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TITLE: **USING ELEVATED PH FOR IMPROVED ANALYSIS OF SAMHSA PANEL AND RELATED DRUGS OF ABUSE**

MARKET SEGMENT: CLINICAL/TOXICOLOGY

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ABSTRACT

When performing an LC analysis of drugs of abuse, the typical functional groups used for separations in reversed-phase include biphenyl, C18, phenyl hexyl, and PFP. Typically the separation is run at acidic pHs, posing problems with peak shape and retention due to the weakly basic nature of many of the compounds in the panel. To address this, separations with mobile phases at different pHs were investigated to understand how it affects the peak shape and separation of illicit drugs. Through this study, it was shown that when using a pH above the pKa of the analyte of interest, the compound is neutralized, improving both peak shape and increasing retention.

INTRODUCTION

Substance use disorders and the detection of their related compounds are important in various settings to ensure the safety and proper treatment for the individual involved. The Substance Abuse and Mental Health Services Administration (SAMHSA) has set guidelines indicating certain drugs and their metabolites to be included in clinical and workplace drug testing (1). The illicit drug classes identified for testing by SAMHSA include opioids, amphetamines, cocaine, cannabinoids, and phencyclidine.

When looking to separate these compounds for confirmatory testing, a reversed-phase LC column paired with acidic pH conditions are the typical chosen parameters (2-7). Reasons for this combination include: first, when performing detection by MS, formic acid, a typical acidic modifier, aids in the ionization of compounds due to its enhanced volatility; second, improved peak shape from the reduced ionization of the surface silanols on the stationary phase, reducing secondary interactions and reducing peak tailing; third, to shift the pH away from the pKa of the compounds of interest, reducing the change in selectivity and retention for a given compound (8). Unfortunately, these conditions are frequently chosen for many different separations, but should instead be catered to the specific set of compounds studied. In the case of the panel of drugs studied here, 17 of the 18 compounds have weakly basic functionality, with one showing acidic functionality and one showing both basic and acidic. With this, their pKa's are around 8-10 (9) as shown in Table 1, indicating them to be effectively 100% protonated and in their positively charged form under these acidic pH conditions. This results in two issues, increased charged compound repulsions within a column separating based on polarity (resulting in peak tailing), and reduced overall retention due to the large differences in polarity and charge between the analyte and the hydrophobic stationary phase.

Compound	pKa	Compound	pKa
Morphine	8.18	Oxycodone	8.53
Hydromorphone	8.2	6-Acetylmorphine	9.08, 10.19
Oxymorphone	8.17	Norfentanyl	10.03
Codeine	8.2	Fentanyl	8.43
4-Hydroxy Xylazine	9.61, 10.33	MDA	9.67
Xylazine	6.94	MDMA	9.9
d-Amphetamine	9.94	Benzoylecgonine	3.15, 9.54
Methamphetamine	9.99	PCP	8.29
Hydrocodone	8.23	THC-COOH	4.02, 9.48

Table 1. SAMHSA drugs and metabolites pKa's

KEY WORDS:

SAMHSA drugs of abuse, high pH separations, HALO 120 Å Elevate C18 column, LC-UV, LC-MS, fentanyl, xylazine, drug metabolites

To address these issues, there are several potential solutions. Higher ionic strength can be used to alleviate the charged compound repulsions between charged molecules, reducing the intermolecular repulsions, improving peak tailing (8). Introducing an ion-pairing reagent would form an equilibrium between the positively charged base analytes and the negatively charged pairing reagent, improving analyte retention and peak shape (10). However, a major drawback to these two options is the frequent incompatibility with mass spectrometric detection due to nonvolatile salts used here, suppressing ionization and contaminating the detector. Alternatively, increasing the pH to ≥2 units above the pKa's of these compounds would result in effective neutralization of the compounds (11), eliminating the charge repulsions seen at low pH, and in turn improving both peak tailing and analyte retention. Additionally, utilizing a volatile high pH additive, such as 0.1% ammonium hydroxide, allows for improved ionization and is MS compatible. As such, implementing the HALO® Elevate C18 phase, a high pH compatible reversed-phase column, alongside increasing the mobile phase pH to improve peak shape and retention of weakly basic drugs of abuse was studied here. It should be clear through this study, that as the pH of the mobile phase is increased, retention increases and the overall peak shape of the analytes improves.

EXPERIMENTAL:

A Shimadzu Nexera X2 UHPLC (Columbia, MD) and an LCMS-8040 triple quadrupole mass spectrometer were used for the experiments. The drugs of abuse standards were obtained from Cerilliant (Round Rock, TX), MilliporeSigma (Burlington, MA) and Cayman Chemicals (Ann Arbor, MI). A HALO® Elevate C18 (Advanced Materials Technology, Wilmington, DE) column was used for the experiments. Solvents and additives were purchased through MilliporeSigma (Burlington, MA). DryLab® modeling software (Molnár Institute, Berlin, Germany) was utilized for the chromatographic method optimization.

