Drugs of Abuse and Metabolite Screening Using High Efficiency LC Columns

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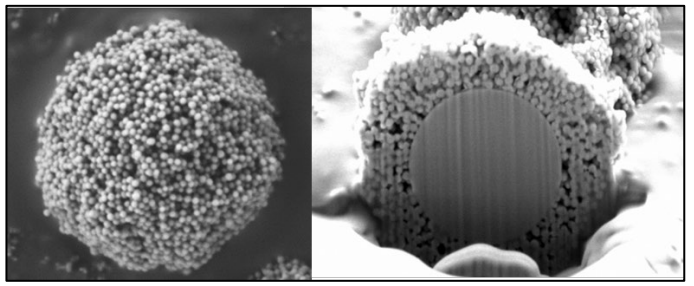
Advanced Materials Technology, Inc.

Pittcon

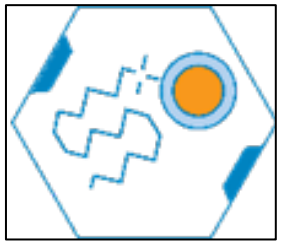
February 28, 2024

Outline

- Background
- Superficially Porous Particles (SPPs)



- HALO® Column Chemistries used in the Screenings



- Results
- Summary

Background

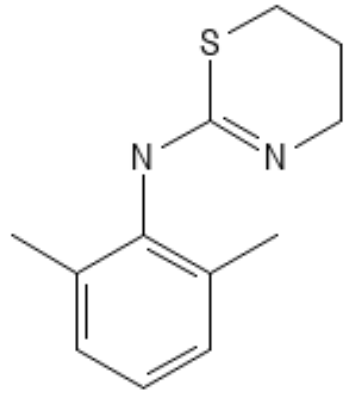
- Goal to develop an LC-MS/MS method containing fentanyl and xylazine

- Motivation:
  - Significant increase in fentanyl overdose deaths where xylazine is detected from January 2019 to June 2022 in the US

  - April 2023: White House Office of National Drug Control Policy designated fentanyl adulterated with xylazine as an emerging threat

Xylazine

- Horse tranquilizer that is not Schedule I restricted
  - In June 2023, Pennsylvania added it to Schedule III making it a controlled substance
- No reversal agent for humans
- Known as “tranq” or “tranq dope”



- Central nervous system depressant that can cause the following:
  - Drowsiness
  - Amnesia
  - Slow breathing, heart rate, and blood pressure to dangerously low levels
- Increases the risk of wounds that will not heal, which could lead to amputations and even death if left untreated

Xylazine

know what's in your drugs

TRANQ | XYLAZINE

Xylazine is a veterinary tranquilizer that is cut in dope to give fentanyl longer legs. It's known as "anestesia de caballo" in Puerto Rico and "tranq"in Philly.

Tranq was found in over

90%

of dope samples tested in Philly in 2021

Dope with tranq was first seen in Puerto Rico. Today, it is being found in more and more places across the US.

When tranq is mixed with another drug (like fentanyl, heroin, or a benzo), the chance of overdose increases. If someone is overdosing administer naloxone like you normally would. If the person starts breathing again but is still sedated, they don't need more naloxone. Put them in rescue position and keep an eye on them.

Tranq has been associated with severe wounds, which spread and worsen very quickly.

These wounds are seen regardless of how people use: smoking, snorting, or injecting. It's very difficult for these wounds to heal on their own so it is important to get medical attention if you have one.

What can you do if you think there is tranq in your dope?

First, try to ask around and see how the drug is making other people feel before you buy or use it. Since tranq can cause a really heavy nod, try to use somewhere that you will be safe and won't fall and hurt yourself. Finally, if you think there is tranq in your dope let others know--including someone at your local exchange program--so folks know to be careful.

Never Use Alone

English hotline: 800-484-3731

Spanish hotline: 800-928-5330

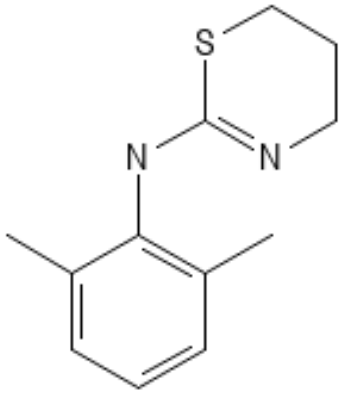
The Brave App

download in the app store

Department of Public Health CITY OF PHILADELPHIA

Created by the Substance Use and Harm Reduction (SUPHR) division

For more information on tranq in Philadelphia visit substanceusephilly.com/tranq



23 Compounds Included in Screening

Opioid Polysubstance Mix Kit	Description
6-Acetylmorphine	metabolite of heroin
Alpha-hydroxyalprazolam	metabolite of alprazolam
Alprazolam	drug of abuse
(±)-Amphetamine	drug of abuse
Benzoylecgonine	metabolite of cocaine
Cocaethylene	metabolite formed when cocaine and ethanol are taken together
Cocaine	drug of abuse
Diazepam	drug of abuse
Diphenhydramine HCl	drug of abuse
Ecgonine HCl	metabolite of cocaine
Fentanyl	drug of abuse
Heroin	drug of abuse
Hydrocodone	drug of abuse
(-) Levamisole HCl	cocaine adulterant
(±)-Methadone	used to treat opioid addiction
(±)-Methamphetamine	drug of abuse
Morphine	drug of abuse
Naloxone	used to treat opioid overdose
Noroxycodone HCl	metabolite of oxycodone
Oxycodone	drug of abuse
Oxymorphone	metabolite of oxycodone
Tetrahydrocannabinol ((-)-delta9-THC)	drug of abuse
Xylazine	fentanyl adulterant

Opioid Polysubstance Mix Kit - standards from Cerilliant
Xylazine - Supelco

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12 drugs of abuse

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7 metabolites

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Xylazine - Supelco

2 adulterants

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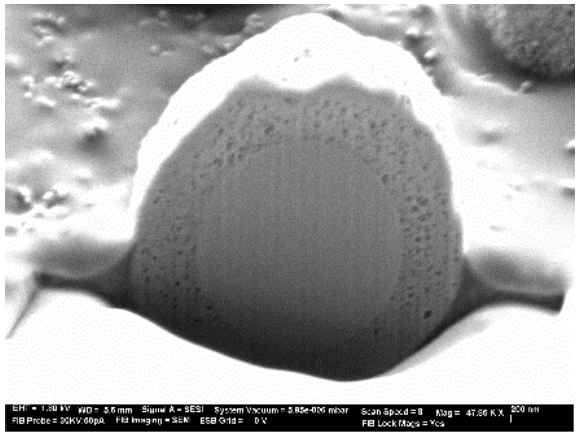
Opioid Polysubstance Mix Kit - standards from Cerilliant
Xylazine - Supelco

2 treatments

Superficially Porous Particles

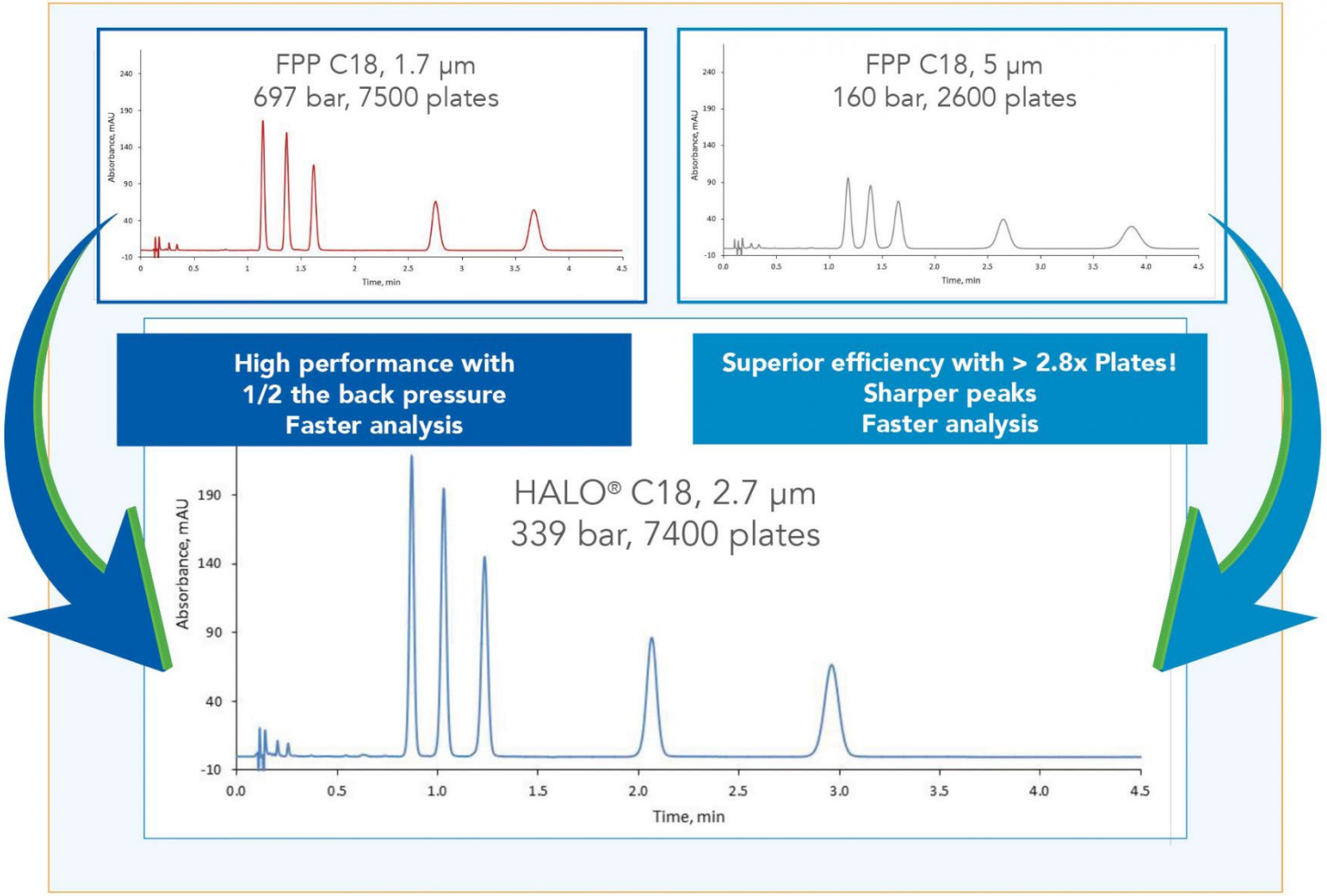
HALO

HALO 90 Å, 2.7 µm

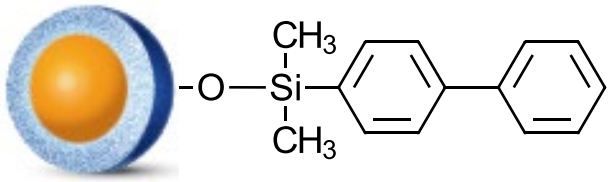


HALO® Advantages

- Fast separations
- Lower back pressure compared to sub-2-µm particle columns
- Increased efficiency/resolution



