

Appendix 1

Ropivacaine Plasma Concentration Measurements

Samples were prepared according to a modified method reported previously by Björk et al.⁴⁰. To 1 mL of plasma enriched with 20 μL of a 40-mM solution of echothiophate, 1 μg of tetracaine was added as an internal standard, followed by 375 μL of sodium carbonate 10%, and extracted with 5 mL of n-hexane:methylene chloride (4:1 v/v) by gentle agitation for thirty minutes. After centrifugation, the organic layers were transferred to other tubes and evaporated to dryness. Residues were reconstituted with 250 μL of mobile phase (70 mM sodium sulphate in 1.25 mM sulphuric acid:ACN 65:35 v/v) and were analyzed with high performance liquid chromatography, a separation technique reported by Arvidsson et al.^{41,42} (precision of 11%), with some modifications. Aliquots of 100 μL were injected into the analytical column (Hichrom S5 ODS 1), maintained at 40°C, with ultraviolet detection at 210 nm. The calibration curve for ropivacaine ranged from 15.62 to 2,000 ng/mL^{-1} plasma.