Exploring Secondary Interactions in Mixed-Mode Chromatography for Analysis of Pancuronium and Bromide Counterion

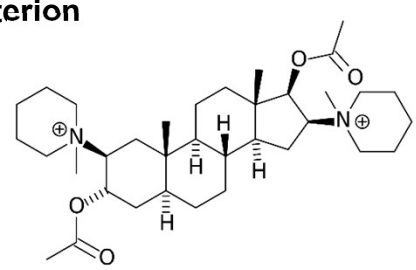
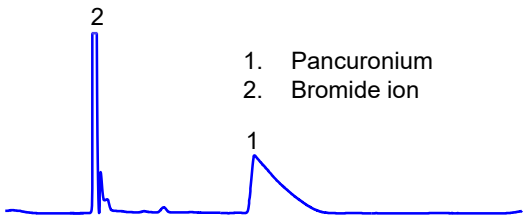
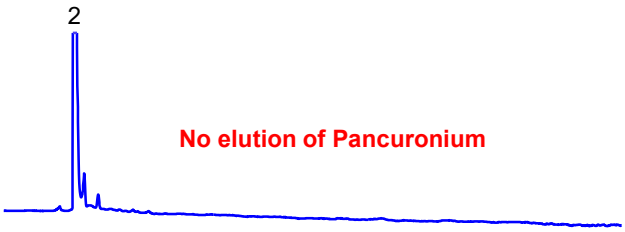


Fig. 1



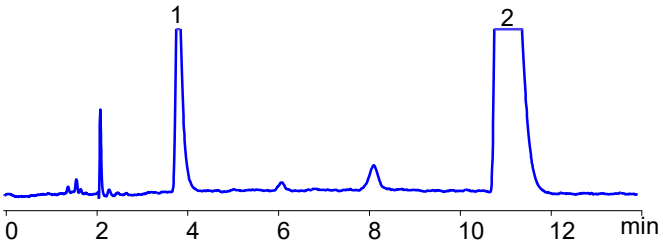
Column: C18 Leading brand
Mobile phase: 40% ACN with 0.1% H_2SO_4

Fig. 2



Column: Coresep 100
Mobile phase: 40% ACN with 0.2% H_2SO_4

Fig. 3



Column: Heritage MA
Mobile phase: 10% ACN with 0.05% H_2SO_4

Column: various
Dimensions: 4.6x150 mm
Mobile Phase: various
Flow Rate: 1 ml/min
Detection: UV 220 nm

Application Notes

Mixed-mode chromatography usually adds secondary and tertiary interactions which help to achieve controlled retention, unique selectivity and allow to adjust retention time of various compounds by changing the amount of organic, buffer concentration, buffer pH, and buffer nature. The presence of ionizable groups on the column also helps improve peak shape for various analytes, which is impossible to achieve with single-mode columns.
Pancuronium bromide is a hydrophobic compound with two strong basic groups. It shows a poor peak shape on RP columns. The poor peak shape is attributed to secondary interaction between analyte and silanols on the surface of RP columns (Fig. 1). In the case of pancuronium bromide, the addition of the cation-exchange mechanism did not work and the target compound never eluted (Fig. 2) due to three strong interactions (reversed-phase and two cation-exchange interactions). The problem of elution and peak shape was addressed by changing RP/cation-exchange interaction to RP/cation-exclusion interaction. This allowed us to retain and analyze both the drug and counterion with simple isocratic conditions (Fig. 3)