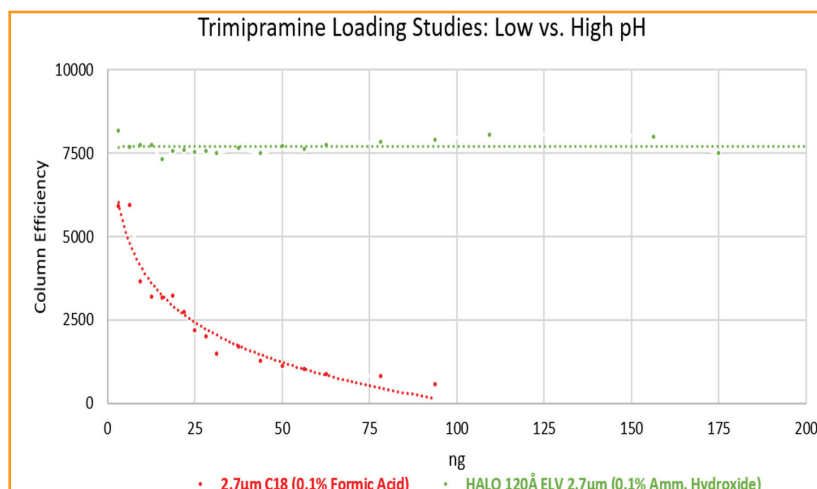




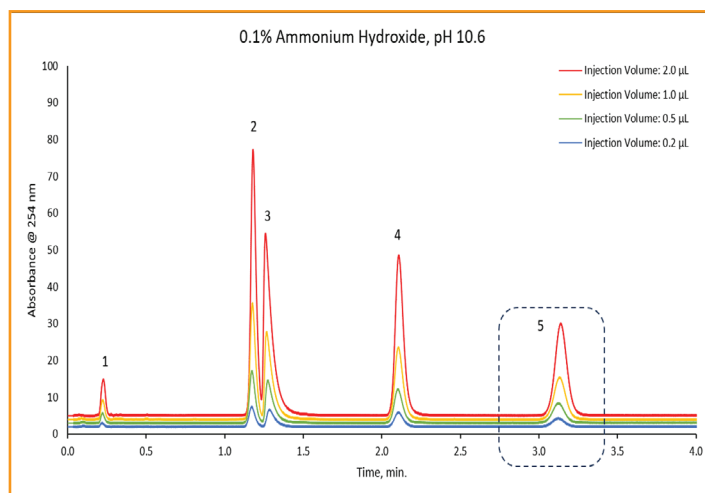
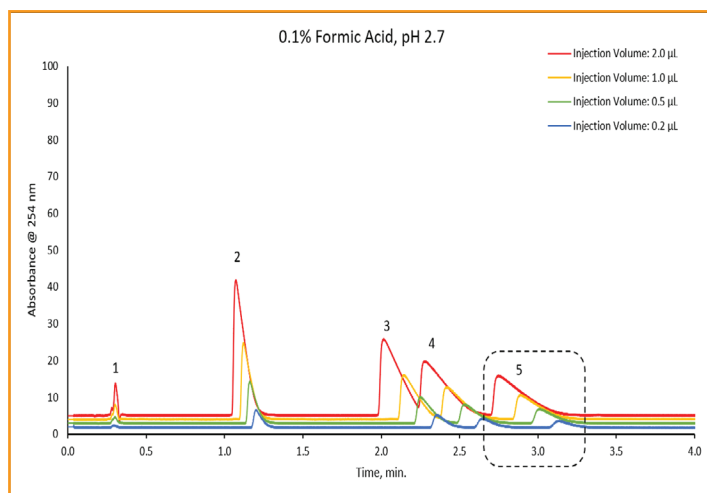
Advantage of HALO® ELEVATE Loading Capability: High vs. Low pH

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PEAK IDENTITIES:

1. Uracil
2. Doxepin
3. Nortriptyline
4. Amitriptyline
5. Trimipramine



TEST CONDITIONS:

Column: HALO 120 Å ELV C18, 2.7 µm, 2.1 x 50mm
 Part Numbers: 92272-402
 Mobile Phase A: see below
 Mobile Phase B: Acetonitrile
 Isocratic: 54% B (pH 10.6)
 26% B (pH: 2.7)
 Flow Rate: 0.4 mL/min.
 Temperature: 30 °C
 Back Pressure: 144 bar
 Sample Solvent: 50/50 Acetonitrile/ Water
 Wavelength: PDA, 254 nm
 Flow Cell: 1 µL
 Data Rate: 100 Hz
 Response Time: 0.025 sec.
 LC System: Shimadzu Nexera X2

At low pH, basic compounds become positively charged which allow for unwanted interactions between the stationary phase/ silanols on the silica surface. Under high pH conditions, basic molecules become deprotonated, increasing retention (becoming less polar) and significantly improving chromatographic peak shape/ efficiency. This allows for much higher sample loading capacities compared to low pH conditions as seen in the figure below. In general, the pH of the mobile phase should be set to 2 units above the analyte's pKa value, allowing the base to become deprotonated (neutral). Below demonstrates trimipramine, a common tricyclic antidepressant (base) observed under low and high pH conditions at different sample loads on column.