# LC-MS Method Development and Column Screening for Pharmaceutical and Personal Care Products (PPCPs) in the Environment

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## Introduction

Common pharmaceutical and personal care products (PPCPs), such as over-the-counter medications, veterinary prescriptions, soaps, lotions, and even insect repellents, have become a growing concern to our environment. PPCPs can be detected at low levels in municipal wastewater, polluted ground water, and even drinking water and come from a variety of different sources. LC-MS method development and LC column screening is performed based on EPA 542 for PPCPs to determine whether phase chemistries other than the method recommended C18 will provide improved chromatographic resolution and a more accurate analysis.

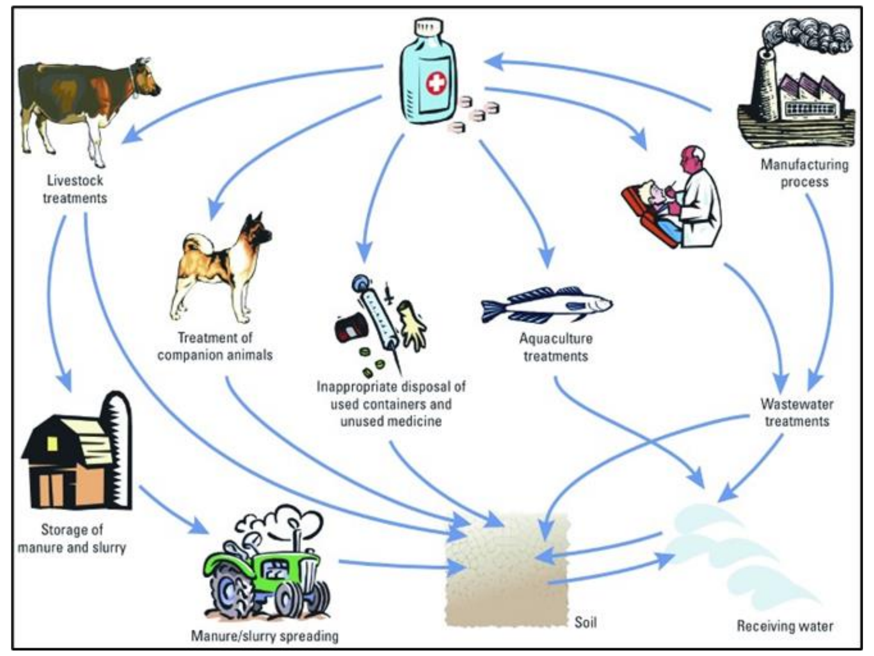



Figure 1: Major pathways of PPCP release into the environment. (nih.gov)

## EPA 542: Determination of PPCPs in Drinking Water by LC/ESI-MS/MS

**Table 1. HPLC Conditions (Positive ion electrospray)**

HPLC
Column: Waters Xterra® MS C18, 2.1 x 150 mm, 3.5 µm
Column temperature: 30 oC
Column flow rate: 0.200 mL/min
Autosampler temperature: 10 oC
Injection volume: 10 µL
Gradient:

| Time (min) | %5 mM ammonium acetate in 10% MeOH/90% reagent water[a] | %MeOH |
|---|---|---|
| 0.00 | 90 | 10 |
| 0.50 | 90 | 10 |
| 0.51 | 50 | 50 |
| 8.00 | 25 | 75 |
| 8.01 | 0 | 100 |
| 10.00 | 0 | 100 |
| 14.00 | 90 | 10 |
| 24.00 | 90 | 10 |

[a] Preparation of 5 mM ammonium acetate in 10% MeOH/90% reagent water: Combine 385 mg ammonium acetate and reagent water in a 1 L volumetric flask. Add 100 mL MeOH and dilute to volume.

**Table 2. HPLC Conditions (Negative ion electrospray)**

HPLC
Column: Waters Xterra® MS C18, 2.1 x 150 mm, 3.5 µm
Column temperature: 30 oC
Column flow rate: 0.200 mL/min
Autosampler temperature: 10 oC
Injection volume: 50 µL
Gradient:

| Time (min) | %5 mM ammonium acetate in 10% MeOH/90% reagent water[a] | %MeOH |
|---|---|---|
| 0.00 | 90 | 10 |
| 0.50 | 90 | 10 |
| 0.51 | 40 | 60 |
| 8.00 | 0 | 100 |
| 11.00 | 0 | 100 |
| 15.00 | 90 | 10 |
| 25.00 | 90 | 10 |

[a] Preparation of 5 mM ammonium acetate in 10% MeOH/90% reagent water: Combine 385 mg ammonium acetate and reagent water in a 1 L volumetric flask. Add 100 mL MeOH and dilute to volume.