HALO® Column Chemistries Selected for Screenings

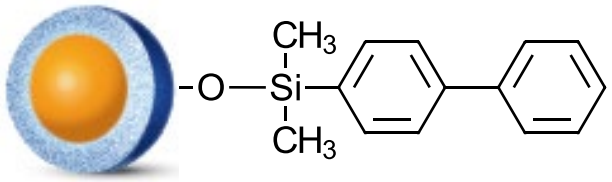


HALO® Biphenyl

Features and Benefits

- Resistant to dewetting, making it 100% aqueous mobile phase compatible (can start gradients at 0% organic)
- Enhanced retention and selectivity for polar molecules

HALO® Column Chemistries Selected for Screenings



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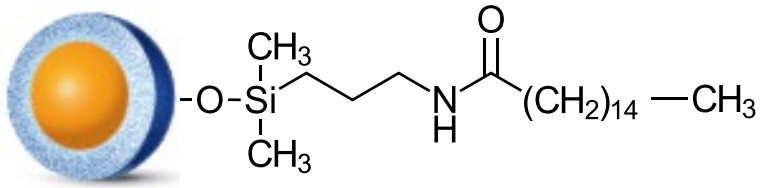

HALO® RP-Amide

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- Alternate selectivity to alkyl phases

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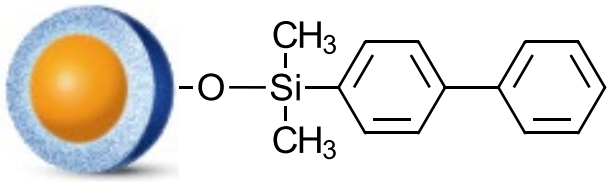

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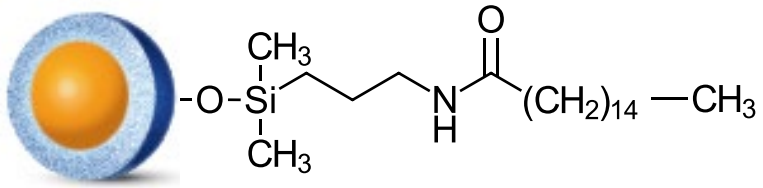

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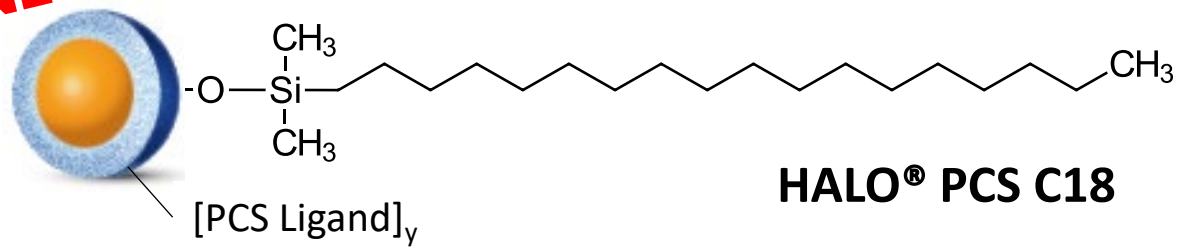
NEW



HALO® PCS C18

- Optimized for low ionic strength mobile phases
- Improved peak shape and sample loading capacity for basic compounds

HALO® Column Chemistries Selected for Screenings

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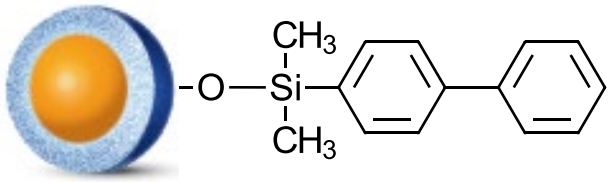

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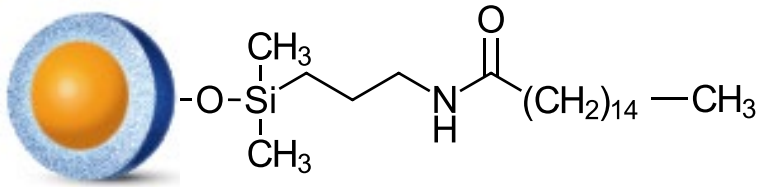

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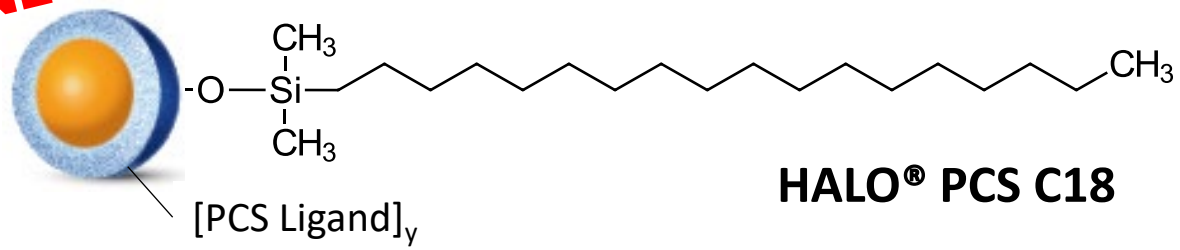

HALO® PCS C18

- Optimized for low ionic strength mobile phases
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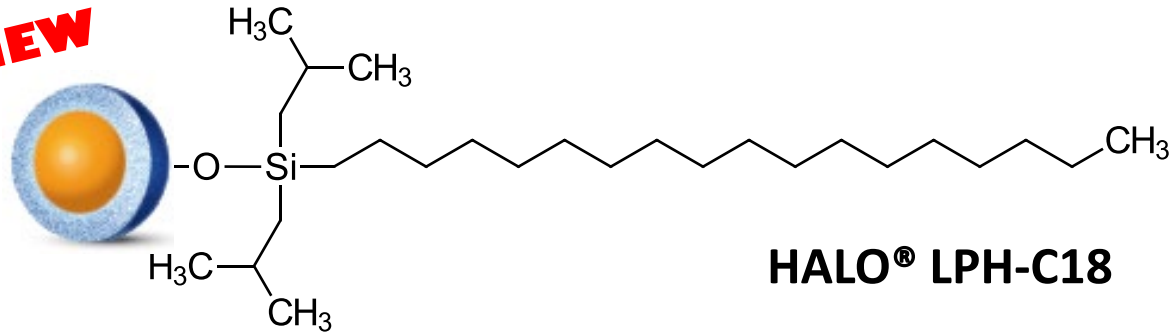
NEW

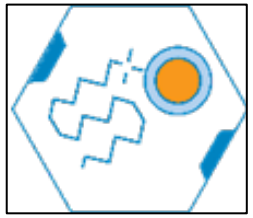


HALO® LPH-C18

- Optimized for small molecule reversed-phase HPLC separations using low pH mobile phases and elevated temperature for acidic and neutral compounds
- Unendcapped

Drugs of Abuse/Metabolites Screening with HALO® Phases

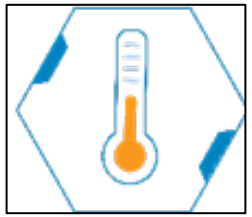
- Stationary phases (all 2.7 µm; 90 Å in 2.1 x 100 mm)
  - Biphenyl
  - RP-Amide
  - PCS C18
  - LPH-C18



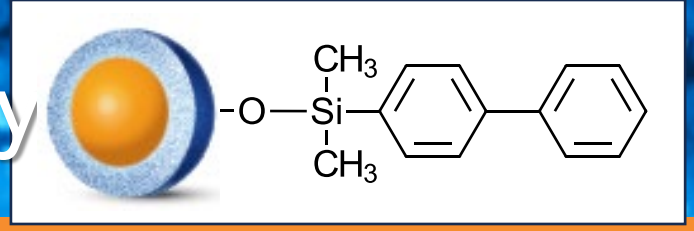
- Method Conditions
  - Flow Rate: 0.5 mL/min
  - Mobile Phase A: water/0.1% formic acid
  - Mobile Phase B: methanol/0.1% formic acid
  - Gradient: 1 min. hold at 3% B; 3-100% B in 9 min; 1 min hold at 100% B
  - Temperature: 35 ^{o}C
  - Injection: 5 µL of 50 µg/mL
  - Sample: Mix of 23 drugs and metabolites
  - Sample Solvent: Mobile Phase A
  - Instrument: Shimadzu Nexera and LCMS 8040

MS Conditions

- Spray Voltage (kV): 4.5
- Nebulizer Gas Flow: 2 L/min
- DL Temperature: 250 °C
- Heat Block Temperature: 400 °C
- Drying Gas Flow: 15 L/min



