Abstract

We have developed a sensitive, selective and reproducible reversed-phase high-performance liquid chromatography method coupled with electrospray ionization mass spectrometry (HPLC-ESI-MS) for the simultaneous quantification of midazolam (MDZ) and its major metabolite, 1'-hydroxymidazolam (1'-OHM) in a small volume (200 microl) of human plasma. Midazolam, 1'-OHM and 1'-chlordiazepoxide (internal standard) were extracted from alkalinised (pH 9.5) spiked and clinical plasma samples using a single step liquid-liquid extraction with 1-chlorobutane. The chromatographic separation was performed on a reversed-phase HyPURITY Elite C18 (5 microm particle size; 100 mm x 2.1mm i.d.) analytical column using an acidic (pH 2.8) mobile phase (water-acetonitrile; 75:25% (v/v) containing formic acid (0.1%, v/v)) delivered at a flow-rate of 200 microl/min. The mass spectrometer was operated in the positive ion mode at the protonated-molecular ions [M+l]+ of parent drug and metabolite. Calibration curves in spiked plasma were linear (r2 > or = 0.99) from 15 to 600 ng/ml (MDZ) and 5-200 ng/ml (1'-OHM). The limits of detection and quantification were 2 and 5 ng/ml, respectively, for both MDZ and 1'-OHM. The mean relative recoveries at 40 and 600 ng/ml (MDZ) were 79.4+/-3.1% (n = 6) and 84.2+/-4.7% (n = 8), respectively; for 1'-OHM at 30 and 200 ng/ml the values were 89.9+/-7.2% (n = 6) and 86.9+/-5.6% (n = 8), respectively. The intra-assay and inter-assay coefficients of variation (CVs) for MDZ were less than 8%, and for 1'-OHM were less than 13%. There was no interference from other commonly used antimalarials, antipyretic drugs and antibiotics. The method was successfully applied to a pharmacokinetic study of MDZ and 1'-OHM in children with severe malaria and convulsions following administration of MDZ either intravenously (i.v.) or intramuscularly (i.m.)