Daptomycin is the first member of the class of cyclic lipopeptide antibiotic drugs with a broad spectrum of activity against Gram-positive bacteria. Unlike traditional antibiotics, daptomycin is active in both the presence and absence of cell wall precursors and potentiates the lysis of bacteria when given in combination with other antibiotics.

**Background**

Daptomycin, a lipopeptide antibiotic, has become an important treatment option for Gram-positive infections. It acts by disrupting the bacterial cell membrane, leading to cell lysis and death. However, its pharmacokinetics are complex, and the development of methods to accurately measure its plasma concentrations is crucial for optimizing treatment regimens.

**Results**

A new HPLC-UV method was developed for the quantification of daptomycin in plasma samples. The method was validated according to the International Conference on Harmonization guidelines, ensuring its reliability and accuracy. The validated method was used to measure daptomycin plasma concentrations in patients undergoing treatment, providing insights into the drug's pharmacokinetic profile.

**Instruments, samples and methods**

**INSTRUMENTS:** The HPLC-UV method was developed using a Waters Alliance 2695 separations module equipped with a Waters 2487 Dual Absorbance Detector and a Waters 426 Controller, and controlled by the Empower software (version 3.0, Waters Corporation). The LC/MS/MS system was operated by Masslytics software (version 4.1, Waters Corporation).

**CALIBRATION AND QUALITY CONTROL SAMPLES:** A daptomycin stock solution was prepared by dissolving 10 mg of daptomycin in 10 ml of water at final concentration 1000 μg/ml. From this solution, 100 μl was diluted with 990 μl of blank human plasma (obtained from healthy volunteers), obtaining a working solution of 100 μg/ml. A gentamicin stock solution was prepared diluting 10 μg of gentamicin 400 mg/l in 4 ml of water (final concentration 400 μg/ml). From this solution, 100 μl were diluted in 900 μl of blank human plasma from healthy volunteers, obtaining a working solution 10 μg/ml.

**SAMPLE PREPARATION:** Protein precipitation was achieved by dissolving 10 mg of daptomycin in 40 ml of water at final concentration 250 μg/ml. From this solution, 100 μl were diluted with 990 μl of blank human plasma (obtained from healthy volunteers), obtaining a working solution of 250 μg/ml. Further, a gentamicin stock solution was prepared diluting 10 μg of gentamicin 400 mg/l in 4 ml of water (final concentration 400 μg/ml).

**HPLC-UV SETTINGS:** The HPLC mobile phase consisted of organic solvent (ACN and/or MeOH) plus buffer (KH₂PO₄, pH 3.2). The choice of final pH was dependent on the chemical structure of daptomycin and gentamicin in order to optimize the interaction of analytes with stationary phase. The stationary phase: Higgins Analytical C18 5 μm (250 mm x 4.6 mm) at 35 °C.

**Flow:** 1.0 ml/min; Injection volume: 20 μl; UV Wavelength: 262 nm

**LC/MS/MS ANALYSIS:** plasma samples were analyzed by using a commercial kit (Eureka - Lab Division) for LC/MS.

**VALIDATION STUDIES:**

- **Linearity range:** daptomycin and gentamicin 5, 10, 50, 100, 500 μg/ml.
- **Precision intra-day:** 2:1:0.05LogConc=(1:2:1-0.05LogConc) Inter-day: 2:1:0.05LogConc.
- **Accuracy:** method was evaluated analyzing interdays peaks at the same retention times of daptomycin and gentamicin: LOQ: signal-to-noise ratio ≥ 3 LOQ: D:0.4xLOD
- **Precision:** method was evaluated analyzing interdays peaks at the same retention times of daptomycin and gentamicin: LOQ: signal-to-noise ratio ≥ 3 LOQ: D:0.4xLOD

**STATISTICAL ANALYSIS:** the software Graph Pad Prism 7 (Graph Pad Software®, USA) was used. Correlation analyses were done between daptomycin concentrations of HPLC-UV data and LC/MS/MS reference method. Level of significance was set at p<0.05.

**Results**

The better recovery after sample analyte extraction was obtained by using ACN-H₂PO₄ 85% (95:5 v/v) (Table 1). The mobile phase was chosen on the basis of retention time values and optimal separation, which were achieved by ACN-MeOH-H₂PO₄ 80:20:20 (pH 3.2) 4.62:54.62:54.62. Retention times were 4.1 and 5.6 min for daptomycin and gentamicin, respectively (Figures 1 and 2). Tables 2 and 3 report the complete list of analytical parameters for method validation.

The performance of the present method was compared with an international commercial LC/MS/MS method on 122 human plasma samples. The correlation analysis of measured plasma concentrations returned an r² value of 0.9474, a slope of 1.052 and a y-intercept of 1.89 ng/ml/injection (Figure 3). A significant difference between laboratories was found (Mann-Whitney and unpaired t-test with Welch's correction, p<1.000 and 0.9927, respectively).

**Conclusions**

In conclusion, a reliable and rapid HPLC-UV method was validated to measure daptomycin plasma concentrations in clinical practice for better accuracy and precision over the range of drug concentrations expected after the administration of daptomycin at standard doses. Furthermore, to our knowledge, this is the first HPLC-UV method for daptomycin that has been validated with a LC/MS/MS reference method. Moreover, the simple preanalytical preparation of samples and the reduced costs of HPLC platform certainly ensure a wide diffusion of the present method, optimal for routine in laboratories. For these reasons, the method is currently used to monitor all plasma samples dispatched to our Clinical Pharmacology Unit.

**References**


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