Enhancing LC and LC-MS Separations of Basic Compounds with Novel High pH Stable SPP Columns

Stephanie A. Schuster, Conner McHale, Peter Pellegrinelli, Mark Haynes

Advanced Materials Technology, Inc.

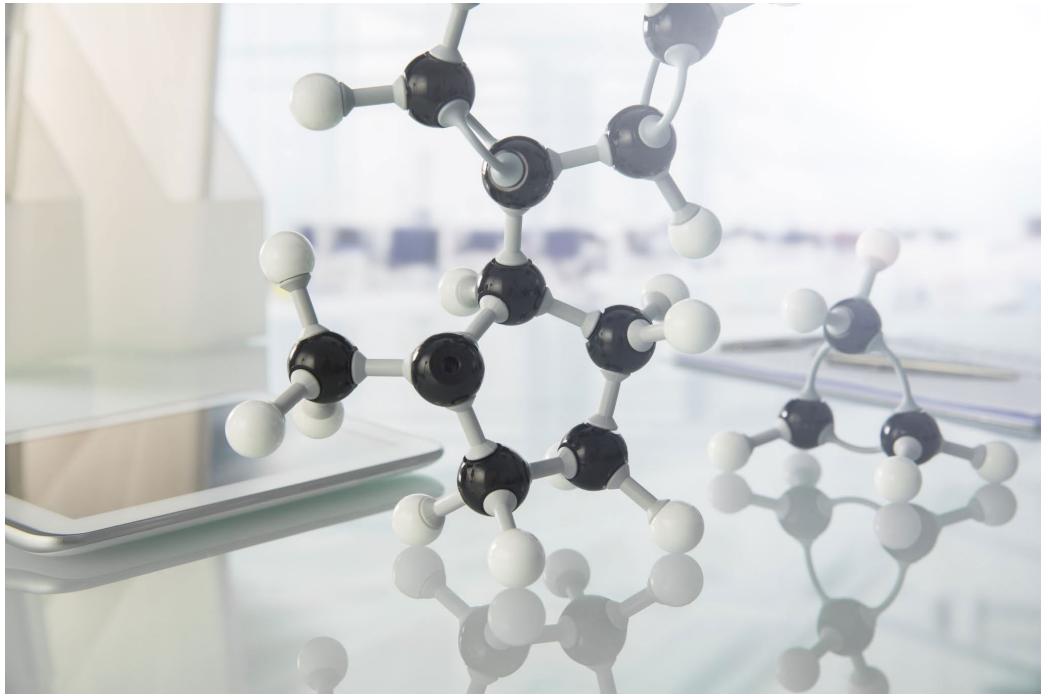
advancedmaterialstechnology

EAS

November 19, 2024

Solutions to Improve Tailed Peak Shape of Basic Compounds

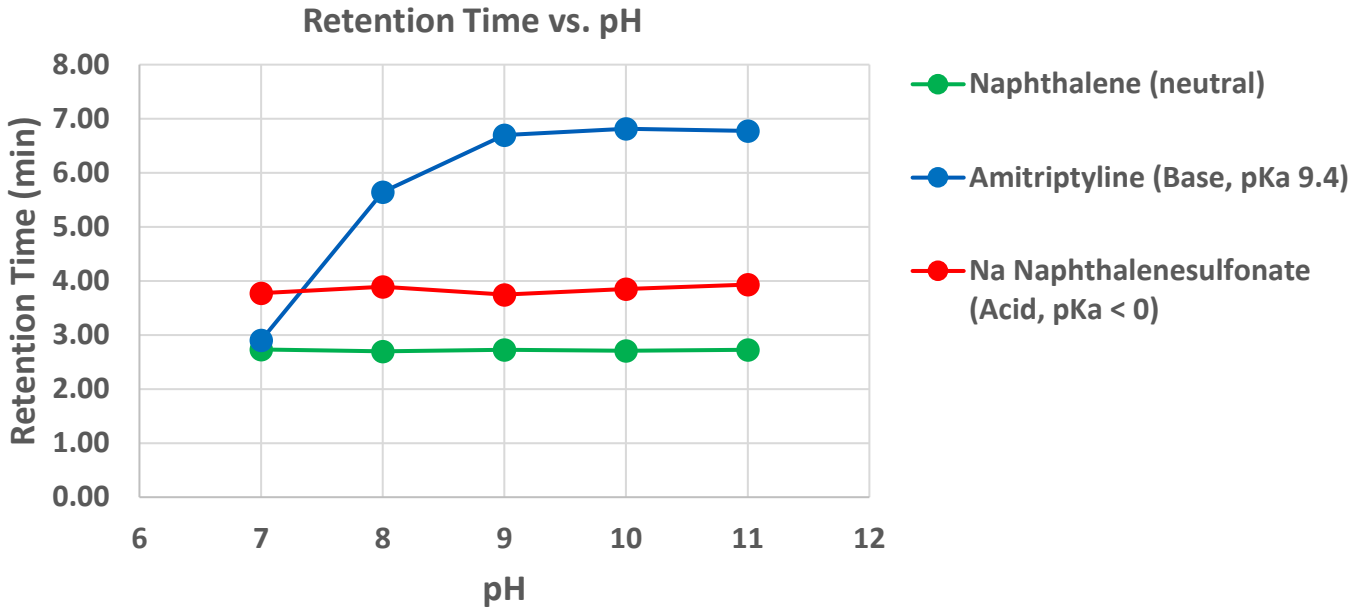
1. Increase the ionic strength of the mobile phase by adding salt or buffer
2. Use an ion pair agent
3. Use a non-silica based column
4. Elevate the pH
5. Use a different stationary phase

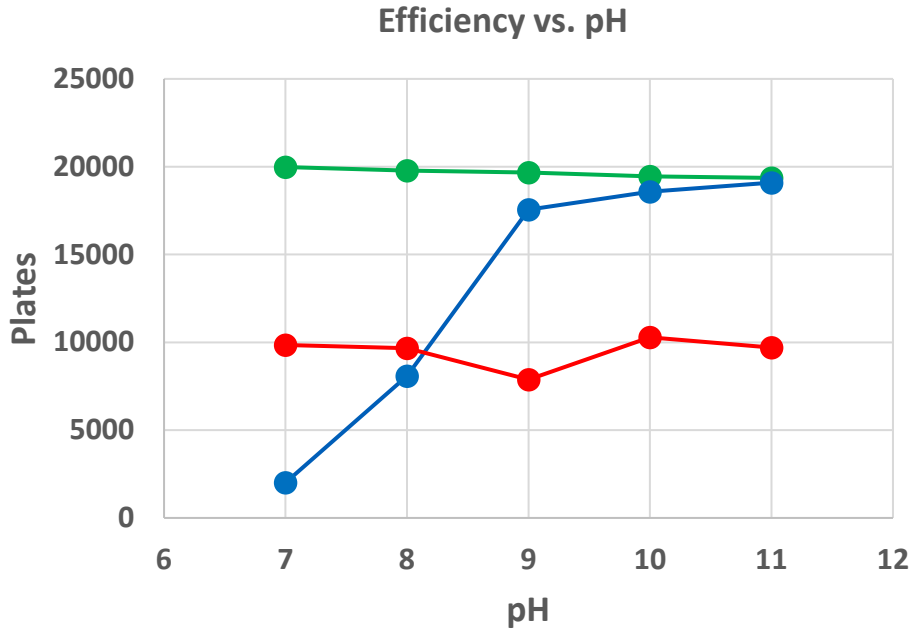


Effect of pH on Retention and Efficiency

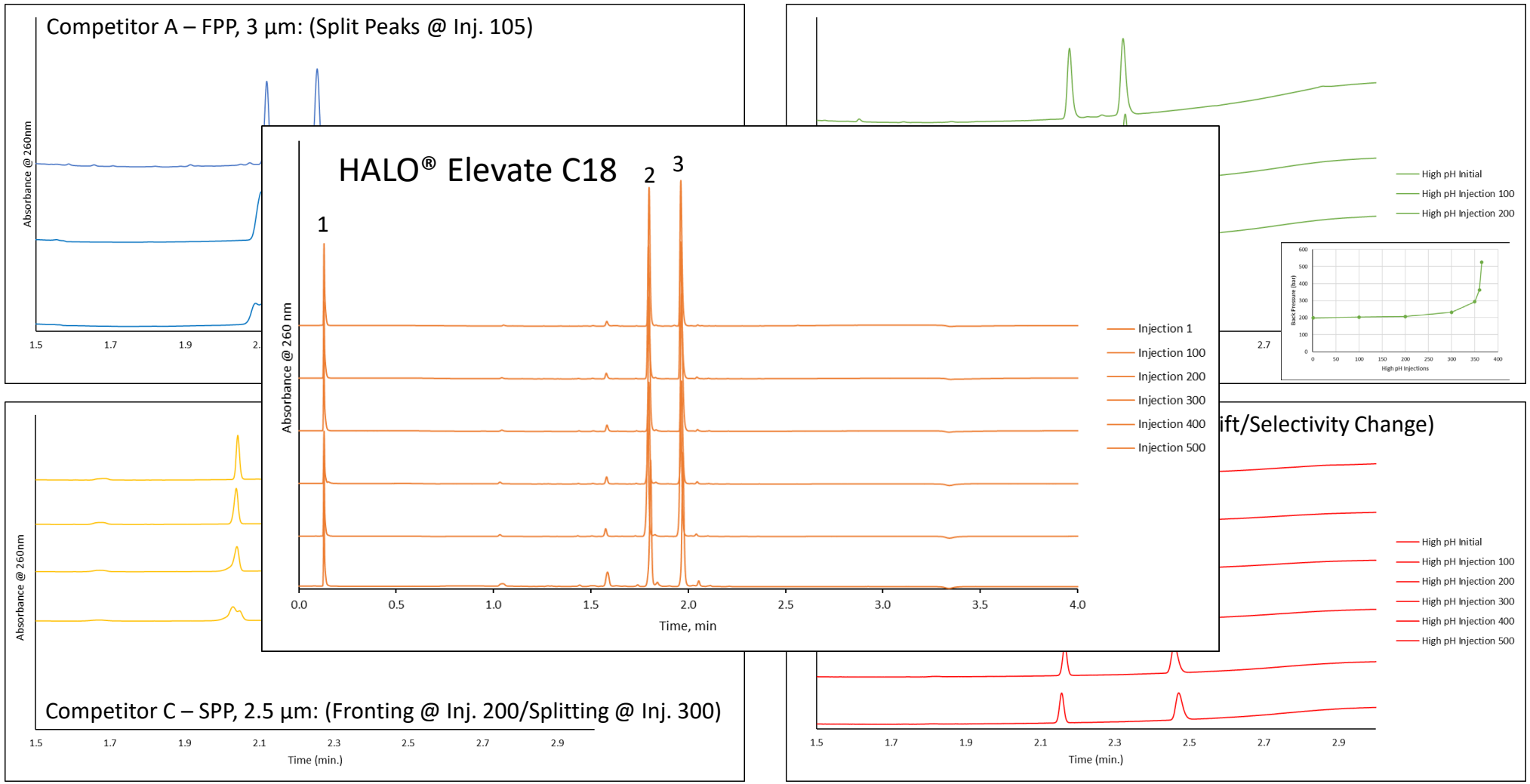
Testing Conditions:
Column: HALO 120 Å ELV C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92272-602
Mobile Phase A: 20 mM potassium phosphate
B: Acetonitrile
Isocratic: 10% B for acid and 50% B for neutral and base
Instrument: Nexera
Injection: 1 µL (50 ng on column)
Temperature: 35 °C
Flow Rate: 0.3 mL/min

