

Novel High pH Stable SPP Columns for Enhanced LC and LC-MS Separations of Basic Compounds

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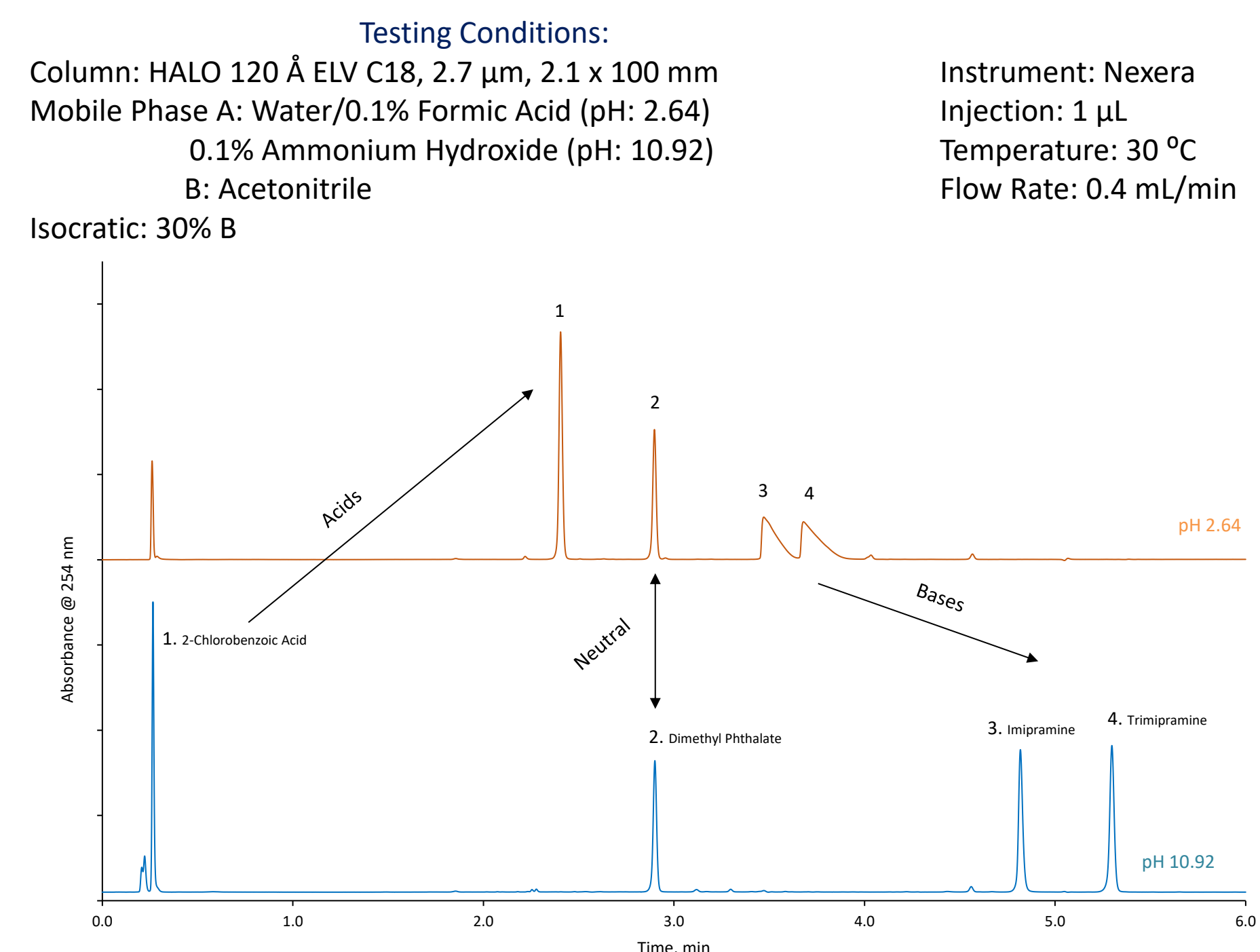


HPLC 2025 Bruges, Belgium
STP-06

Introduction

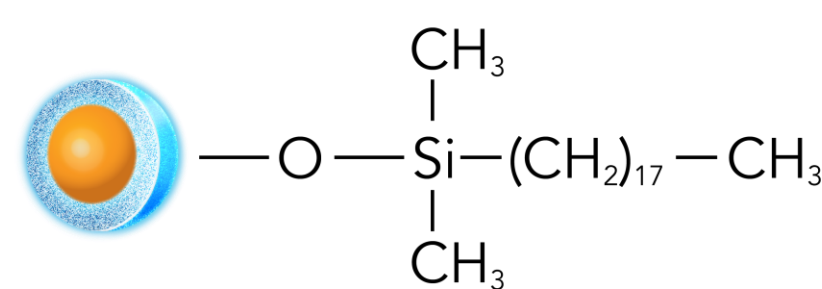
Problem: Low retention and poor peak shape for basic compounds when run under low pH/acidic conditions

Solution: Use high pH/basic conditions with a column packed with high pH stable particles



- Retention increases for basic compounds as pH is increased while retention is unaffected for the neutral compound (dimethyl phthalate)
- Efficiency also increases for basic compounds as pH is increased