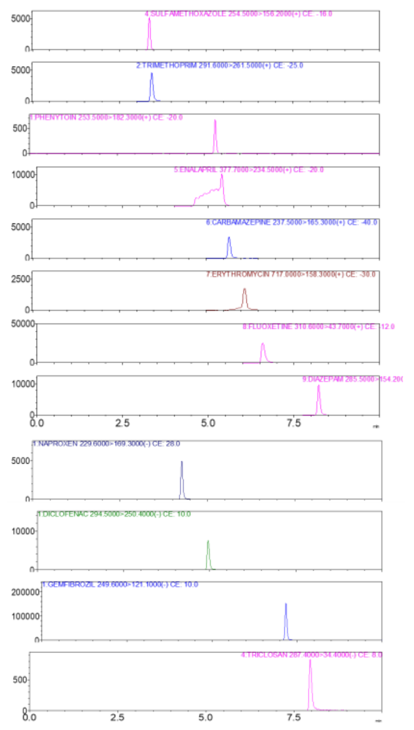
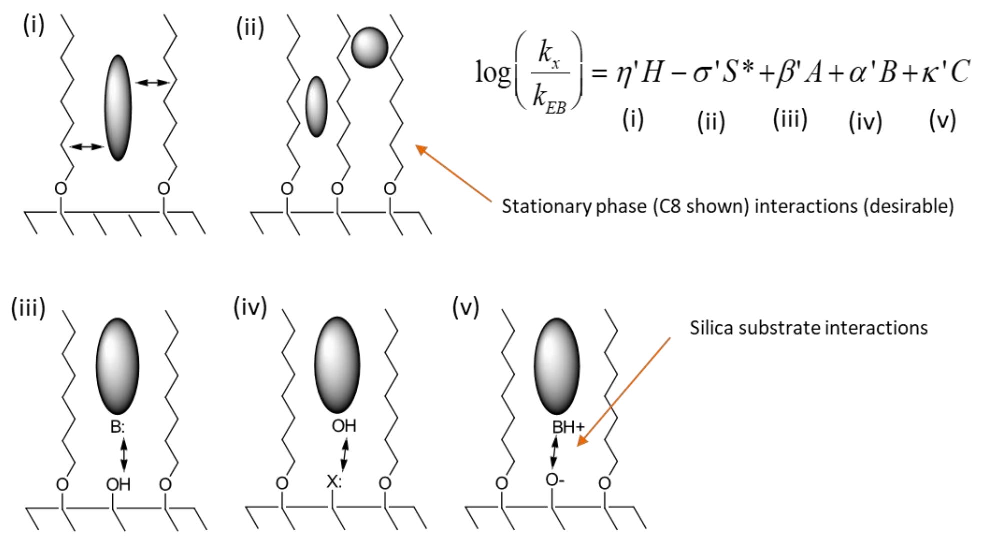


Figure 2: EPA 542 using a HALO 90 Å C18, 2.7µm 2.1x150mm

EPA 542 recommends using two different gradients with ammonium acetate and methanol as the mobile phases. The column of choice is a C18 column. Chromatogram shows the separation of 12 PPCP compounds performed on a HALO 90 Å C18 column.

## Hydrophobic Subtraction Model/ Phase Screening




$$\log\left(\frac{k_x}{k_{EB}}\right) = \eta' H - \sigma' S^* + \beta' A + \alpha' B + \kappa' C$$

(i) (ii) (iii) (iv) (v)

2. Lloyd R. Snyder, Joseph J. Kirkland and John W. Dolan, *Introduction to Modern Liquid Chromatography*, John Wiley and Sons, Inc. (2010).

