Poster

[P25-2] P25-2: Anti-infective drugs (2): Beta-lactams

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[P25-2-4] Tandem LC-MS/MS method for the determination of Piperacillin (PIP) and Tazobactam (TAZ) in human plasma of patients in intensive care units who are critically ill: pitfalls and potential solutions

Carsten Mueller[1], Niels Winkelmann[2], Thomas Streichert[3], Martinh.J. Wiesen[4], Thorsten Annecke[5], Guido Michels[6], Andreas Hohn[7] (1.University Hospital of Cologne, 2.University Hospital of Cologne, 3.University Hospital of Cologne, 4.University Hospital of Cologne, 5.University Hospital of Cologne, 6.University Hospital of Cologne, 7.University Hospital of Cologne)

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Background

Therapeutic drug monitoring of β-lactams: piperacillin (PIP) in combination with tazobactam (TAZ) in particular is based on drug-level control in biological matrices and serves as a diagnostic approach for individualization of antibiotic dosing and drug safety in intensive care medicine. Factors influencing drug-levels of antibiotics (PIP/TAZ) in patients who are critically ill are diverse and in this context TDM a challenge. There is currently no common methodology for the determination of PIP/TAZ antifungal blood levels using isotopically labled substances. We have therefore developed a feasible method for simultaneous quantification of piperacillin and tazobactam.

Methods

An UHPLC-MS/MS method was developed and validated for simultaneous determination of PIP and TAZ in serum and plasma matrix. Extraction of serum samples consisted of simple protein precipitation using acetonitrile. Stable isotope labeled analogues for each analyte were obtained for internal standardization and quantitative analysis ([$^{2}H_{5}$]-PIP, ([$^{13}C_{2}$, $^{15}N_{3}$]-TAZ). Calibrators and quality controls were prepared in plasma matrix of normal individuals. Sample preparation: protein precipitation with acetonitrile and addition of internal standard-mix.

Results

All analytes were eluted within a runtime of 3.5 minutes. Linearity experiments were demonstrated in plasma over a concentration range from 1 - 130 mg/l: PIP and 0.1 - 50 mg/l: TAZ, (R^{2}>0.99).
Chromatographic separation was achieved using a C18 column (50x2.1mm, 1.9m particle size) and a gradient elution procedure. LC-MS/MS analyses were performed on a triple quadrupole mass spectrometer using positive electrospray ionization in selected reaction monitoring (SRM) mode. Ion transitions monitored for quantitation were m/z 518.1 →115.0/143.0 for PIP, m/z 523.2 →148.1 for TAZ. For all analytes, inter- and intra-day *precisions* (CV, %) varied between 1.62 –12.3, inter- and intra-day *accuracy values* ranged from -19.6 –11.8%. The lower limits of detection (LOD) were 0.004 mg/l for PIP and 0.06 mg/l for TAZ. The lower limits of quantification (LLOQ) were and 0.013 mg/l for PIP and 0.17 mg/L and TAZ respectively.

Conclusions

We have established this method with isotopically labled pure substance as standard practice for the measurement of PIP/TAZ plasma concentrations in critically ill patients on ICU. This method might also be used to determine all relevant antibiotics within only one method, hence replacing all the different methods that are currently in use, thus optimizing the cost effectiveness of the therapeutic drug monitoring.