LC-MS Screening of Nitrosamines using Superficially Porous Particle Columns

Stephanie Schuster, Joshua McBee, Conner McHale, Peter Pellegrinelli

Advanced Materials Technology, Inc.

advancedmaterialstechnology

EAS

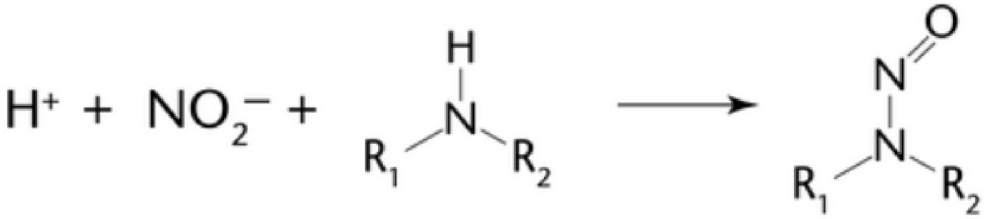
November 17, 2025

Outline

- Review of Nitrosamines & Superficially Porous Particle Columns
- Column Stationary Phase Screening Experiments
- DryLab® Optimization
- 1.5 mm ID Advantage
- Summary
- Future Work

Nitrosamines

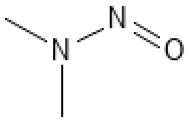
- Nitrosamine formation



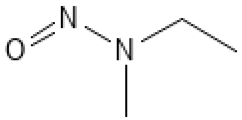
- Why are we concerned about nitrosamines?
  - Known carcinogens
  - Have been found in pharmaceuticals, food, water, and consumer products
  - Have environmental persistence
  - Levels are strictly regulated and must be tested for

Nitrosamines Included in this Study

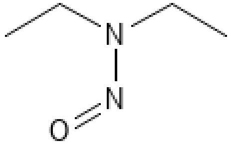
Standard from LGC: GB/T 24153-2009 Nitrosamines Mixture 137 100 µg/mL in Methanol
Part Number: DRE-A50000137ME
Number of nitrosamines: 12



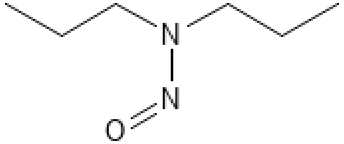
N-nitrosodimethylamine (NDMA)



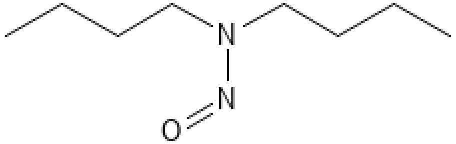
N-nitrosomethylethylamine (NMEA)



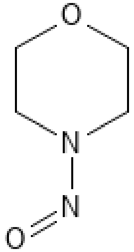
N-nitrosodiethylamine (NDEA)



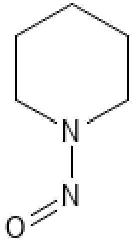
N-nitroso-n-propylamine (NDPA)



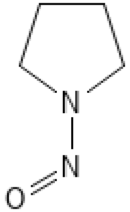
N-nitrosodi-n-butylamine (NDBA)



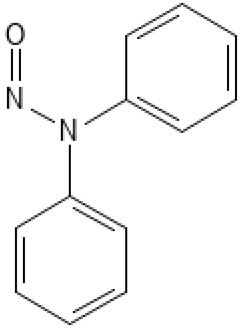
N-nitrosomorpholine (NMOR)



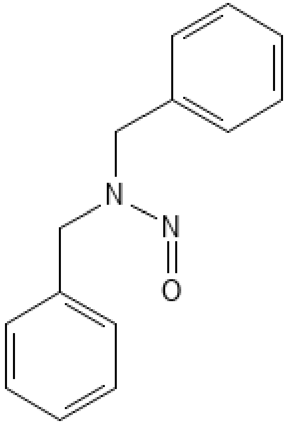
N-nitrosopiperidine (NPIP)



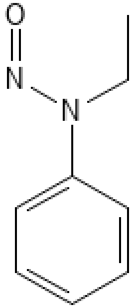
N-nitrosopyrrolidine (NPYR)



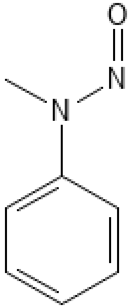
N-nitrosodiphenylamine (NDPhA)



N-nitrosodibenzylamine (NDBzA)



N-nitroso-N-ethylaniline

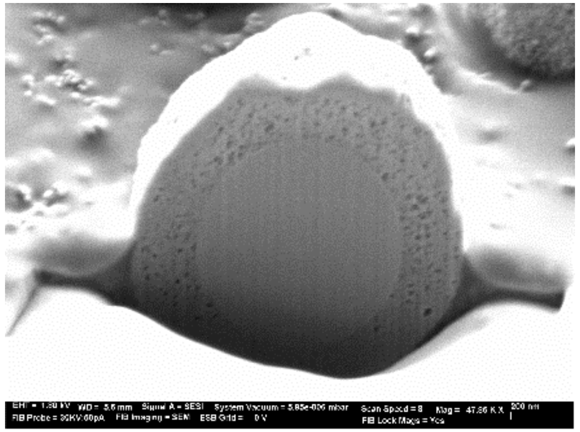


N-nitroso-N-methylaniline

HALO® Fused-Core® Particle HPLC Columns

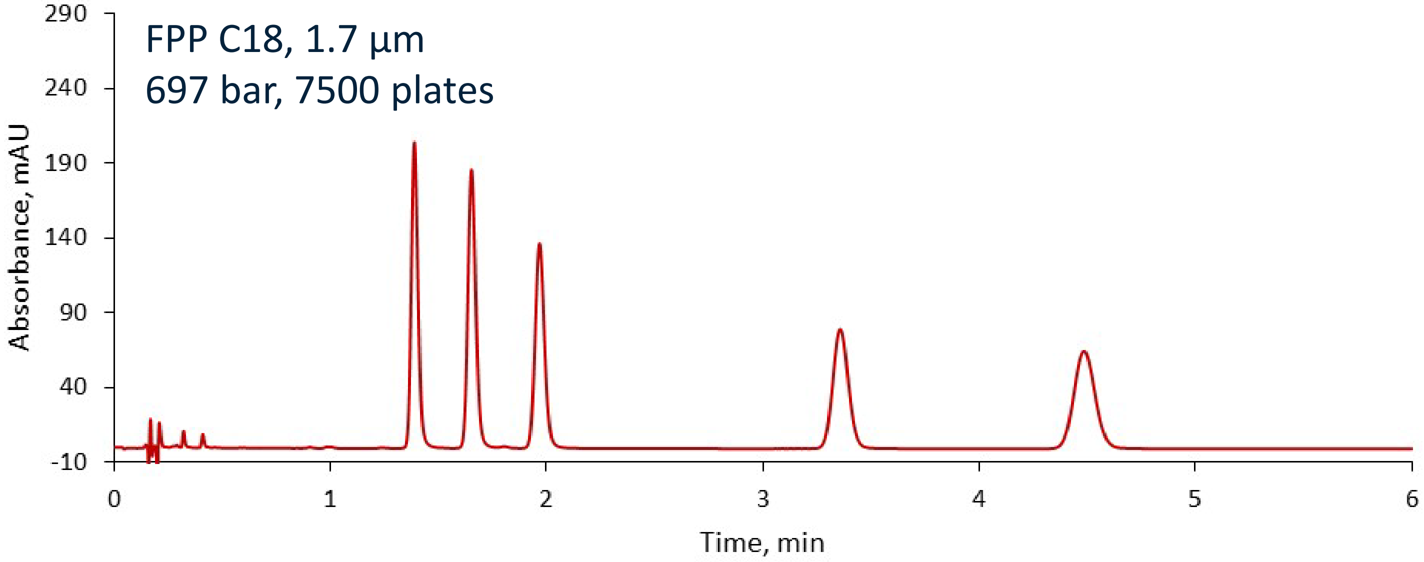
- Manufactured by Advanced Materials Technology (AMT) in Wilmington, DE
- Fused-Core® or superficially porous particles consist of a solid silica core surrounded by a porous silica shell