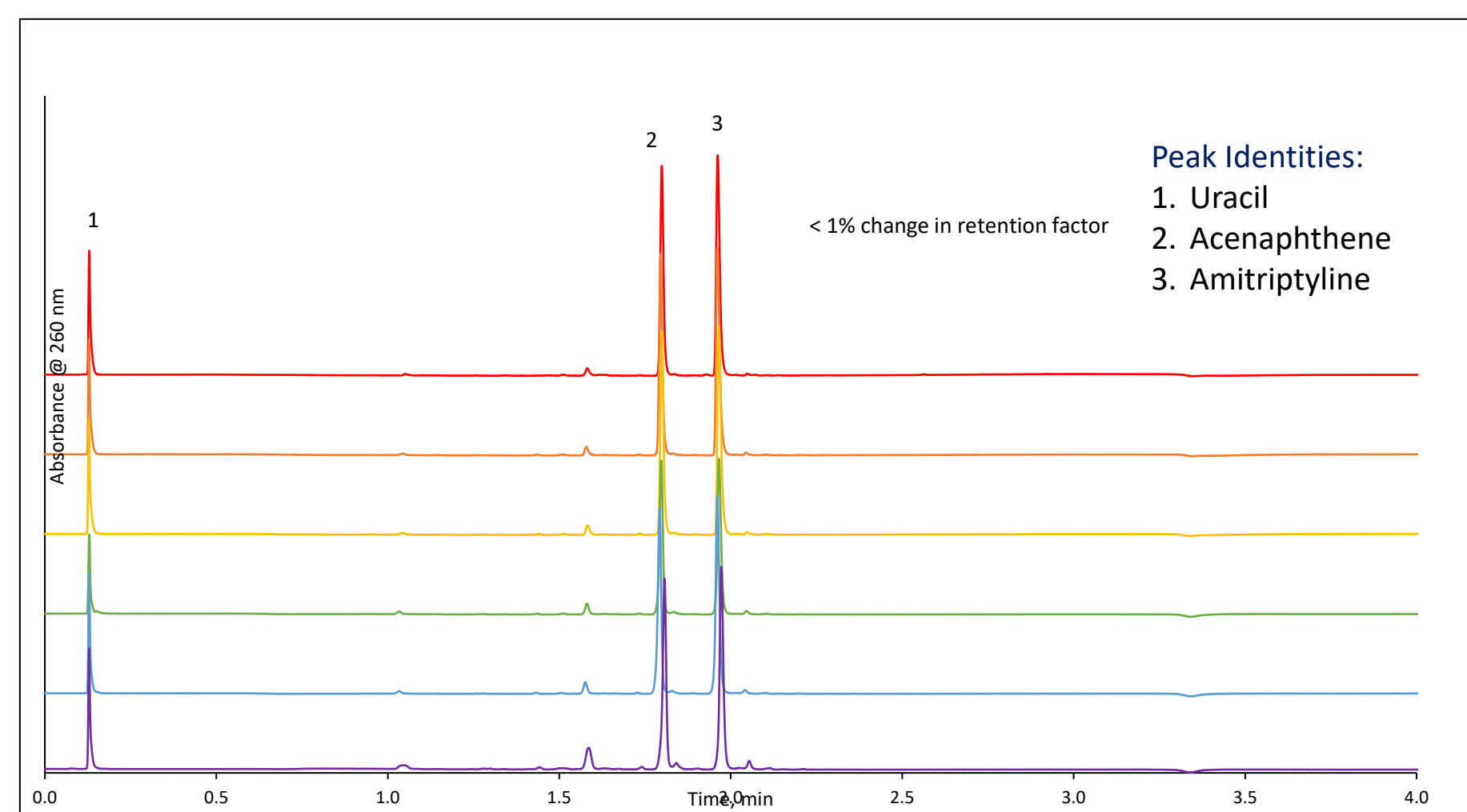
HALO® ELEVATE C18



- Surface modified organo-silane technology for resistance to alkaline conditions
- 2 and 2.7 µm particle size with 0.5 µm thick shell and 120 Å pore size
- Excellent stability under high pH conditions
- Excellent peak shape and increased loading capacity for basic compounds
- Wide operational use range of pH 2-12 and 60 °C for robust method development

