DEVELOPMENT AND VALIDATION OF A RAPID HIGH PERFORMANCE CHROMATOGRAPHY METHOD (HPLC) FOR THE DETERMINATION OF LINEZOLID IN HUMAN PLASMA

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BACKGROUND AND IMPORTANCE

Linezolid is an oxazolidinone antibiotic with time-dependent activity and moderate post-antibiotic effect used as a treatment against multidrug-resistant gram-positive pathogens. Recent guidelines recommend therapeutic drug monitoring (TDM) of linezolid in critically ill patients, who often manifest great inter- and intra-individual pharmacokinetic variability. The latter is the reason why in this kind of patients conventional dosing may not achieve optimal efficacy and safety concentrations (2-7 mcg/mL).

OBJECTIVES

The aim of this study was the development and validation of a high-performance liquid chromatography (HPLC) method for measuring linezolid in human plasma using tedizolid as an internal standard (IS).

MATERAIL AND METHODS

The Agilent® 1260 Infinity system with UV-DAD and a Thermo Scientific® BDS HYPERSIL C18 4.6X250 mm, 5 µm column was used for isocratic analysis lasting 8 minutes. The method was validated according to FDA bioanalytical guidelines, with details in Table 1. Plasma drug extraction involved mixing 100 µL of IS (tedizolid 25 µg/mL) with 250 µL of plasma (QC or samples) and 500 µL of 50/50 acetonitrile/methanol in a test tube, vortexing for 1 min, centrifuging at 15000 rpm for 5 min, and injecting 300 µL of supernatant into the HPLC.

Table 1. Chromatographic conditions and calibration methods.

Analyte	Mobile Phase	λ nm	Calibration range	Flow (mL/min)	T (ºC)	Injection volume (µL)
Linezolid	75% BUFFER (KH2PO4 20 nM Ph=3.5)/25% acetonitrile	260	- 6 calibration levels: range 1-14 µg/mL -n=5 replicates/calibration level) - Blank (no analyte, no IS) - Zero calibrator (blank + IS)	1.5	25ºC	50

RESULTS

Table 2. Validation parameters according FDA guidance.

Analyte	Rt (min)	Equation	R^2	Within-day mean (µg/mL) (%CV) (n=15)			Between-day mean (µg/mL) (%CV) (n=15)		
				Low (3 µg/mL)	Medium (7 µg/mL)	High 10 µg/mL)	Low (3 µg/mL)	Medium (7 µg/mL)	High (10 µg/mL)
Linezolid	4,3	y= 0.2451x-0.0179	0.9995	-3.39 (1.57)	-3.25 (0.56)	1.46 (6.89)	-6,18 (2,21)	-5,45 (1,97)	-2,78 (7,54)

CONCLUSIONS

A method has been validated for the determination of linezolid by HPLC in human plasma. This will allow in future to improve therapeutic outcomes in critically ill adult patients, limiting the risk of dose-related adverse effects and avoiding suboptimal concentrations.