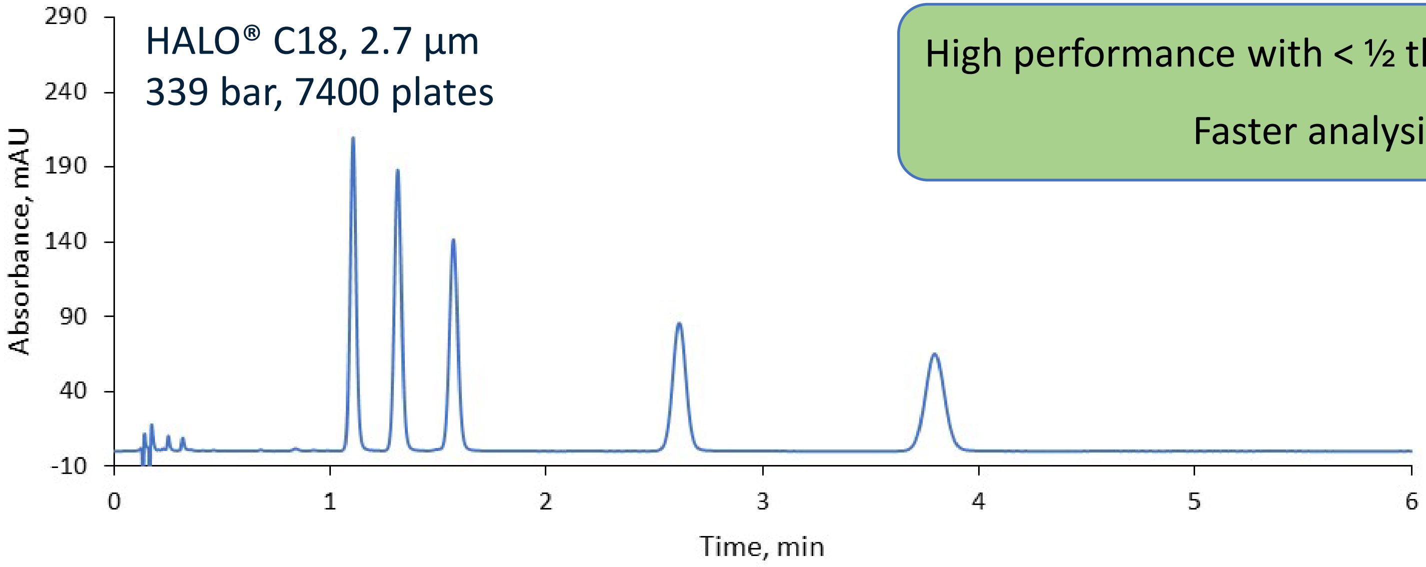
HALO 90 Å, 2.7 µm



- HALO columns offer the advantages of high efficiency and fast separations without high back pressures from sub-2-µm particle size columns

Power of HALO® Fused-Core® Particles





High performance with < ½ the back pressure

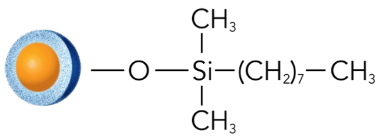
Faster analysis

Selected Stationary Phases

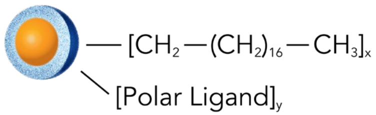
HALO®

Alkyl Phases

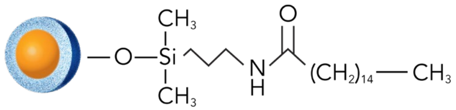
C18



AQ-C18

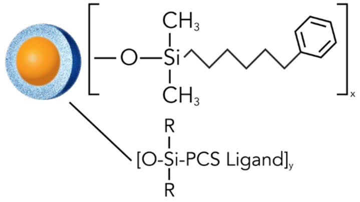


RP-AMIDE

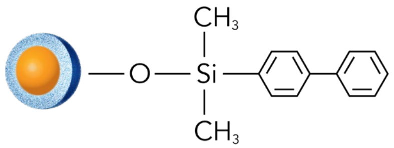


Phenyl Phases

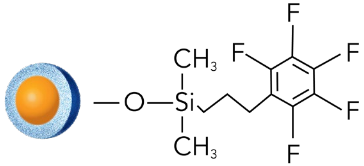
PCS PHENYL-HEXYL



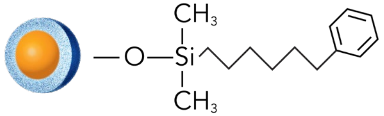
BIPHENYL



PFP



PHENYL-HEXYL



All of the phases listed here are 100% aqueous compatible except C18

Mobile Phase Considerations

SOLVENT	ADVANTAGES	DISADVANTAGES
Acetonitrile	Low viscosity → high efficiency UV transparency Good miscibility with water Stable over wide pH range	More expensive than methanol Toxic and flammable Supply shortages can occur
Methanol	Inexpensive and widely available Good UV transparency Miscible with water Less toxic than acetonitrile	Higher viscosity → lower efficiency Weaker elution strength → may require gradient adjustments Can cause high backpressure
THF	Strong elution power for nonpolar compounds Miscible with water and organic solvents Useful for polymer analysis	Peroxide formation risk → requires stabilizers Toxic and volatile Not ideal for reversed-phase HPLC due to UV cutoff

Screening Method

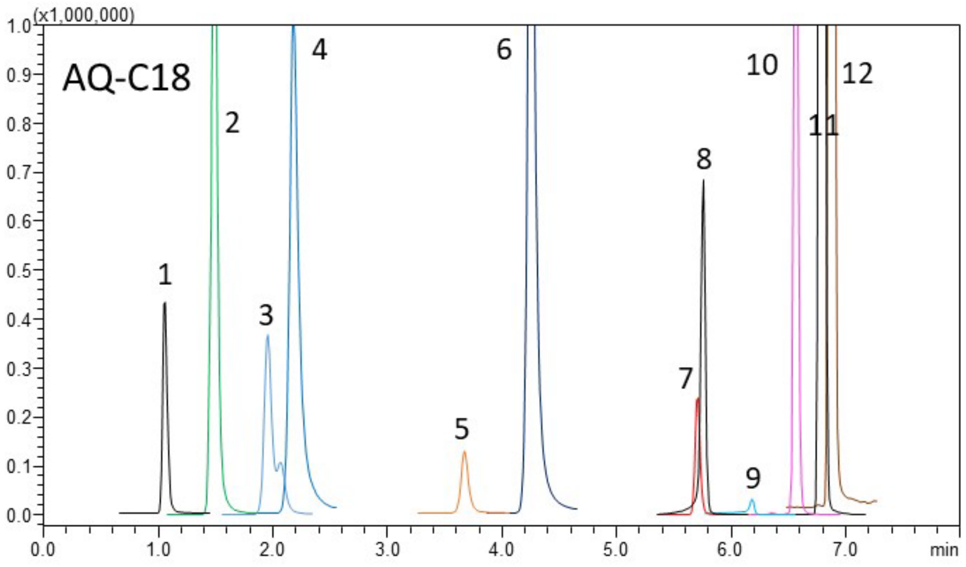
Mobile Phase A: Water/0.1% Formic Acid
Mobile Phase B: Methanol/0.1% Formic Acid

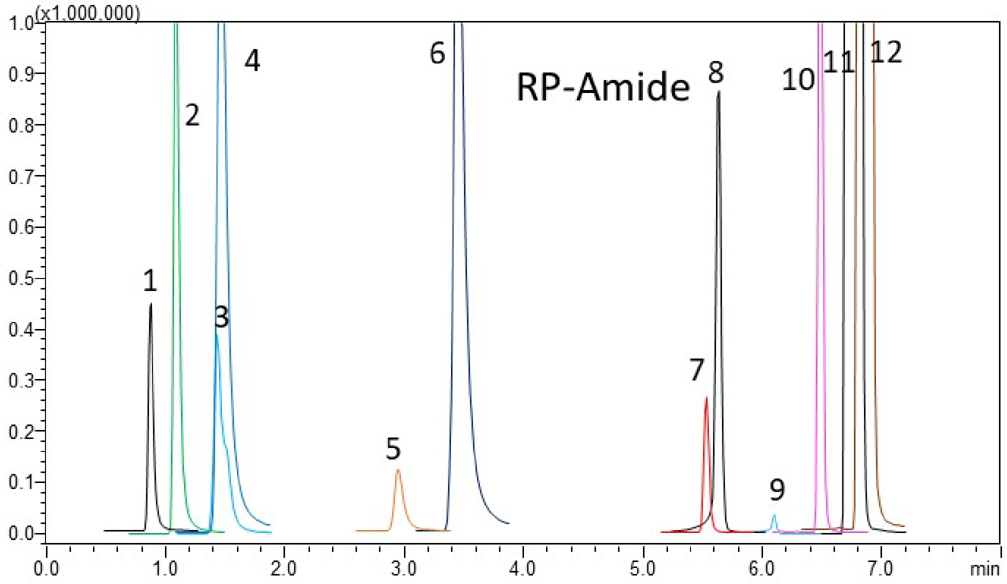
Gradient:

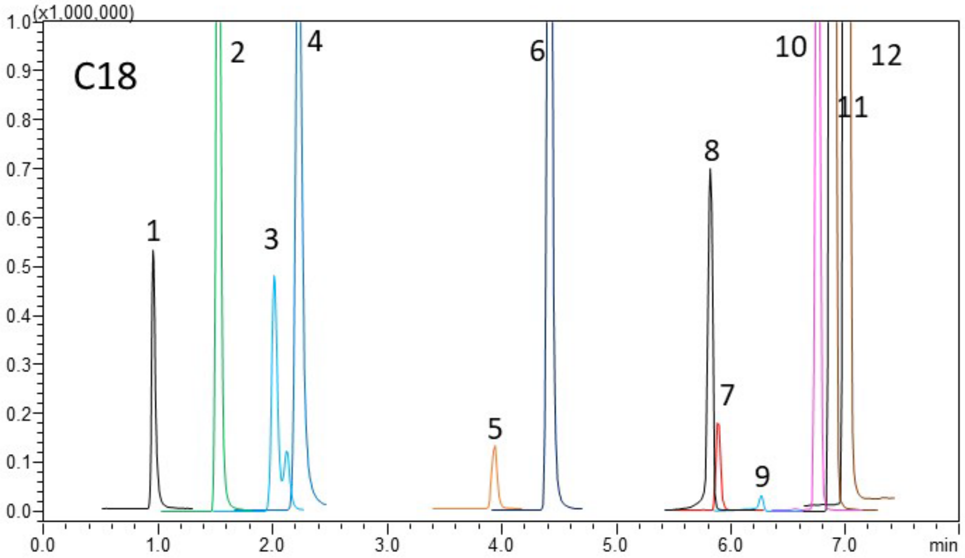
Time	%B
0.0	5
1.0	5
3.0	20
7.0	100
9.0	100

Instrument: Shimadzu Nexera/Triple Quad 8060NX
Injection: 1 μL
Temperature: 30 ^{0}C
Flow Rate: 0.4 mL/min.
Column: HALO 90 Å, 2.7 μm, 2.1 x 100 mm
Nebulizing Gas: 3 L/min.
Heating Gas: 15 L/min.
Interface Temperature: 400 °C
DL Temperature: 250 °C
Heat Block Temperature: 400 °C
Drying Gas Flow: 3 L/min.

Screening Results: AQ-C18 vs. C18 vs. RP-Amide



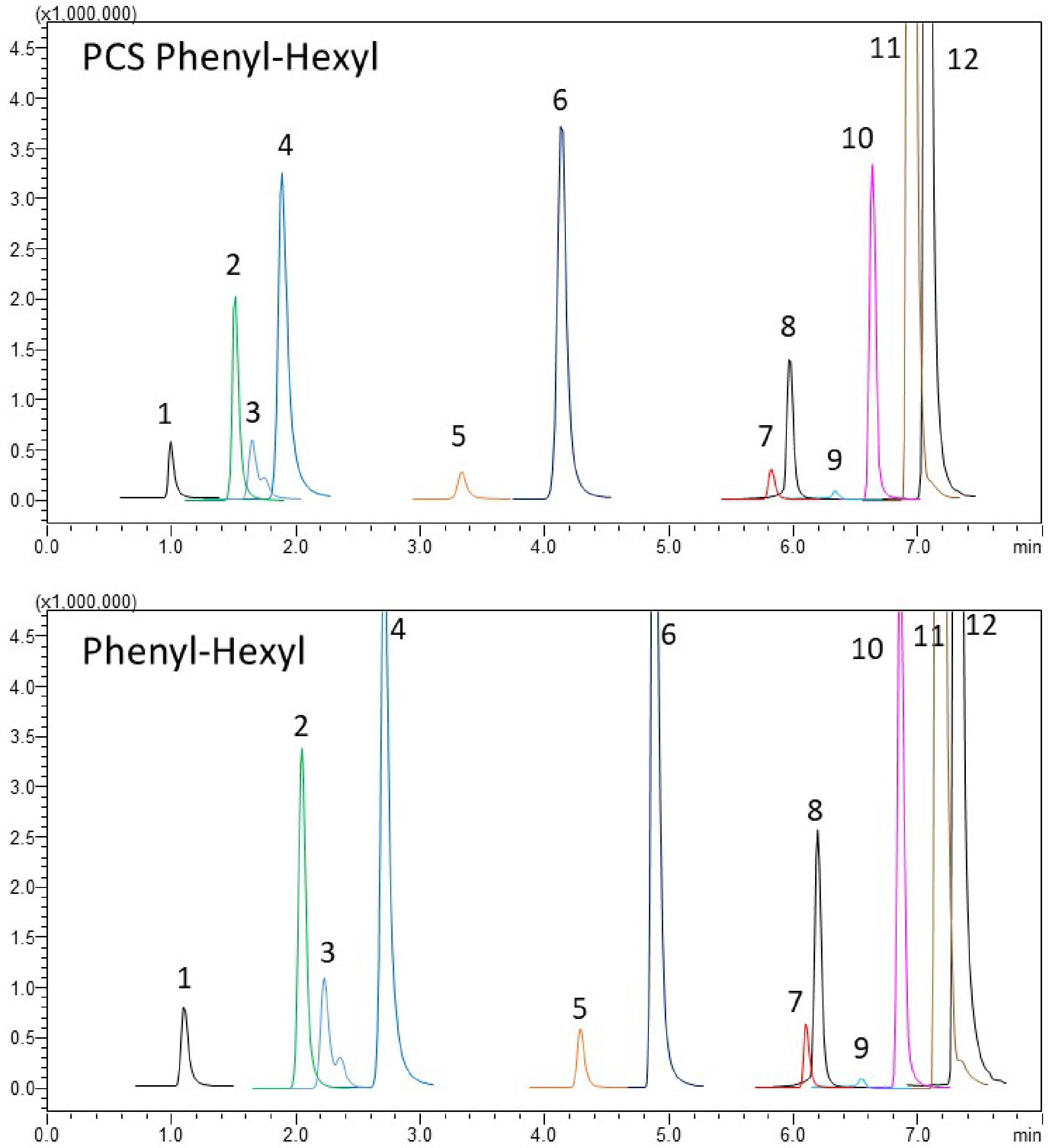




1. N-nitrosodimethylamine
2. N-nitrosomorpholine
3. N-nitrosomethylethylamine
4. N-nitrosopyrrolidine
5. N-nitrosodiethylamine
6. N-nitrosopiperidine
7. N-nitroso-n-propylamine
8. N-nitroso-N-methylaniline
9. N-nitroso-N-ethylaniline
10. N-nitrosodi-n-butylamine
11. N-nitrosodiphenylamine
12. N-nitrosodibenzylamine

- Peak elution order is slightly different between AQ-C18 and C18 (peaks 7 & 8)
- Peaks 3 & 4 are coeluted on RP-Amide
- C18 shows slightly more retention and narrower peak widths over AQ-C18 and RP-Amide

