Antimicrobial Agents and Chemotherapy

Development of a novel multi-penicillin assay and assessment of the impact of analyte degradation: lessons for scavenged sampling in antimicrobial pharmacokinetic study design

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Running Head: Issues on beta-lactam scavenged sampling.

Supplementary data
**Carry-over**

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

**Matrix effect**

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

**Dilution integrity**

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