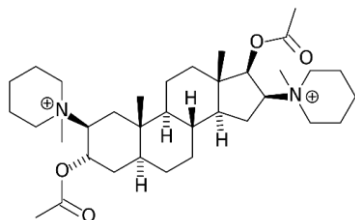
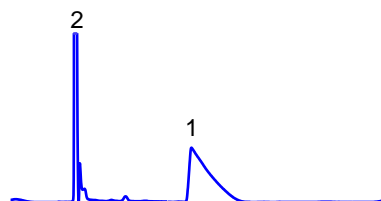


Exploring Secondary Interactions in Mixed-Mode Chromatography for Analysis of Drugs and Counterions

1. Pancuronium
2. Bromide ion

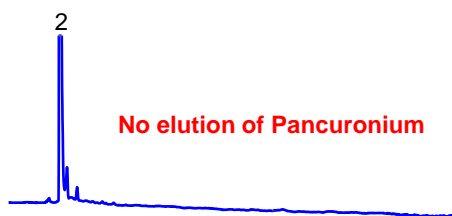


Dimensions: 4.6x150 mm
Flow Rate: 1 ml/min
Detection: UV 220 nm



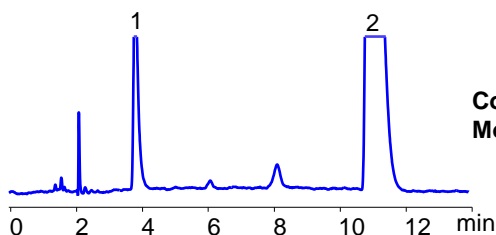
Column: C18 Leading brand
Mobile phase: 40% ACN with 0.1% H₂SO₄

Fig. 1



Column: Coresep 100
Mobile phase: 40% ACN with 0.2% H₂SO₄

Fig. 2



Column: Heritage MA
Mobile phase: 10% ACN with 0.05% H₂SO₄

Fig. 3

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 sometimes 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 when RP columns are used. The poor peak shape is attributed to secondary interaction between analyte and silanols on the surface of RP columns (Fig. 1). Sometimes you need to understand what secondary interaction to add to improve your chromatography. In the case of pancuronium bromide, the addition of the cation-exchange mechanism did not work and the target compound never eluted (Fig. 2) even with a very strong mobile phase from the column 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). Learn more at www.helixchrom.com.