TEST CONDITIONS:

Column: HALO 120 Å Elevate C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92272-602
Mobile Phase A1: Water + 0.1% Formic Acid (pH = 2.75)
Mobile Phase A2: Water + 10 mM Ammonium Acetate (pH = 4.99)
Mobile Phase A3: Water + 0.1% Ammonium Hydroxide (pH = 11.02)
Mobile Phase B: Methanol
Gradient 1: (Time - %B) 0 min – 5%, 15 min – 95%, 16 min – 95%, 16.1 min – 5%, 21 min – 5%
Temperature: 30 °C

Gradient 2:(Time - %B) 0 min – 10%, 1 min – 10%, 6.3 min – 40%, 8.5 min – 46%, 12 min – 100%, 13 min – 100%, 13.1 min – 10%, 19 min – 10%
Temperature: 50 °C

MS SOURCE CONDITIONS:

Spray Voltage: 4.5 kV
Nebulizing gas: 2 L/min
Drying gas: 10 L/min
DL Temperature: 200 °C
Heat Block Temperature: 300 °C

DryLab testing conditions:

15 min gradient, 45 min gradient (at 30 °C)
15 min gradient, 45 min gradient (at 60 °C)

RESULTS:

pH Comparison

The selected SAMHSA drugs of abuse panel was run on a HALO® Elevate C18 at three different pHs, 2.75 (A1), 4.99 (A2), and 11.02 (A3), utilizing a scouting gradient from 5% to 95% methanol over 15 minutes (Gradient 1). A comparison of the separations at these pHs is shown in Figure 1.

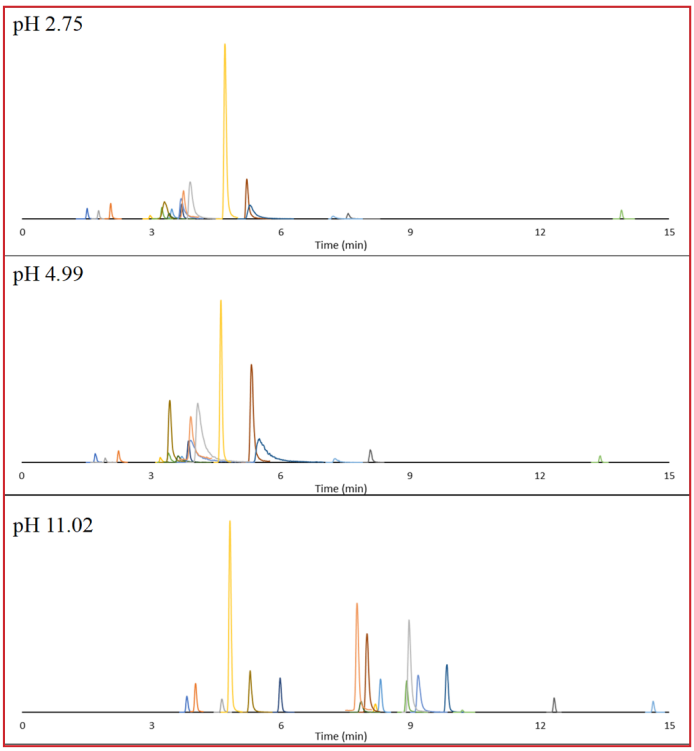

Figure 1. LC-MS/MS separation and detection of SAMHSA drugs of abuse panel on HALO® Elevate C18 column at different pHs. Peak identities and retention times are listed in Table 2 on the next page.

Compound	MRM Transition Q1->Q3	Retention Time (min) pH 2.75	Retention Time (min) pH 4.99	Retention Time (min) pH 11.02
Morphine	286.2->152.1	1.504	1.706	3.815
Hydromorphone	286.2->185.2	2.051	2.242	4.021
Oxymorphone	302.2->284.0	1.769	1.934	4.627
Codeine	300.2->152.3	2.966	3.208	8.182
Hydrocodone	300.2->199.2	3.466	3.713	8.307
Oxycodone	316.2->241.3	3.238	3.406	8.912
6-Acetylmorphine	328.2->165.1	3.704	3.873	5.972
Norfentanyl	233.4->84.1	5.233	5.328	7.991
Fentanyl	337.5->188.0	7.564	8.076	12.327
4-Hydroxy Xylazine	237.2->137.2	3.298	3.435	5.286
Xylazine	220.9->164.0	5.271	5.514	9.848
d-Amphetamine	136.1->91.0	3.403	3.63	7.856
Methamphetamine	150.1->119.1	3.682	3.917	9.175
MDA	180.0->163.0	3.735	3.92	7.761
MDMA	194.0->163.0	3.897	4.078	8.964
Benzoylecgonine	290.1->168.2	4.702	4.615	4.811
PCP	244.3->90.9	7.207	7.265	14.629
THC-COOH	345.0->299.2	13.896	13.407	10.204

Table 2. MRM transitions and retention times for the 18 illicit drugs and metabolites analyzed.