Screening Results: Biphenyl



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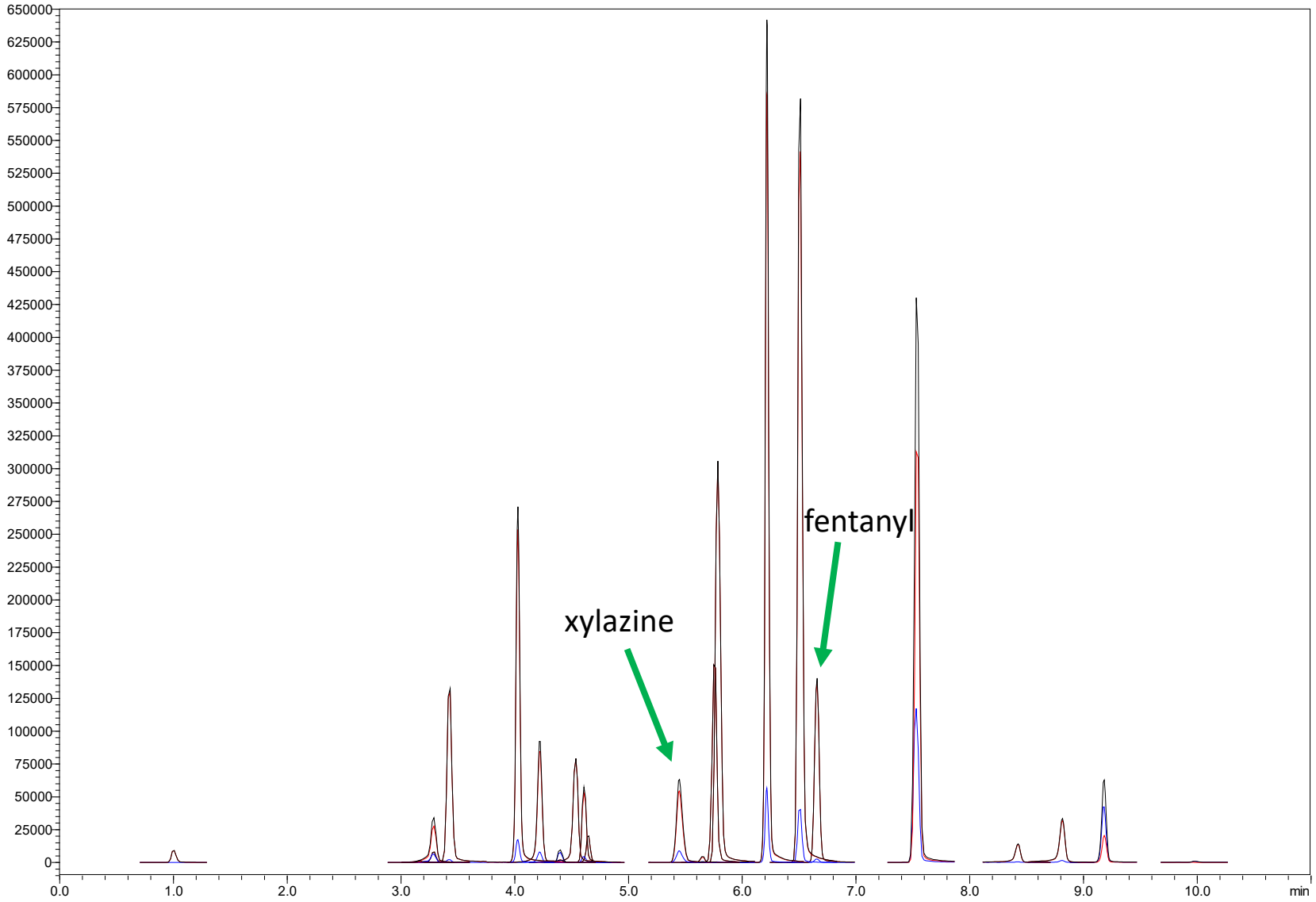
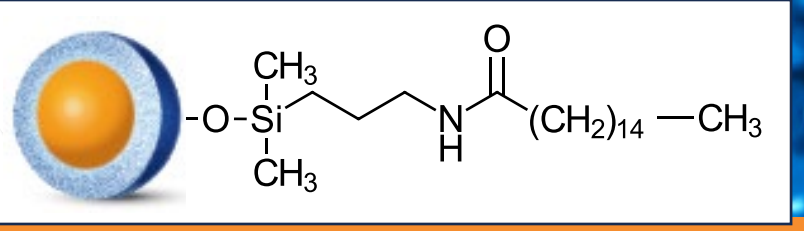


Name	Retention Time (min)
Ecgonine HCl	1.00
Morphine	3.17
Noroxycodone HCl	3.29
Oxymorphone	3.29
(±)-Amphetamine	3.42
(±)-Methamphetamine	4.03
Naloxone	4.22
6-Acetylmorphine	4.40
Oxycodone	4.53
(-) Levamisole HCl	4.61
Hydrocodone	4.65
Xylazine	5.45
Heroin	5.65
Benzoylecgonine	5.76
Cocaine	5.78
Cocaethylene	6.22
Diphenhydramine HCl	6.51
Fentanyl	6.66
(±)-Methadone	7.54
Alpha-hydroxyalprozalam	8.42
Alprazolam	8.81
Diazepam	9.18
Tetrahydrocannabinol ((-)-delta9-THC)	9.98

Screening Results: RP-Amid



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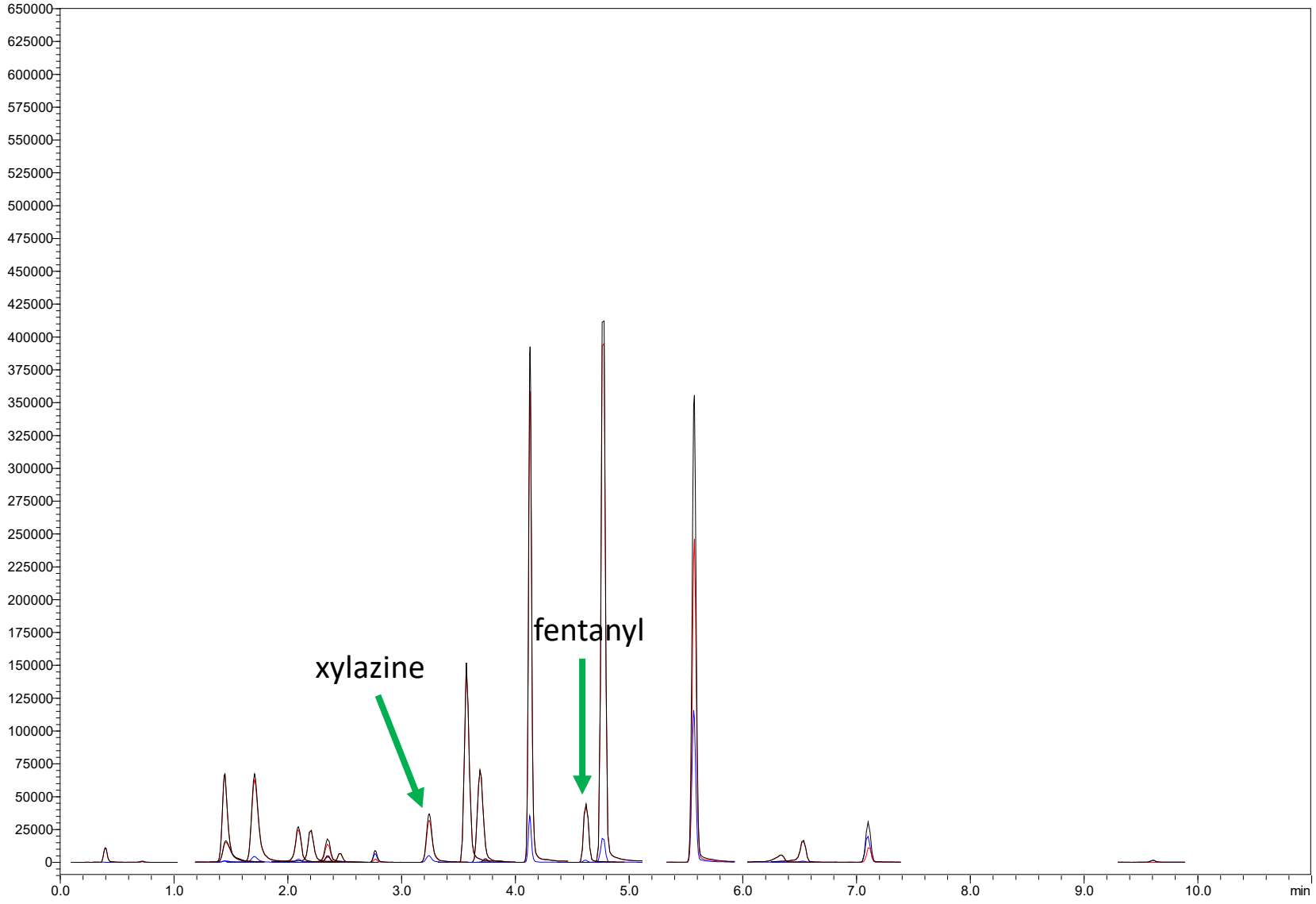
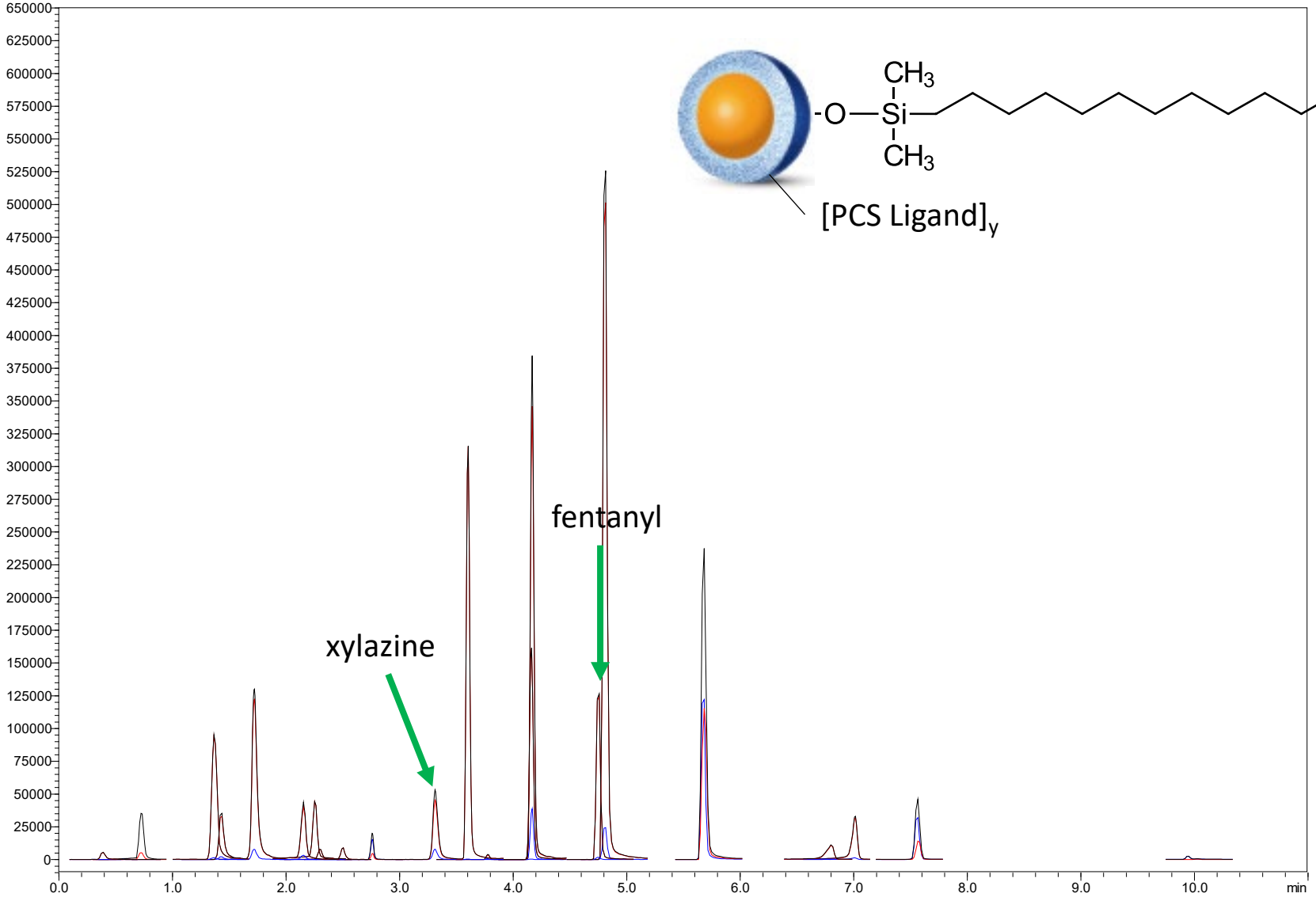