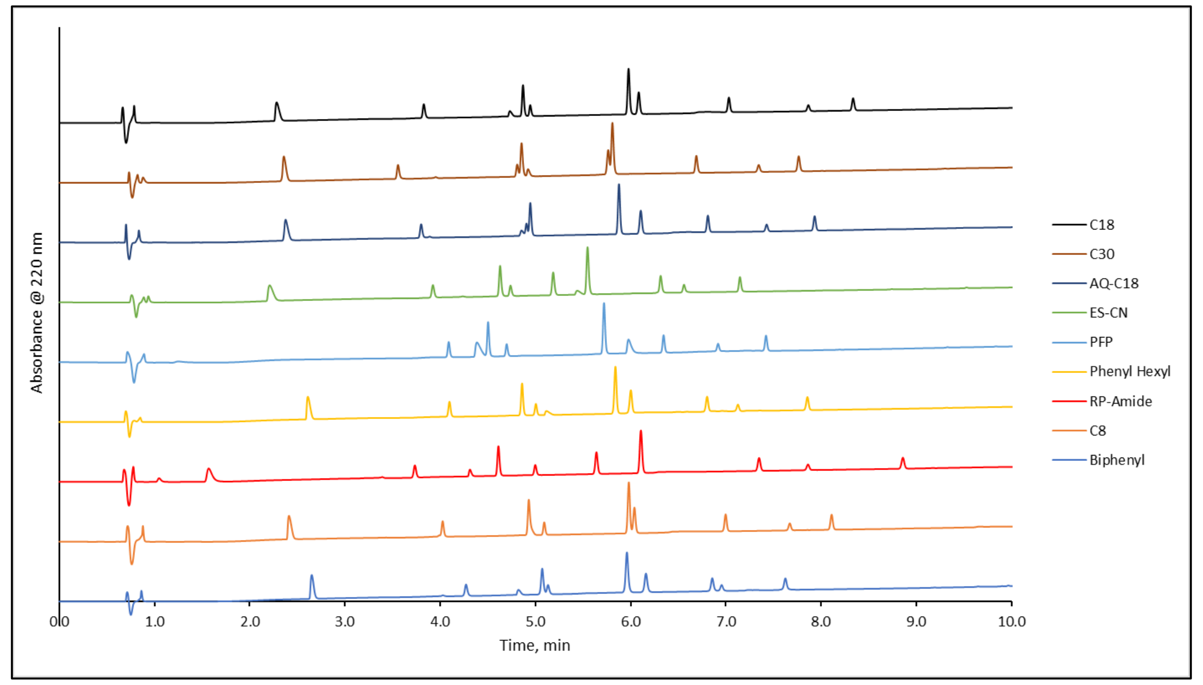
- H measures the hydrophobicity of the phase.
- S* measures the resistance of the stationary phase to penetration by a solute molecule.
- A measures the hydrogen-bond acidity of the phase.
- B measures the hydrogen-bond basicity of the phase.
- C measures the interaction of the phase with ionized solute molecules and is measured at pH 2.8 and 7.0.

Table 1. HSM values

| Fs | Phase | USP type | H | S* | A | B | C (pH 2.8) | C (pH 7.0) |
|---|---|---|---|---|---|---|---|---|
| 0 | HALO C18 | L1 | 1.100 | 0.040 | 0.000 | -0.050 | 0.050 | 0.040 |
| 10.04 | HALO C8 | L7 | 0.910 | 0.020 | -0.130 | 0.000 | -0.010 | 0.180 |
| 12.07 | HALO AQ-C18 | L1 | 1.000 | -0.036 | 0.099 | -0.048 | 0.156 | 0.864 |
| 17.35 | HALO Phenyl-Hexyl | L11 | 0.780 | -0.090 | -0.230 | 0.000 | 0.100 | 0.450 |
| 17.43 | HALO C30 | L62 | 0.938 | -0.046 | -0.140 | 0.023 | 0.170 | 0.350 |
| 22.78 | HALO ES-CN | L10 | 0.566 | -0.110 | -0.344 | 0.021 | 0.126 | 1.150 |
| 26.76 | HALO Biphenyl | L11 | 0.708 | -0.183 | -0.279 | 0.028 | 0.047 | 0.990 |
| 52.83 | HALO RP-Amide | L60 | 0.850 | 0.080 | -0.380 | 0.190 | -0.410 | 0.310 |
| 94.45 | HALO PFP | L43 | 0.702 | -0.117 | -0.073 | -0.062 | 1.170 | 0.972 |

Decreased Similarity to C18

*Note HSM values listed are for 2.7 µm particle size.