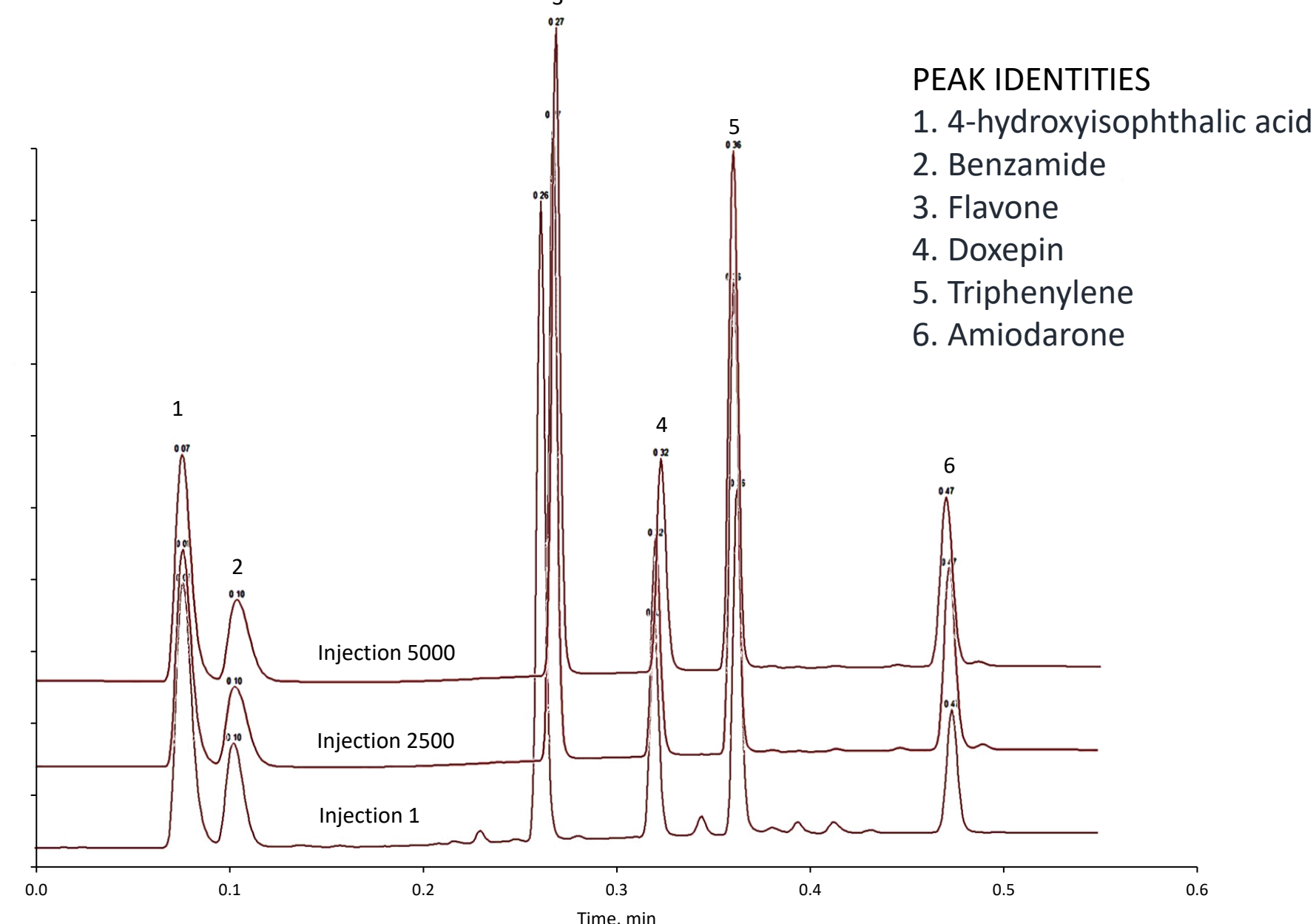
Stability at High pH Conditions

HALO 120 Å ELV C18, 2.7 µm, 2.1 x 50 mm, A: 95/5 10mM Ammonium Bicarbonate, pH:10/ Acetonitrile; B: Acetonitrile; Gradient: 0-95% B in 2.5 min., hold at 95% B for 0.5 min., ramp to 0% B for 0.1 min., hold at 0% B for 1.9 min.
Flow Rate: 0.8 mL/min; Back Pressure: 220 bar; Temperature: 60 °C; Injection: 1.0 µL
Wavelength: PDA, 260 nm, LC System: Shimadzu Nexera X2



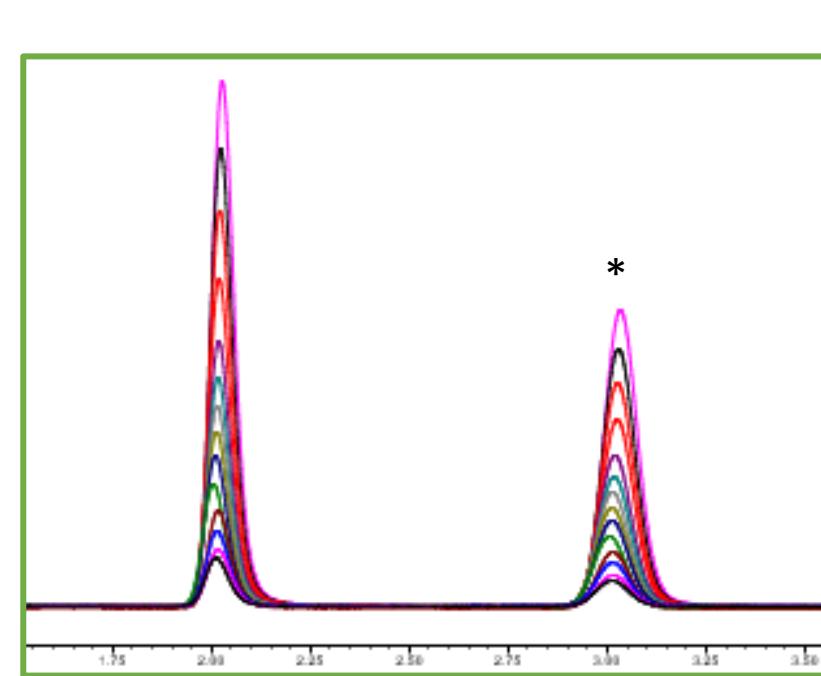
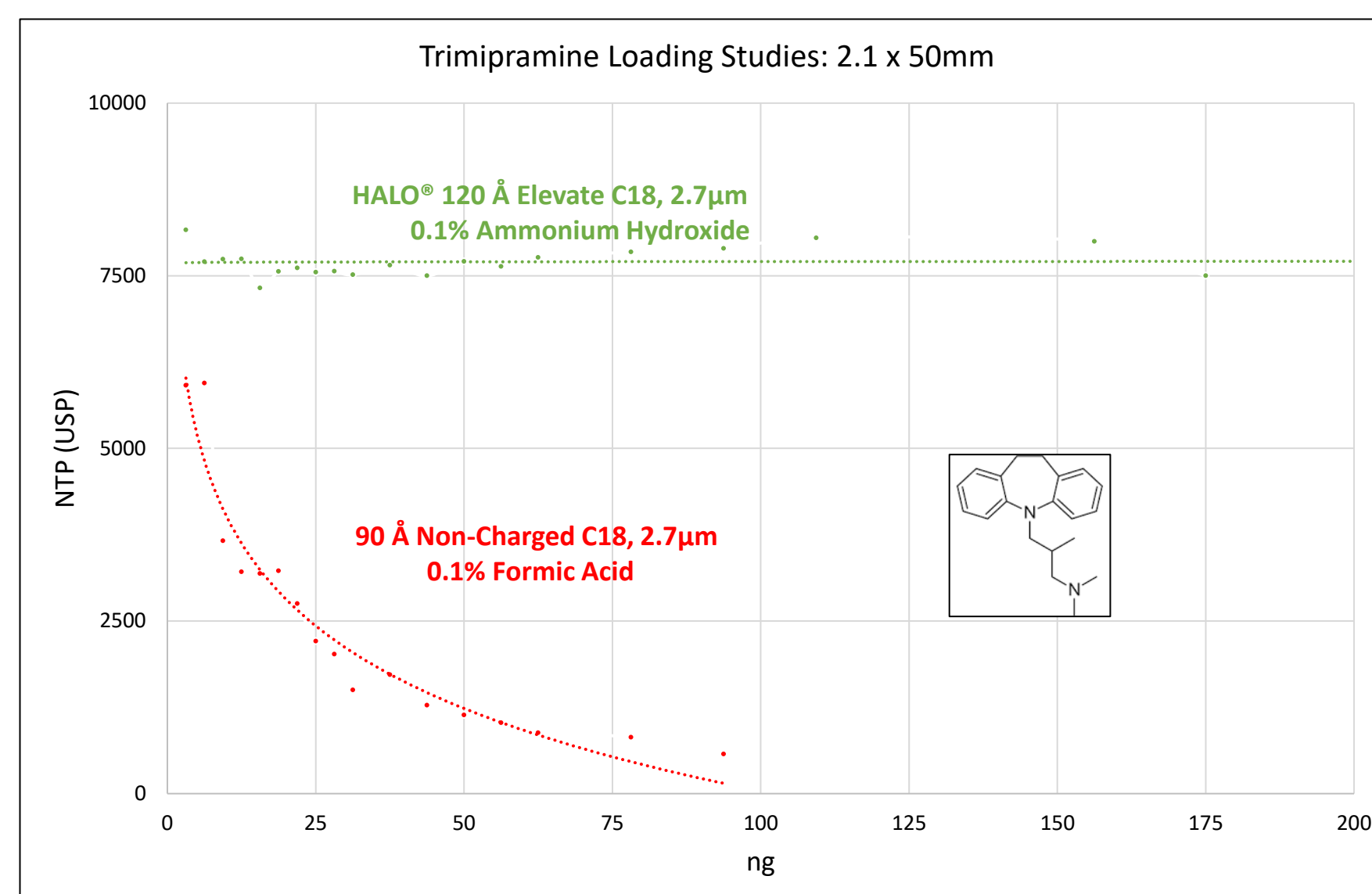
- Less than a 1% change in retention is achieved over 20,000 column volumes.