Retention and Tailing Factor Changes

Overall retention for basic compounds increased by over 100%, from an average of 3.87 minutes under acidic conditions (pH 2.75) to 7.98 minutes under basic conditions (pH 11.02). This change in retention corresponding to pH is highlighted in Figure 2. There is a slight increase in retention for the basic compounds when moving from pH 2.75 (blue) to 4.99 (red), but a significant change occurs once the pH is ~2 units above the basic pKa of these compounds, showing the large increase for pH 11.02 (yellow). Exceptions to the trend are those which contain acidic pKa's, THC-COOH and benzoylecgonine, both containing a carboxylic acid functional group.

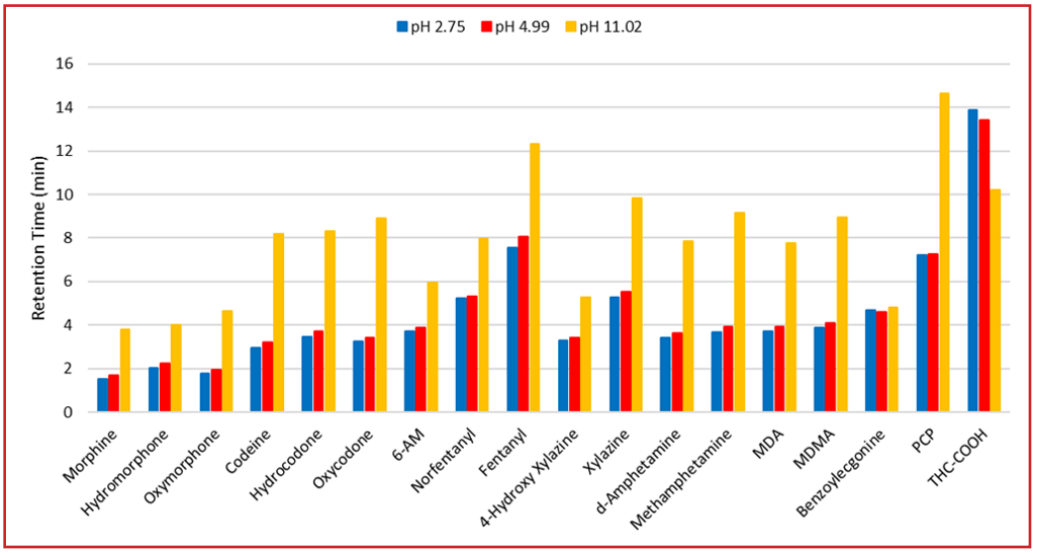

Figure 2. Retention time for the compounds across three different pHs tested.

Similarly, there was an improvement in the tailing factor when increasing the pH, shifting from an overall average of 1.83 at acidic conditions to 1.36 at basic conditions. The amphetamines frequently have large tailing factors when separated by reversed phase, where when run with the HALO® Elevate C18 column, the average for the four was 2.21 under acidic conditions, improving to 1.66 under basic. Considering the pKa of this drug class is around 10, performing a separation at a pH 2 units above their pKa could allow for a more symmetric peak shape.