- Retention increases for basic compound as pH is increased while retention is unaffected for neutral and acidic compounds
- Efficiency also increases for basic compounds as pH is increased





HALO® Elevate C18 Stability vs. Competitors: pH: 10, 60 °C, 500 injections



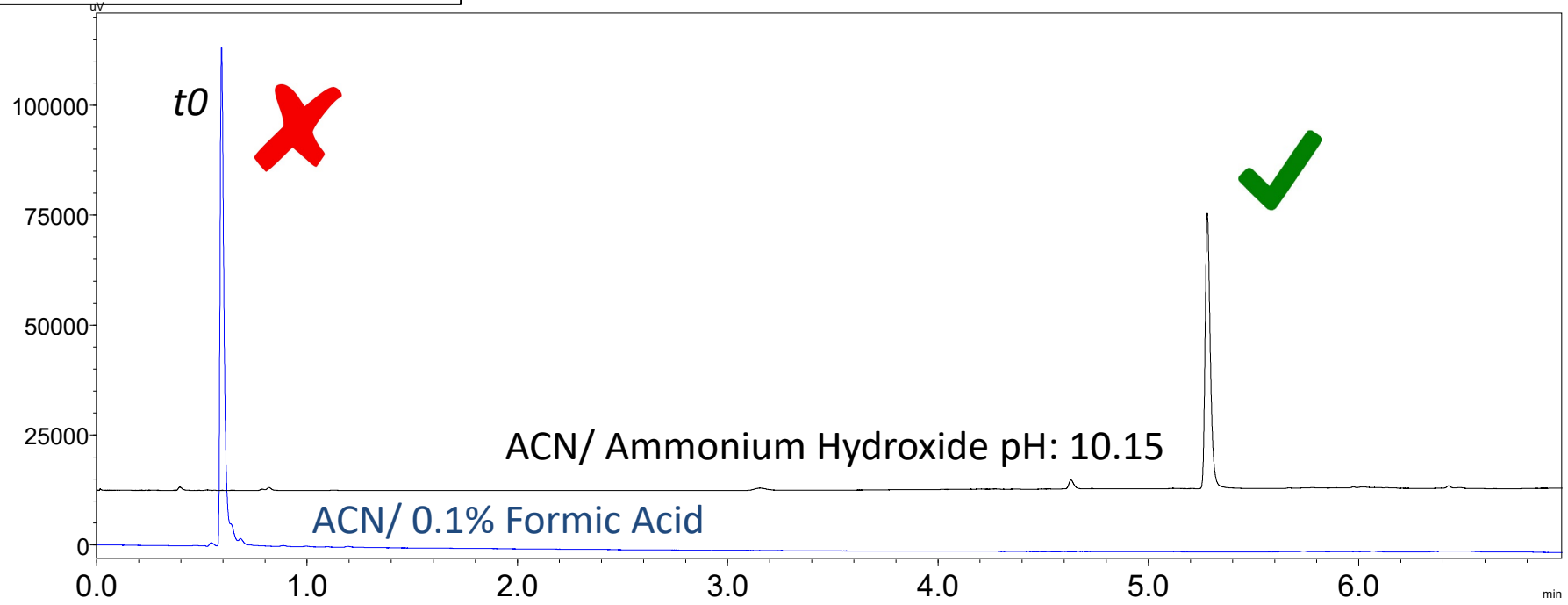
PEAK IDENTITIES
1. Uracil
2. Acenaphthene
3. Amitriptyline

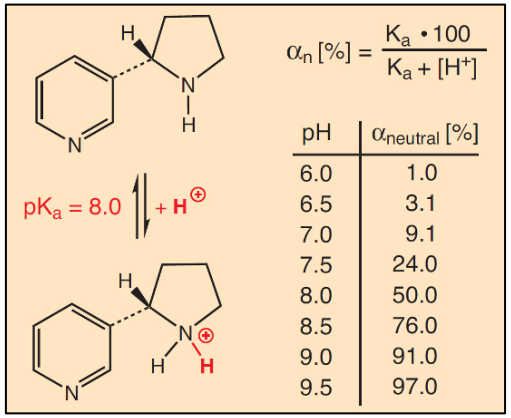
Conditions
2.1 x 50 mm
A: 95/5 10 mM Ammonium Bicarbonate, pH 10/ACN
B: ACN
0-95% B in 2.5 min
0.8 mL/min
60 °C
260 nm

HALO 120 Å Elevate: Nicotine

Testing Conditions:
Column: HALO 120 Å ELV C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92272-602
Mobile Phase A: Water/ as listed
B: Acetonitrile
Gradient: 5-60%B in 10min (2 min. hold)
Instrument: Nexera
Injection: 0.1 µl Nicotine (50 ng)
Temperature: 30°C
Flow Rate: 0.4 mL/min.

- No retention using low pH/0.1% formic acid
- Great retention using pH 10.15 since pKa is 8.0

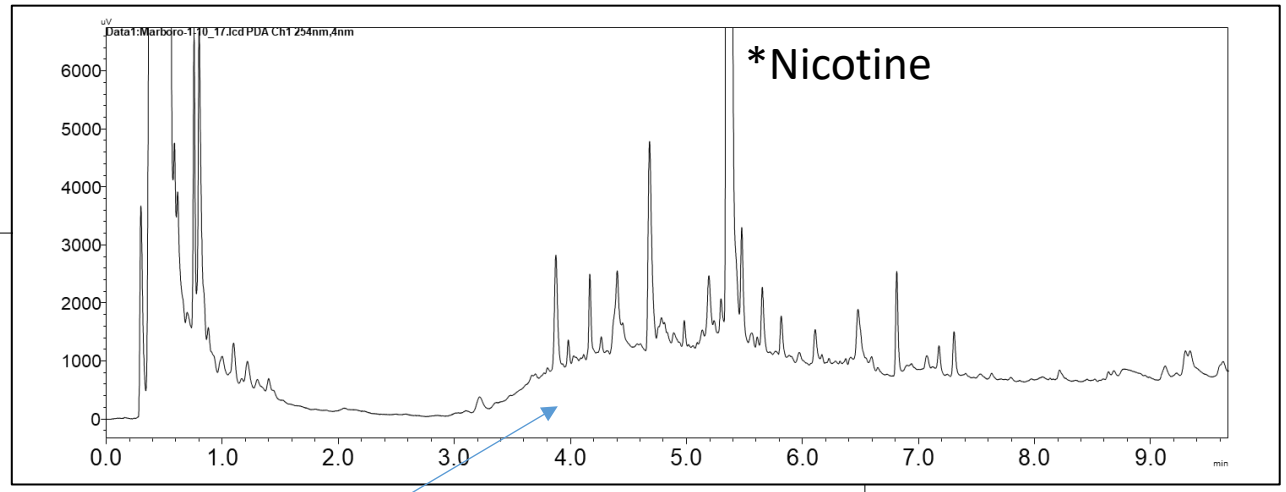


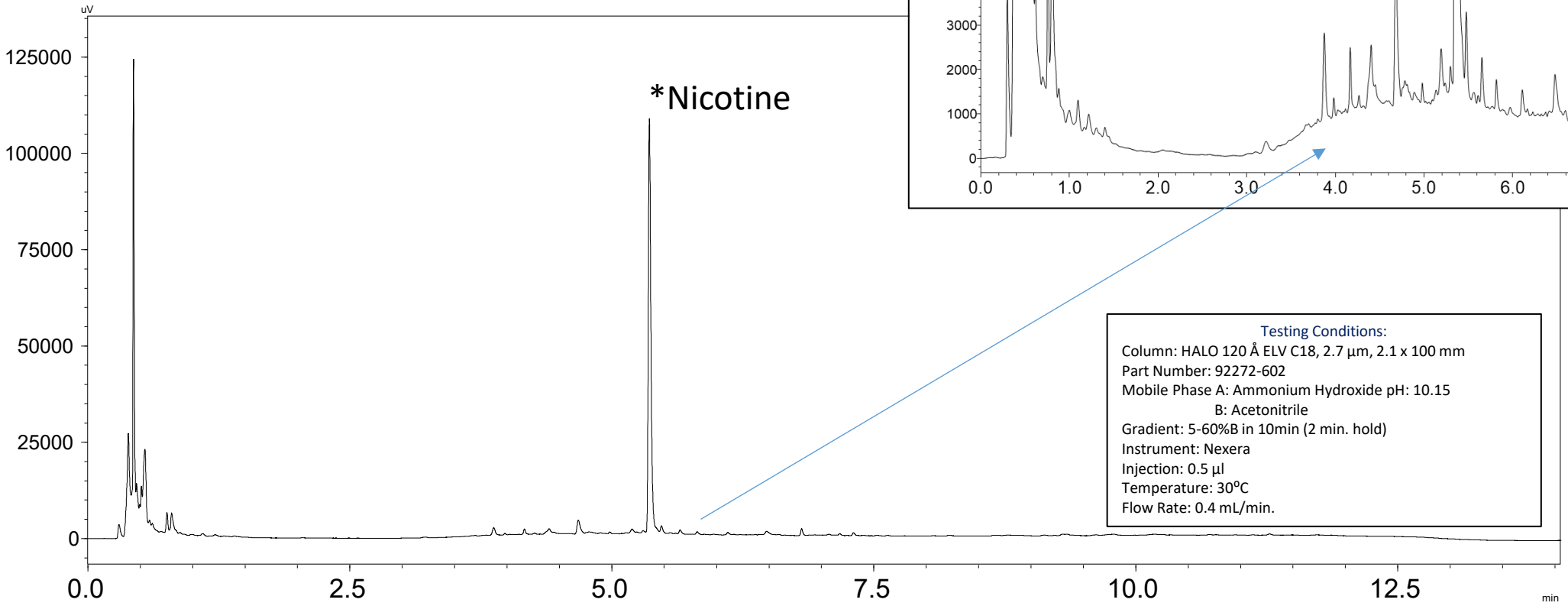


pH	$\alpha_{neutral}$ [%]
6.0	1.0
6.5	3.1
7.0	9.1
7.5	24.0
8.0	50.0
8.5	76.0
9.0	91.0
9.5	97.0