HALO 120 Å ELV C18, 2.7 µm, 1.5 x 50 mm, A: 0.1% NH4OH, pH:11
B: Acetonitrile; Gradient: 20-95% B in 0.25 min., 95-100% B in 0.2 min., hold at 100% B for 0.1 min.
Flow Rate: 1.0 mL/min; Max Back Pressure: 550 bar; Temperature: 60 °C; Injection: 1.0 µL
Detection: UV Wavelength Range: 210-400 nm; MS Scan Range: 100-1250 Da
LC System: Waters Acquity H-Class
Data Courtesy of: Boehringer Ingelheim (Biberach, Germany)

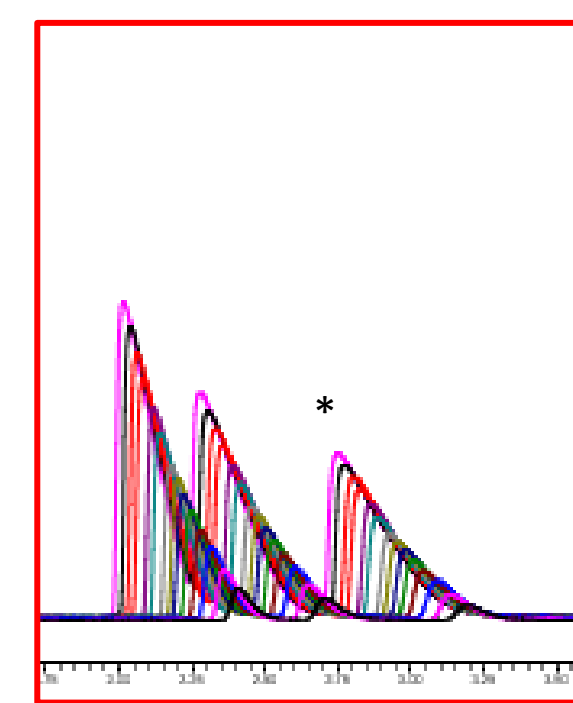


- 1.5 mm ID HALO® Elevate C18 column run at 5 times the optimum flow rate demonstrated excellent reproducibility and stability for retention time, peak shape, and back pressure over the course of 5000 injections

Improved Sample Loading of Basic Compounds



Trimipramine Peak Shape on HALO® Elevate C18 at high pH



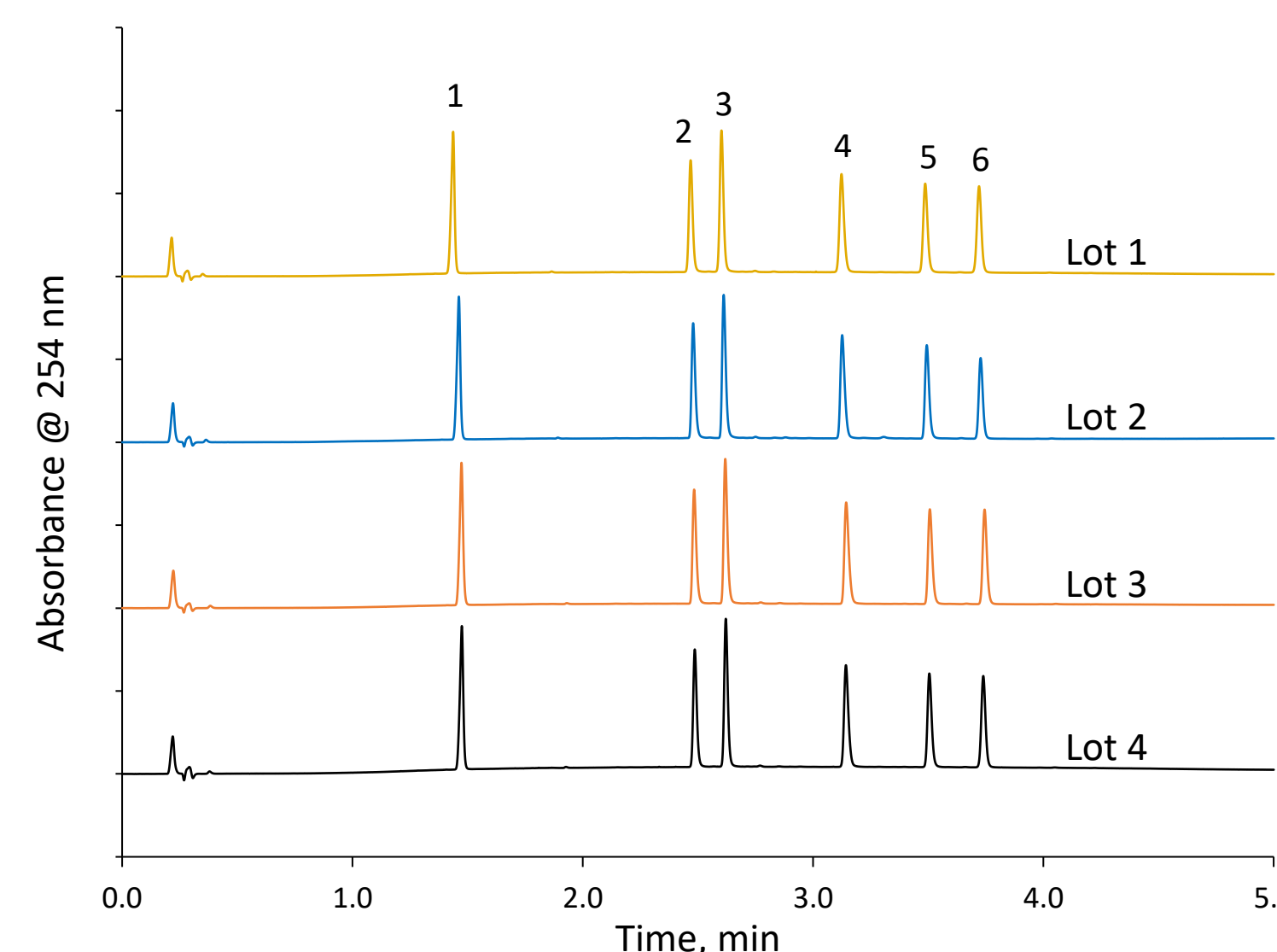
Trimipramine Peak Shape on Non-Charged C18 at low pH