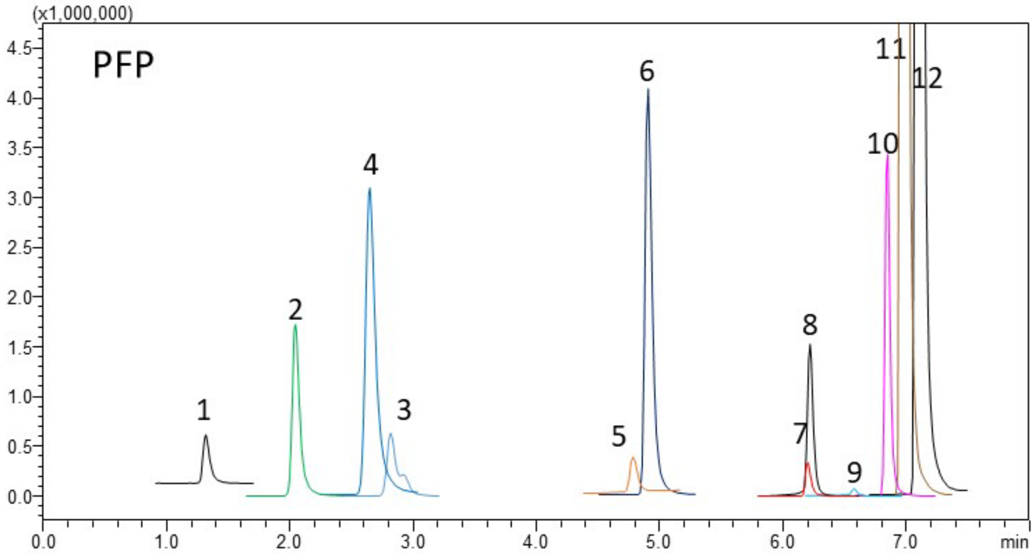
Screening Results: PCS Phenyl-Hexyl vs. Phenyl-Hexyl

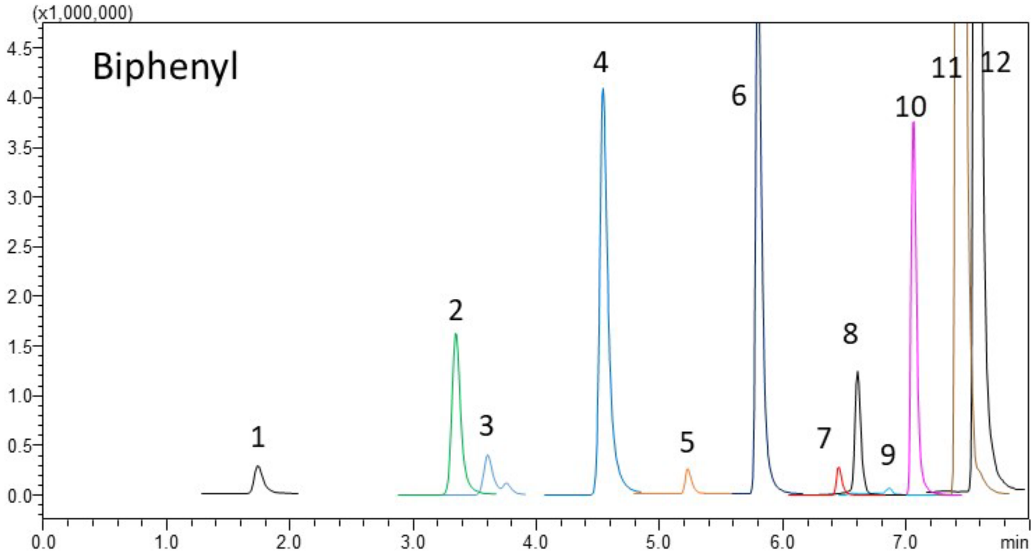


- Peak elution order and peak shape very similar between PCS Phenyl-Hexyl and Phenyl-Hexyl
- Phenyl-Hexyl shows increased retention over PCS Phenyl-Hexyl

1. N-nitrosodimethylamine
2. N-nitrosomorpholine
3. N-nitrosomethylethylamine
4. N-nitrosopyrrolidine
5. N-nitrosodiethylamine
6. N-nitrosopiperidine
7. N-nitroso-n-propylamine
8. N-nitroso-N-methylaniline
9. N-nitroso-N-ethylaniline
10. N-nitrosodi-n-butylamine
11. N-nitrosodiphenylamine
12. N-nitrosodibenzylamine

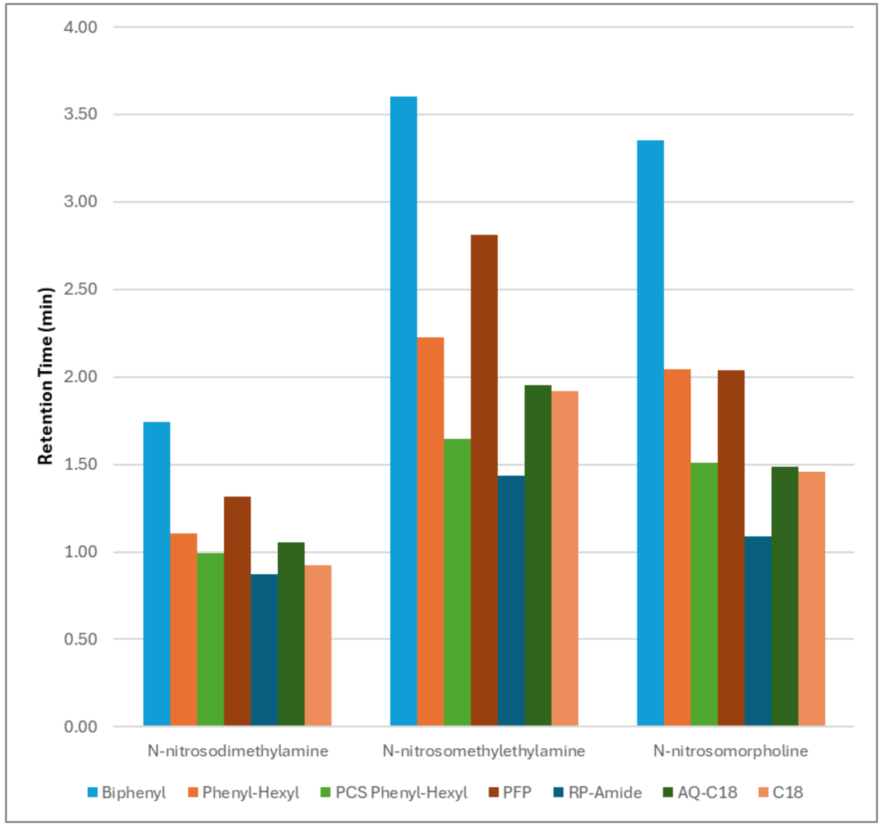
Screening Results: PFP vs. Biphenyl





- Peak elution order is different between PFP and Biphenyl
- Biphenyl shows increased retention over PFP

1. N-nitrosodimethylamine
2. N-nitrosomorpholine
3. N-nitrosomethylethylamine
4. N-nitrosopyrrolidine
5. N-nitrosodiethylamine
6. N-nitrosopiperidine
7. N-nitroso-n-propylamine
8. N-nitroso-N-methylaniline
9. N-nitroso-N-ethylaniline
10. N-nitrosodi-n-butylamine
11. N-nitrosodiphenylamine
12. N-nitrosodibenzylamine



DryLab® Optimization

- DryLab® is a method optimization software that speeds up method development
- Can be used for 2 or 3 parameter optimizations
  - Gradient Time
  - Temperature
  - pH
  - Ternary mixes of mobile phases
- Input dwell volume, retention times, peak areas, tailing factor and peak widths

- t_G = 10 min and T = 30 °C
- t_G = 10 min and T = 50 °C
- t_G = 30 min and T = 30 °C
- t_G = 30 min and T = 50 °C

- Gradient = 5 - 100%
- Mobile Phase A: water/0.1% formic acid
- Mobile Phase B: methanol/0.1% formic acid
- Flow rate: 0.4 mL/min.
- Column = HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 100 mm

Optimized Conditions

LC CONDITIONS:

Column: HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-611
Mobile Phase A: Water/0.1% formic acid
Mobile Phase B: Methanol/0.1% formic acid
Gradient:

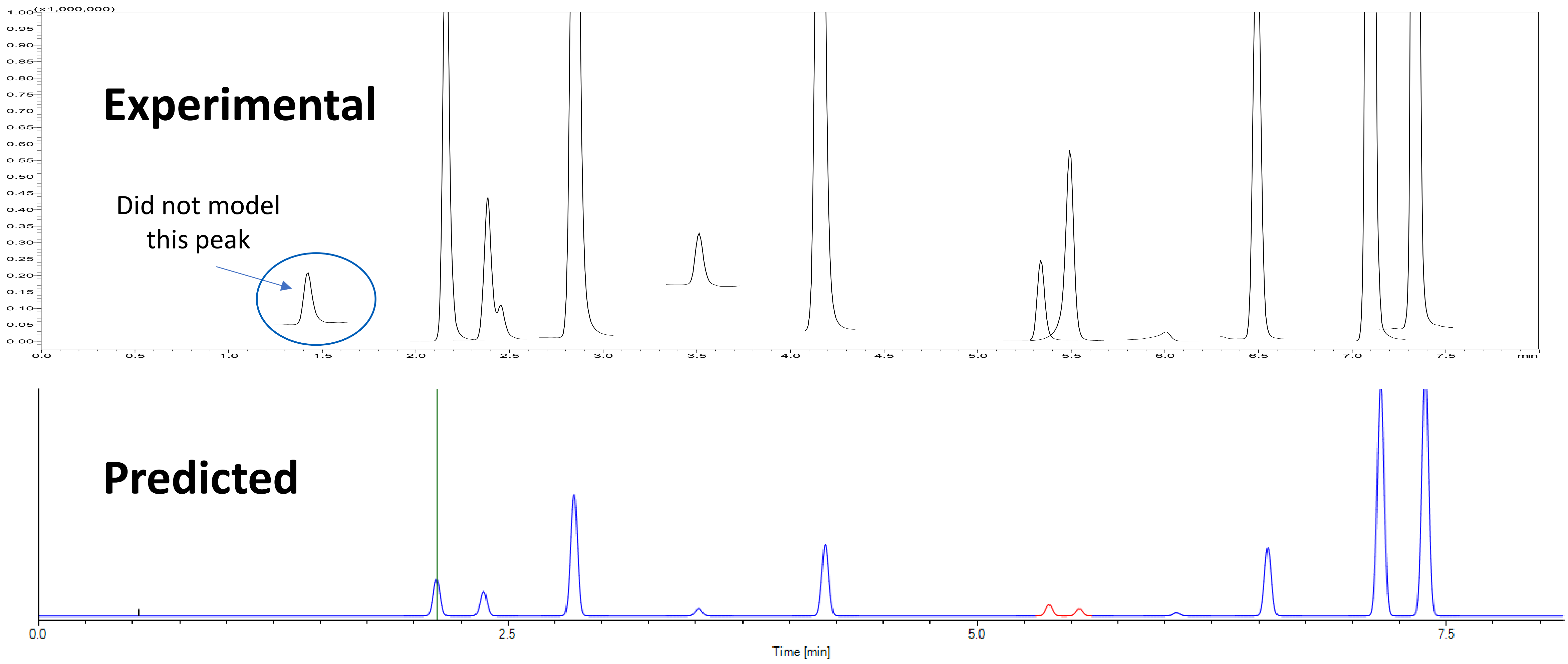
Time	%B
0.00	5
8.00	100
9.00	100
9.01	5
12.00	5

Flow Rate: 0.4 mL/min.
Pressure: 180 bar
Temperature: 45 °C
Injection Volume: 0.6 µL
Sample: 10 µg/mL each analyte
Sample Solvent: 90/10 water/methanol
LC System: Shimadzu Nexera X2

MS CONDITIONS:

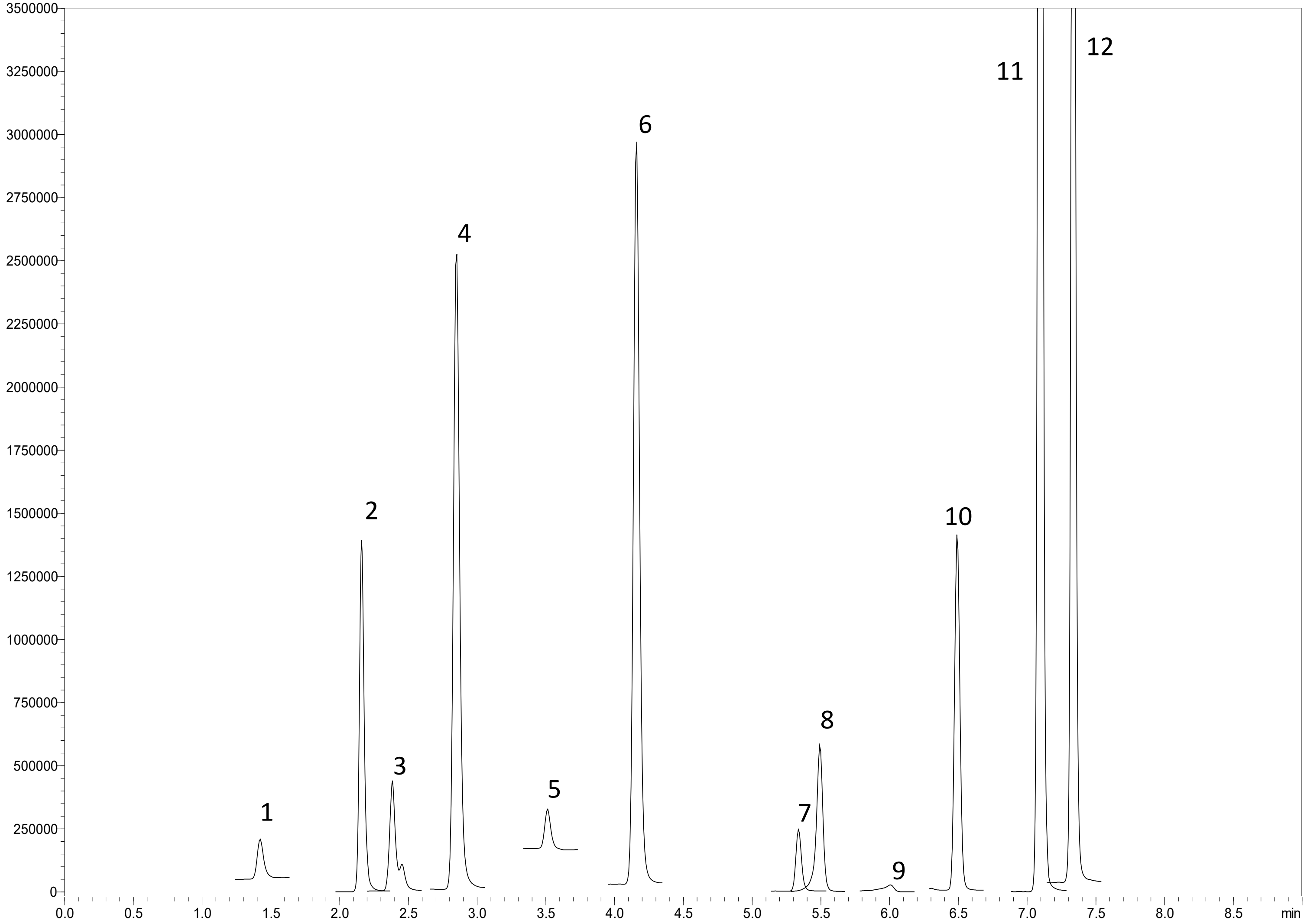
System: Shimadzu 8060NX
Detection Mode: DUIS ESI + 1 kV; Corona Needle 3.5 kV
Nebulizer Gas Flow: 3 L/min.
Interface Temperature: 300 °C
DL Temperature: 200 °C
Heat Block Temperature: 200 °C
Drying Gas Flow: 5 L/min.

