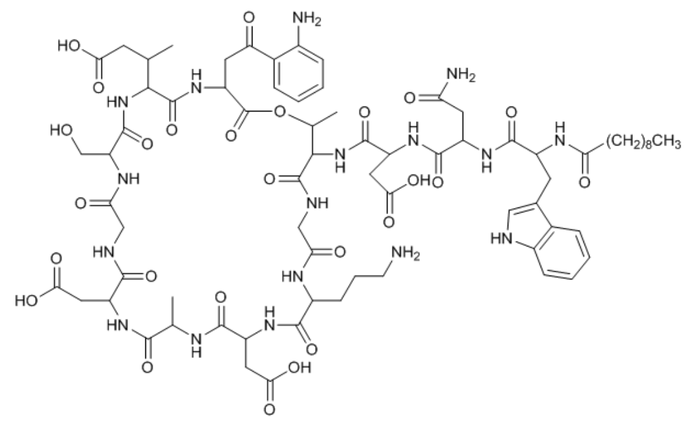
A NEW HPLC-UV METHOD COMPARED WITH HPLC-MS FOR DAPTOMYCIN LEVELS IN HUMAN PLASMA SAMPLES

Giacomo Luci, Federico Cucchiara, Marianna Lastella, Laura Ciofi, Romano Danesi, Antonello Di Paolo

Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
Unit of Clinical Pharmacology and Pharmacogenetics, Pisa University Hospital, Pisa, Italy

Background

Daptomycin is the first member of the class of cyclic lipopeptide antibiotic drugs with a broad spectrum of activity against Gram-positive bacteria [1]. The outcome of a therapy with daptomycin in clinical practice is comparable with standard antibiotic treatment and has a linear pharmacokinetics in healthy volunteers with a plasma half-life of about 9h, with drug excretion via kidneys [2]. However, daptomycin shows additional activity against multi-resistant bacterial strains like methicillin-resistant *Staphylococcus aureus* (MRSA) [3], vancomycin-resistant enterococci (VRE) [4] or penicillin-resistant *Streptococcus pneumoniae* [5] and it is therefore a viable alternative for the treatment of persisting infections. Standard doses ranged from 4 to 6 mg/kg, in several cases physicians are prescribing higher dosages up to 10 or 12 mg/kg [6]. Its efficacy and safety has been evaluated in a variety of infectious conditions, especially in critical care settings. Some factors may influence daptomycin PK and its PK/PD, especially in hospitalized patients (CKD, GFR < 30 ml/min) [7]. Therefore, therapeutic drug monitoring protocols are highly recommended and they can be performed by high performance liquid chromatography (HPLC) systems. Literature and guidelines recommend therapeutic doses for daptomycin [8] to avoid side effects, such as renal failure, hepatotoxicity and rhabdomyolysis [9-10].



Aim

Application of a new HPLC-UV method on 122 plasma samples of patients from various AOUP wards, after analytical method settings, validation and comparison with a reference LC-MS/MS method. All research activities were performed according to the "Daptolin" protocol (approved by the Pisa University Hospital Ethics Committee, prot. num. 55945)