Name	Retention Time (min)
Ecgonine HCl	0.39
Morphine	0.72
(±)-Amphetamine	1.46
(-) Levamisole HCl	1.47
(±)-Methamphetamine	1.74
Naloxone	2.11
Oxycodone	2.22
Oxymorphone	2.37
Noroxycodone HCl	2.37
Hydrocodone	2.47
6-Acetylmorphine	2.78
Xylazine	3.27
Cocaine	3.59
Benzoylecgonine	3.69
Heroin	3.75
Cocaethylene	4.15
Fentanyl	4.66
Diphenhydramine HCl	4.81
(±)-Methadone	5.61
Alpha-hydroxyalprazolam	6.34
Alprazolam	6.53
Diazepam	7.10
Tetrahydrocannabinol ((-)-delta9-THC)	9.61

Screening Results: PCS C18



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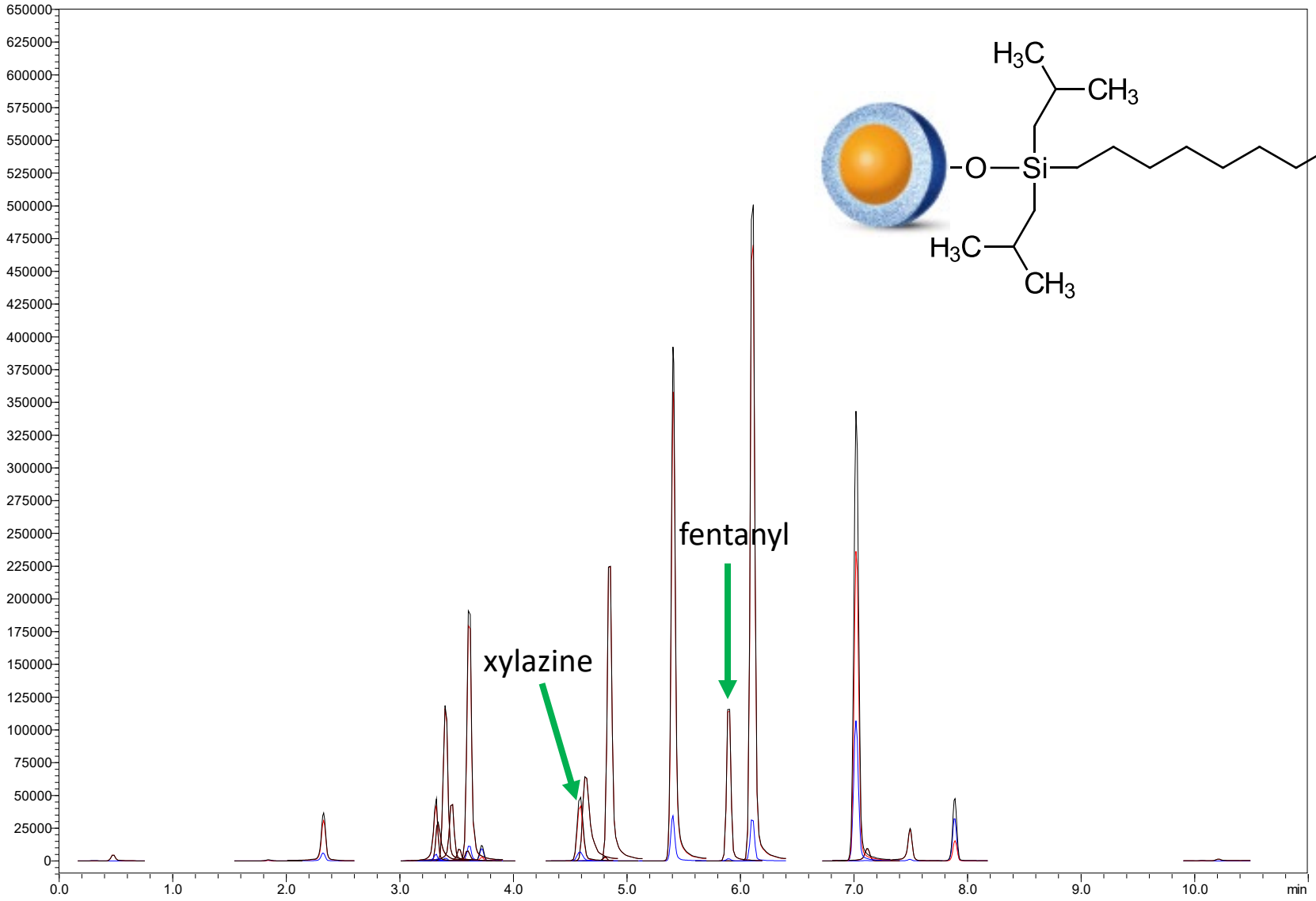
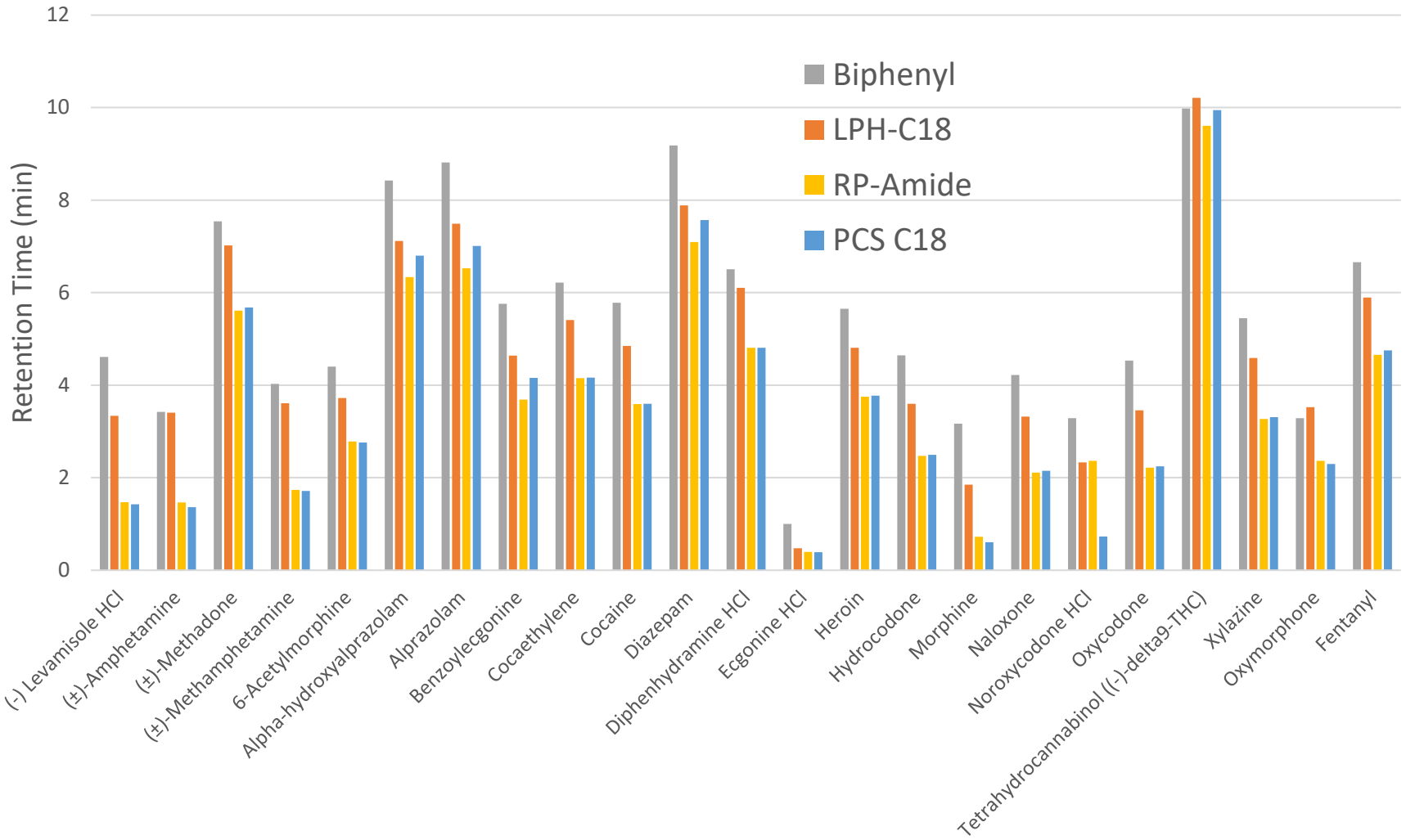
Ecgonine HCl	0.39
Morphine	0.60
Noroxycodone HCl	0.73
(±)-Amphetamine	1.36
(-) Levamisole HCl	1.42
(±)-Methamphetamine	1.71
Naloxone	2.15
Oxycodone	2.25
Oxymorphone	2.29
Hydrocodone	2.50
6-Acetylmorphine	2.76
Xylazine	3.31
Cocaine	3.60
Heroin	3.78
Benzoylecgonine	4.16
Cocaethylene	4.16
Fentanyl	4.75
Diphenhydramine HCl	4.81
(±)-Methadone	5.68
Alpha-hydroxyalprozalam	6.80
Alprazolam	7.01
Diazepam	7.57
Tetrahydrocannabinol ((-)-delta9-THC)	9.94