DryLab® Optimization: Experimental Compared to Predicted



Optimized Nitrosamines Method

HALO®



Peak #	Compound	m/z Transition	Retention Time (min)
1	N-nitrosodimethylamine	75.10>43.25	1.42
2	N-nitrosomorpholine	117.10>87.10	2.16
3	N-nitrosomethylethylamine	89.10>61.10	2.38
4	N-nitrosopyrrolidine	101.10>55.10	2.85
5	N-nitrosodiethylamine	103.10>75.05	3.51
6	N-nitrosopiperidine	115.10>69.05	4.16
7	N-nitroso-n-propylamine	131.20>43.10	5.34
8	N-nitroso-N-methylaniline	137.00>107.00	5.49
9	N-nitroso-N-ethylaniline	151.00>121.00	6.01
10	N-nitrosodi-n-butylamine	159.20>57.15	6.49
11	N-nitrosodiphenylamine	199.22>169.05	7.10
12	N-nitrosodibenzylamine	227.00>91.00	7.34

Advantages of 1.5 mm ID Columns

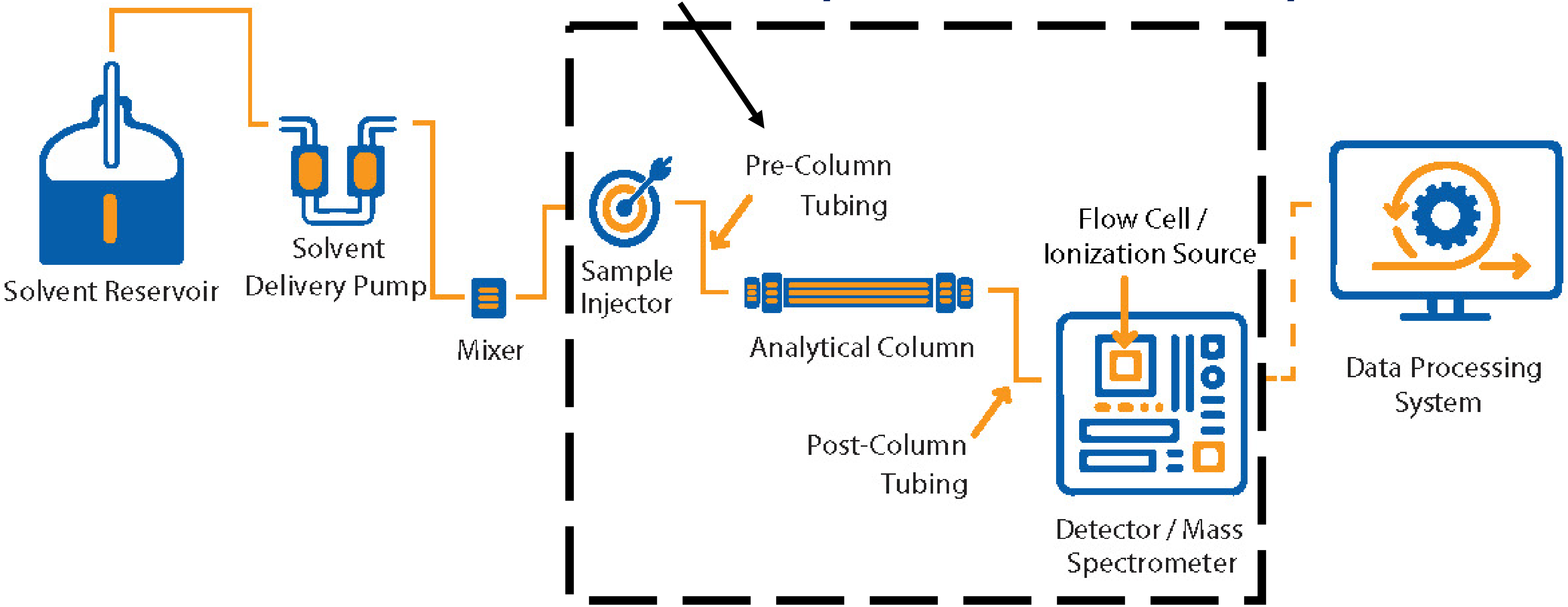
HALO®

- More sensitivity from conventional UHPLC systems
- Higher ionization efficiencies from LCMS systems
- Reduced solvent consumption compared to 2.1 mm ID columns (and greater)
- Easy to implement microflow solution with existing systems

Considerations for Moving to Smaller Column IDs

- Smaller column IDs are more susceptible to extra-column band broadening

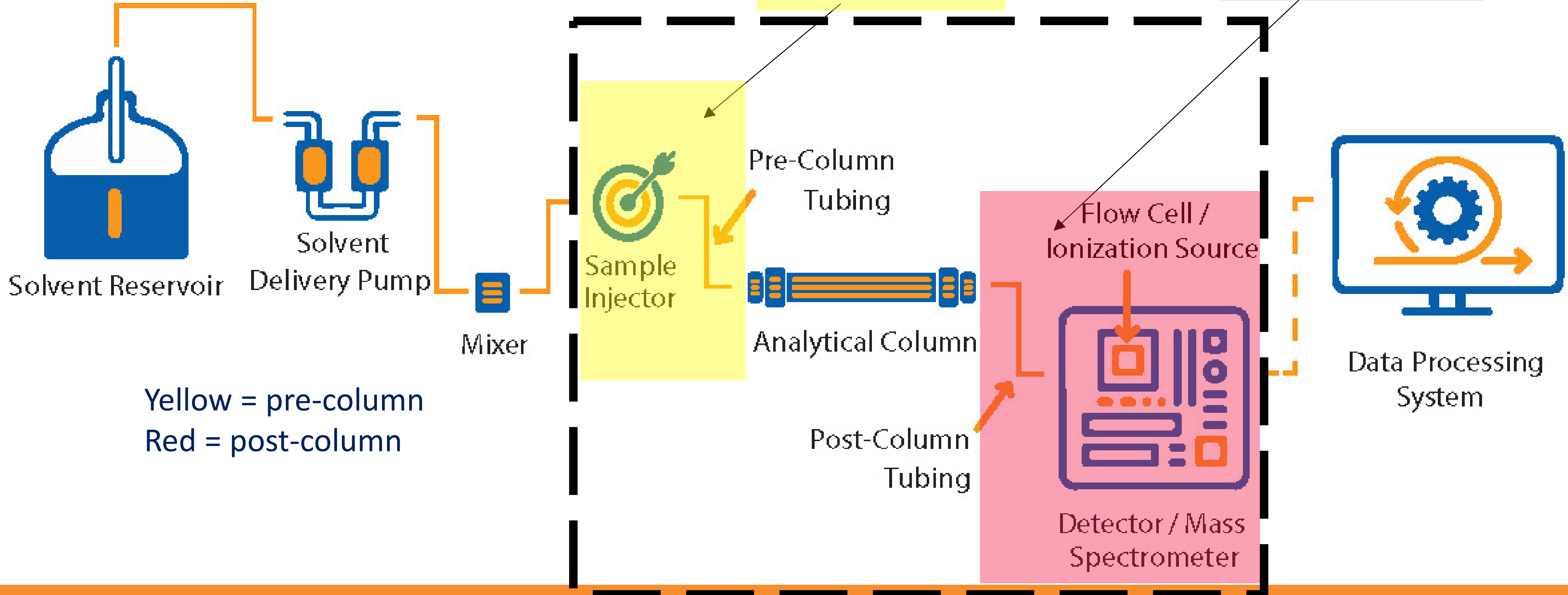
$$\sigma^2_{V,tot} = \sigma^2_{V,pre-col} + \sigma^2_{V,col} + \sigma^2_{V,post-col}$$



Considerations for Moving to Smaller Column IDs

- Smaller column IDs are more susceptible to extra-column band broadening

$$\sigma^2_{V,tot} = \sigma^2_{V,pre-col} + \sigma^2_{V,col} + \sigma^2_{V,post-col}$$