Instruments, samples and methods

INSTRUMENTS: The HPLC-UV method was developed using a Waters Alliance 2695 Separations Module equipped with a Waters 2487 Dual λ Absorbance Detector (Waters Corporation, Milford, CT) and controlled by the Empower software (version Pro, Waters Corporation).
The LC-MS/MS kit was applied using a Acquity UPLC Binary Solvent Manager pump with Sample Manager autosampler equipped with a TQ Detector (Waters Corporation,). LC-MS/MS system was controlled by MassLynx software (version V4.1, Waters Corporation, USA).
CALIBRATION AND QUALITY CONTROL SAMPLES: A daptomycin stock solution was prepared by dissolving 10 mg of daptomycin in 10 ml of water (final concentration, 1000 mg/l). From this stock solution, 100 µl was diluted with 900 µl of blank human plasma (obtained from healthy volunteers), obtaining a working solution of 100 mg/l. A gentamicin stock solution was prepared diluting 10 µl of gentamicin 40 mg/ml in 1 ml of water (final concentration 400 mg/l). From this solution, 100 µl were diluted in 900 µl of blank human plasma from healthy volunteers, obtaining a working solution of 40 mg/l.
SAMPLE PREPARATION Protein precipitation = 20 µl of gentamicin 100 mg/l such Internal Standard, were added to at 200 µl of plasma sample. After vortexing for 30 sec, plasma proteins were precipitated by additing organic solvent. Different tests: Acetonitrile, Methanol, Acetonitrile + H_3PO_4 conc. 5% (v/v).
HPLC-UV SETTINGS: The HPLC mobile phase consisted of organic solvent (i.e., ACN and/or MeOH) plus buffer KH_2PO_4 20 mM pH=3.2. The choice of final pH was dependent on the chemical structure of daptomycin and gentamycin in order to optimize the interaction of analytes with stationary phase. Stationary phase: Higgins Analytical C18 5 µm (250 mm x 4.6 mm) at 35 °C.
Flow: 1,0 ml/min; Injection volume: 50 µl; UV Wavelenght: 262 nm
LC-MS/MS ANALYSIS: plasma samples analyzed by using a commercial kit (Eureka – Lab Division) for LC/MS.
VALIDATION STUDIES [11]:
Linearity range concentrations: daptomycin and gentamicin 5, 10, 25, 50, 100 mg/l;
Precision intra-day: 2(1-0.5logConc)×2/3; Inter-day: 2(1-0.5logConc)
Specificity was evaluate analyzing interfering peaks at the same retention times of daptomycin and gentamicin. LOD: signal-to-noise ratio ≥ 3 LOQ: 3.04 x LOD
SAMPLES: From November 2018 to February 2019, 122 plasma samples from AOUP Pisa Hospital wards (Infectious diseases, cardiovascular diseases, orthopaedics, general medicine and neurosurgery) were analyzed.
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 $p<0.05$.

Figure 1. Analytes RT at different mobile phases

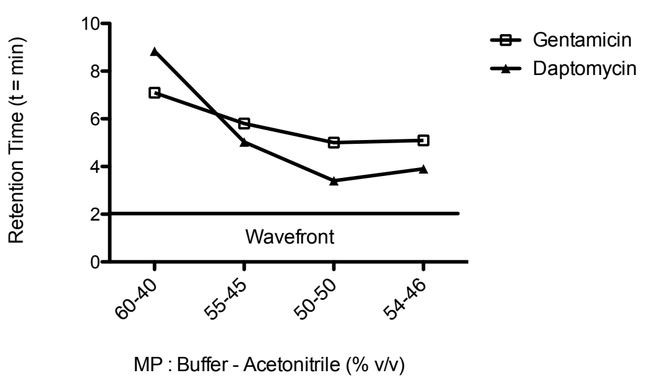


Table 1. Solvent for extraction

Solvents	Rec % (mean ± SD, n = 10)
ACN - H_3PO_4 85% (95:5 v/v)	98.2 ± 4.57
ACN	85.1 ± 5.47
CH_3OH	70.6 ± 3.67
TCA 10 %	45.9 ± 4.65

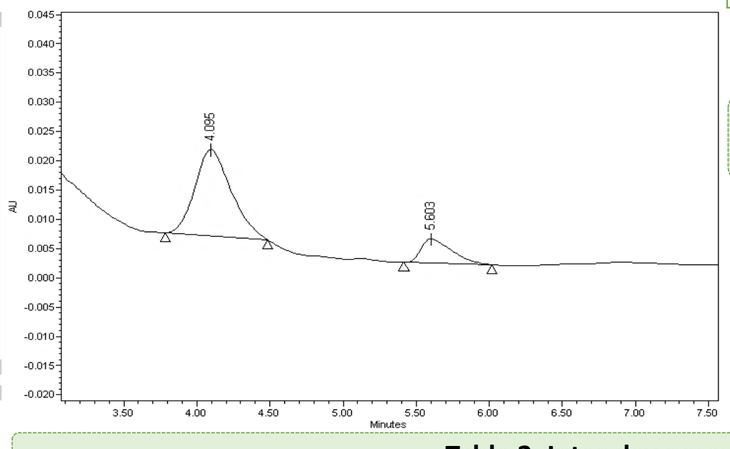


Figure 2. HPLC-UV chromatogram of plasma extracted samples:

Peaks:
- Daptomycin 4,10 min
- Gentamicin 5,60 min (Internal Standard)

Table 2. Inter-day parameters

DAY	Concentration (mg/L) Theoretical	Concentration (mg/L) Measured	Accuracy (%)	Precision (%)	CV%
1	5	4.77 ± 0.70	4.64	0.88	14.61
	50	49.32 ± 2.26	2.20	0.20	0.00
	100	98.29 ± 2.78	1.71	0.05	2.83
2	5	4.77 ± 0.69	4.58	0.88	14.39
	50	48.80 ± 2.26	2.40	0.21	4.64
	100	97.76 ± 3.99	2.24	0.01	4.08
3	5	4.62 ± 0.46	1.41	0.87	0.00
	50	50.41 ± 3.34	-0.81	0.20	6.62
	100	99.75 ± 0.76	0.25	0.001	0.77

Table 3. Intra-day parameters

Concentration (mg/L) Theoretical	Concentration (mg/L) Measured	Acc. (%)	Prec. (%)	CV%	LOD (mg/L)	LOQ (mg/L)	r^2
5	4.77 ± 0.83	3.19	1.32	11.65	1.26	3.85	0.9975 ± 0.0009
50	49.74 ± 2.39	0.53	0.30	4.80			
100	98.60 ± 2.86	1.40	0.01	2.90			

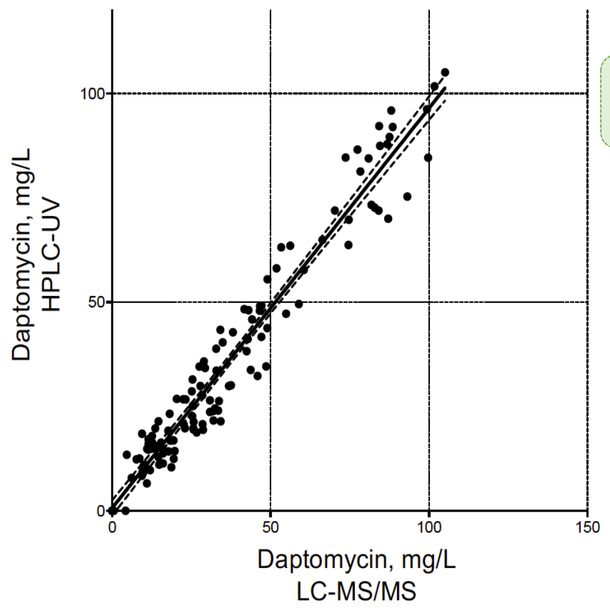


Figure 3. Linear correlation HPLC-UV and LC-MS/MS

Linear correlation of daptomycin concentration values obtained with HPLC-UV and LC-MS/MS methods in 122 plasma samples. The linear correlation analysis returned a r^2 value of 0.9474 ($p < 0.0001$), slope of 1.052 and y-intercept of 0.8543 ± 0.9368 mg/l. Linear regression line is perfectly included within the 95% confidence interval.

Results

The better recovery after sample analytes extraction was obtained by using ACN - H_3PO_4 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-buffer KH_2PO_4 20 mM pH=3.2 46:54, v/v. 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 a commercial LC-MS/MS method on 122 human plasma samples. The correlation analysis of measured plasma concentrations returned an r^2 value of 0.9474, a slope of 1.052 and a y-intercept of 0.8543 ± 0.9368 mg/L (**Figure 3**), without significant differences between the two methods (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 concentrations in plasma samples using an internal standard 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 compared 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 laboratory. For these reasons, the method is currently used to monitor all plasma samples dispatched to our Clinical Pharmacology Unit.

References

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Contacts

Dott. Giacomo Luci, ChemPharmD, PhD – e-mail: **giacomo.luci@for.unipi.it**
Prof. Antonello di Paolo, MD, PhD – e-mail: **antonello.dipaolo@med.unipi.it**

Department of Clinical and Experimental Medicine
University of Pisa
Via Roma 55
56126 Pisa - Italy