HALO®

TECHNICAL REPORT

TITLE: **LC-MS METHOD DEVELOPMENT AND COLUMN SCREENING FOR PHARMACEUTICAL AND PERSONAL CARE PRODUCTS (PPCPS) IN THE ENVIRONMENT**

MARKET SEGMENT: ENVIRONMENTAL

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ABSTRACT:

Pharmaceutical and personal care products (PPCPs) have been a growing concern to our environment which include prescription and over-the-counter medications, veterinary drugs, soaps, lotions, and even insect repellents. These products have entered the environment through various sources which permeate the water table, contaminating wastewater, ground water, and even drinking water. Validated LC-MS methods have been completed in order to screen for these wide range of chemical compounds which can further be optimized in order to achieve better resolution and selectivity. LC-MS method development is performed based on the EPA 542 PPCP method in order to achieve an improved chromatographic resolution and selectivity for environmental applications.

INTRODUCTION:

Pharmaceutical and personal care products that are a concern to the environment range from a wide variety of compounds and come from a variety of different sources. PPCPs include prescription and non-prescription human drugs, illegal drugs, and veterinary drugs, as well as their subsequent metabolites and conjugates, including antibiotics, hormones, anticonvulsants, antidepressants, lipid regulators, antihypertensives, and nonsteroidal anti-inflammatory drugs. PPCPs also include sunscreen, soaps, moisturizers, lipsticks, fragrances, insect repellent, and shampoo.[1] There are many different ways that these chemicals can enter the environment. Whether through a manufacturing process, aquaculture treatments, inappropriate disposal of unused medicine, treatment of animals (pets), and livestock treatments these chemicals eventually enter the soil or wastewater treatment plants which then leads to receiving water. Figure 1 represents the variety of sources where these chemicals can come from.

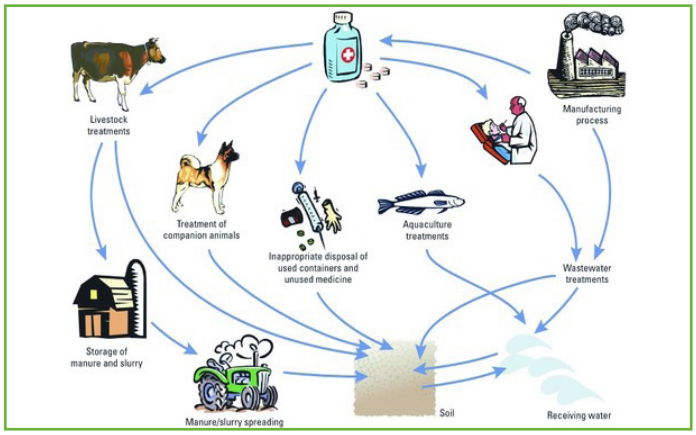


Figure 1: Common sources of PPCP in the environment (nih.gov)

KEY WORDS:

pharmaceutical, personal care products, superficially porous particles, HPLC

These broad range of chemical compounds lead to a wide variety of chemical structures and can make it challenging to analyze, especially at very low levels around the parts per trillion (ng/L) range. Because of this, LC-MS detection is needed. Choosing the right method conditions such as mobile phases, acidic modifiers, gradient, and column selection can all lead to an overall better separation. Column screening was performed in order to choose the best stationary phase along with the use of method optimization software to further improve the method.

EXPERIMENTAL DATA:

Method development is based on the EPA method 542: Determination of Pharmaceuticals and Personal Care Products in Drinking Water by Solid Phase Extraction and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS). This method screens twelve common PPCP compounds that are listed in Figure 2.