- Much higher sample loading capacities with HALO® Elevate C18 at basic conditions compared to low pH conditions on a non-charged C18 column
- Peak shapes shown in boxes demonstrate how much narrower and sharper the results are with HALO® Elevate compared to non-charged C18 over the entire range of sample loads tested (3.125 – 175 ng)

HALO® Elevate C18 Lot-to-Lot Reproducibility

2.1 x 50 mm, A: 0.1% NH₄OH, pH 10.7; B: Acetonitrile; Gradient: 5-95% B in 4 min;
Flow Rate: 0.4 mL/min; Back Pressure: 134 bar; Temperature: 40 °C; Injection: 0.5 µL
Sample Solvent: 65/35 0.1% NH₄OH/ACN; Wavelength: PDA, 254 nm, LC System: Shimadzu Nexera X2

Peak Identities:
1. Butyl Paraben (neutral)
2. Doxylamine (base)
3. Chlorpheniramine (base)
4. Doxepin (base)
5. Amitriptyline (base)
6. Trimipramine (base)



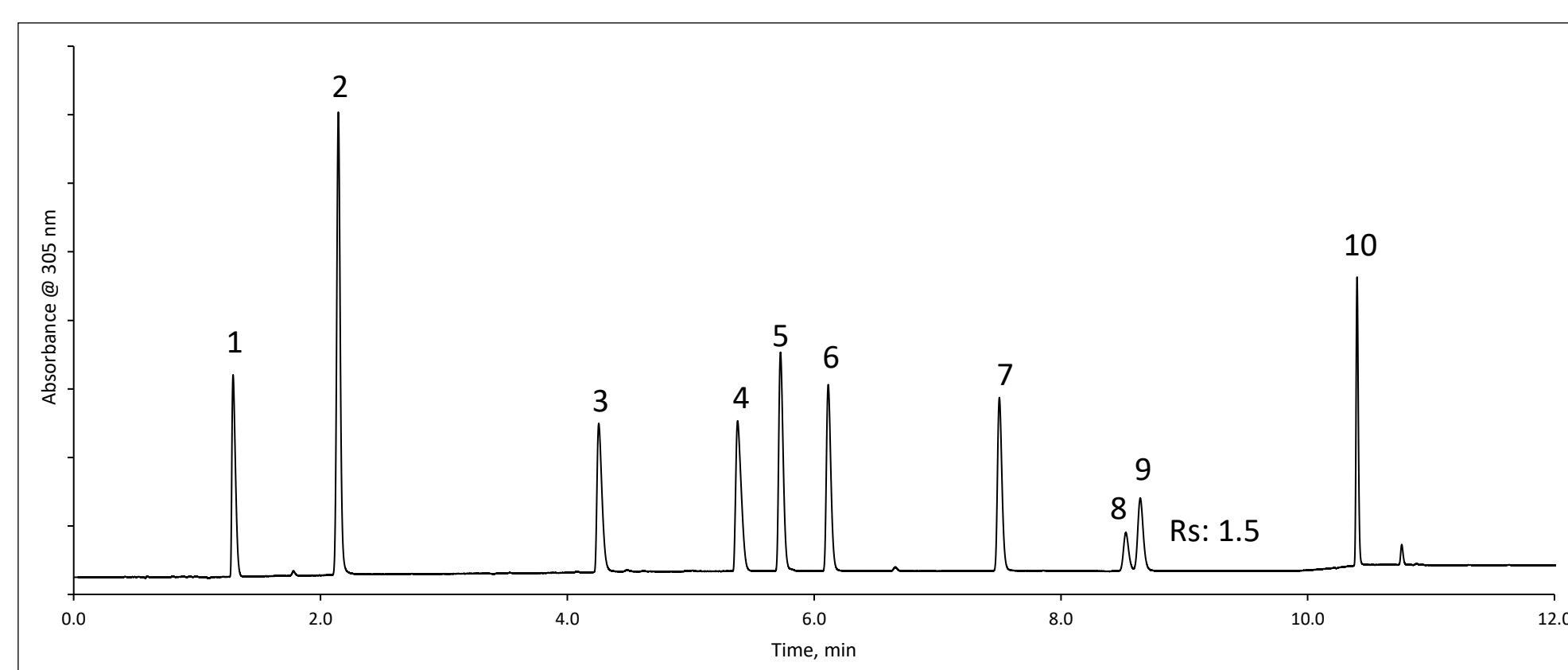
- Four different lots of HALO® Elevate C18 were tested using a mix containing a neutral compound and 5 basic compounds
- Average %RSD across all of the compounds for retention time was 0.4%

HALO® Elevate C18, 2 µm: Omeprazole and Related Impurities

2.1 x 100 mm, A: Water, 0.03% Ammonium Hydroxide (pH 10.6); B: Methanol; Gradient: 12-45% B in 7 min, hold at 45% B for 2 min, 45-70% B in 0.5 min, hold at 70% B for 1.5 min; Flow Rate: 0.4 mL/min;
Temperature: 60 °C; Injection: 1.0 µL; Wavelength: PDA, 305 nm, LC System: Shimadzu Nexera X2