Screening Results: LPH-C18



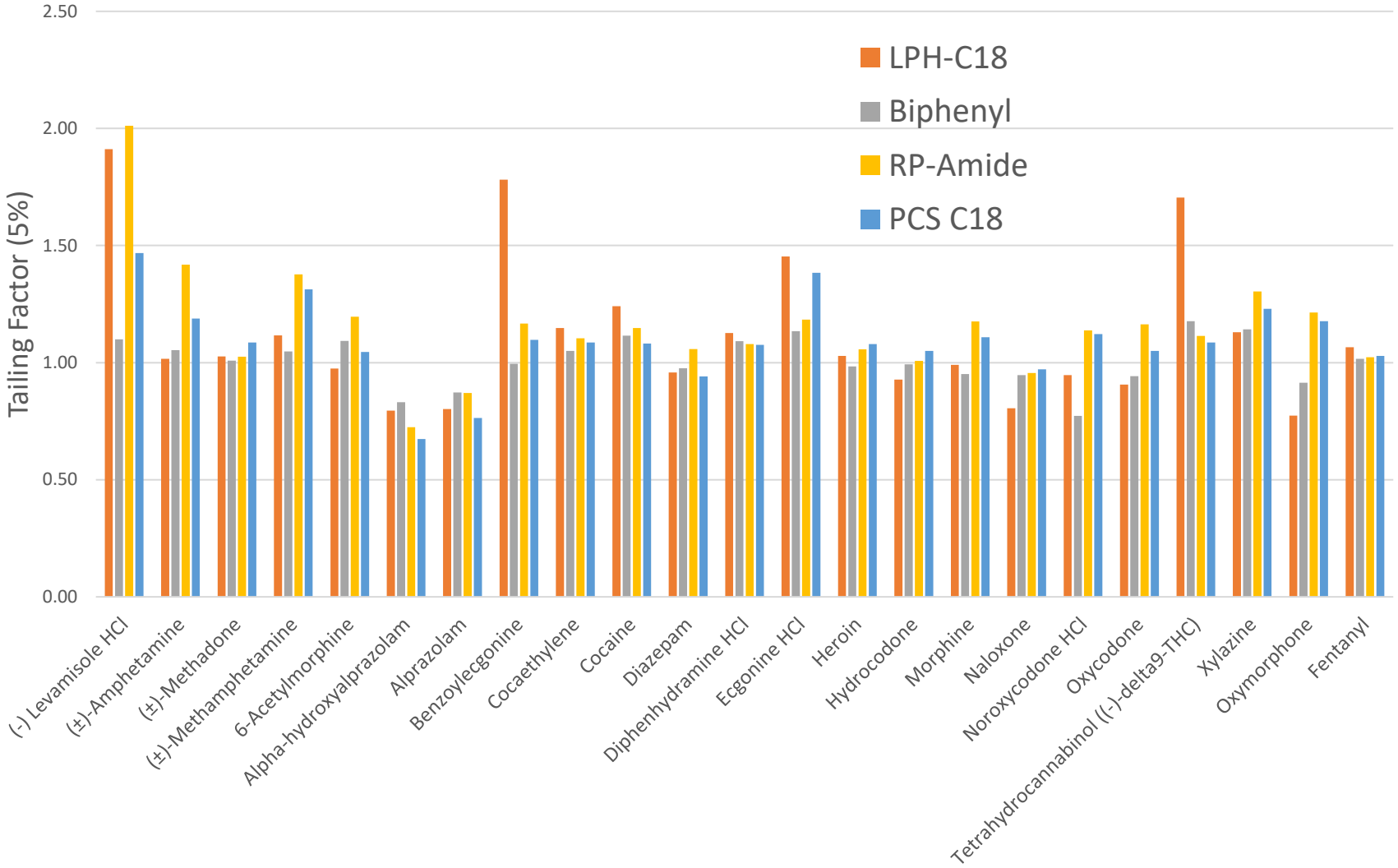
Name	Retention Time (min)
Ecgonine HCl	0.48
Morphine	1.85
Noroxycodone HCl	2.33
Naloxone	3.32
(-) Levamisole HCl	3.34
(±)-Amphetamine	3.40
Oxycodone	3.46
Oxymorphone	3.52
Hydrocodone	3.60
(±)-Methamphetamine	3.61
6-Acetylmorphine	3.72
Xylazine	4.59
Benzoylecgonine	4.64
Heroin	4.81
Cocaine	4.85
Cocaethylene	5.41
Fentanyl	5.90
Diphenhydramine HCl	6.11
(±)-Methadone	7.02
Alpha-hydroxyalprozalam	7.12
Alprazolam	7.49
Diazepam	7.89
Tetrahydrocannabinol ((-)-delta9-THC)	10.21

Comparison of Retention Times



- Biphenyl was most retentive for the majority of compounds
- LPH-C18 was the second highest retentive phase
- For RP-Amide and PCS C18, retention was similar for some compounds and higher with PCS C18 for other compounds such as alprazolam and diazepam

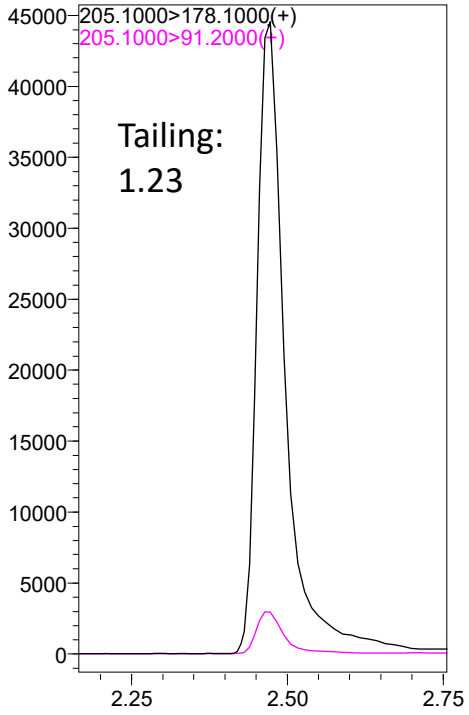
Comparison of Tailing Factors



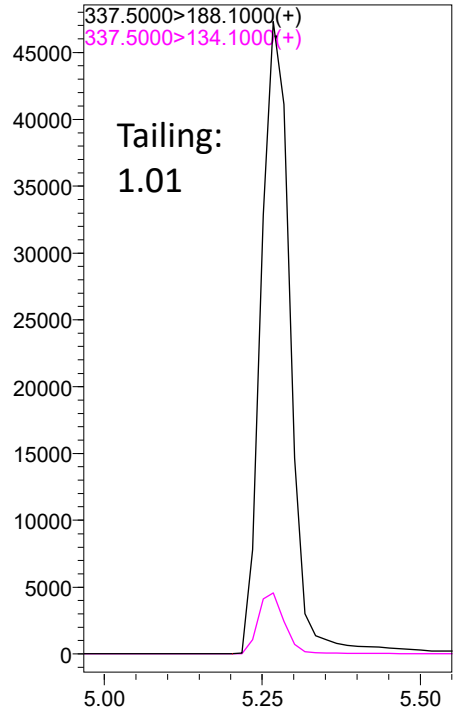
- Best tailing observed with Biphenyl and PCS C18