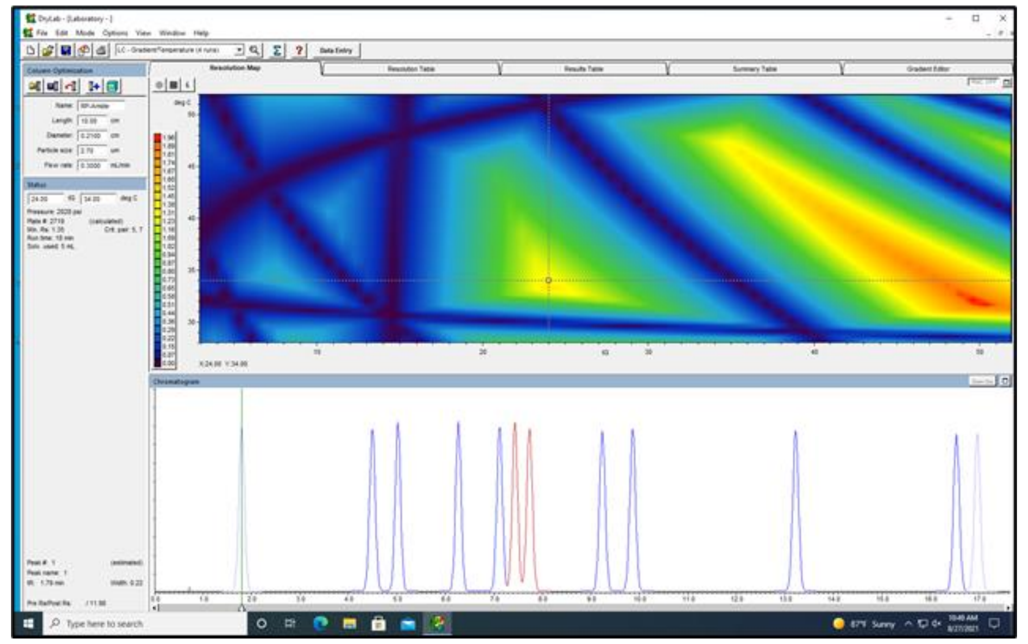



Column screening was performed using 9 different stationary phases (2.1x100mm) in order to increase resolution between PPCP compounds. Screening gradients were used (10-100%B in 10 min) with Water/ 0.1% formic acid and Acetonitrile/ 0.1% formic acid, 0.3 mL/min at 30˚C. The HALO RP-Amide stationary phase showed best overall resolution/ peak shape.

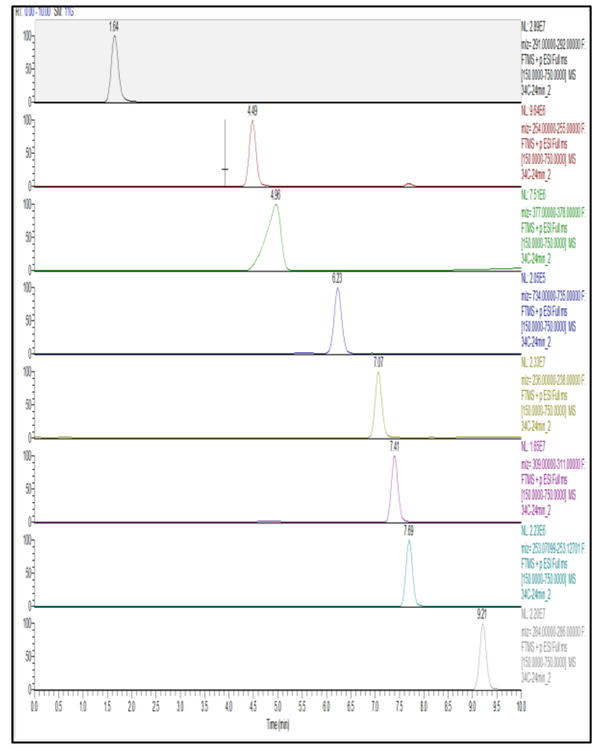
## EPA 542: Compound Table

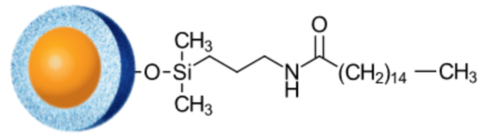
| | Analyte | Drug Category |
|---|---|---|
| 1 | Carbamazepine | Anticonvulsant |
| 2 | Diazepam | Anxiolytic and Sedative |
| 3 | Diclofenac | NSAID |
| 4 | Enalapril | ACE Inhibitor Medication |
| 5 | Erythromycin | Antibiotics and Gut Motility Stimulator |
| 6 | Fluoxetine | Selective Serotonin Reuptake Inhibitor |
| 7 | Gemfibrozil | Cholesterol Medication |
| 8 | Naproxen | NSAID |
| 9 | Phenytoin | Anticonvulsant |
| 10 | Sulfamethoxazole | Antibiotics |
| 11 | Triclosan | Antibacterial and Antifungal Agent |
| 12 | Trimethoprim | Antibiotics and Folate Synthesis Inhibitor |