PEAK IDENTITIES:

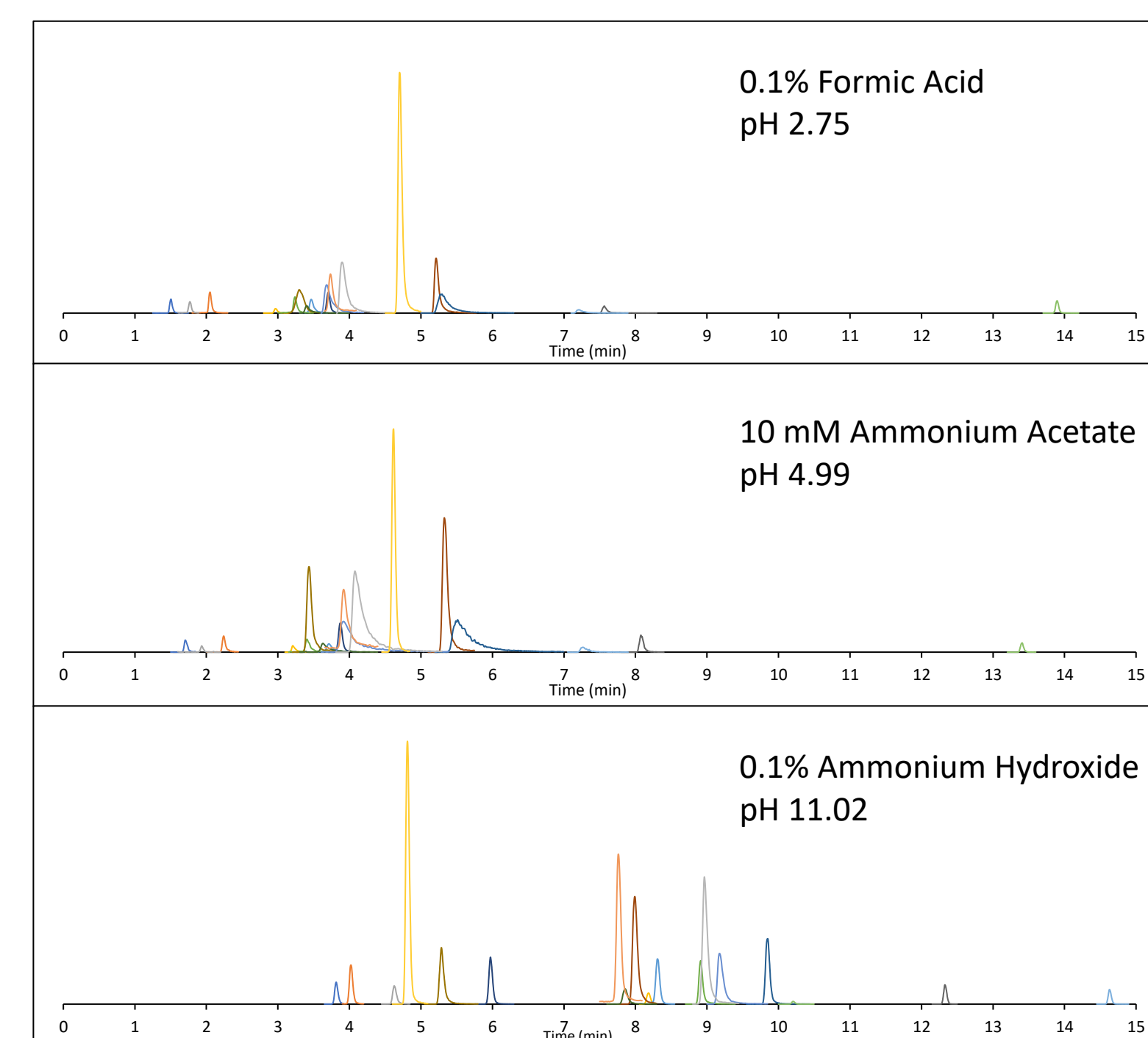
1. Related Compound F & G
2. Related Compound B
3. Related Compound E
4. Related Compound A
5. Impurity B
6. Omeprazole
7. Impurity H
8. N'-Methyl Omeprazole isomer 1
9. N'-Methyl Omeprazole isomer 2
10. Impurity C



- Omeprazole: one of the most frequently prescribed proton pump inhibitors and among the top ten prescribed drugs in the United States
- Stability of omeprazole is maximized at pH 11 so most methods are run at elevated pH
- Baseline separation of 9 different related compounds and impurities of omeprazole is complete within 11 minutes
- Method developed with the HALO® Elevate C18 column is ~2 times faster than the current USP method for impurities of omeprazole delayed release capsules