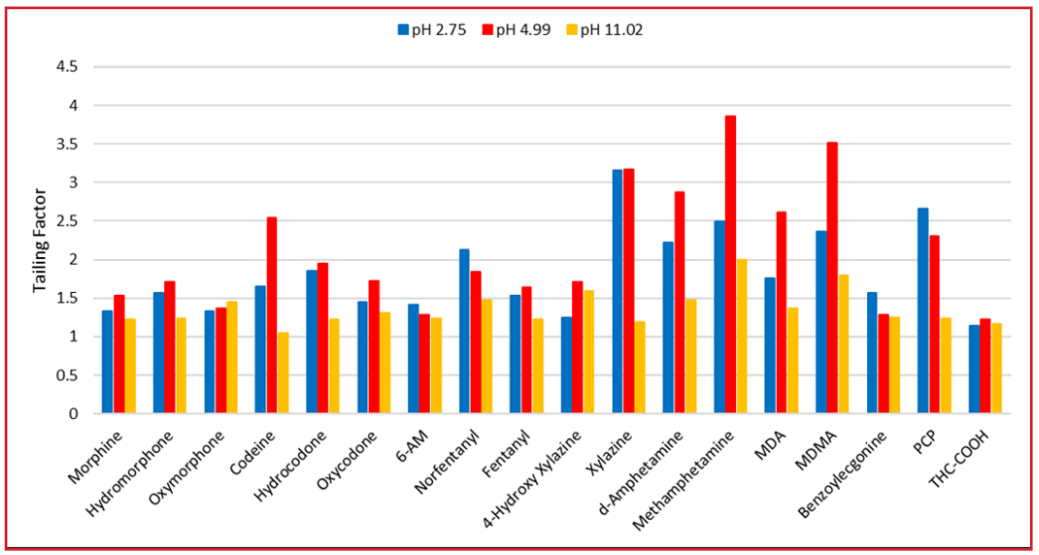

Figure 3. Tailing factor for the compounds across three different pHs tested.

Method Optimization

An optimization for the separation of these compounds was done by using the DryLab® chromatography modeling software (Gradient 2). Compared to the predicted separation modeled by DryLab®, the experiment strongly matched, with consistent compound elution order and one coelution seen in both. Figure 4 shows the optimized separation, resulting in baseline resolution (>1.5) for 16 of the 18 compounds.

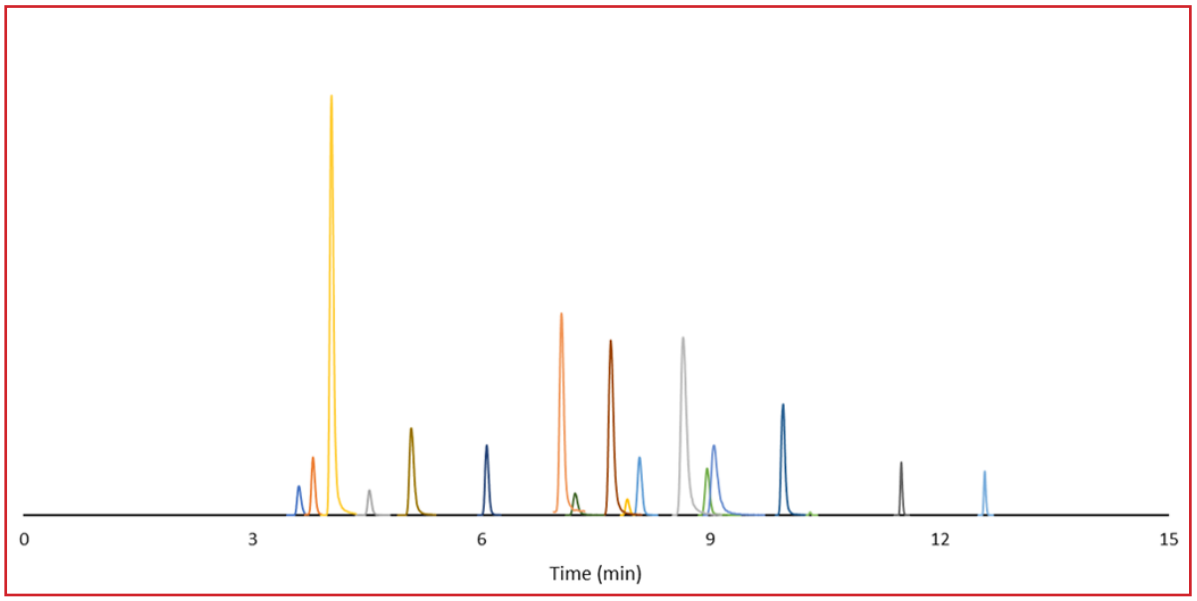

Figure 4. Optimized chromatographic method for separation of the drugs of abuse compounds. Compound elution order: morphine, hydromorphone, benzoylecgonine, oxymorphone, 4-hydroxy xylazine, 6-acetylmorphine, MDA, d-amphetamine, norfentanyl, codeine, hydrocodone, MDMA, oxycodone, methamphetamine, xylazine, THC-COOH, fentanyl, PCP.

CONCLUSION:

Separation of drugs of abuse and their metabolites is critical for the safety and proper treatment of the affected individual. Typically, an acidic pH separation of these compounds with a reversed-phase column is used, which frequently shows peak tailing and low retention for these compounds. By using the HALO® Elevate C18 column under high pH conditions, a method was developed that separated and detected 18 drugs of abuse and metabolites in the SAMHSA panel by LC-MS/MS, which solves the problems of high tailing and low retention. When comparing to acidic conditions with the same column, there was improved peak tailing and increased retention for compounds with basic functionality in the mix. With the instrumentation and column ability to run mobile phases at a high pH, this separation shows the potential for further high pH separations of compounds with basic functionality.

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