gel separator (Terumo Europe, Leuven, Belgium). One mL of whole blood from tube B was removed to an Eppendorf cup and stored at 4°C (postponed centrifugation condition, tube C). Tube A and the remaining part of tube B were both centrifuged [8 min, 1885 g, room temperature (RT)]. Tube A (plasma in contact with the gel separator) was first stored at RT for 4 h and was then placed at 4°C for further storage (mimicking the worst case scenario in our laboratory). Tube B (plasma in contact with the blood cells) was stored at 4°C immediately after centrifugation. The tubes on the bench at room temperature were not protected from light. An aliquot

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of plasma was taken from tube A and B. For tube C, an aliquot of whole blood was transferred to a new cup which was centrifuged. The resulting plasma was transferred to a new cup. Collection of aliquots occurred at serial time points up to 72 h (0, 4, 6, 8, 24, 48 and 72 h) and were stored at -80°C until analysis. The aliquots were analyzed in duplicate using an adapted and optimized version of an inhouse developed ultra high performance liquid chromatography tandem mass spectrometric method [11]. In brief, 15 μL plasma was precipitated with 100 μL acetonitrile containing the internal standard (a deuterated analog of each of the antibiotics) at a concentration of 1.5 mg/L, which was then vortexed and centrifuged. One hundred μL of the supernatant was diluted in 400 μL of water and 40 μL was injected onto the chromatographic column. Imprecision was $<10\%$ at all concentrations. The influence of one freeze thaw cycle was investigated during validation and no significant degradation occurred.

The drug was considered stable if the mean recovery was $\geq 95\%$ of the reference condition. The aliquot immediately sampled after centrifugation of tube B was considered the reference condition. In 5% of the samples, the analysis could not be performed because of too small sample volume.

For the different storage conditions and tested β -lactam antibiotics, mean recovery and mean percentage

degradation (\pm standard deviation) are shown in Figure 1 and Table 1, respectively. Meropenem was stable for 8 h in whole blood or plasma in contact with cells at 4°C , while amoxicillin and piperacillin were stable for 48 h under this condition. The tube containing a gel separator stored for 4 h at RT followed by storage at 4°C was stable up to 8 h for amoxicillin, but only 6 h for meropenem and piperacillin. We first assumed that the limited stability of piperacillin might be caused by adsorption of piperacillin to the gel barrier. However, our initial experiment was not appropriate to test this, as the storage conditions during the first 4 h were different (RT for samples with separator gel and 4°C for plasma without gel barrier) and storage at a higher temperature could possibly also explain the higher instability of piperacillin in tube A. Therefore, we carried out an additional gel-adsorption experiment and compared six piperacillin plasma concentrations which were sampled with and without gel barrier and were stored at identical conditions (4°C). The recovery was calculated as the ratio of the piperacillin concentration in the sample to the reference (concentration of piperacillin immediately sampled in the tube without gel separator). This recovery was compared for each time point using the related samples Wilcoxon-signed rank test and the difference in recovery between the tube with and without gel separator reached statistical significance after 48 h ($p=0.046$) and