Analyte	Drug Category	Use
Carbamazepine	Anticonvulsant	can treat seizures, nerve pain, and bipolar disorder
Diazepam	Anxiolytic and Sedative	can treat anxiety, muscle spasms, and seizures
Diclofenac (sodium salt)	NSAID	can treat pain, migraines, and arthritis
Enalapril (maleate salt)	ACE inhibitor medication	can treat high blood pressure, diabetic kidney disease, and heart failure
Erythromycin	Antibiotics and Gut motility stimulator	can treat infections/ acne
Fluoxetine (HCl)	Selective Serotonin Reuptake Inhibitor (SSRI)	can treat depression, OCD, bulimia nervosa, and panic disorder
Gemfibrozil	Cholesterol Medication	can lower high cholesterol and triglyceride levels in the blood
Naproxen	NSAID	can treat fever and pain
Phenytoin	Anticonvulsant	can treat and prevent seizures
Sulfamethoxazole	Antibiotics	can treat or prevent infections
Triclosan (Irgasan)	Antibacterial and Antifungal Agent	antibacterial/ antifungal agent present in some consumer products (toothpaste, soap, detergents)
Trimethoprim	Antibiotics and Folate synthesis inhibitor	can treat infections, including urinary tract and ear infections

Figure 2: EPA 542 analyte list

EPA 542 recommends using a C18 column with two separate gradients, one for positive ion electrospray, and the other for negative due to protonation or deprotonation of an analyte. Water and methanol mobile phases using ammonium acetate are used for the analysis. These methods can be seen in Tables 1 and 2 followed by the MS method conditions in Tables 3 and 4.

Table 1. HPLC Conditions (Positive ion electrospray)

HPLC
Column: Waters Xterra® MS C18, 2.1 x 150 mm, 3.5 µm
Column Temperature: 30 °C
Column Flow Rate: 0.200 mL/min
Autosampler Temperature: 10 °C
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 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 °C
Column Flow Rate: 0.200 mL/min
Autosampler Temperature: 10 °C
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.

Table 3. Positive Mode ESI-MS/MS Method Conditions

MS Parameter	HPLC-MS/MS
Polarity	Positive ion electrospray
Capillary Voltage, kV	2.50
Source Temperature, °C	120
N2 Desolvation Temperature, °C	400
N2 Desolvation Gas Flow, L/hr	900
Cone Gas Flow, L/hr	50
Extractor Lens, V	2.00
RF Lens, V	0.2

Table 4. Negative Mode ESI-MS/MS Method Conditions

MS Parameter	HPLC-MS/MS
Polarity	Negative ion electrospray
Capillary Voltage, kV	2.50
Source Temperature, °C	120
N2 Desolvation Temperature, °C	400
N2 Desolvation Gas Flow, L/hr	900
Cone Gas Flow, L/hr	50
Extractor Lens, V	1.00
RF Lens, V	0.1

A HALO 90 Å C18, 2.7 µm, 2.1 x 150 mm column from Advanced Materials Technology, Inc. (Wilmington, DE) was used for the initial analysis. In order to further increase peak resolution, a column screening approach was performed using a scouting gradient and nine different stationary phases (2.7 µm, 2.1 x 100 mm) from Advanced Materials Technology, Inc. The best performing stationary phase as determined by overall best selectivity and resolution was then used for DryLab® optimization to further improve the separation. Scouting gradient conditions used for the column screening experiment are shown in Table 5 followed by DryLab® optimization conditions in Table 6.

Table 5. Scouting Gradient

TEST CONDITIONS:
Column: HALO® 2.7 µm, 2.1 x 100mm
Mobile Phase A: Water, 0.1% FA
Mobile Phase B: Acetonitrile, 0.1% FA
Gradient:

Time	%B
0.0	10
0.5	10
10	100
11	100

Flow Rate: 0.3 mL/min
Temperature: 30°C
Detection: 220 nm, PDA
Injection Volume: 1.0 µL
Sample Solvent: 50/50 Water/MeOH
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL

Table 6. DryLab® optimization method conditions

TEST CONDITIONS:
Column: HALO 90 Å RPA, 2.7 µm, 2.1 x 100mm
Mobile Phase A: Water, 0.1% FA
Mobile Phase B: Acetonitrile, 0.1% FA
Gradient:

Time	%B
0.0	10
18.0	67.5

Flow Rate: 0.3 mL/min
Temperature: 34 °C
Detection: LC-MS/MS
Injection Volume: 1.0 µL
Sample Solvent: 50/50 Water/MeOH

All experiments were conducted on a Shimadzu Nexera HPLC instrument using LabSolutions software (Shimadzu Scientific Instruments, Columbia, MD). A UV diode array detector (1 µL flow cell) was used for the scouting gradient experiments. Initial LC/MS runs were performed on a Shimadzu 8040 LC-MS/MS and finalized using a Thermo Q-Exactive (Waltham, MA). Standards were obtained from Millipore Sigma (St. Louis, MO). Methanol (MS grade), Acetonitrile (MS grade), water (HPLC grade), formic acid, and ammonium acetate were purchased from Millipore Sigma (Burlington, MA).