Nicotine Analysis of Cigarette Tobacco

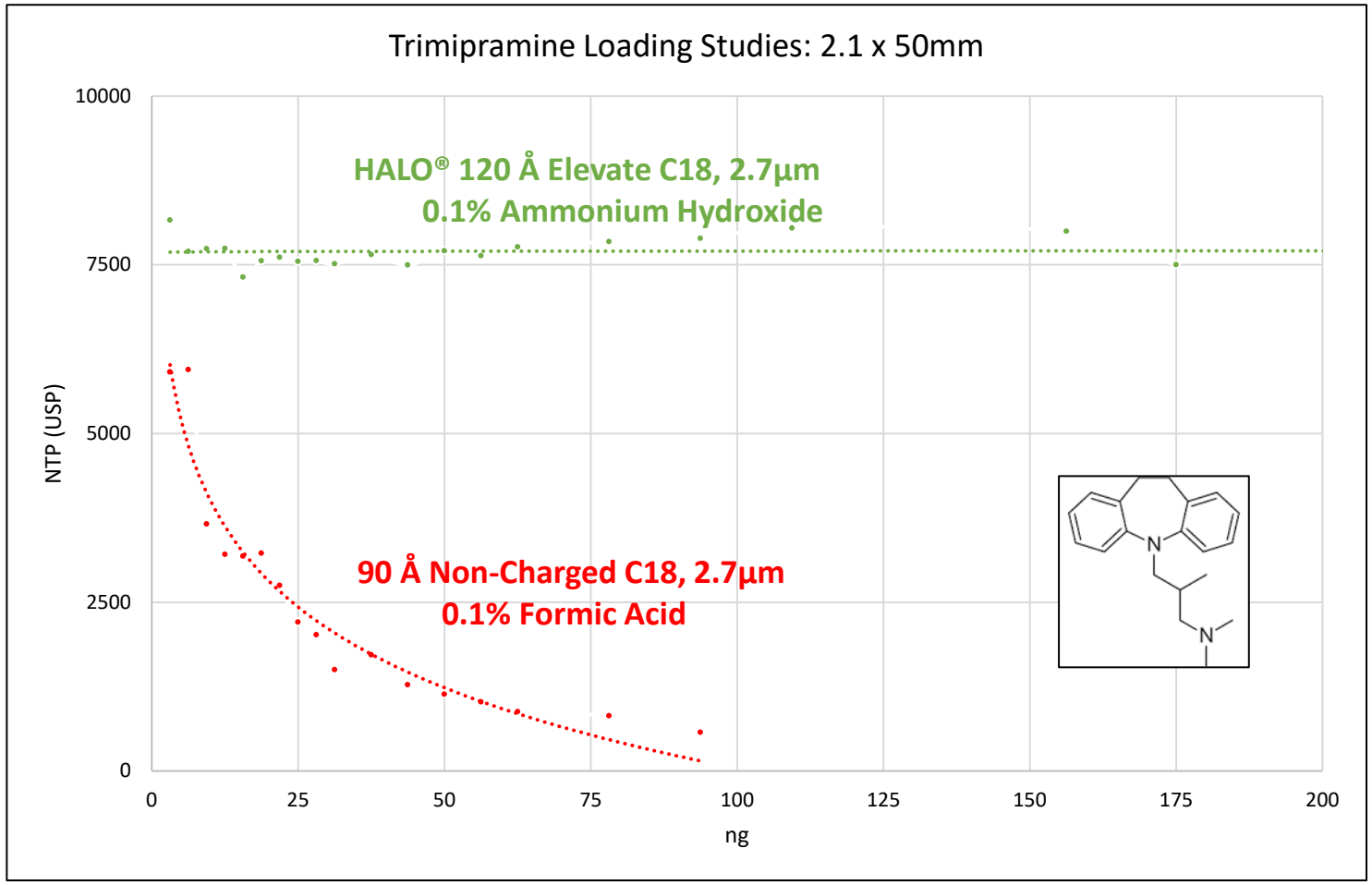
- Many low concentration peaks observed when zoomed in near the baseline
- Work in progress to ID using MS

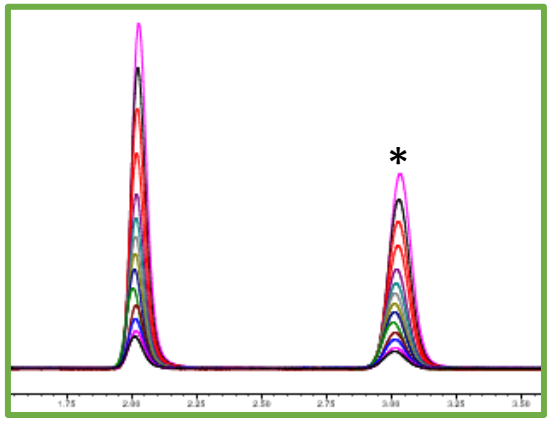


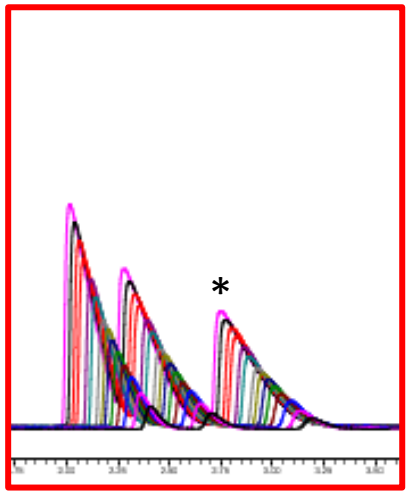


Testing Conditions:
Column: HALO 120 Å ELV C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92272-602
Mobile Phase A: Ammonium Hydroxide pH: 10.15
B: Acetonitrile
Gradient: 5-60%B in 10min (2 min. hold)
Instrument: Nexera
Injection: 0.5 µl
Temperature: 30°C
Flow Rate: 0.4 mL/min.

HALO® Elevate Loading Capacity

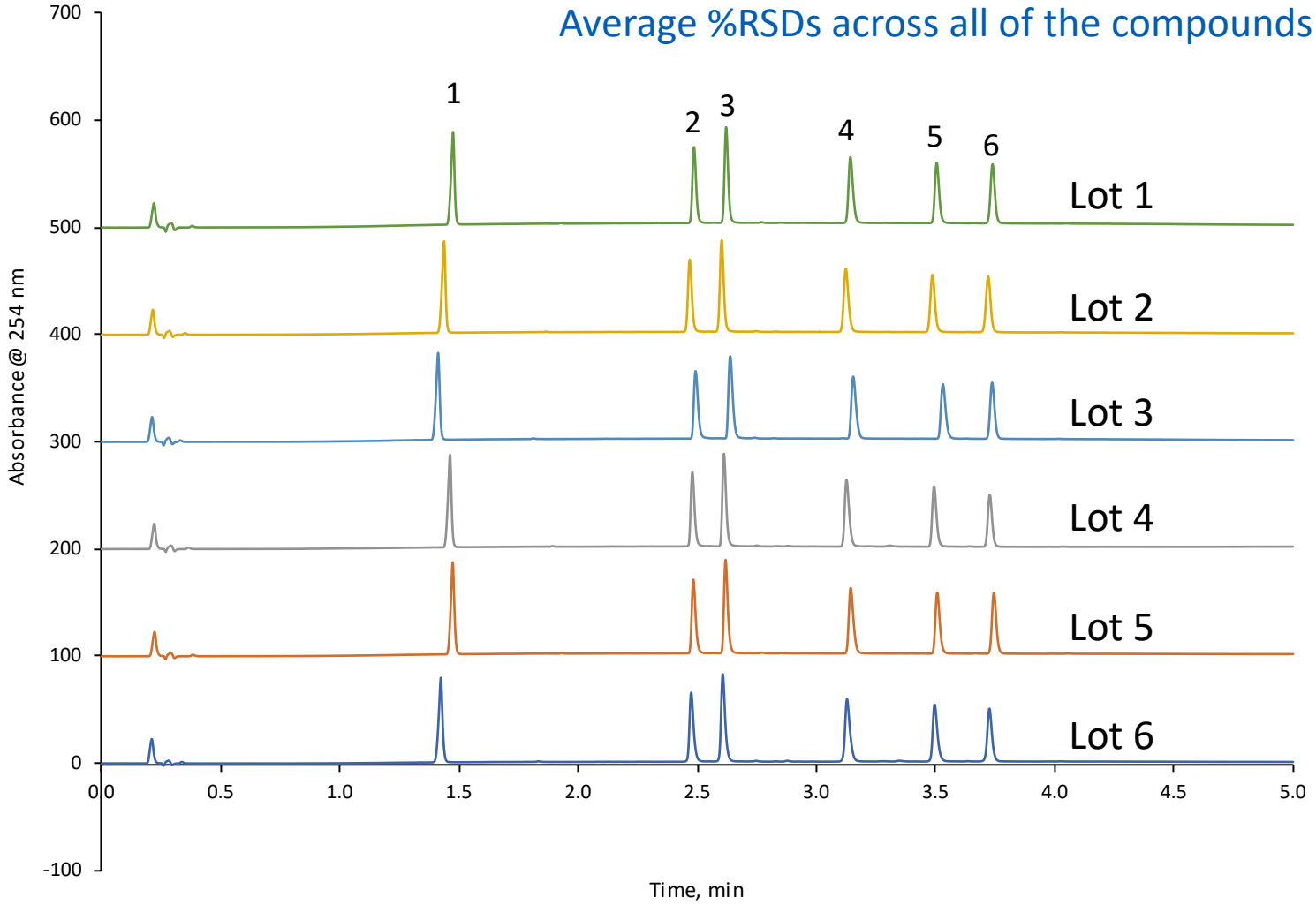






HALO® Elevate C18
Lot-to-Lot Reproducibility

Average %RSDs across all of the compounds was < 0.6%



TEST CONDITIONS:

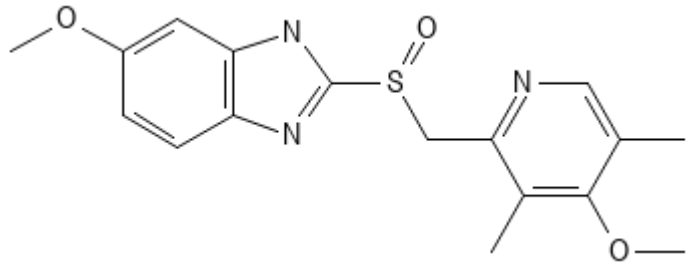
Columns: HALO 120 Å ELV C18, 2.7 µm, 2.1 x 50 mm
Part Number: 92272-402
Mobile Phase A: 0.1% NH_4OH, pH:10.7
Mobile Phase B: Acetonitrile
Gradient: 5- 95% B in 4 min
Flow Rate: 0.4 mL/min
Temperature: 40 °C
Detection: UV/PDA, 254 nm
Injection Volume: 0.5 µL
LC System: Shimadzu Nexera X2

PEAK IDENTITIES

1. Butyl Paraben
2. Doxylamine
3. Chlorpheniramine
4. Doxepin
5. Amitriptyline
6. Trimipramine

Omeprazole

- Classified as a proton pump inhibitor (PPI)
- Most frequently prescribed PPI
- Used to reduce the amount of acid in the stomach
- In 2021, omeprazole was the 9th most prescribed drug in the United States
- Most methods for omeprazole are run at elevated pH and stability of omeprazole is maximized at pH 11