Xylazine and Fentanyl Peak Shapes: PCS C18 and LPH-C18

PCS C18

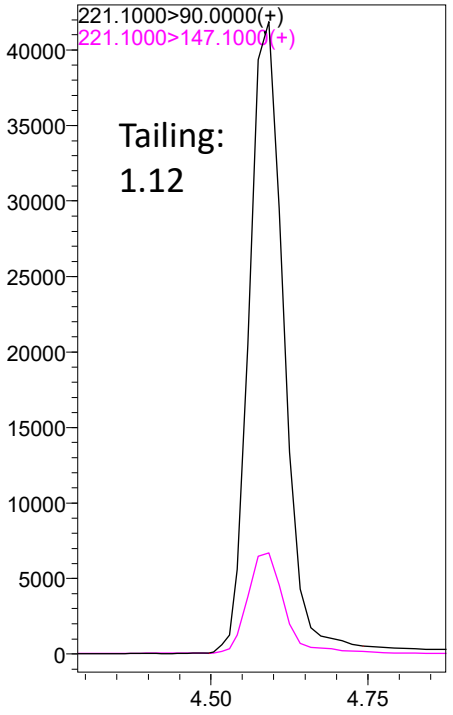


Xylazine

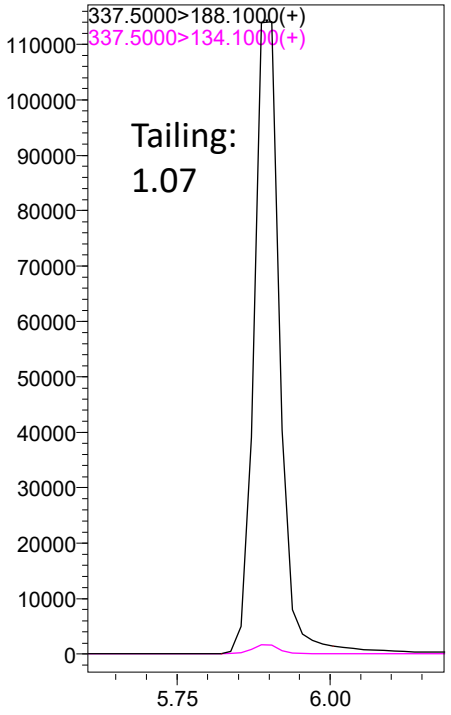


Fentanyl

LPH-C18



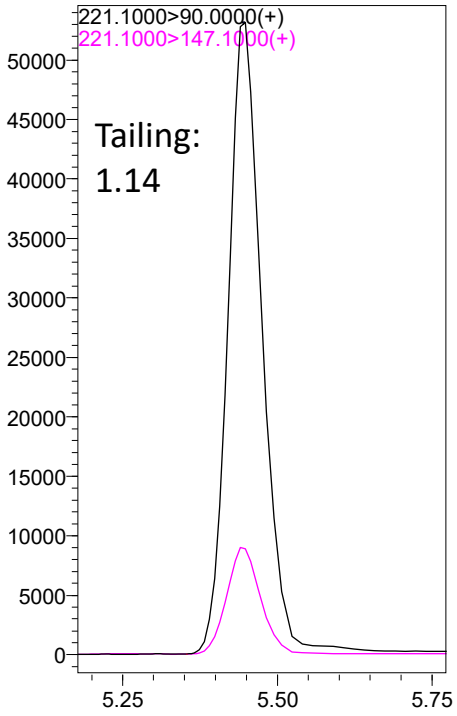
Xylazine



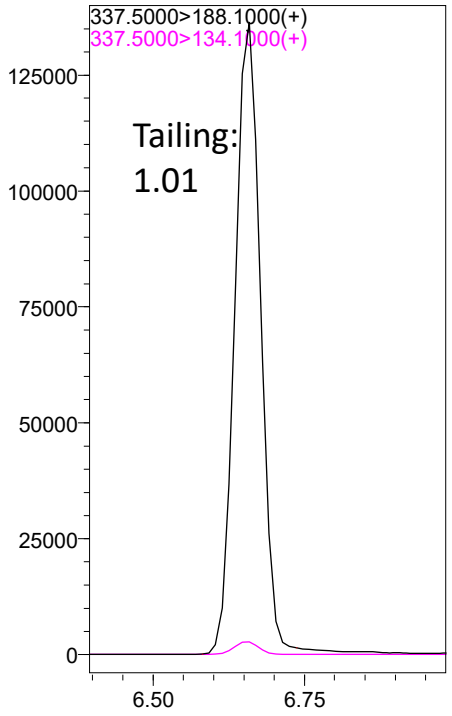
Fentanyl

Xylazine and Fentanyl Peak Shapes: Biphenyl and RP-Amide

Biphenyl

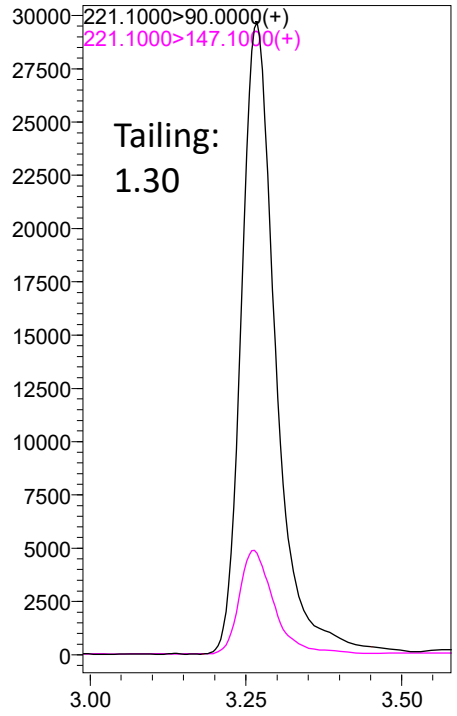


Xylazine

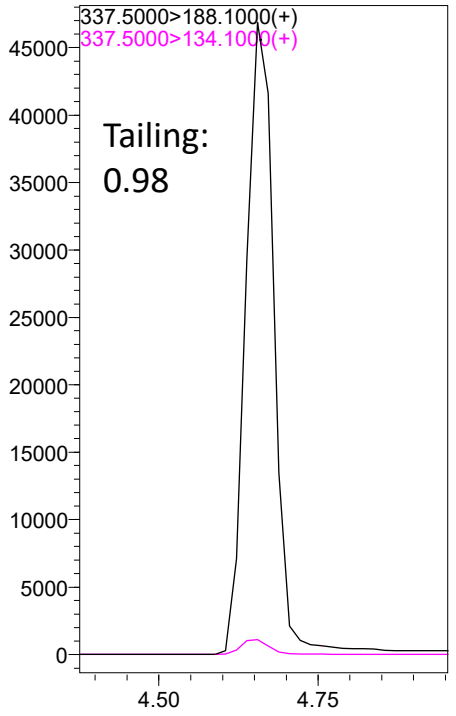


Fentanyl

RP-Amide

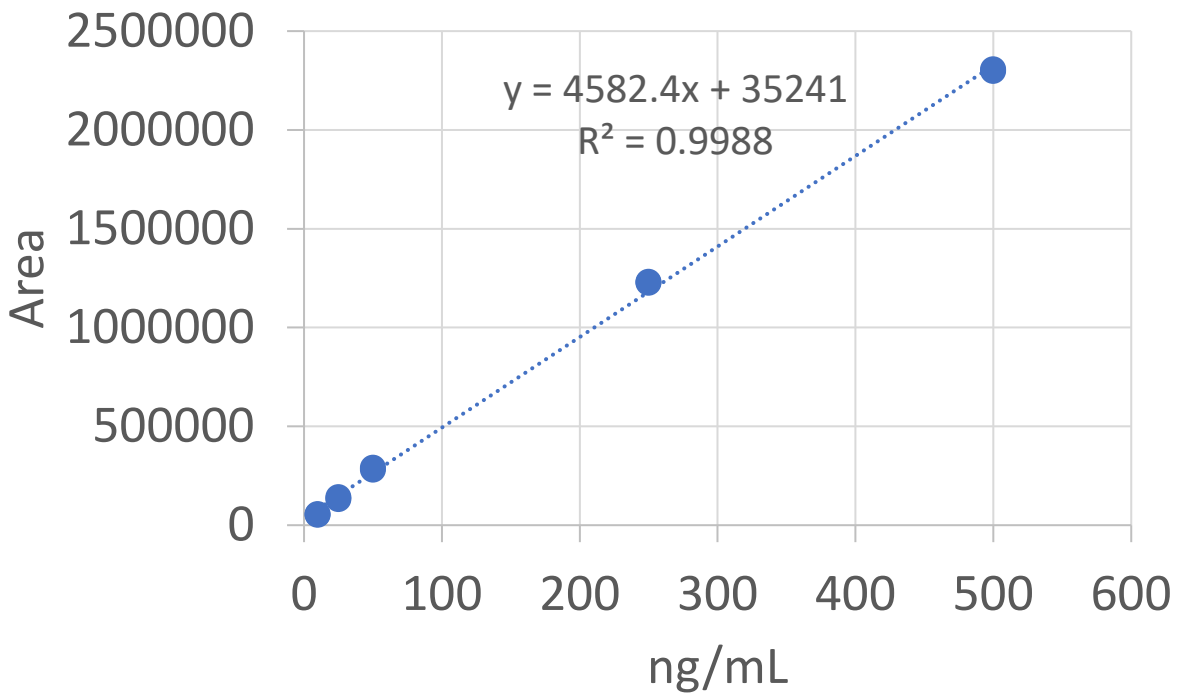


Xylazine

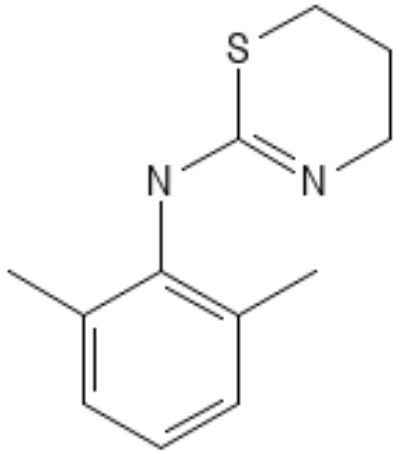


Fentanyl

Xylazine Calibration Curve

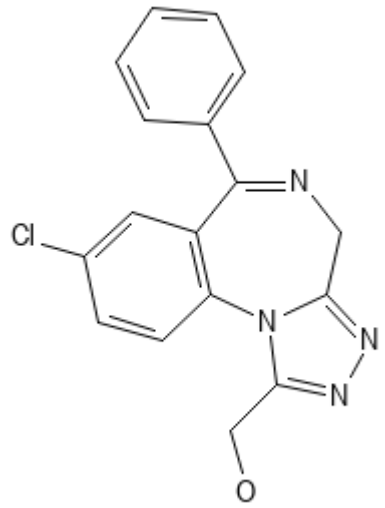


- Calibration curve for xylazine was run using the HALO® Biphenyl column
- Good linearity was observed from 10-500 ng/mL

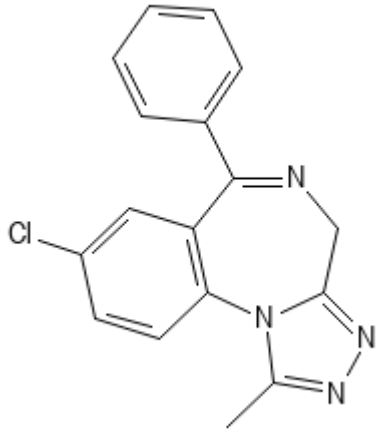


Observed Fronting Peak Shape

- 0.1% formic acid has a pH of 2.7
- The pH is too close to the pKa of alprazolam. It should be far enough away for the alpha-hydroxyalprazolam. Both compounds show fronting.