Analytical standards were prepared at 1000 µg/mL in 50/50 methanol/ water and used as stocks. LC-MS analysis required dilution of standards to 8.33 µg/mL with water for column screening and method development to better serve MS analysis.

RESULTS:

The original EPA 542 method was performed on a HALO 90 Å C18, 2.7 µm 2.1 x 150 mm column. These results can be seen in Figure 3. This method requires two separate multi-step gradients using ammonium acetate and methanol as the mobile phases.

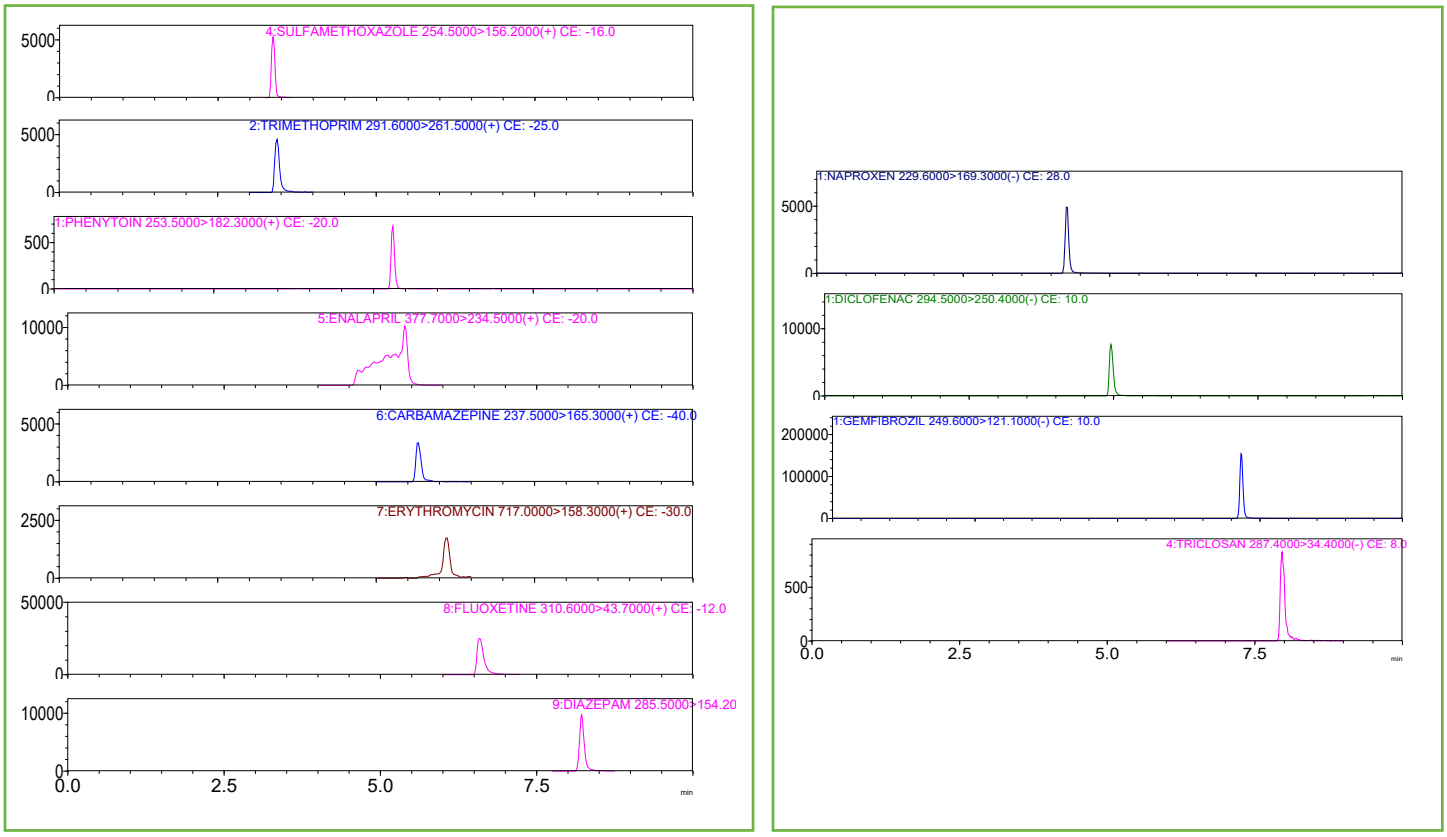


Figure 3: PPCP separation on HALO® C18 following EPA 542

The C18 stationary phase is known for being a very universal phase, ideal for many different types of compounds. However, C18 is not always the best column of choice. Many different phases exist to help improve peak shape and resolution and, in some cases, show advantages compared to C18. During method development, different stationary phases should be screened in order to make sure maximum resolution is achieved. Figure 4 shows a PPCP panel screened on nine different stationary phases using the scouting gradient (Table 5).