Omeprazole Standard @ Low pH

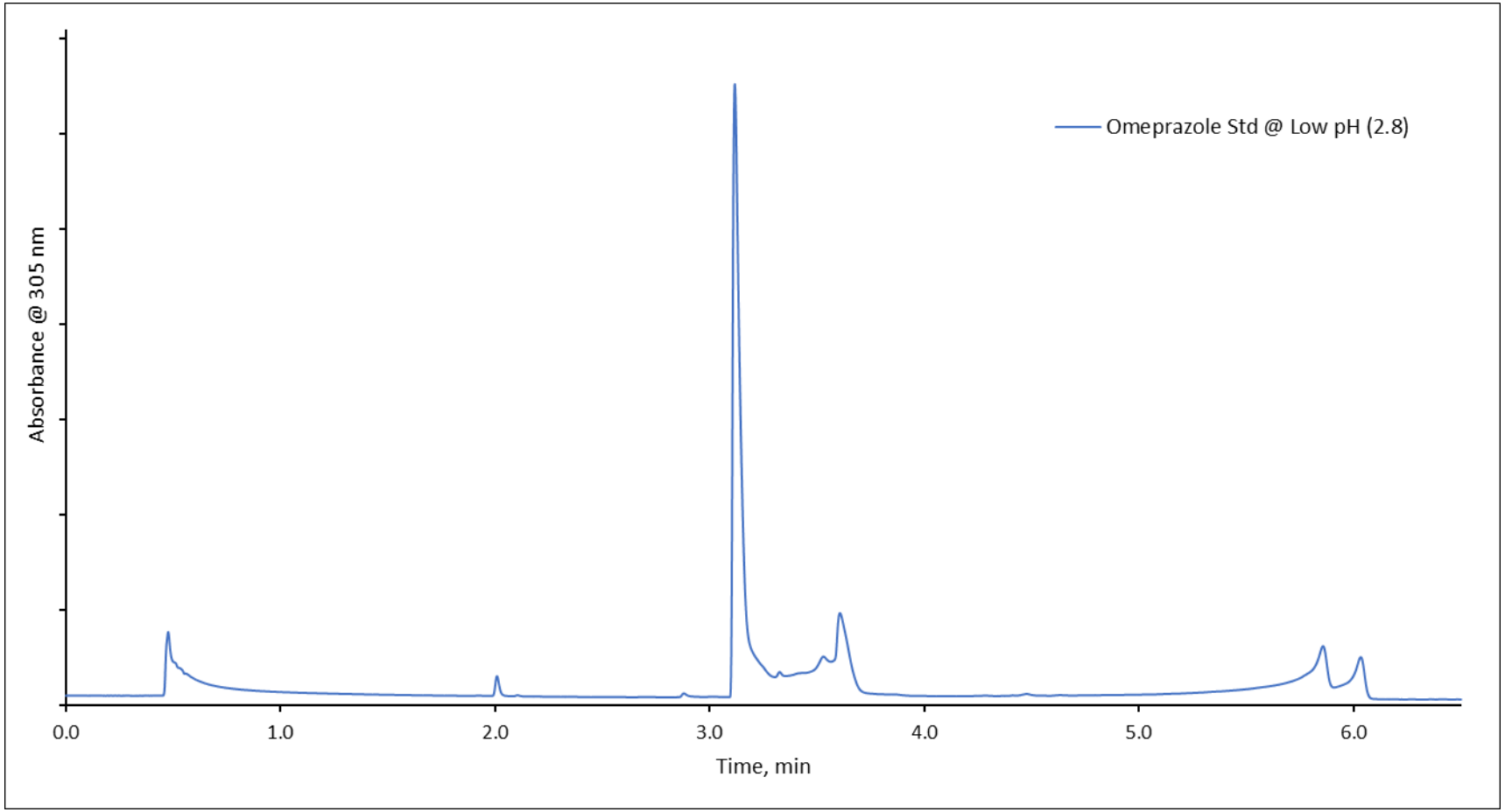
HALO®

Testing Conditions:

Column: HALO 120 Å Elevate, 2.7 µm, 2.1 x 100 mm
Mobile Phase: A: 0.1% Formic (2.8)
B: ACN
Gradient:

Time	%B
0.0	5
6.0	55
7.0	55
7.2	5
12.0	5

Flow Rate: 0.4mL/min
Back Pressure: 174 bar
Temperature: 60 °C
Injection: 1.0 µL
Sample Solvent: USP Diluent
Wavelength: PDA, 305 nm
Flow Cell: 1 µL
Data Rate: 40 Hz
Response Time: 0.05 sec.
LC System: Shimadzu Nexera X2



pH Comparison for Omeprazole & Related Compounds

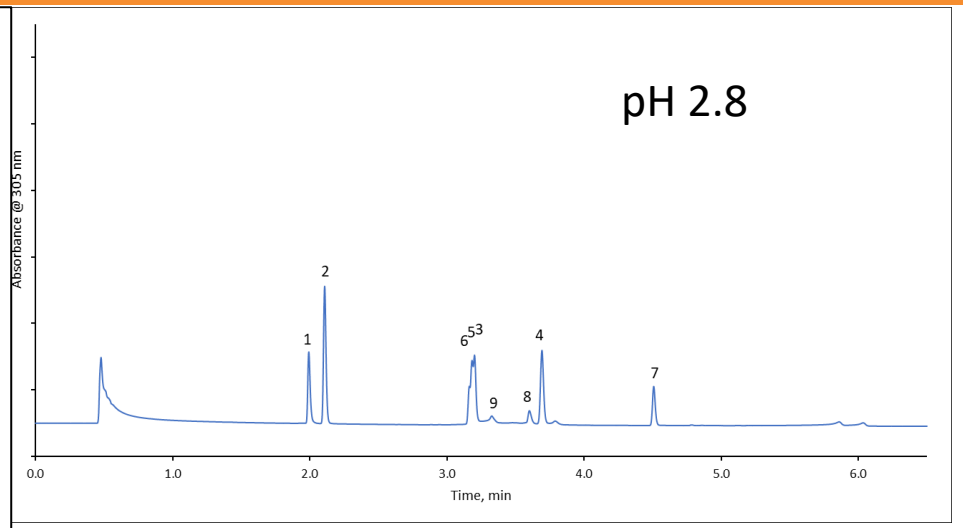
Testing Conditions:

Column: HALO 120 Å Elevate, 2.7 µm, 2.1 x 100 mm
Mobile Phase: A: 0.1% Formic (2.8)
20mM Potassium Phosphate (7.1)
0.03% Ammonium Hydroxide (10.65)
B: ACN

Gradient:

Time	%B
0.0	5
6.0	55
7.0	55
7.2	5
12.0	5

Flow Rate: 0.4mL/min
Back Pressure: 174 bar
Temperature: 60 °C
Injection: 1.0 µL
Sample Solvent: USP Diluent
Wavelength: PDA, 305 nm
Flow Cell: 1 µL
Data Rate: 40 Hz
Response Time: 0.05 sec.
LC System: Shimadzu Nexera X2



PEAK IDENTITIES:

1. Related Compounds 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
9. Impurity C

pH Comparison for Omeprazole & Related Compounds

HALO®

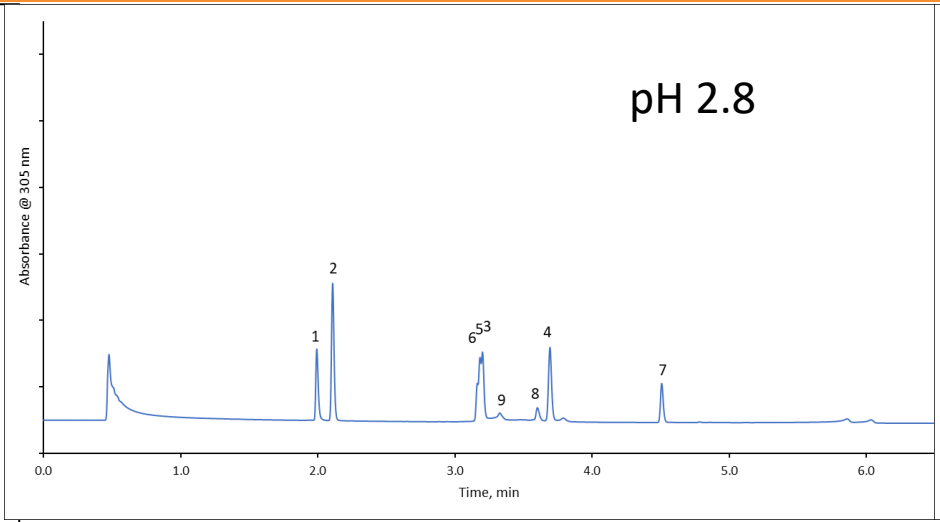
Testing Conditions:

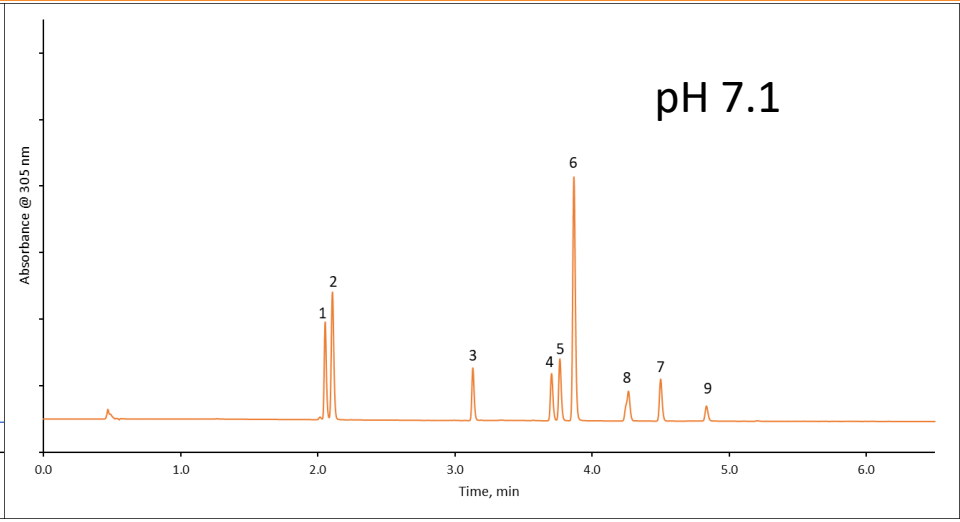
Column: HALO 120 Å Elevate, 2.7 µm, 2.1 x 100 mm
Mobile Phase: A: 0.1% Formic (2.8)
20mM Potassium Phosphate (7.1)
0.03% Ammonium Hydroxide (10.65)
B: ACN

Gradient:

Time	%B
0.0	5
6.0	55
7.0	55
7.2	5
12.0	5

Flow Rate: 0.4mL/min
Back Pressure: 174 bar
Temperature: 60 °C
Injection: 1.0 µL
Sample Solvent: USP Diluent
Wavelength: PDA, 305 nm
Flow Cell: 1 µL
Data Rate: 40 Hz
Response Time: 0.05 sec.
LC System: Shimadzu Nexera X2





PEAK IDENTITIES:

1. Related Compounds 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
9. Impurity C

pH Comparison for Omeprazole & Related Compounds

HALO®

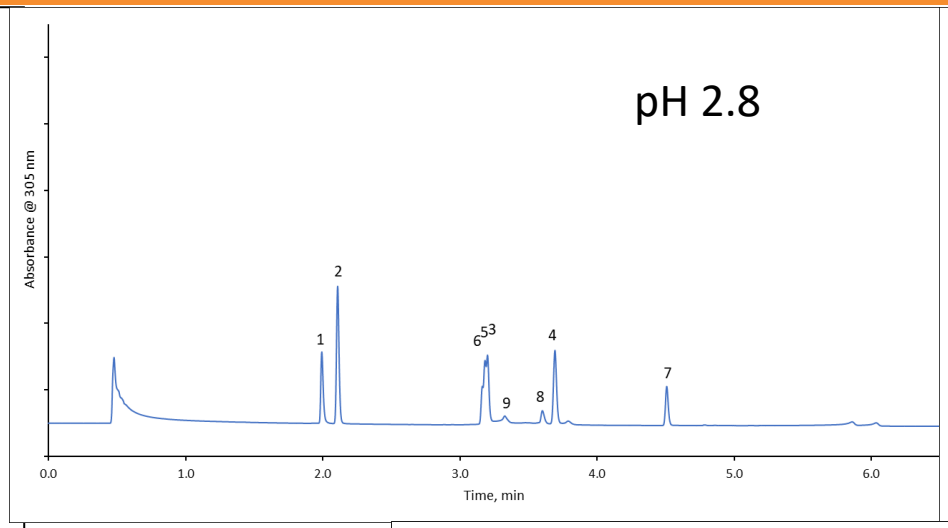
Testing Conditions:

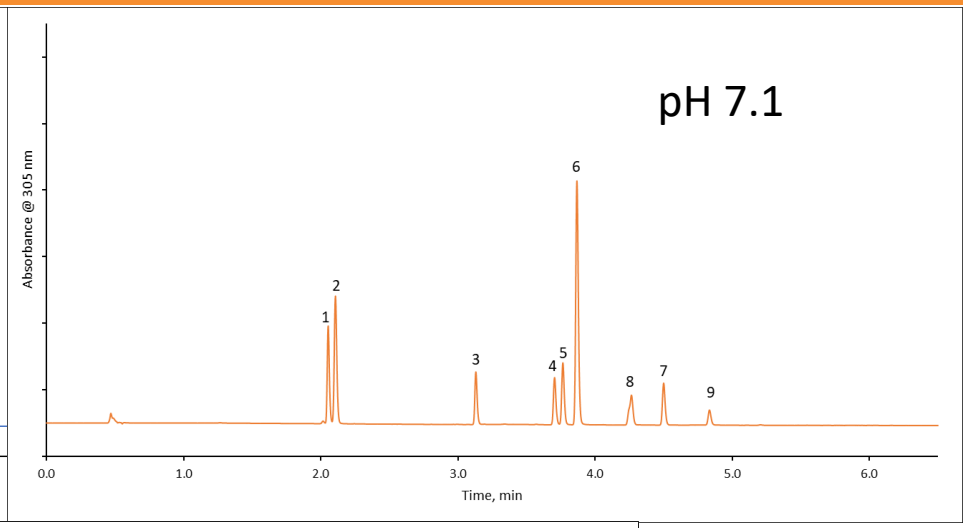
Column: HALO 120 Å Elevate, 2.7 µm, 2.1 x 100 mm
Mobile Phase: A: 0.1% Formic (2.8)
20mM Potassium Phosphate (7.1)
0.03% Ammonium Hydroxide (10.65)
B: ACN

Gradient:

Time	%B
0.0	5
6.0	55
7.0	55
7.2	5
12.0	5

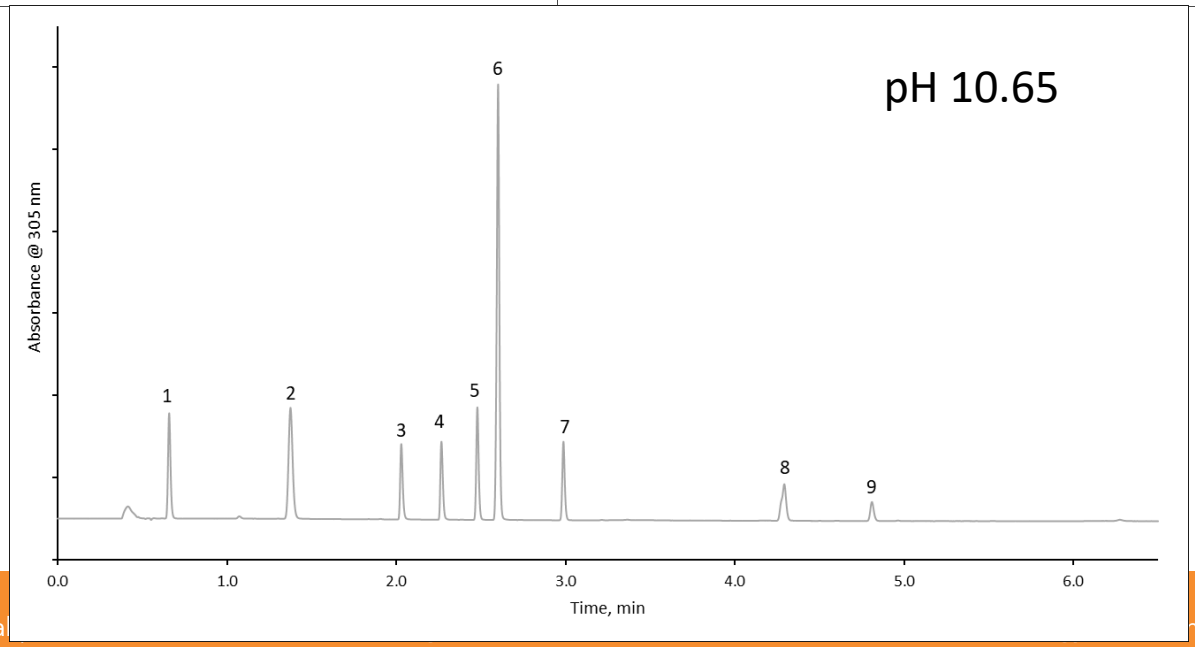
Flow Rate: 0.4mL/min
Back Pressure: 174 bar
Temperature: 60 °C
Injection: 1.0 µL
Sample Solvent: USP Diluent
Wavelength: PDA, 305 nm
Flow Cell: 1 µL
Data Rate: 40 Hz
Response Time: 0.05 sec.
LC System: Shimadzu Nexera X2





PEAK IDENTITIES:

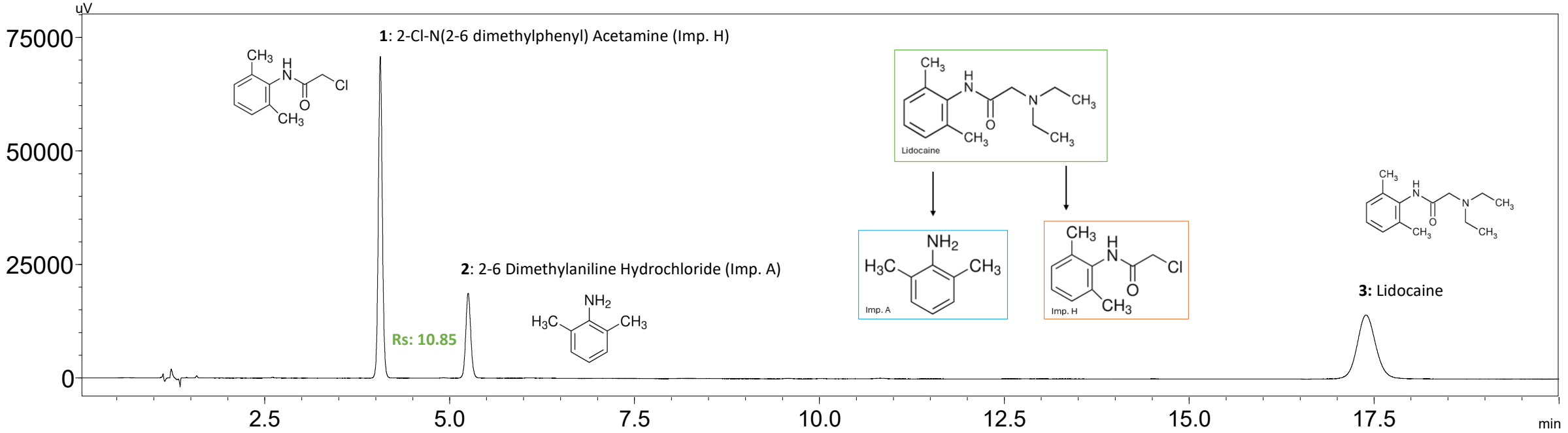
1. Related Compounds F & G
2. Related Compound B
3. Related Compound E
4. Related Compound A
5. Impurity B
6. Omeprazole
7. Impurity H
8. N'-Methyl Omeprazole
9. Impurity C



Separation of Lidocaine and Related Impurities Based on USP Monograph

Testing Conditions:

USP L1 Column: HALO 120Å ELV C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92274-702
Mobile Phase: A: Potassium Phosphate Buffer, pH 8
B: Acetonitrile
Isocratic: 30% B
Injection Volume: 20 µL
Detection (UV): 230 nm
Temperature: 30 °C
Flow Rate: 1.0 mL/min
Instrument: Shimadzu Nexera X2



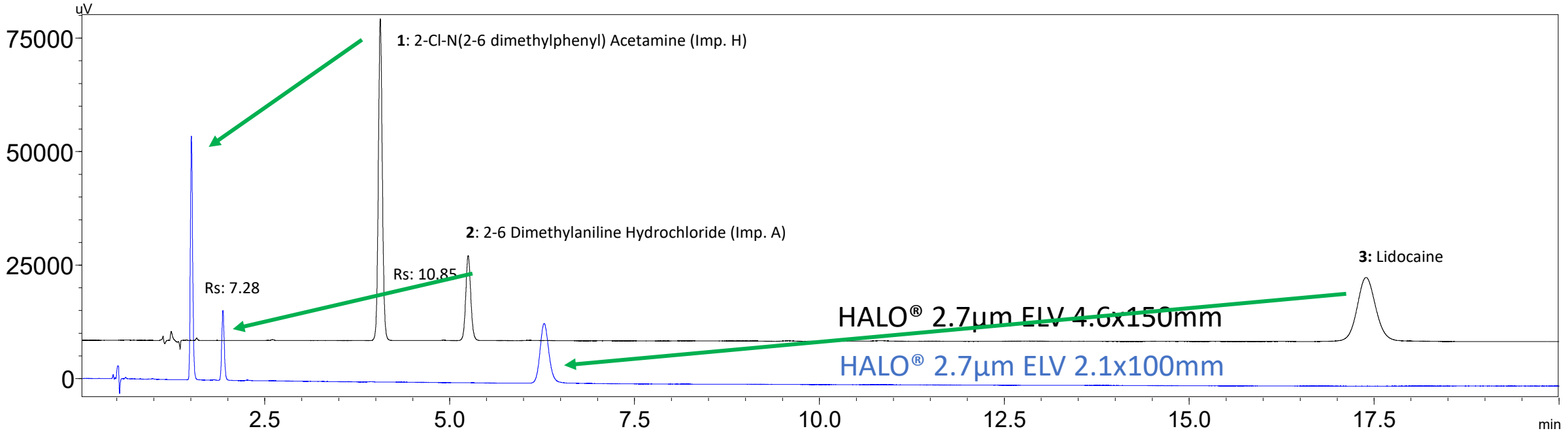
Separation of Lidocaine and Related Impurities Based on USP: Speeding up the Method

HALO®

Testing Conditions:

USP L1 Column: HALO 120Å ELV C18, 2.7µm
Mobile Phase: A: Potassium Phosphate Buffer, pH 8
B: Acetonitrile
Isocratic: 30% B
Injection Volume: 20 µL/ 3.4 µL
Detection (UV): 230 nm
Temperature: 30 °C
Flow Rate: 1.0/ 0.4 mL/min
Instrument: Shimadzu Nexera X2

