Abstract

A simple, sensitive, selective, and reproducible reversed-phase high-performance liquid chromatographic (HPLC) method with UV detection was developed for the determination of lorazepam (LZP) in human plasma, using oxazepam (OZP) as internal standard. LZP and OZP were extracted from alkalinized (pH 9.5) spiked and clinical plasma samples using a single step liquid-liquid extraction with a mixture of n-hexane-dichloromethane (70:30%; v/v). Chromatographic separation was performed on a reversed-phase Synergi Max RP analytical column (150 mmx4.6 mm i.d.; 4 microm particle size), using an aqueous mobile phase (10 mM KH2PO4 buffer (pH 2.4)-acetonitrile; 65:35%, v/v) delivered at a flow-rate of 2.5 ml/min. Retention times for OZP and LZP were 10.2 and 11.9 min, respectively. Calibration curves were linear from 10 to 300 ng with correlation coefficients (r2) better than 0.99. The limits of detection (LOD) and quantification (LOQ) were 2.5 and 10 ng/ml, respectively, using 0.5 ml samples. The mean relative recoveries at 20 and 300 ng/ml were 84.1+/-5.5% (n=6) and 72.4+/-5.9% (n=7), respectively; for OZP at 200 ng the value was 68.2+/-6.8% (n=14). The intra-assay relative standard deviations (R.S.D.) at 20, 150 and 270 ng/ml of LZP were 7.8%, 9.8% (n=7 in all cases) and 6.6% (n=8), respectively. The inter-assay R.S.D. at the above concentrations were 15.9%, 7.7% and 8.4% (n=7 in all cases), respectively. Intra- and inter-assay accuracy data were within the acceptance interval of +/-20% of the nominal values. There was no interference from other commonly co-administered anticonvulsant, antimicrobial, antipyretic, and antimalarial drugs. The method has been successfully applied to a pharmacokinetic study of LZP in children with severe malaria and convulsions following administration of a single intravenous dose (0.1 mg/kg body weight) of LZP