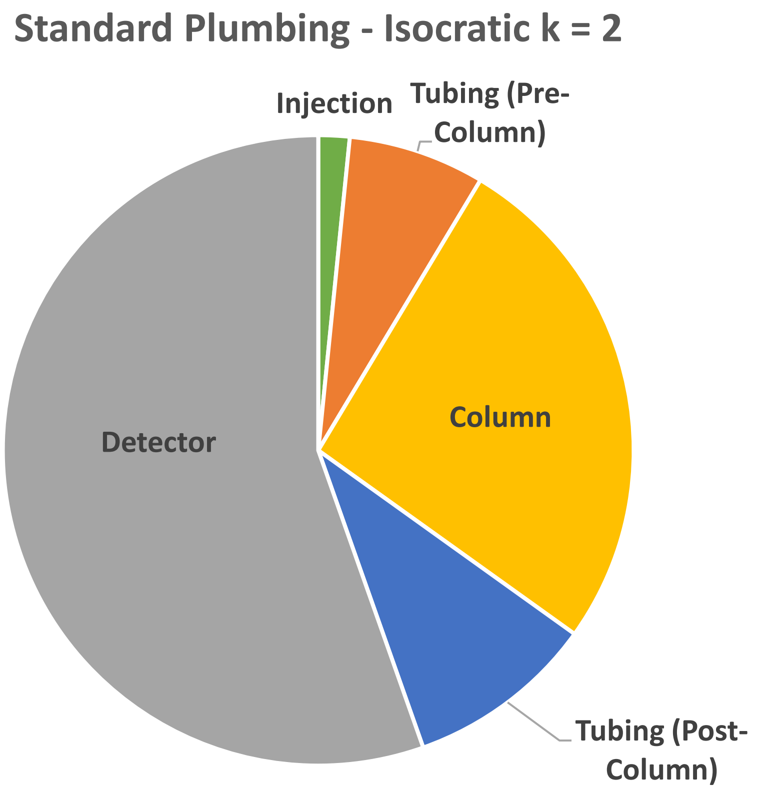


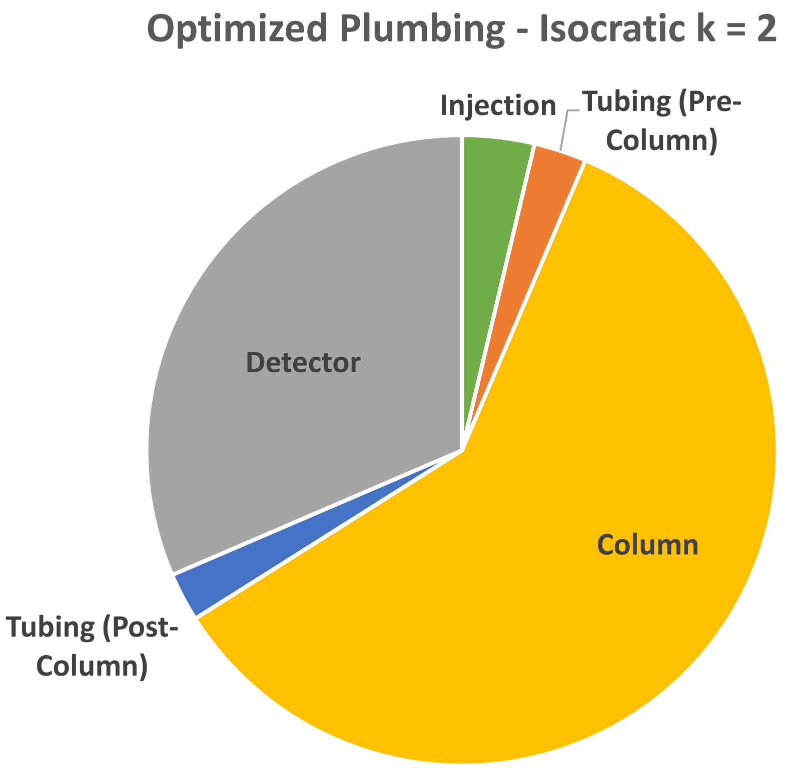
Yellow = pre-column
Red = post-column

How to Estimate Extracolumn Dispersion

HALO®

Where Has My Efficiency Gone? Impacts of Extracolumn Peak Broadening on Performance
4 part series in LCGC North America from Dwight R. Stoll, Thomas Lauer, & Ken Broeckhoven
http://www.multidlc.org/dispersion_calculator

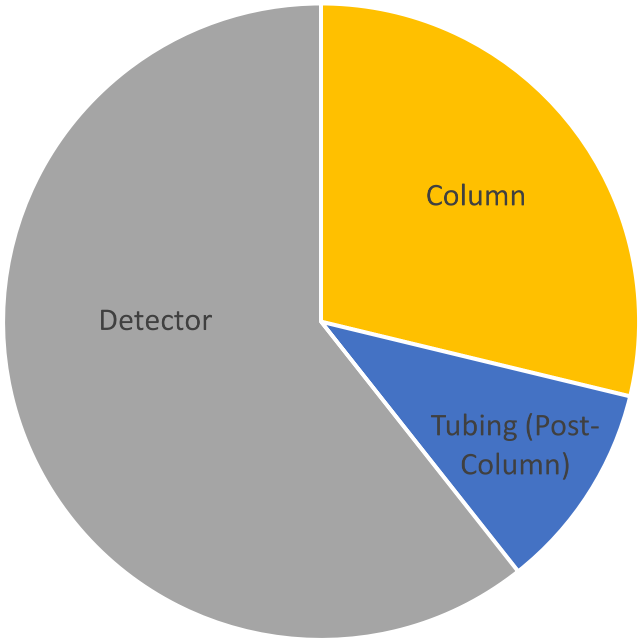




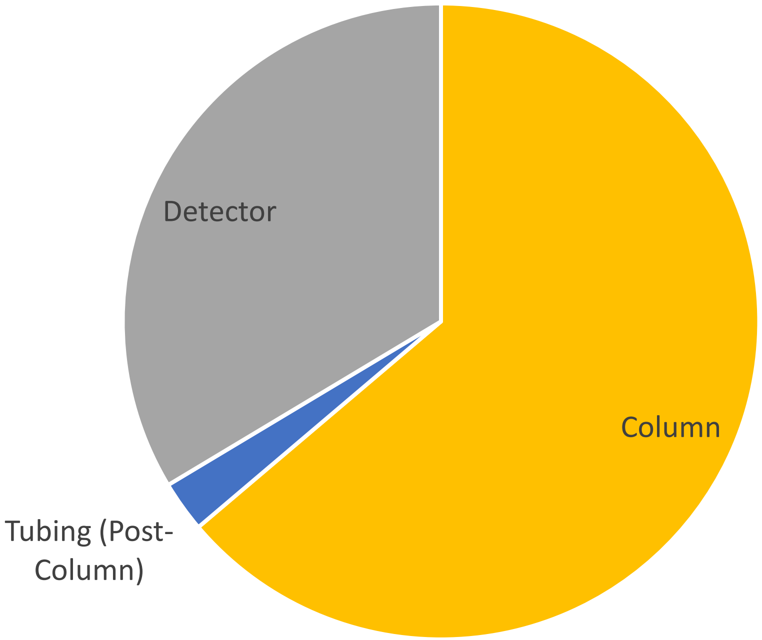
How to Estimate Extracolumn Dispersion

Where Has My Efficiency Gone? Impacts of Extracolumn Peak Broadening on Performance
4 part series in LCGC North America from Dwight R. Stoll, Thomas Lauer, & Ken Broeckhoven
http://www.multidlc.org/dispersion_calculator