- Half the Runtime
- ~150% Solvent Savings

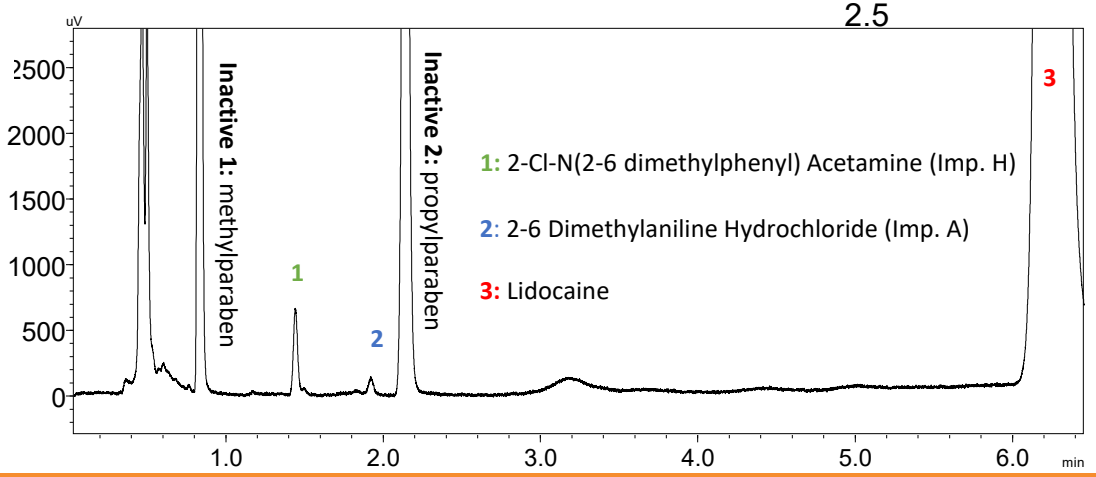


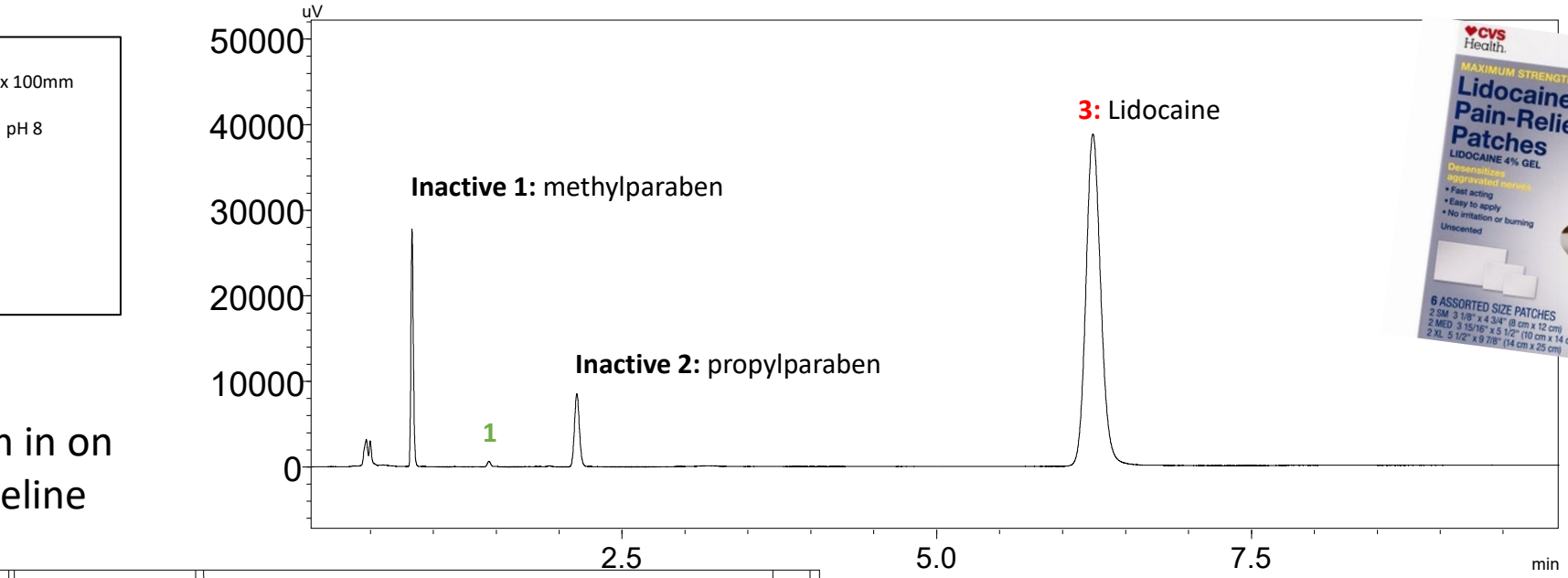
Lidocaine Patch Analysis: 4%

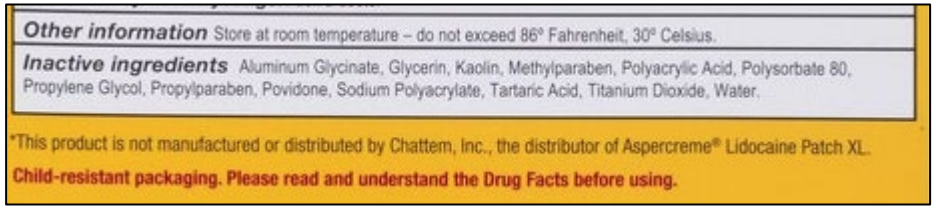
Testing Conditions:

USP L1 Column: HALO 120Å ELV C18, 2.7µm, 2.1x 100mm
Part Number: 92272-602
Mobile Phase: A: Potassium Phosphate Buffer, pH 8
B: Acetonitrile
Isocratic: 30% B
Injection Volume: 0.1 µL
Detection (UV): 230 nm
Temperature: 30 °C
Flow Rate: 0.4 mL/min
Instrument: Shimadzu Nexera X2

Zoom in on baseline







LC-MS of 18 Drugs and Metabolites

HALO®

Opioids

- Codeine
- Morphine
- Hydrocodone
- Hydromorphone
- Oxycodone
- Oxymorphone
- 6-Acetylmorphine
- Fentanyl
- Norfentanyl

Amphetamines

- Methamphetamine
- d-Amphetamine
- MDMA
- MDA

Others

- Benzoylecgonine (cocaine metabolite)
- THC-COOH (THC metabolite)
- Phencyclidine (PCP)
- Xylazine
- 4-Hydroxy Xylazine

HALO Elevate C18: pH Screening

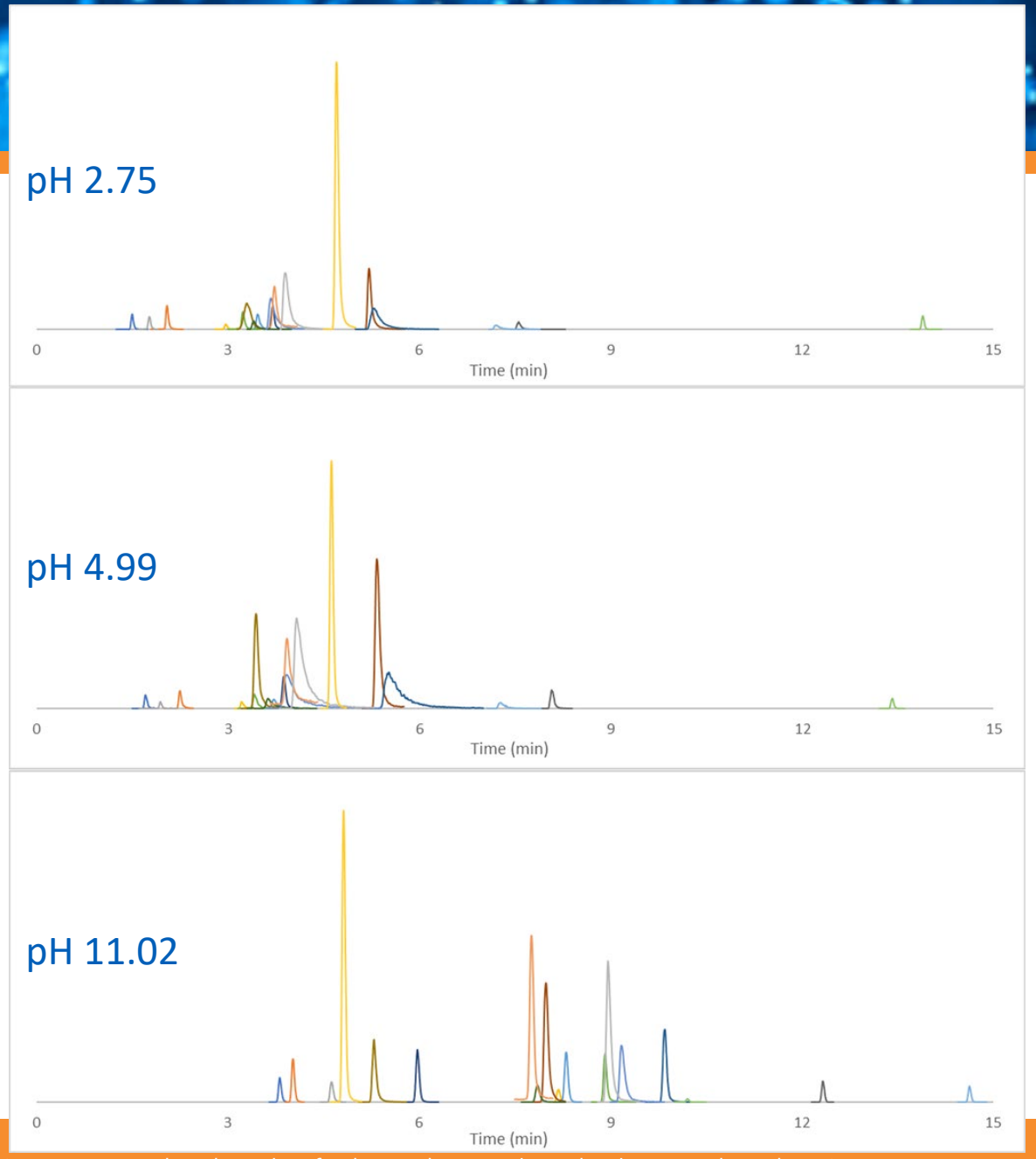
- Low retention, poor selectivity for most analytes at low pH
- Increased retention, improved selectivity & peak tailing at high pH

LC Conditions:

Mobile Phase A1: H_2O + 0.1% Formic Acid (pH 2.75)
A2: H_2O + 10 mM Ammonium Acetate (pH 4.99)
A3: H_2O + 0.1% Ammonium Hydroxide (pH 11.02)
Mobile Phase B: MeOH
MS Acquisition Mode: MRM
Injection Volume: 1 µL
Sample Conc.: 2.5 µg/mL