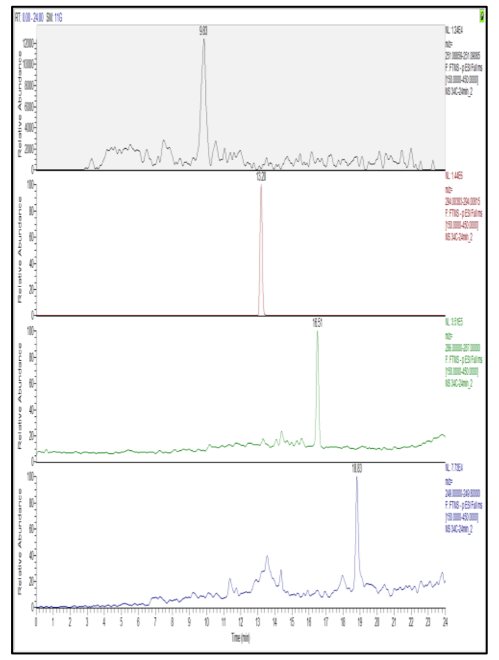
## DryLab® Optimization



## PPCP Analysis Using RP-Amide

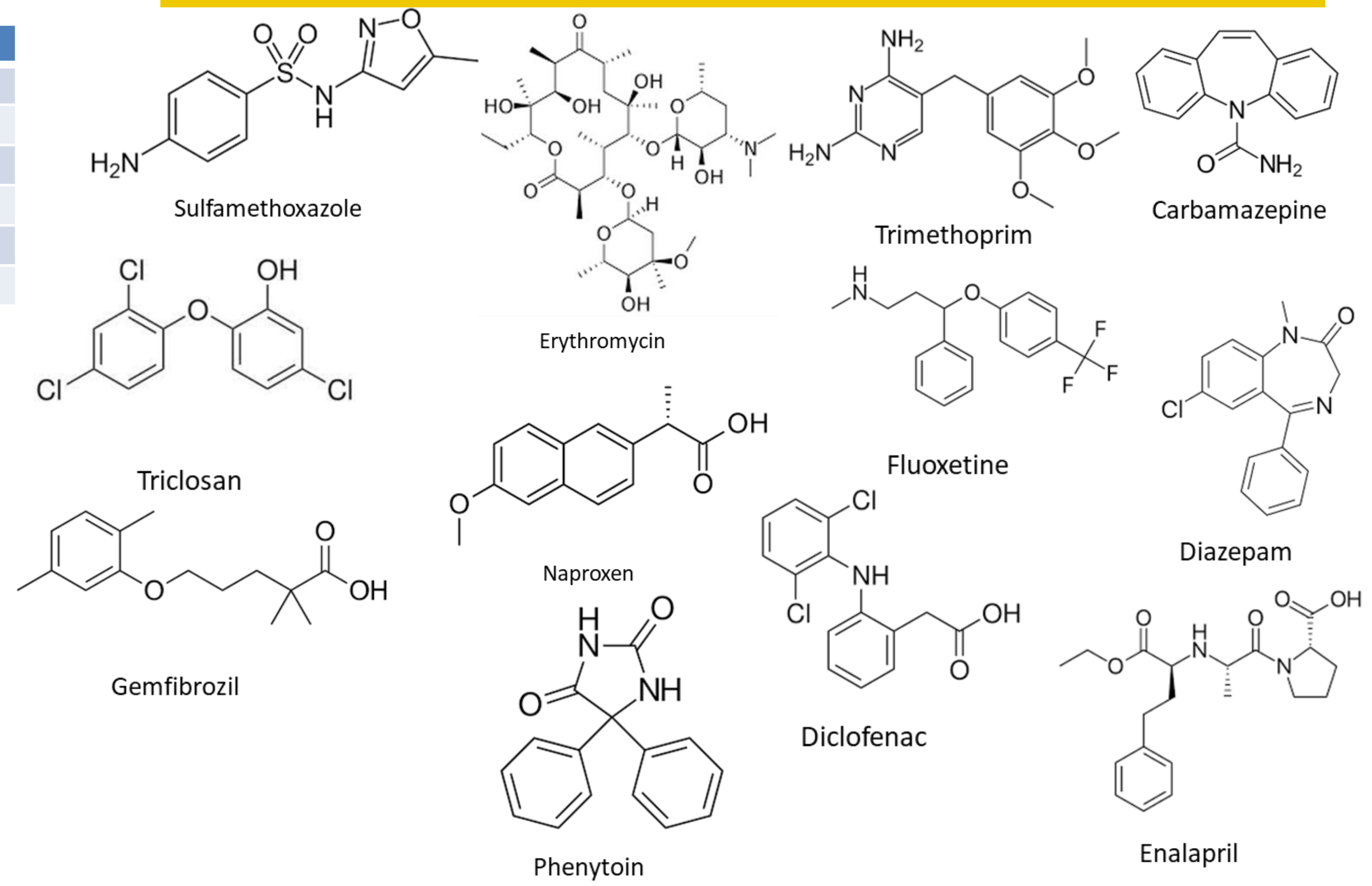




DryLab® method optimization further increased peak resolution using the RP-Amide stationary phase. Methods were combined into one gradient in order to increase throughput. Gradient: 10-100% B in 24 min with Water/ 0.1% formic acid and Acetonitrile/ 0.1% formic acid, 0.3ml/min at 34˚C.

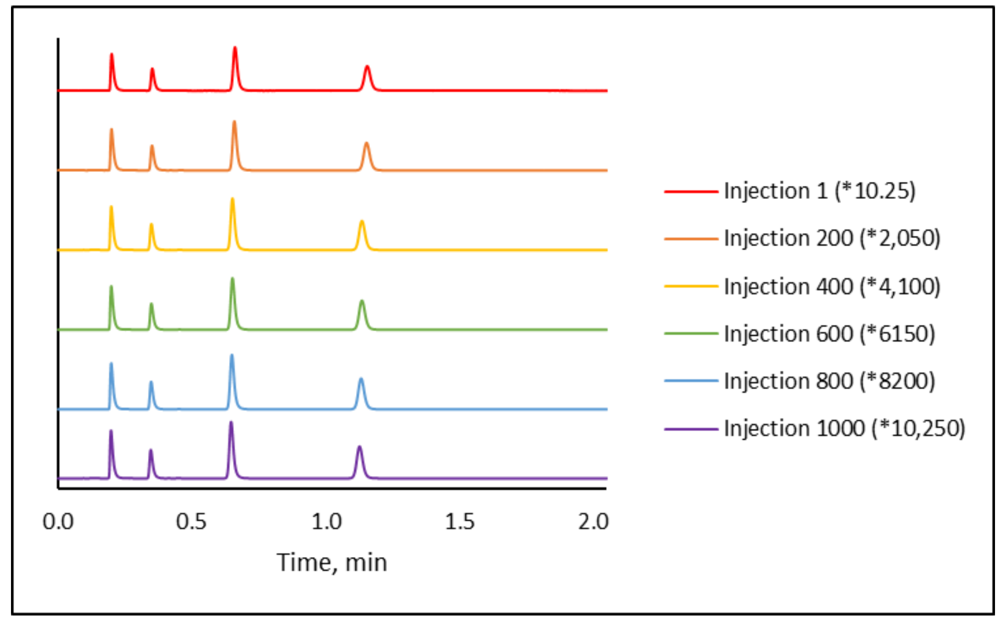
## Structures




## RP-Amide Stability

TEST CONDITIONS:
Column: HALO 90Å RP-Amide, 2.7µm, 2.1x100mm
Part Number: 92812-607
Mobile Phase A: Water
Mobile Phase B: ACN
Flow Rate: 1 mL/min
Temperature: 30 °C
Detection: UV, 254 nm
Back Pressure: 518 bar
Injection Volume: 0.5 µl
Sample: uracil, phenol, 1 Cl-4- nitrobenzene, naphthalene

A HALO 90 Å RP-Amide column shows excellent stability using a mixture of neutral compounds.




## Conclusions

Over the next century, the combination of increasing global population size and potential droughts may result in reduced water availability, increased need for water reuse, and increasing concentrations of PPCP in wastewaters. The current wastewater treatment methods do not remove all PPCP effectively. This, coupled with the possibility that antibiotics may promote the development of antibiotic-resistant bacteria and antibiotic-resistant genes, leads to concerns about the sustainability of global water supplies. Improved chromatographic methods can help this environmental concern by showing higher resolution and efficiency compared to existing validated methods.