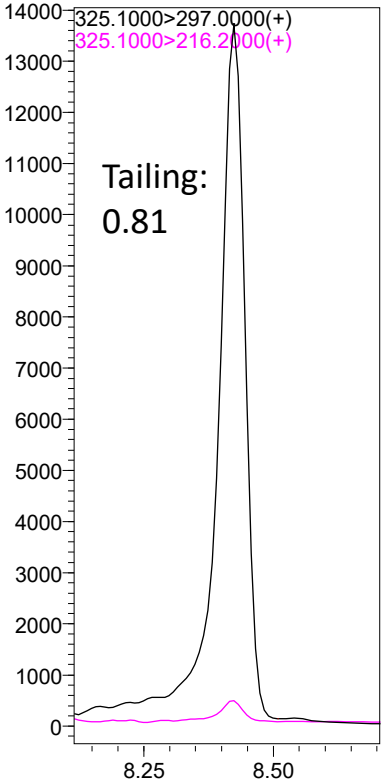
Alpha-hydroxyalprazolam
pKa = 4.9



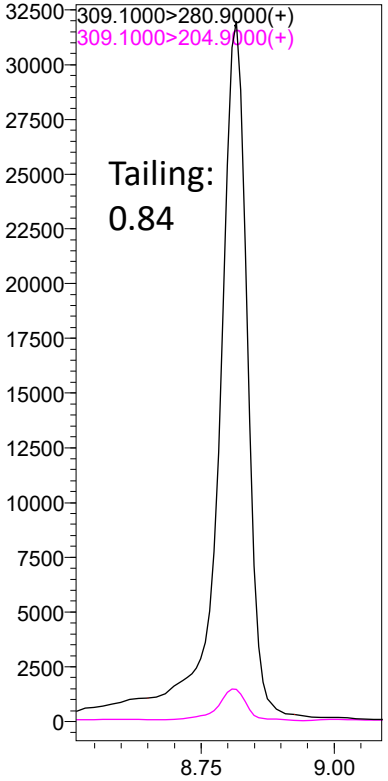
Alprazolam
pKa = 2.4

HALO® Biphenyl

0.1% Formic acid



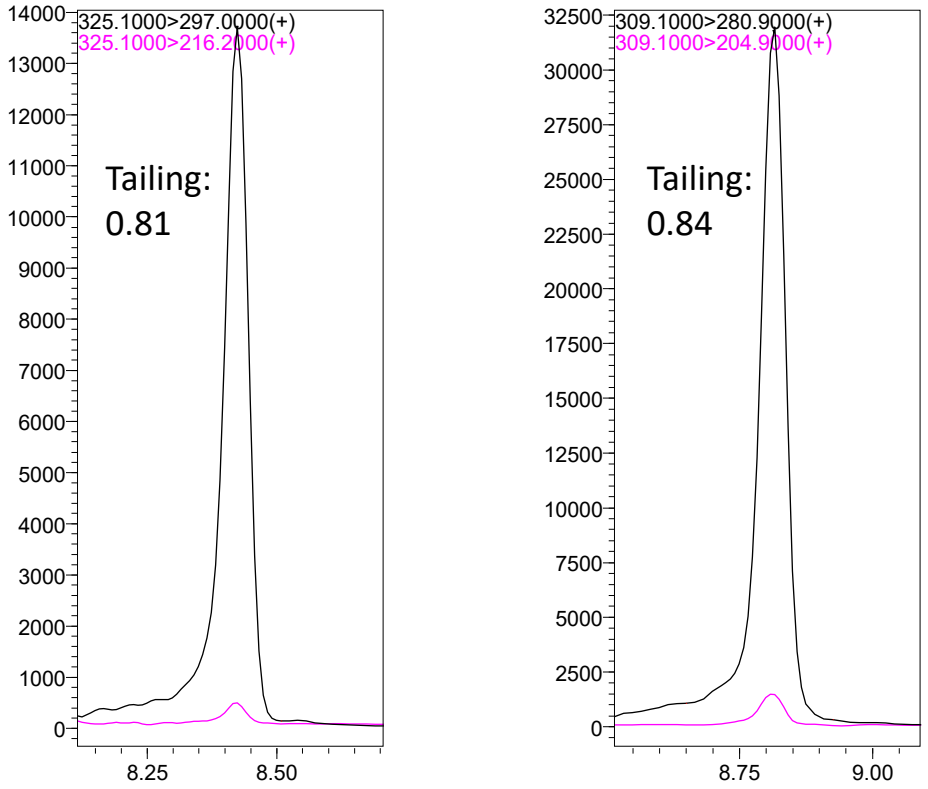
Alpha-hydroxyalprazolam



Alprazolam

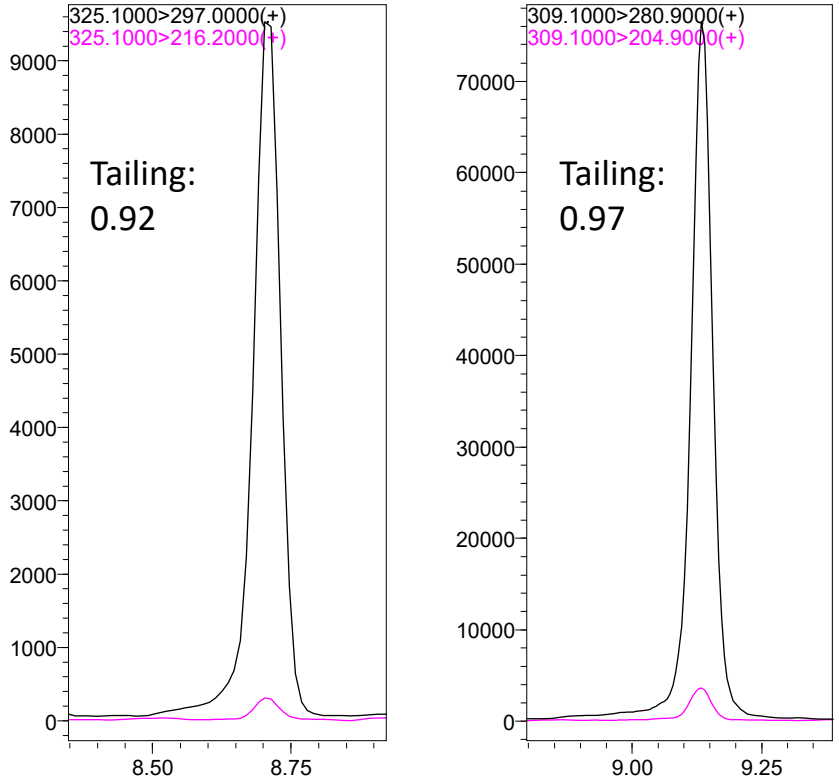
Improved Peak Shape with Increased pH

0.1% Formic acid/**pH 2.7**



Alpha-hydroxyalprazolam | Alprazolam

20 mM Ammonium Formate/ 0.1% Formic acid/**pH 3.6**

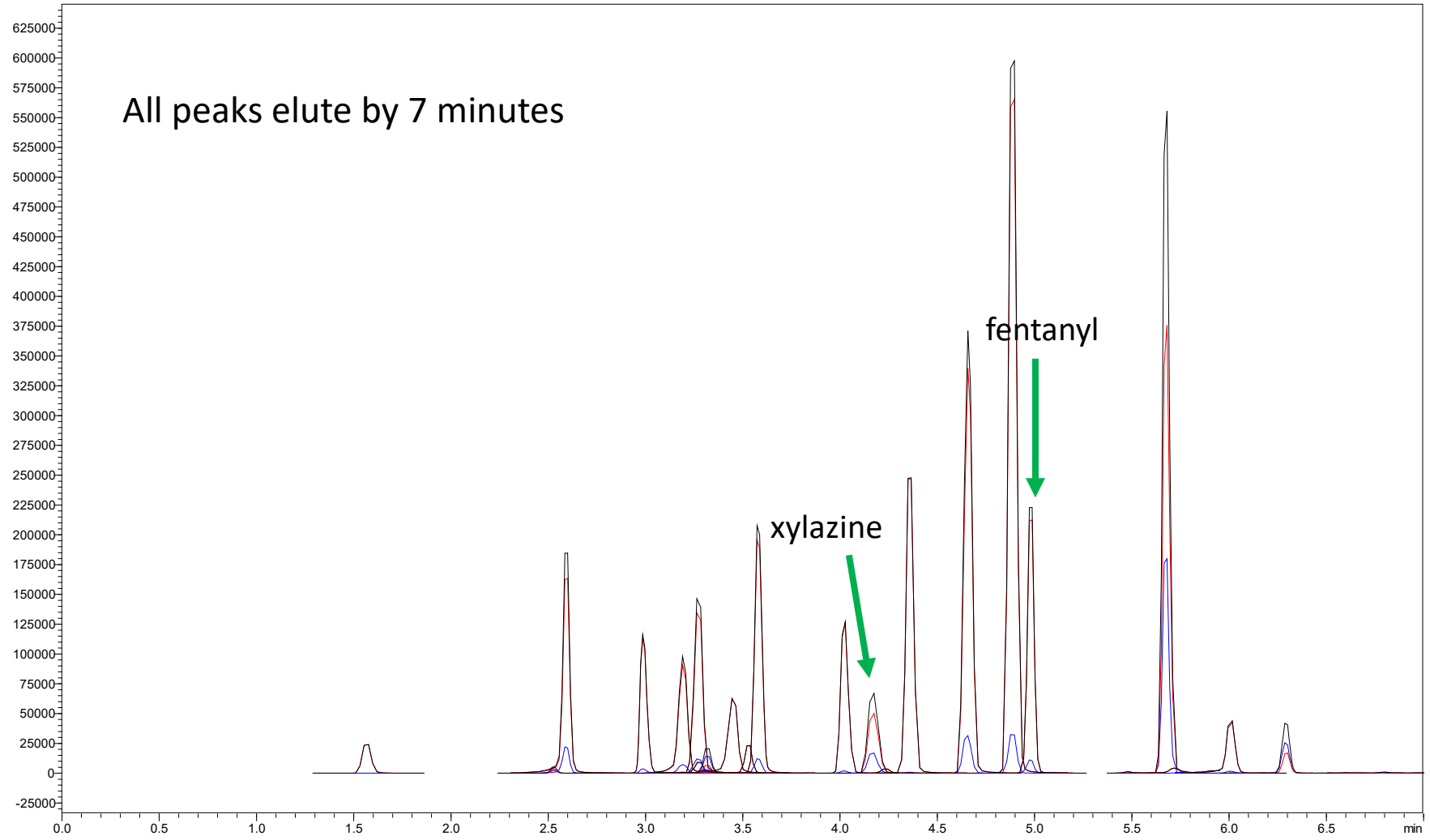


Alpha-hydroxyalprazolam | Alprazolam

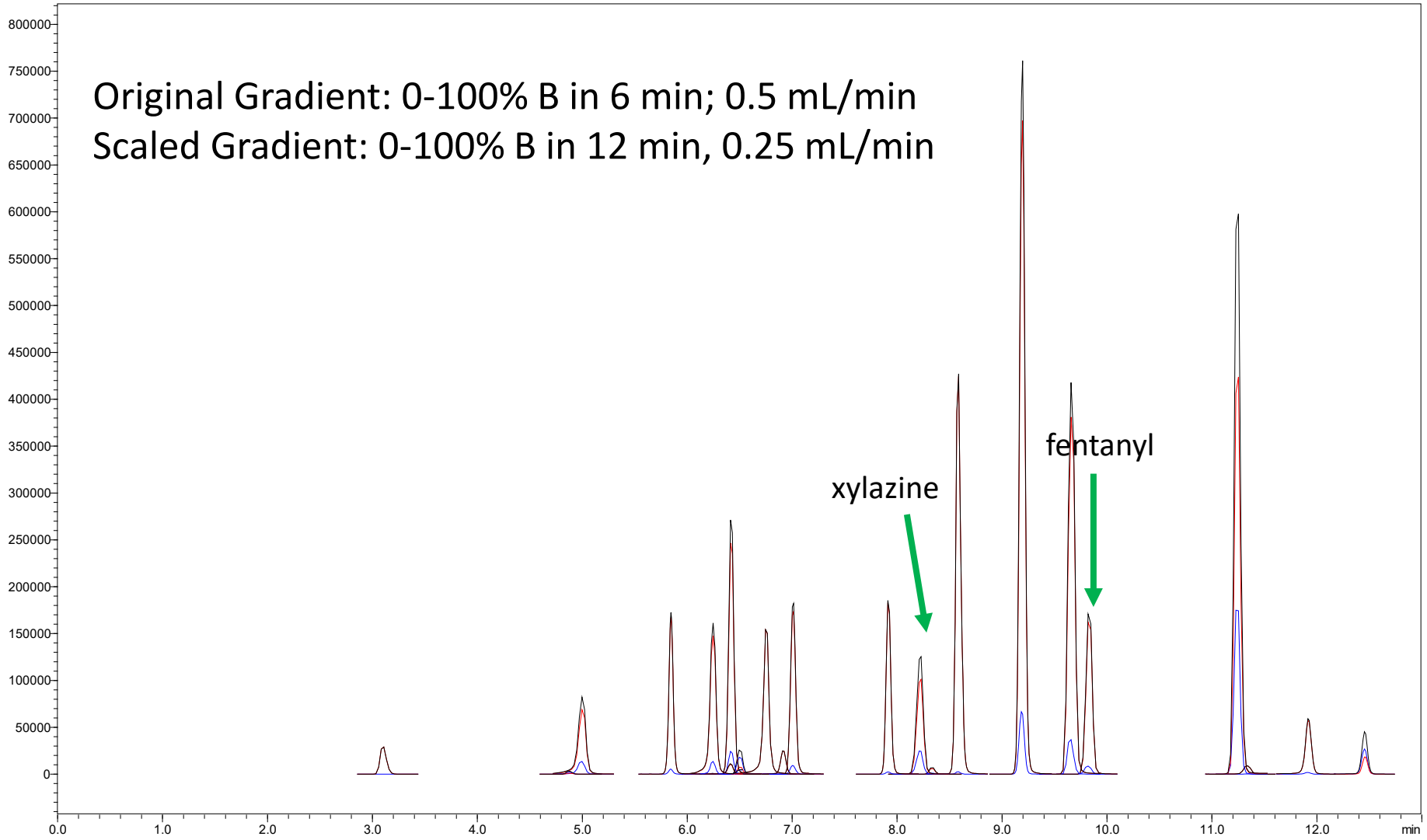
Gradient Optimization with HALO® Biphenyl

- Faster Method
  - Removed the 1 minute hold at 3% B
  - Used a linear gradient from 0-100% B in 6 minutes
  - Kept a 1 minute hold at 100% B from 6-7 minutes
  - Total time with re-equilibration is 12 minutes
- Used the same flow rate of 0.5 mL/min