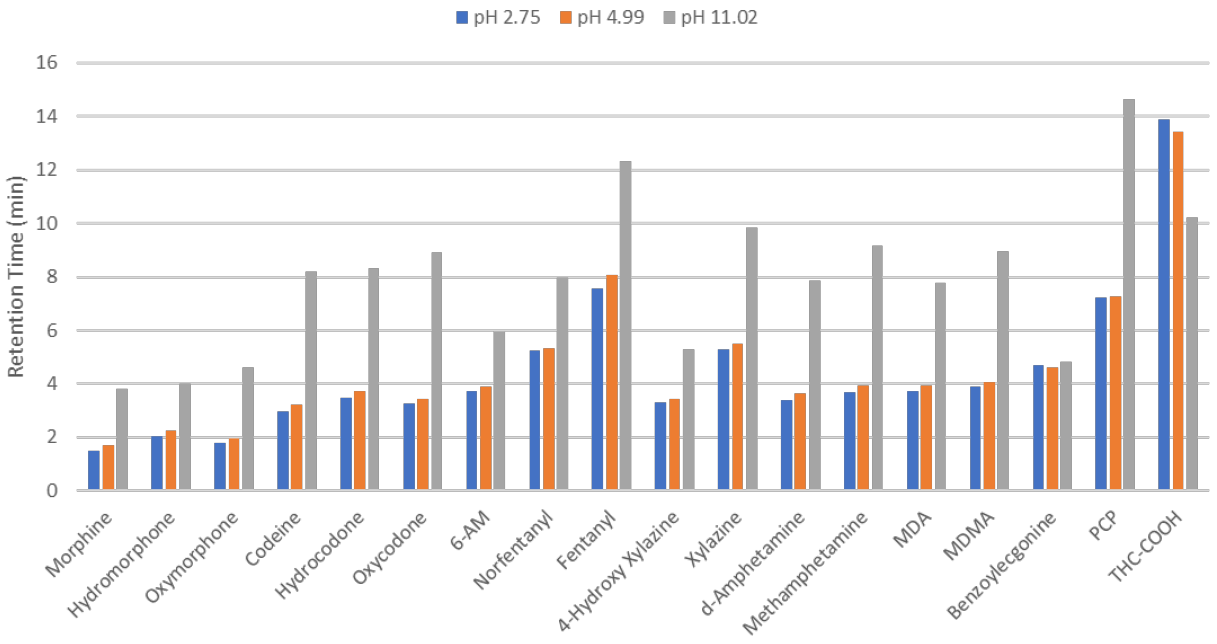
Gradient:

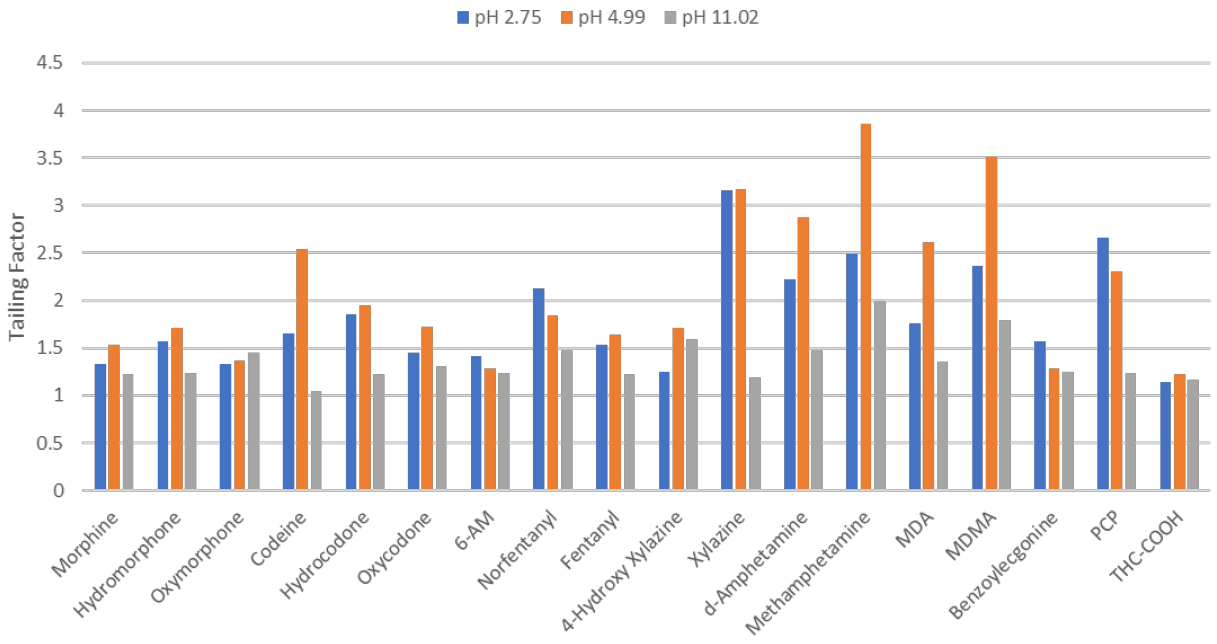
Time	% B
0	5
15	95
16	95
16.1	5
21	5



Retention and Tailing Factor Improvements

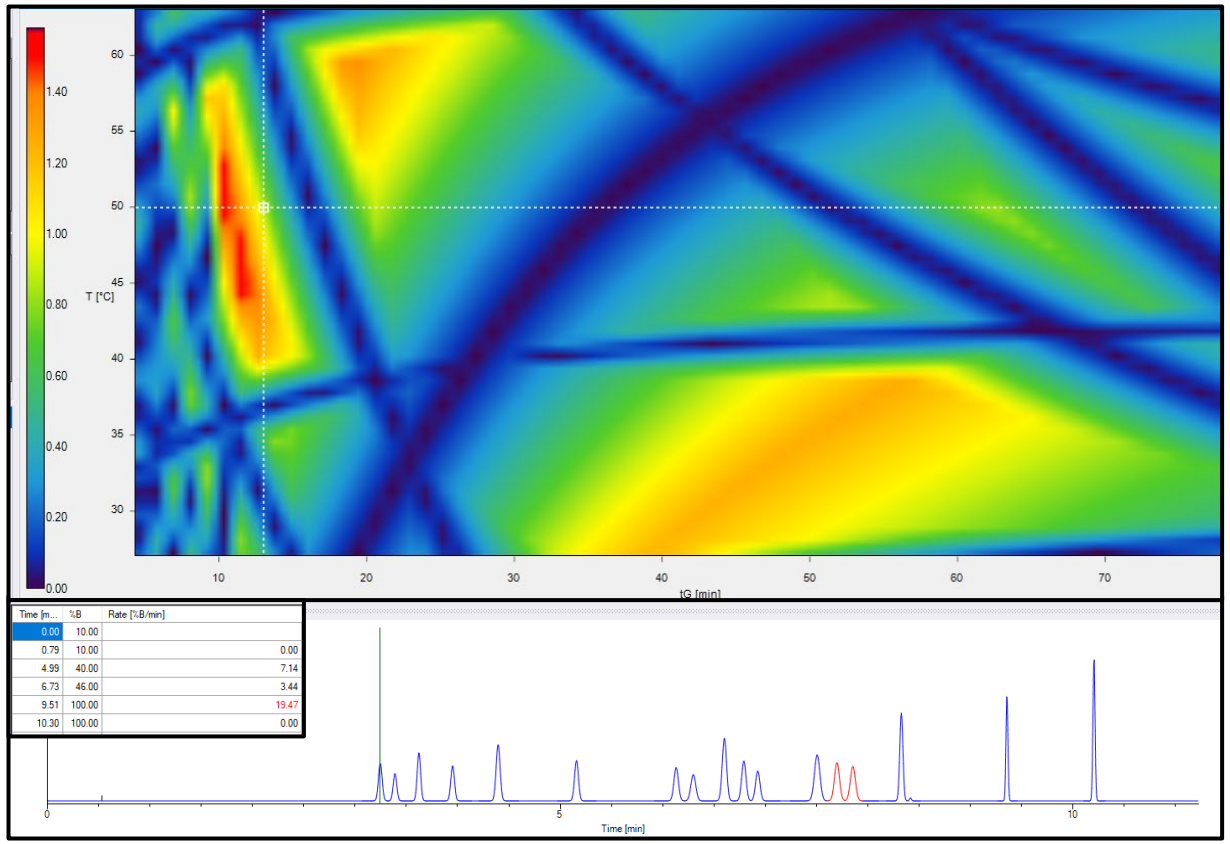
- Retention increases with pH for 16 of 18 compounds
- Tailing factor improves at high vs. low pH for 16 of 18 compounds

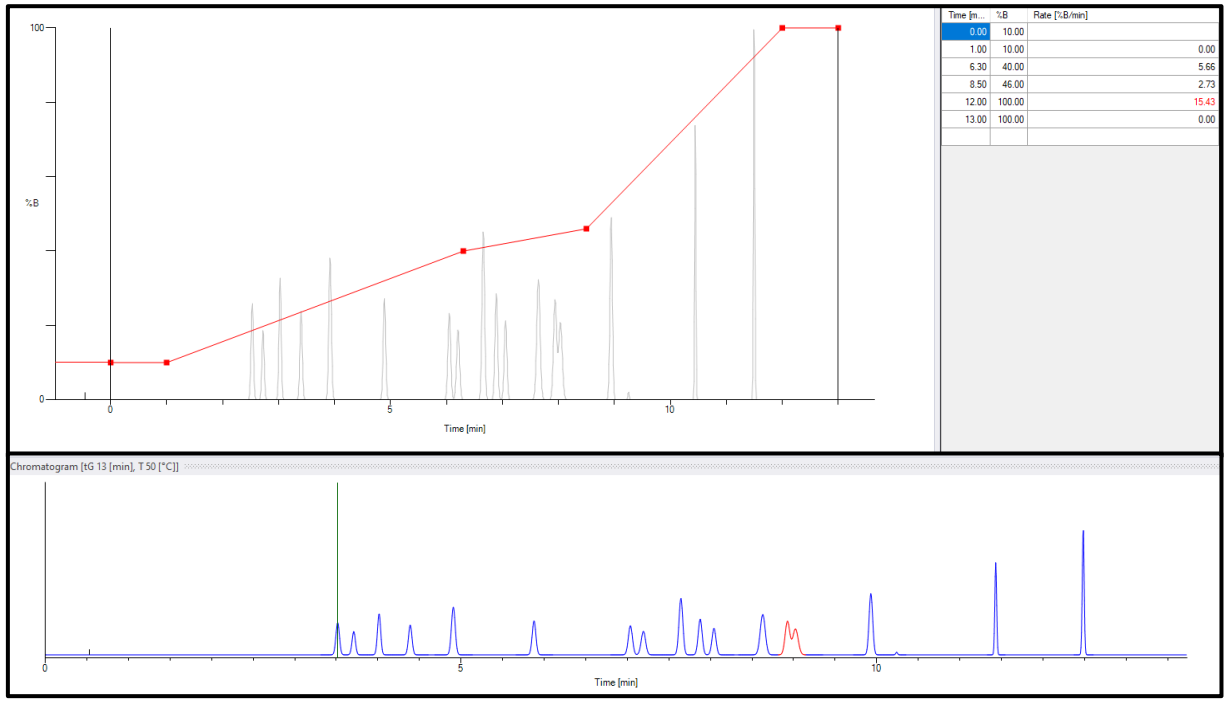




DryLab® Optimization

- 2D: Gradient Time (15 min, 45 min) and Temperature (30 °C, 60 °C)
- 13 min at 50 °C resolved critical pairs