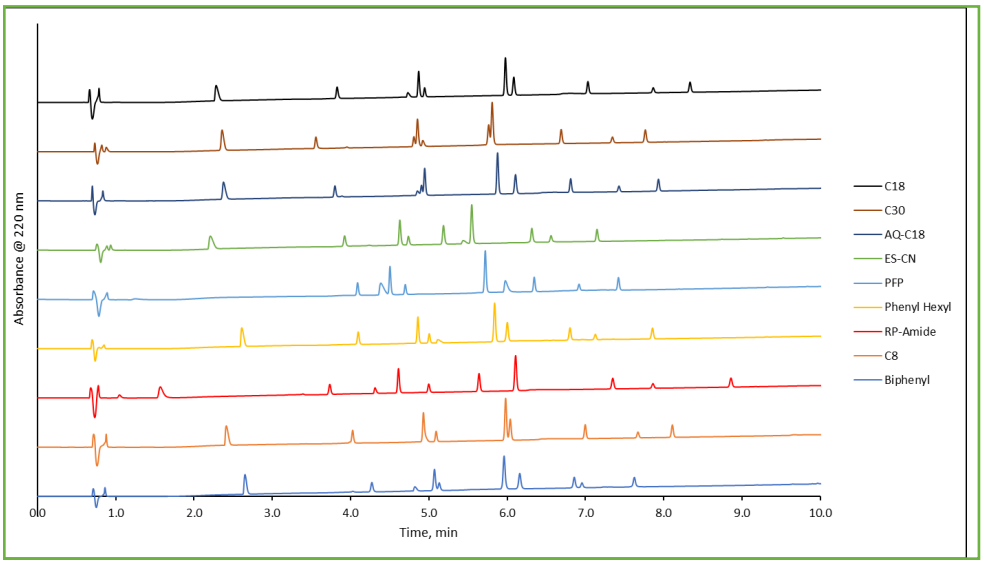


Figure 4: PPCP phase screening using a scouting gradient

The dimethylpalmitamideopropylsilane, better known as the RP-Amide stationary phase showed the overall best selectivity and resolution compared to other available phases. (red trace in Figure 4). This phase is ideal for reversed-phase separations of basic compounds as well as alcohols, acids, phenols, and catechins. The functionality of polar embedded phases can be attributed to the proximity of the polar group to the silica surface, allowing hydrogen bonding to occur with unreacted silanols, deactivating them making the surface base-friendly. Additionally, the presence of the polar group near the surface allows more water in the mobile phase to get near the silica surface making the column less hydrophobic and friendlier to separations of polar solutes. The RP-Amide stationary phase can be seen in Figure 5.