- Added 20 mM ammonium formate/0.1% formic acid to both mobile phase A and mobile phase B for improved peak shape
  - Needed to use 95/5 ACN/water for mobile phase B for solubility of ammonium formate

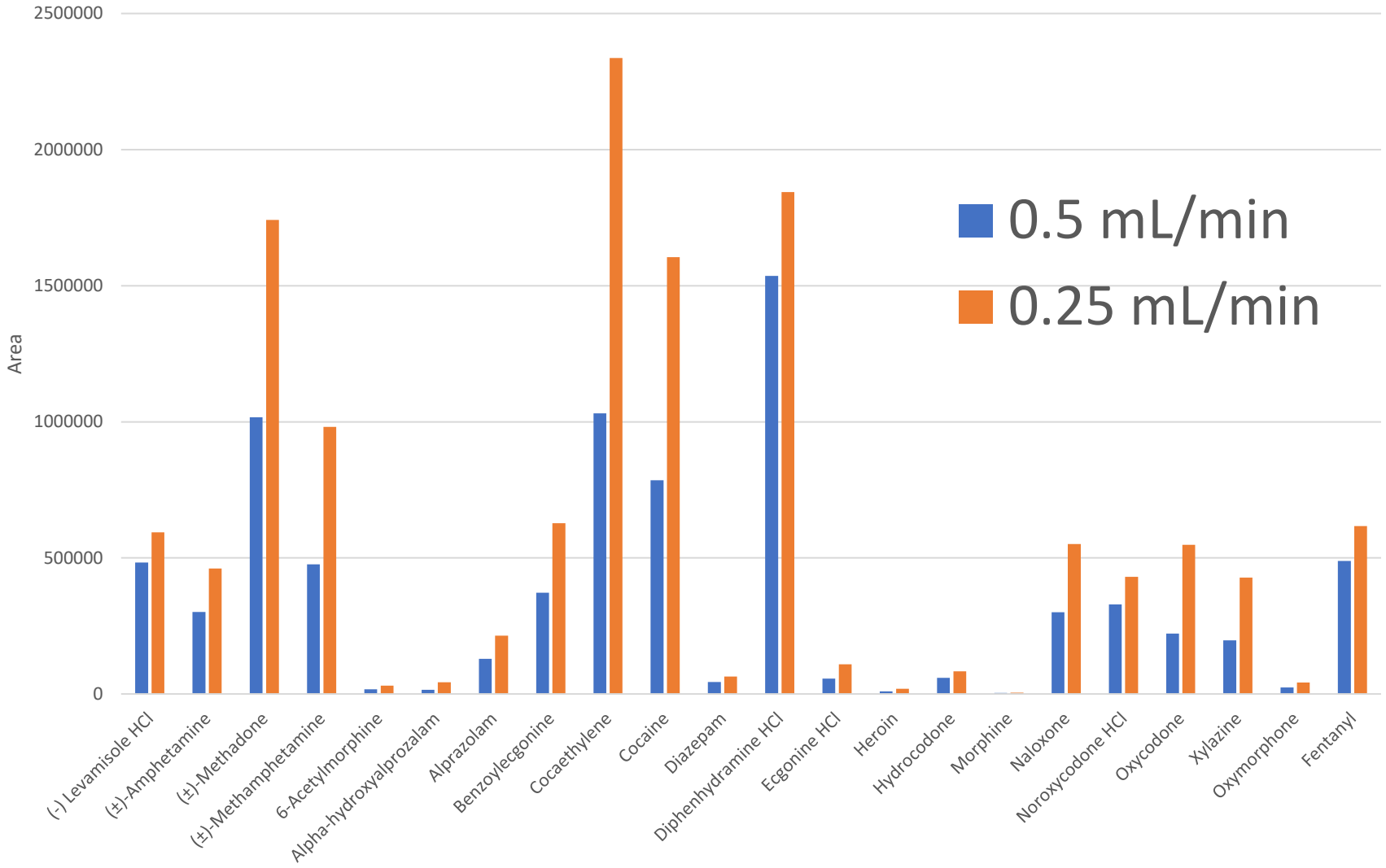
Faster Gradient on HALO® Biphenyl



Impact of Reduced Flow Rate



Larger Peak Areas with 0.25 mL/min compared to 0.5 mL/min



- Increased ionization efficiency with 0.25 mL/min flow rate

Summary

- HALO® Biphenyl showed the best compromise of retention and peak shape of the 4 phases screened
- Xylazine calibration showed a linear response
- 100% aqueous compatibility enables the gradient to begin at 0% B
- HALO® columns can be run at fast flow rates for increased throughput or at reduced flow rates for increased response, depending on the requirements of the analysis

Questions

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