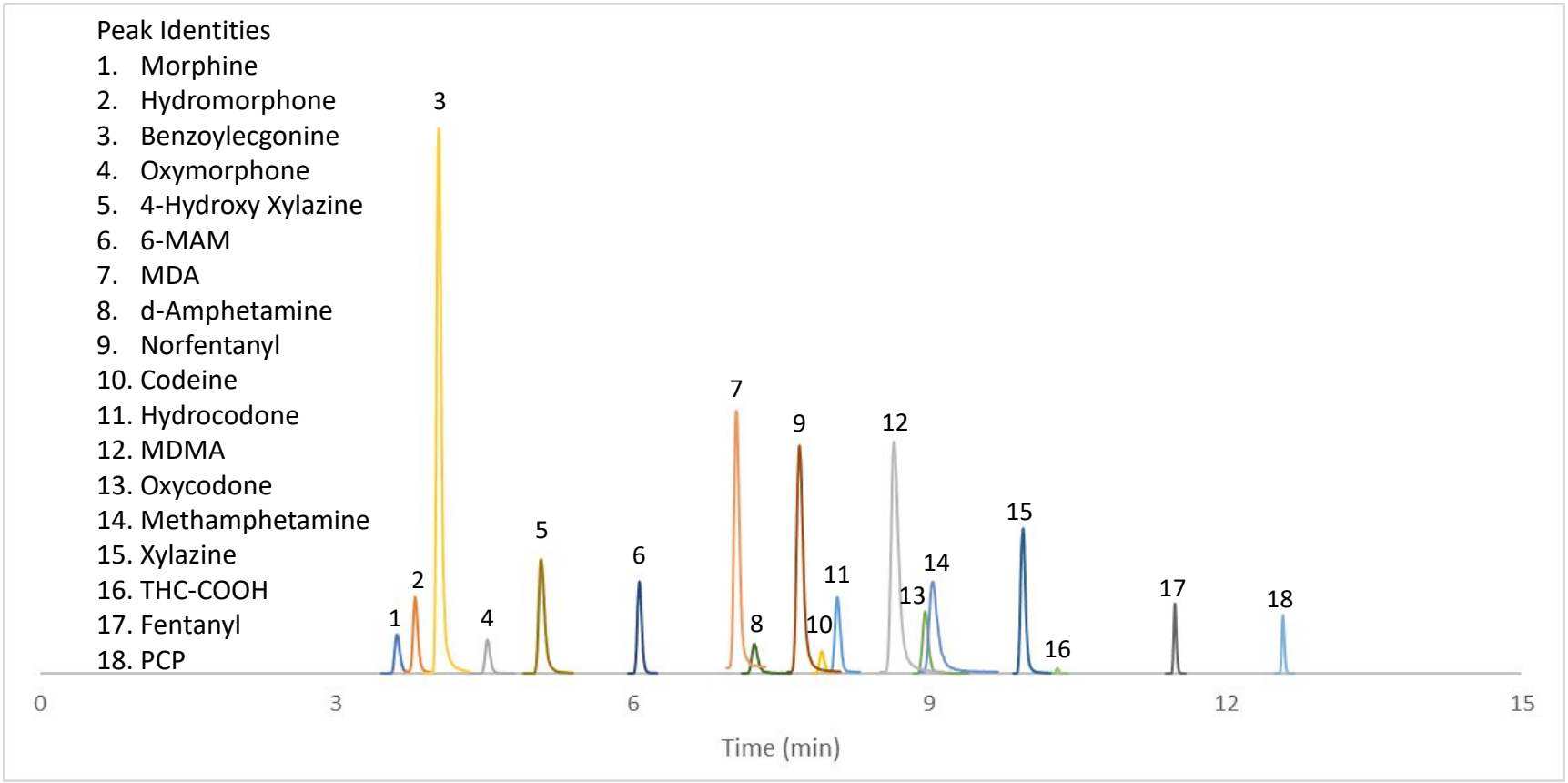
Elevate C18: Optimized Separation of Drugs of Abuse and Metabolites

- Baseline resolution (>1.5) for 16 of the 18 compounds

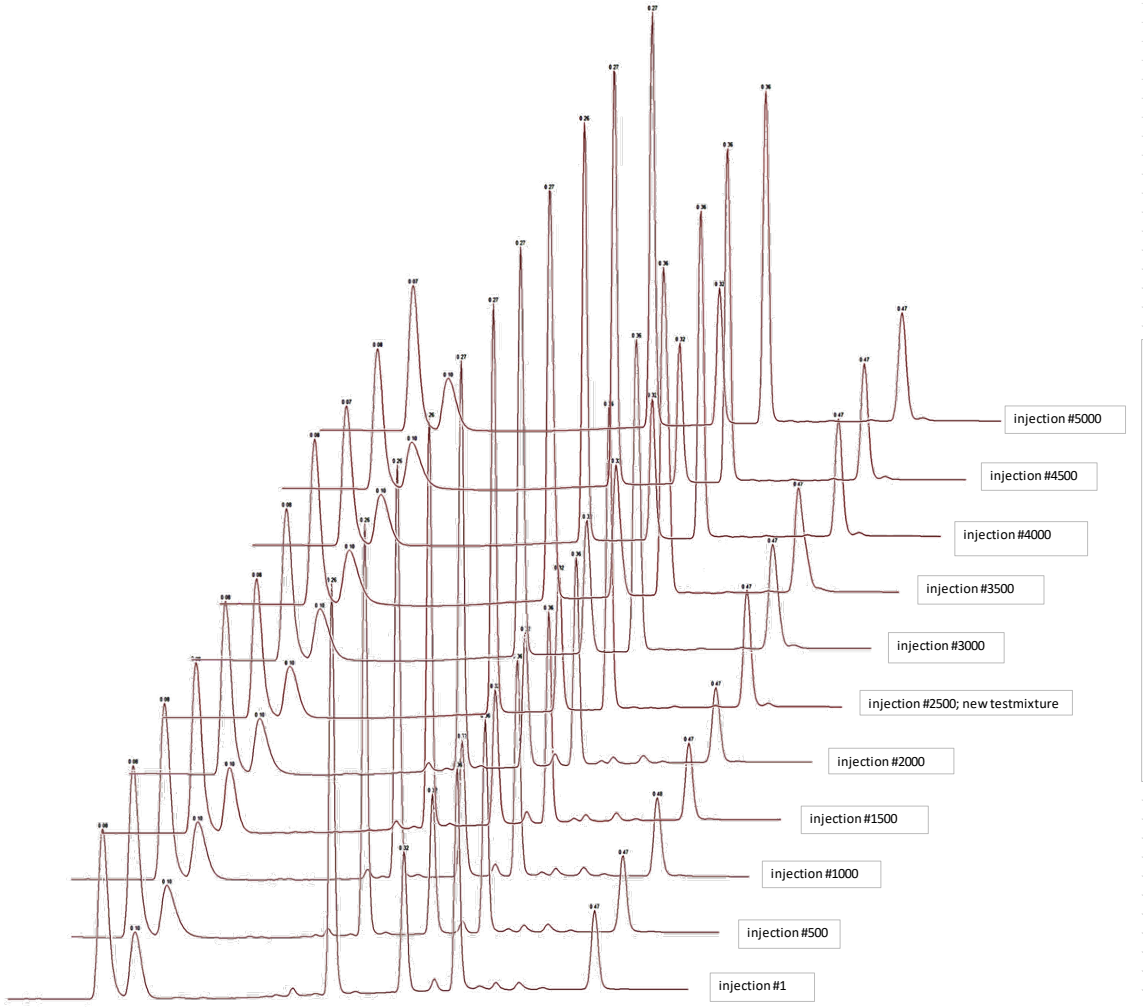
LC Conditions:
Mobile Phase A: H_2O + 0.1% **NH_4OH (pH 11.02)**
Mobile Phase B: MeOH
MS Acquisition Mode: MRM
Injection Volume: 5 µL
Sample Conc.: 2.5 µg/mL

Gradient:

Time	% B
0	10
1	10
6.3	40
8.5	46
12	100
13	100
13.1	10
19	10



HALO Elevate C18: 1.5 x 50 mm Stability



Conditions:
Column: HALO 120 Å, ELV C18, 2.7 µm, 1.5 x 50 mm
Part No. 9227X-402
Flow: 1 mL/min
Temperature: 60°C
pH: 11
Injection volume: 1µL
max. pressure: ~80000 psi (~550 bar)
Solvent A: Water + 0.1 % NH4OH
Solvent B: Acetonitrile

Gradient:

min	% A	% B
0.00	80	20
0.25	5	95
0.45	0	100
0.55	0	100

Instrument: Waters Acquity
Test mixture: new test mixture at 2500 injections; better baseline; fewer degradation products between the main peaks
Elution order: 4-Hydroxyisophthalic acid, Benzamide, Flavone, Doxepin, Triphenylene, Amiodarone

Boehringer Ingelheim

Summary

- HALO® Elevate C18 is a high pH stable column for HPLC, UHPLC, and LC-MS separations that is very reproducible
- 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

Acknowledgements

- Advanced Materials Technology, Inc.
  - Peter Pellegrinelli
  - Conner McHale
  - John Boughton
  - William Miles
  - Barry Boyes
  - Stephanie Rosenberg

Questions

support@advanced-materials-tech.com

Photo by Alexander Grey on Unsplash

advancedmaterialstechnology

halocolumns.com

3521 Silverside Road, Suite 1-K
Quillen Building
Wilmington, DE 19810

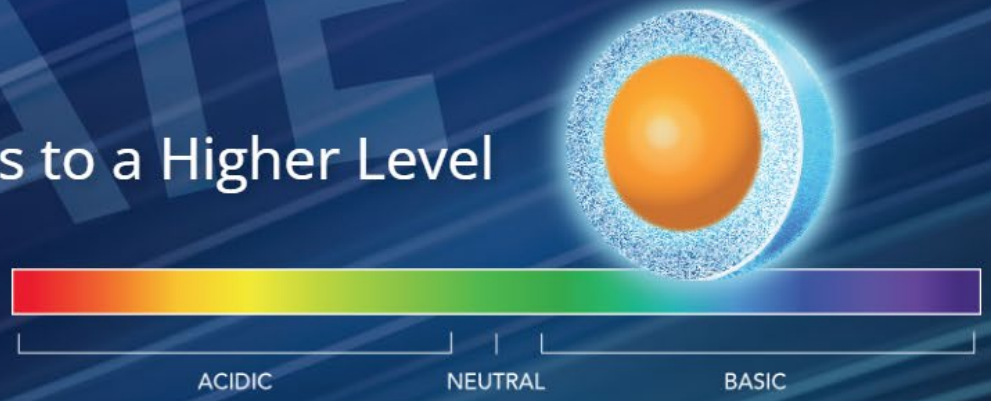
(302) 992-8060

NEW

HALO®

ELEVATE C18

Taking Separations to a Higher Level



Delivering More Performance

- ✓ Excellent Stability for **High pH Environments**
- ✓ Reliability of Proven Fused-Core® Technology for **Highest Efficiencies**
- ✓ Enables Wide Operational Use Range for **Robust Method Development**

Ligand: dimethyloctadecylsilane
Chemical Classification: alkyl
USP: L1
Pore Size: 120 Å
Low pH/T Limit: 2/60 °C
High pH/T Limit: 12/60 °C
Endcapped: Yes