Development and validation of an UHPLC-PDA method for simultaneous quantification of eight antibiotics in human plasma using 96-well µ-elution plates.

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Background: In critically ill patients serious infections and sepsis are a global medical problem: they are associated with considerable morbidity, mortality and costs. Different antibiotic classes including beta-lactams, aminoglycosides, glycopeptides, fluoroquinolones, and oxazolidinones show extensive evidence of sub-therapeutic exposure from standard doses, so optimization of antibiotic dosing is very crucial to achieving a good patient outcome from infections. Therapeutic drug monitoring is a tool used to minimize toxicity in drugs with narrow therapeutic window or complex pharmacokinetics. A single rapid method could optimize laboratory routine activity in order to use therapeutic drug monitoring for individual therapies, than we aimed to develop and validate a new chromatographic method for simultaneous plasma quantification of 8 antibiotics: ceftriaxone (CFTX), ceftaroline (CFTL), ciprofloxacin (CIPRO), daptomycin (DAPTO), ertapenem (ERTP), levofloxacin (LEVO), linezolid (LZD) and moxifloxacin (MOXI).

Method: Oasis-HLB µ-elution 96-well plates were used; plate wells were activated with 200 µL of methanol and then conditioned with 200 µL of KH2PO4 pH 1,9 (phosphoric acid). 125 µL of sample, 125 µL of KH2PO4 pH 1,9 and 50 µL of IS working solution, were put in 1.5 mL microcentrifuge tubes. Each tube sample was loaded in plate wells and 2 different consecutive washes were made: first using 200 µL of 100% HPLC grade water, second with 200 µL of HPLC grade water and methanol (90:10, v/v) solution, acidified with 2% formic acid. After positioning 1 mL collection plate, 2 consecutive elution phases were made with 25 µL of HPLC grade water and acetonitrile (10:90, v/v) solution. Finally, samples were diluted with 200 µL of 100% HPLC grade water and 10 µL were injected in the UHPLC-PDA system. Very important step during sample preparation was mobile phases passage across well resin: a positive pressure of 1-10 psi was generated using the Vacuum Manifold P/N 186001831.

Chromatographic separation was performed at 45 ?C using a column ACQUITY UHPLCTM HSS T3, 1.8 m, 2.1 × 150 mm protected by an ACQUITY UHPLC Column In-Line Filter. Mobile phase was composed by solution A, potassium dihydrogen phosphate buffer 10 mM (pH 5.5 with sodium hydroxide, NaOH) and solution B, acetonitrile. Chromatographic separation was performed at 0.4 mL/min with the following gradient (solution B): 5% from 0 to 0.10 min; 20% at 2.00 min; 50% at 3.50 min; 60% from 3.50 to 4.50 min; 70% from 4.51 to 5.51 min, 5% from 5.52 to 8 min.

Results: Assay validation was done according to Food and Drug Administration and European Medicine Agency guidelines. Following parameters were evaluated: specificity, calibration curve, lower limit of quantification (LLOQ), limit of detection (LOD), intra and interday accuracy and precision, recovery, and stability. This method resulted fast, accurate and precise and allowed us to detect each antibiotic with different retention times and well-separated peaks, with no interference to endogenous compounds and other drugs.

Conclusions: OASIS-HLB reduced significantly pre-analytical time, in fact preparation of complete plate requires about two hours. 96 samples can be analyzed in a single session, optimizing instrumental time and a small volume of sample plasma (125 uL) was required; this was an important advantage especially for hospitalized patients undergoing numerous withdrawals or for pediatric patients. At last this new method allows us to give results to clinicians in very lower time than previously.

All these aspects, conjugated to possible automation, make this method faster, more robust, reproducible and generally allow a significant improvement in performance, with a good economical competition.