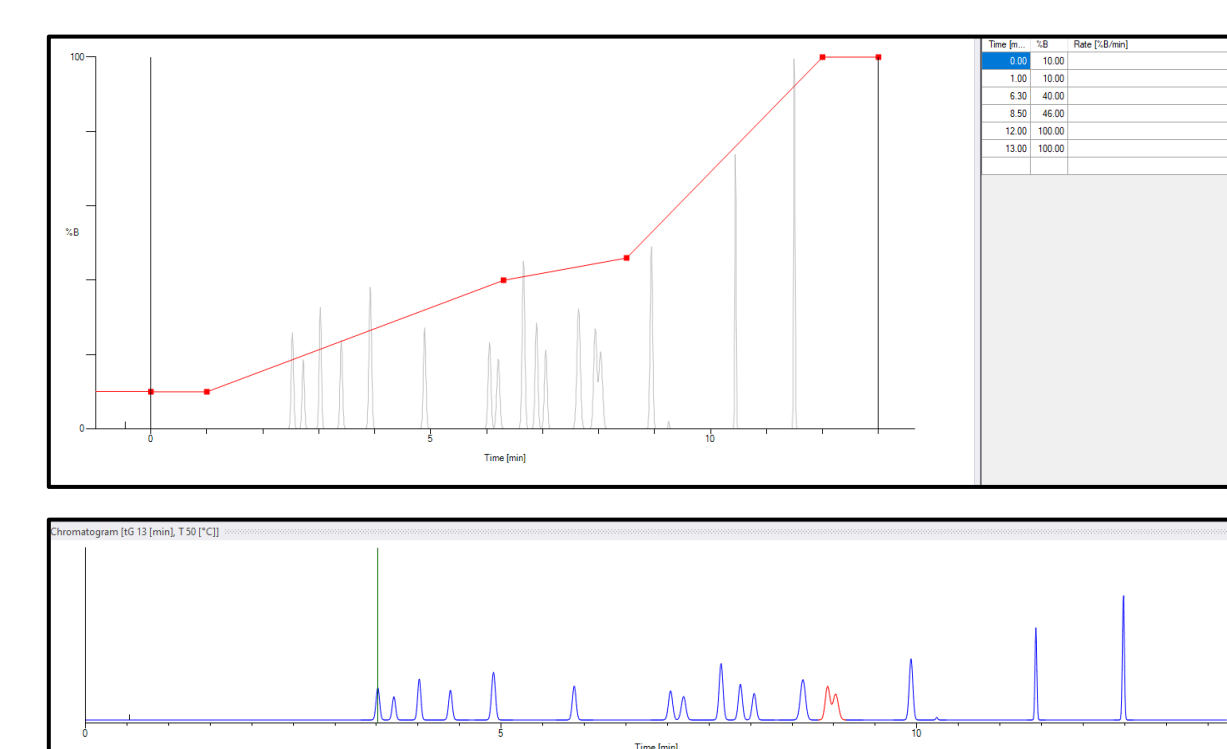
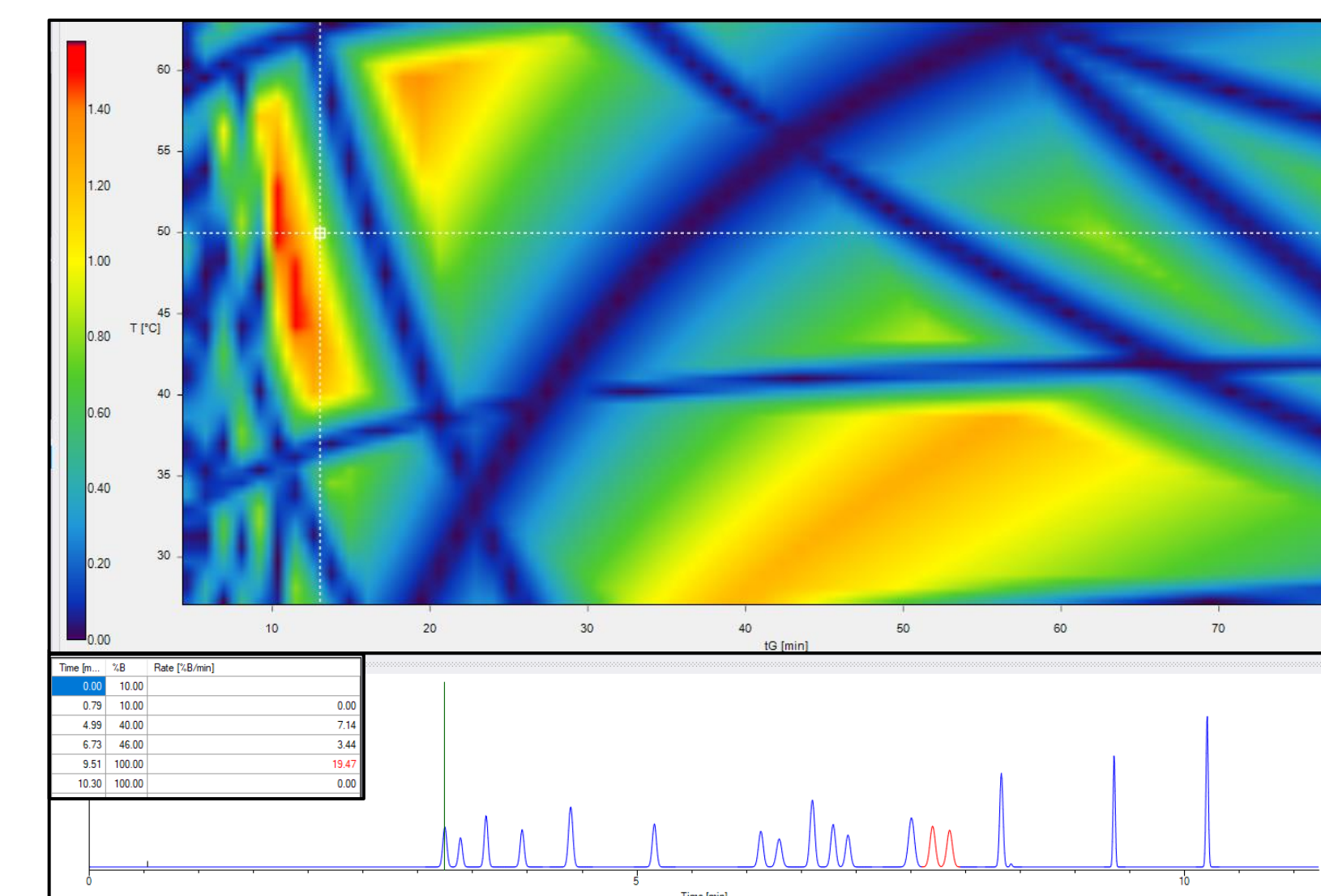
HALO® Elevate C18: pH Screening of 18 Drugs and Metabolites

2.1 x 100 mm, A1: Water/0.1% Formic Acid (pH 2.75); A2: Water/10 mM Ammonium Acetate (pH 4.99); A3: Water/0.1% Ammonium Hydroxide (pH 11.02); B: Methanol; Gradient: 5-95 %B in 15 min.;
Flow Rate: 0.4 mL/min; Temperature: 30 °C; Injection: 1.0 µL; Detection: Shimadzu LCMS 8040