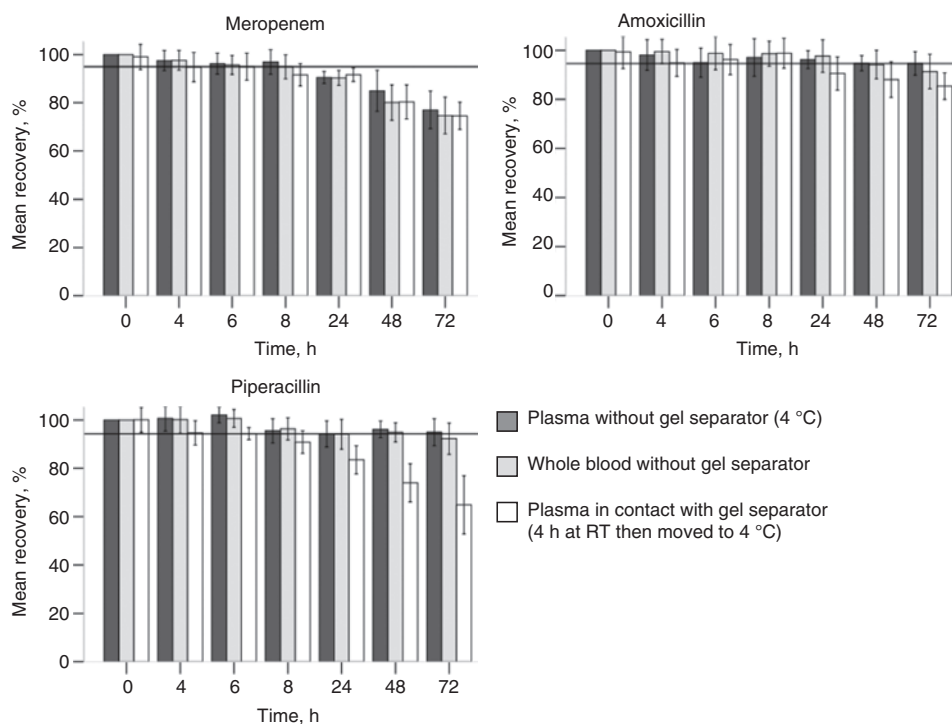


Figure 1 Mean recovery in function of time and storage conditions for meropenem, amoxicillin and piperacillin. Error bars: ± 1 standard deviation. Black line: limit of stability (95% recovery). RT, room temperature.

Table 1 Mean recovery (\pm standard deviation) in function of time and storage conditions for meropenem, amoxicillin and piperacillin.

	Condition A, mean recovery %						Condition B, mean recovery %						Condition C, mean recovery %					
	4 h	6 h	8 h	24 h	48 h	72 h	4 h	6 h	8 h	24 h	48 h	72 h	4 h	6 h	8 h	24 h	48 h	72 h
Amoxicillin	95 \pm 5	96 \pm 6	97 \pm 8	91 \pm 7	87 \pm 7	85 \pm 5	98 \pm 6	95 \pm 6	97 \pm 5	96 \pm 4	95 \pm 3	94 \pm 4	100 \pm 5	99 \pm 7	100 \pm 5	98 \pm 7	95 \pm 6	92 \pm 7
Meropenem	96 \pm 7	95 \pm 6	93 \pm 7	93 \pm 4	81 \pm 6	75 \pm 7	98 \pm 4	96 \pm 4	97 \pm 7	89 \pm 3	83 \pm 7	76 \pm 7	97 \pm 4	96 \pm 4	95 \pm 7	90 \pm 3	79 \pm 6	73 \pm 6
Piperacillin	95 \pm 4	95 \pm 4	91 \pm 5	84 \pm 6	74 \pm 9	65 \pm 13	101 \pm 5	102 \pm 3	96 \pm 5	95 \pm 5	96 \pm 3	94 \pm 6	100 \pm 6	101 \pm 4	96 \pm 5	95 \pm 5	95 \pm 4	92 \pm 6

Condition A: plasma in contact with gel separator, stored for 4 h (not protected from light) at room temperature after which it was placed at 4 °C (protected from light); Condition B: plasma without gel separator in contact with cells stored at 4 °C (protected from light); Condition C: postponed centrifugation: whole blood stored at 4 °C (protected from light).

after 72 h ($p=0.028$) as shown in Figure 2. The difference in recovery between gel and no gel was around 10% after 48 and 72 h. As the percentage recovery for the gel tube in the first experiment was much lower than the second after 24, 48 and 72 h, this is due to the period stored at RT.

These experiments were performed using Venosafe heparin tubes from Terumo®, and the results are therefore only applicable on these tubes. Although only a limited number of samples were used in our experiment, we believe they give already a good estimation on the stability of the different compounds tested.

In conclusion, this study shows that the pre-analytical stability of these selected β -lactam antibiotics is relatively good and is dependent on the compound. Meropenem is slightly less stable than amoxicillin and piperacillin. Labor intensive measures, now often taken to prevent degradation, such as transportation on ice, immediate centrifugation and stabilization of meropenem using non-nucleophilic buffers, may be unwarranted. This can considerably simplify storage and transportation to the

laboratory and therefore facilitate the implementation of TDM in clinical practice. Tubes not containing a gel separator are preferred, as there seems to be some adsorption of piperacillin to the gel barrier if the plasma is in contact with the gel for more than 24 h.

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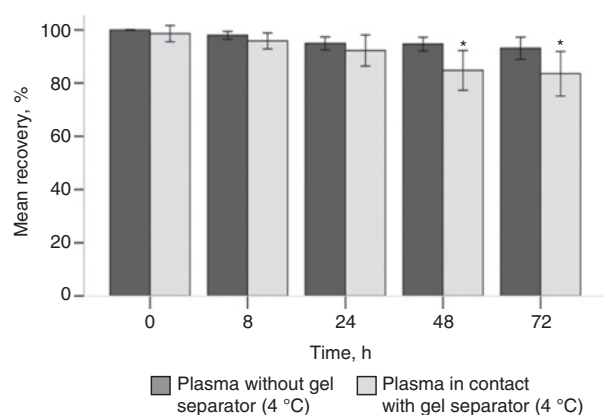


Figure 2 Mean recovery of piperacillin concentrations (compared to the reference condition: concentration in plasma without gel separator immediately sampled) in function of time and blood sample tube from six different patients.

Error bars: ± 1 standard deviation. * $p<0.05$.

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