

1 ***Antimicrobial Agents and Chemotherapy***

2 Development of a novel multi-penicillin assay and assessment of the impact of analyte  
3 degradation: lessons for scavenged sampling in antimicrobial pharmacokinetic study design  
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16 Running Head: Issues on beta-lactam scavenged sampling.

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**Supplementary data**

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19 ***Carry-over***

20 The extent of the auto-sampler carry-over was evaluated by injecting the prepared ULOQ (upper  
21 limit of quantification) calibrator with the concentration 200 mg/L, the extracted blank matrix  
22 sample and the LLOQ (lower limit of quantification) calibrator with the concentration of 0.1 mg/L.  
23 Carry-over (signal in the blank sample after ULOQ sample compared with the LLOQ sample) for  
24 amoxicillin was 0.03 %, for ampicillin 0.32%, for penicillin G and piperacillin 0.38% and for  
25 flucloxacillin 4.64%. Carry-over for the IS was 2.1%. Carry-over was considered acceptable for  
26 all analytes and the IS.

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28 ***Matrix effect***

29 Matrix effects were determined for amoxicillin, ampicillin, penicillin G, piperacillin and  
30 flucloxacillin using pre- and post-extraction spike and standard solutions. Penicillins were tested  
31 over the calibration concentration range and the matrix influence was evaluated. Peak area  
32 measurements obtained from post-extraction plasma spiked with ampicillin, amoxicillin, penicillin  
33 G, piperacillin and flucloxacillin at the same concentrations as the calibration range samples were  
34 compared to the peak area measurements obtained from the standard solutions. The matrix effect  
35 in 6 plasmas was 96-101.2% for amoxicillin, 98.3-102.1% for ampicillin, 97.5-104.8% for  
36 penicillin G, 98.3-107.6 for piperacillin, and 96.5-106.7% for flucloxacillin

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38 ***Dilution integrity***

39 The accuracy and precision of the diluted QCs after including the  
40 dilution factor were within the 15% limit.