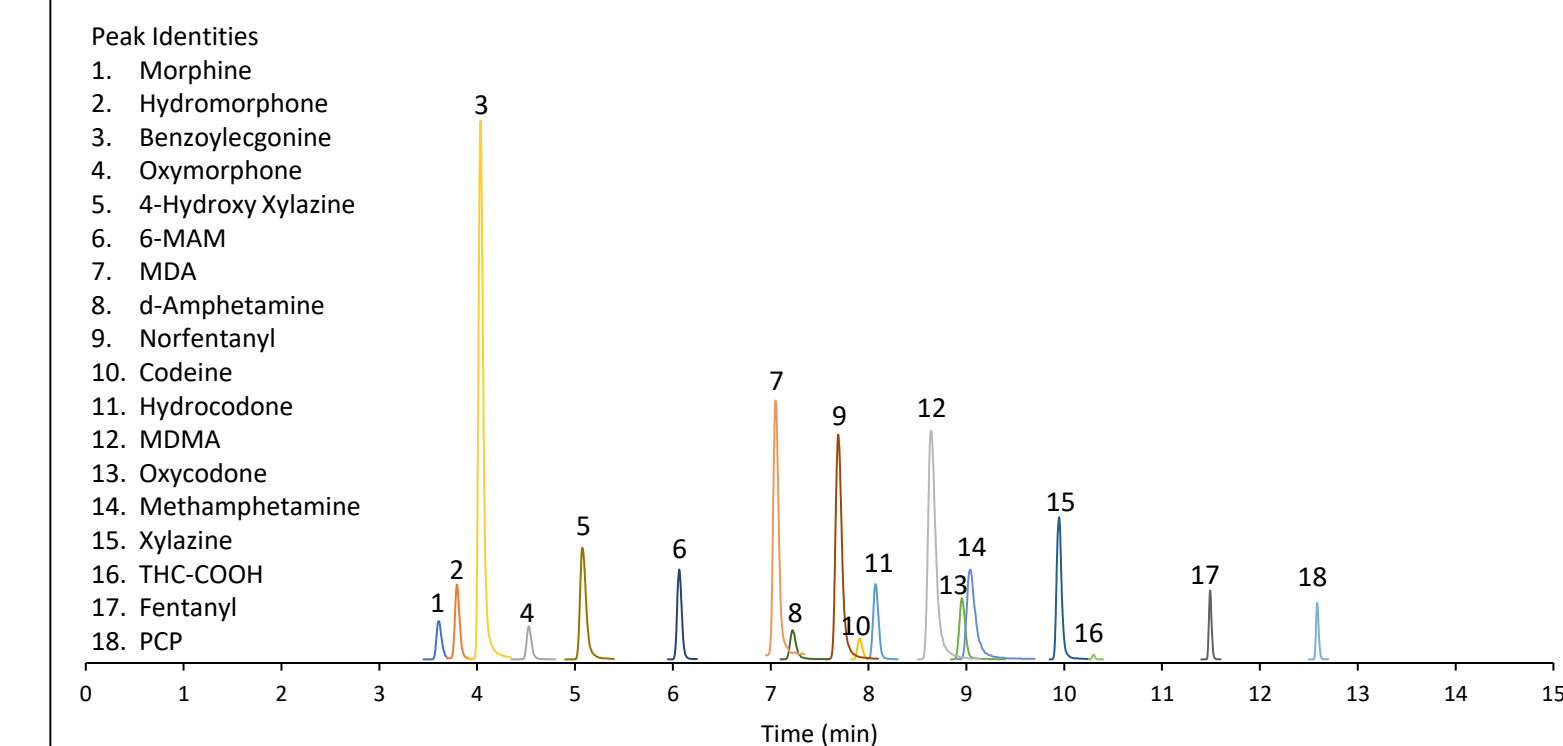
Compound	MRM Transition Q1->Q3	Retention Time (min) pH 2.75	Retention Time (min) pH 4.99	Retention Time (min) pH 11.02
Morphine	286.2->152.1	1.504	1.706	3.815
Hydromorphone	286.2->185.2	2.051	2.242	4.021
Oxycodone	302.2->284.0	1.769	1.934	4.627
Codeine	300.2->152.3	2.966	3.208	8.182
Hydrocodone	300.2->199.2	3.466	3.713	8.307
Oxycodone	316.2->241.3	3.238	3.406	8.912
6-Acetylmorphine	328.2->165.1	3.704	3.873	5.972
Norfentanyl	233.4->84.1	5.233	5.328	7.991
Fentanyl	337.5->188.0	7.564	8.076	12.327
4-Hydroxy Xylazine	237.2->137.2	3.298	3.435	5.286
Xylazine	220.9->164.0	5.271	5.514	9.848
d-Amphetamine	136.1->91.0	3.403	3.63	7.856
Methamphetamine	150.1->119.1	3.682	3.917	9.175
MDA	180.0->163.0	3.735	3.92	7.761
MDMA	194.0->163.0	3.897	4.078	8.964
Benzoylcegonine	290.1->168.2	4.702	4.615	4.811
PCP	244.3->90.9	7.207	7.265	14.629
THC-COOH	345.0->299.2	13.896	13.407	10.204

HALO® Elevate C18: DryLab® Optimized Separation of Drugs of Abuse and Metabolites



- 2D: Gradient Times (15 min, 45 min) and Temperatures (30 °C, 60 °C)
- 13 min gradient at 50 °C resolved critical pairs
- resolution between two sets of critical peaks (morphine/hydromorphone and hydrocodone/codeine) was not baseline (<1.5) using MS. Switch to longer isocratic hold and gradient gave baseline resolution

2.7 µm, 2.1 x 100 mm, A: Water/0.1% Ammonium Hydroxide (pH 11.02); B: Methanol; Gradient: Hold at 10% for 1 min, 10-40% B in 5.3 min, 40-46% B in 2.2 min; 46-100% B in 3.5 min, hold at 100% B for 1 min; Flow Rate: 0.4 mL/min; Temperature: 50 °C; Injection: 5 µL; Detection: Shimadzu LCMS 8040



Summary

- HALO® Elevate C18 is a high pH stable column for HPLC, UHPLC, and LC-MS separations that is very reproducible.
- Sample loading is improved for basic compounds when high pH conditions are used.
- Using pH as method development tool for basic compounds can be very successful provided the pH is > pKa of the compounds.
- Applications using pharmaceuticals, drugs of abuse, and metabolites show high efficiency, rapid separations.

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