Standard Plumbing - Gradient k* = 2

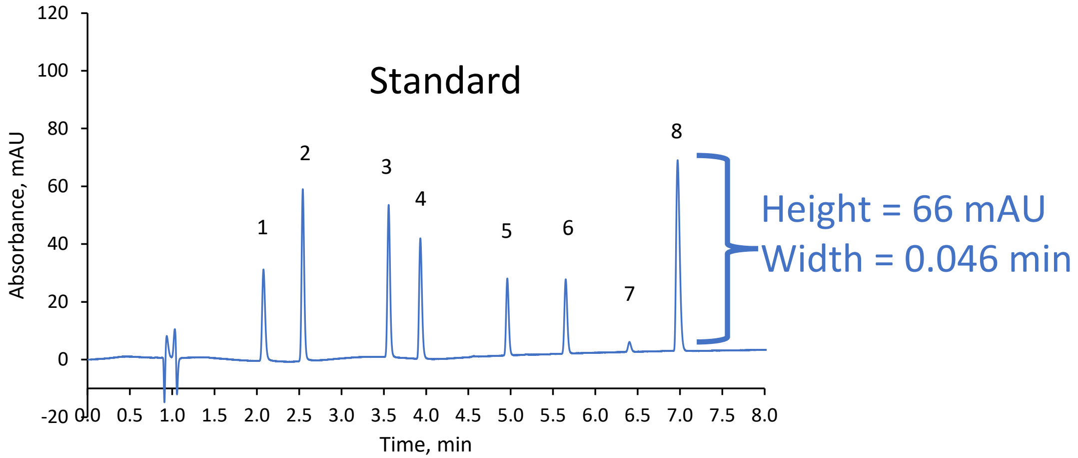


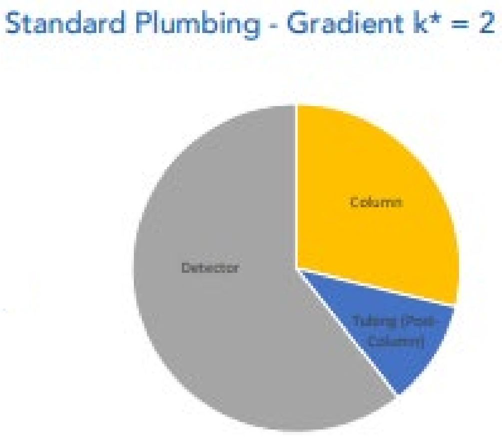
Optimized Plumbing - Gradient k* = 2

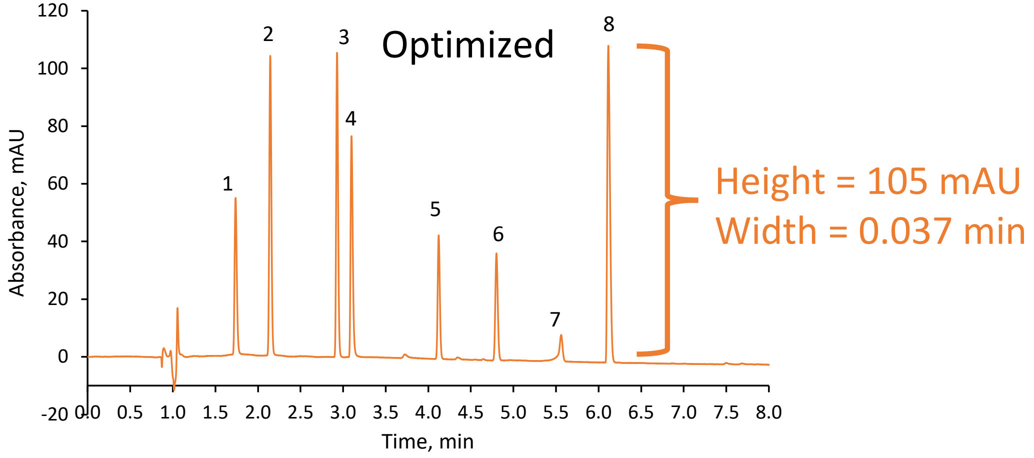


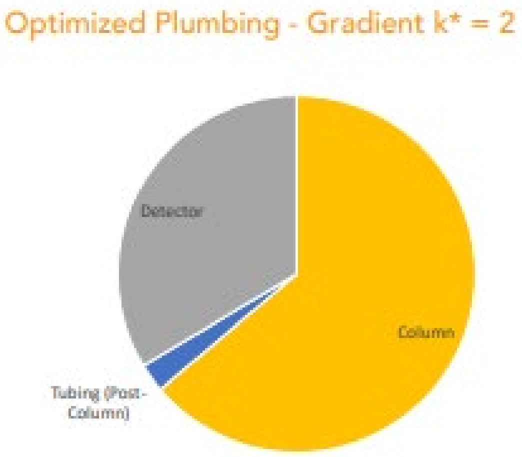
How to Estimate Extracolumn Dispersion?

Where Has My Efficiency Gone? Impacts of Extracolumn Peak Broadening on Performance
4 part series in LCGC North America from Dwight R. Stoll, Thomas Lauer, & Ken Broeckhoven
http://www.multidlc.org/dispersion_calculator

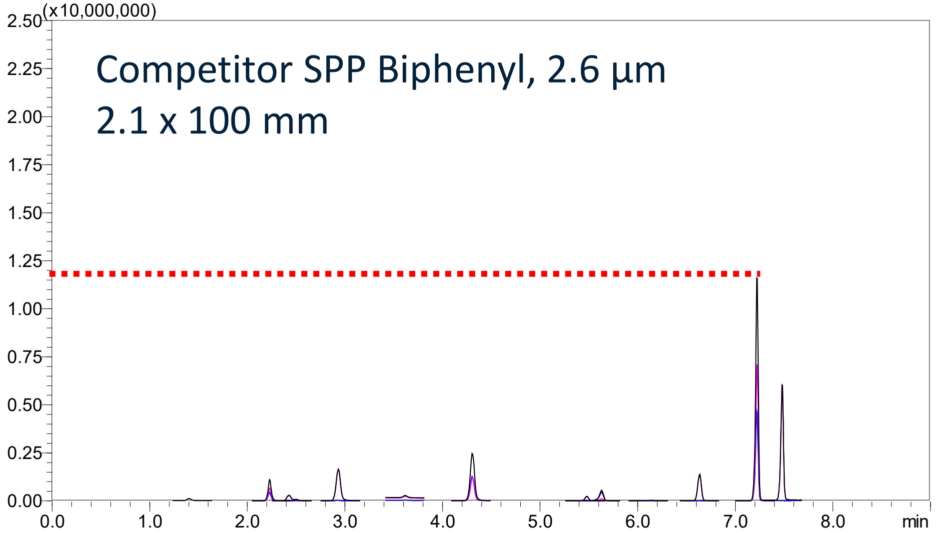


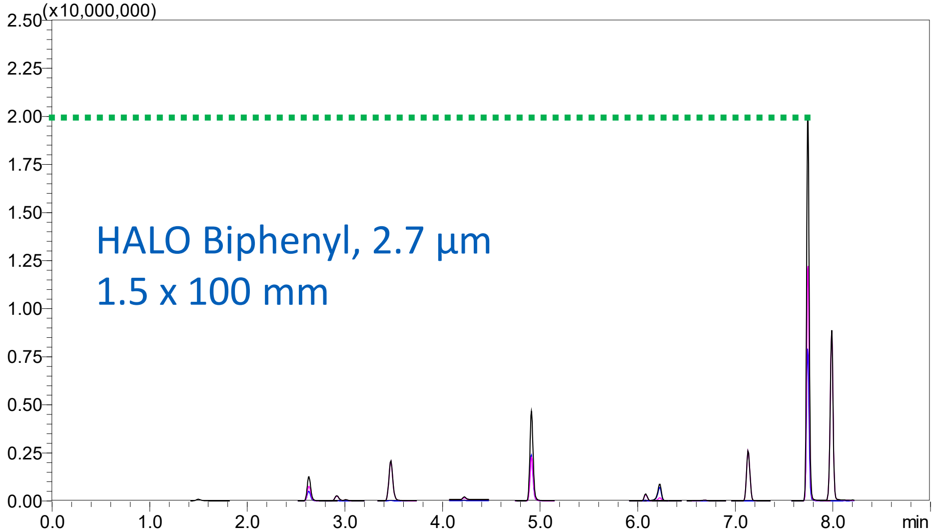






HALO 1.5 mm ID compared to Competitor 2.1 mm ID





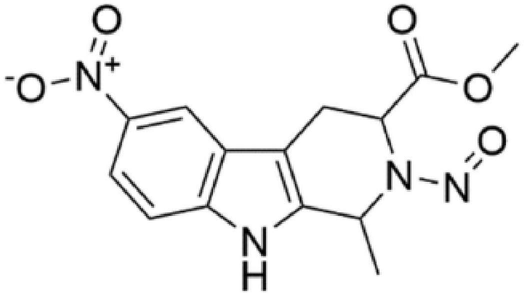
- Same method run on both columns except for flow rate
  - 0.4 mL/min on 2.1 mm ID
  - 0.2 mL/min on 1.5 mm ID
- MarvelXACT™ connectors used to reduce extracolumn volume
  - 75 µm x 350 mm from column outlet to ground
  - 75 µm x 150 mm from ground to source
- On average, 34% larger peak heights observed with the 1.5 mm ID HALO Biphenyl column
- On average, 14% narrower peak widths observed with the 1.5 mm ID HALO Biphenyl column

Summary

- Of the 7 HALO® phases screened, the HALO® Biphenyl gave the best combination of retention, peak shape, and resolution
- DryLab® was used to optimize the separation on the HALO® Biphenyl column
- HALO® Biphenyl in 1.5 mm ID gives sharper peaks and increased peak height over 2.1 mm ID columns
- 1.5 mm ID columns offer 50% solvent savings over 2.1 mm ID columns for greener, more sustainable methods

Future Work

- Compare ESI and APCI using calibration curves
- Run an active pharmaceutical drug with matrix
- Thousands of nitrosamines – would like to screen to test if Biphenyl is still the recommended column



methyl 1-methyl-6-nitro-2-nitroso-1,3,4,9-tetrahydropyrido(3,4)-b-indole-3-carboxylate (positive)
2 hindered (iPr) sites
aromatic nitro alerting feature

Computational Toxicology
Volume 20, November 2021, 100186

Questions

HALO®

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