A NEW HPLC-UV METHOD COMPARED WITH HPLC-MS FOR DAPTOMYCIN LEVELS IN HUMAN PLASMA SAMPLES

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Introduction
Daptomycin is the first member of the class of cyclic lipopeptide antibiotic drugs with a broad spectrum of activity against Gram-positive bacteria. The outcome of a therapy with daptomycin in clinical practice is comparable with standard antibiotic treatment and has a linear pharmacokinetics. However, daptomycin shows additional activity against multi-resistant bacterial strains like methicillin-resistant Staphylococcus aureus, vancomycin-resistant enterococci or penicillin-resistant Streptococcus pneumoniae and it is therefore a viable alternative for the treatment of persisting infections. Because of its efficacy and safety in a variety of infectious conditions, this is especially true in critical care settings. The plasma half-life of daptomycin is relatively long with about 9 h and the excretion of the drug occurs mainly via the kidneys. Daptomycin has PK/PD variability in hospitalized patients, therefore it requires a therapeutic drug monitoring with a High Performance Liquid Chromatography (HPLC) system. Guidelines recommend therapeutic doses for daptomycin. The issue needs rapid, simple and validated method to measure drug levels in human plasma.

Materials and methods
The HPLC-UV (Ultraviolet) and MS (Mass Spectrometry) system: 2695 comprising a binary pump, an autosampler, a thermostated column compartment, 2487 UV-vis detector, MS TQD Triple Quadrupole Detector (Waters Corporation, USA). A C18 250 mm x 4.6 mm 5 mm analytical column was used (Higgins analytical, USA).
KH_2PO_4, daptomycin, gentamicin and acetonitrile were purchased (Sigma Aldrich, USA) and EUREKA Daptomycin Mass Spectrometry kit (Eureka S.r.l.)
Chromatographic conditions: stationary phase thermostatically at 35 °C; mobile phase was KH_2PO_4 20 mM pH 3.2 – acetonitrile (54-46 v/v); flow 1.0 ml/min; volume injection, 50 ml and wavelenght used, 262 nm.
Patient samples: 100 EDTA-plasma sampling tube, from hospitalized patients in various hospital AOUP departments in Pisa.
Sample preparation: 200 ml of plasma sample added with 20 ml of Gentamycin (as Internal Standard) and 200 ml of acetonitrile-H_3PO_4 solution for plasma proteins precipitation. Samples was mixed for 30 sec and centrifuged for 15 min x 14000 rpm.
Method validation: Linearity, inter and intra-day mean accuracies and precision were calculated at three different concentrations (5, 50 and 100 mg/l - mean±SD) in triplicated sessions, based on therapeutic range of daptomycin, as analytical FDA guidelines.
Statistical analysis: made by GraphPad Prism 5.0a.

Results
Average recovery of 95%. Daptomycin linearity (r^2) was 0.9975; LOD = 0.81 mg/l; LOQ = 3.85 mg/l. The levels found in plasma samples ranged between 0.00 – 101.00 mg/l. These validation parameters were calculated at three different concentrations (in triplicated sessions) based on therapeutic range of daptomycin at 5, 50 and 100 mg/l concentration values (mean±SD) in extracted samples: 4.77 ± 0.69; 48.80 ± 2.26 and 97.76 ± 3.99 mg/L, respectively. Further validation parameters were inter and intra-day mean accuracies (1.70% and 1.40%, respectively) and precision (6.45% and 7.70%, respectively). The method respected all validation parameters of FDA and it is routinely used in laboratory activities for daptomycin TDM. In particular, sample preparation and the running time are short enough to allow the analysis of at least 5 samples per hour.

Discussions and conclusions
The method respected all validation parameters of FDA. This method was compared with analytical gold standard (HPLC-MS), and differences from results didn't report statistically significant differences, so the method can be used in routine in laboratory activities for daptomycin TDM, for hospitalized patients affected by Gram-positive bacterial infections.