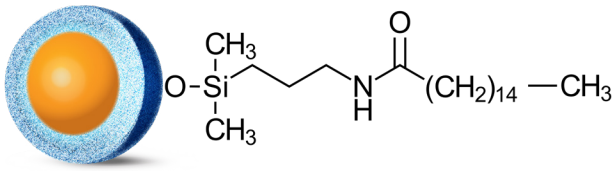
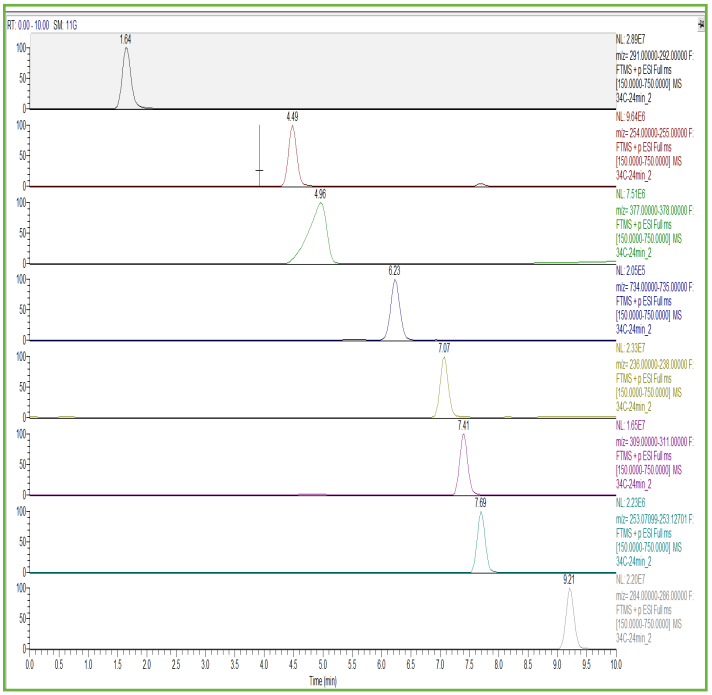


Figure 5: HALO® RP-Amide stationary phase

Method optimization software such as DryLab® can be used in order to further increase method performance. This software helps predict chromatograms under a wide range of experimental conditions and allows for quicker method development for complex samples while further improving method validation. Figure 6 shows the PPCP panel under DryLab® recommended conditions.



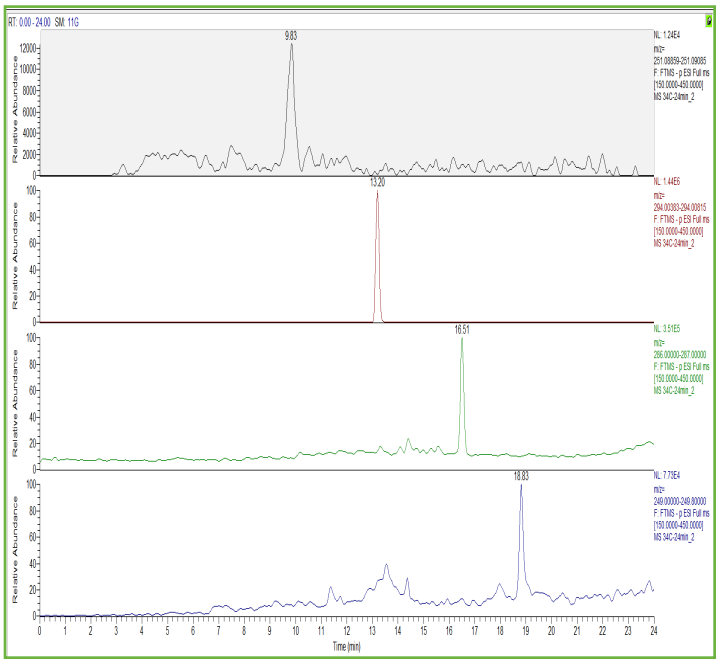


Figure 6: DryLab® optimization of separation with RP-Amide stationary phase

CONCLUSION:

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 PPCPs in water systems. The current wastewater treatment methods do not remove all PPCPs 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.[1] This work serves to show how screening columns and conducting method development with available software tools for optimal method conditions can lead to improved and faster separations.

REFERENCES:

1. Cizmas L, Sharma VK, Gray CM, McDonald TJ. Pharmaceuticals and personal care products in waters: occurrence, toxicity, and risk. Environ Chem Lett. 2015 Dec;13(4):381-394. doi: 10.1007/s10311-015-0524-4. Epub 2015 Aug 26. PMID: 28592954; PMCID: PMC5459316.