A high performance liquid chromatography (HPLC) assay for linezolid in continuous ambulatory peritoneal dialysis fluid (CAPDF)


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Sir,

Linezolid, a new oxazolidinone, has a unique mode of action.1 The antimicrobial agent has a wide spectrum of activity against Gram-positive organisms, including methicillin-resistant Staphylococcus aureus,2,3 penicillin-resistant pneumococci1 and both vancomycin-resistant Enterococcus faecalis and Enterococcus faecium.3,4 Linezolid is available in oral and intravenous (iv) preparations and no dosage modification is required when switching formulation, as the drug has 100% bioavailability.5 In a study with critically ill patients, a mean peak serum concentration of 12.8 mg/L and a trough of 4.7 mg/L were reported after multiple twice daily iv 600 mg linezolid dosages.6 This trough concentration was close to the breakpoint (≤4.0 mg/L) recommended by the British Society for Antimicrobial Chemotherapy for S. aureus, suggesting a possible need for serum monitoring in this patient group.7 Linezolid may also be a suitable agent for the treatment of continuous ambulatory peritoneal dialysis (CAPD) patients with Gram-positive infection. Previously, we developed an HPLC method to quantify linezolid in serum.8 However, in order to investigate the pharmacokinetics of linezolid fully in this group of patients, we report here a modification of our original method to permit the assay of linezolid in CAPDF fluid (CAPDF).

The stationary phase was Hypersil 5 ODS in a stainless steel column, 100 × 4.6 mm (ThermoQuest, Runcorn, UK). The mobile phase was 1% ortho-phosphoric acid, 30% methanol, 2 g/L heptane sulphonic acid, adjusted to pH 4.5 with 0.1 N sodium hydroxide. The flow rate was 1.0 mL/min. Detection was by UV absorbance at 254 nm (Waters model 480 detector, Elstree, UK). Pooled CAPDF (from 12 patients) was used for all the investigations. The CAPDF was prepared for assay by mixing (50:50) with acetonitrile; it was then allowed to stand for 5 min before centrifuging at 25 000g for 5 min. Twenty microlitres of the supernatant was injected.

The linezolid retention time was ~6 min. Single-point calibration was used for quantification. The reproducibility of the assay, expressed as the percentage coefficient of variation (%CV), was <6% after the repeated assay (n = 6) of CAPDF samples containing 2, 25 and 45 mg/L linezolid. The detection limit (in CAPDF), defined as the linezolid concentration equivalent to a peak three times the height of the chromatographic baseline noise, was 0.2 mg/L. Linearity and CAPDF recovery were investigated by assaying aqueous and CAPDF specimens containing 0, 1, 5, 10, 15, 20, 30 and 50 mg/L linezolid. The linezolid peak height was plotted against drug concentration and a regression analysis performed. The correlation between drug concentration and peak height was good for the aqueous and CAPDF specimens (r = 0.999 and 0.996, respectively). The percentage recovery of linezolid from CAPDF (serum peak height/aqueous peak height × 100) was 100% at each drug concentration. The assay accuracy was investigated by the assay of CAPDF samples containing 11, 23 or 37 mg/L linezolid using a single 45 mg/L calibrator. The accuracy, expressed as the percentage error [(measured concentration−target concentration)/target concentration × 100] was 1.8% (11 mg/L sample), 0.0% (23 mg/L sample) and −4.9% (3.5 mg/L sample). To test assay specificity, 23 commonly used antibiotics and 10 clinical CAPDF samples were assayed. Only benzyl-penicillin eluted closely to linezolid and could potentially interfere. However, this was not considered to be a significant problem. A 20 mg/L benzyl-penicillin sample resulted in a chromatographic peak equivalent to 0.35 mg/L linezolid, and at therapeutically relevant concentrations, benzyl-penicillin is unlikely to cause major interference. Other β-lactams investigated (including ampicillin, amoxicillin, azlocillin and flucloxacinil) did not elute closely to linezolid.

We conclude that this simple, rapid, accurate and reproducible assay for linezolid in CAPDF is ideal for drug monitoring and pharmacokinetic studies in the clinical laboratory. It can readily be employed using basic HPLC equipment.

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References


