APPLICATIONS

# High pH Chiral Separations of Amphetamine and Substituted Amphetamines with the Polysaccharide-based Lux® 3 µm AMP Column

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## Abstract
*In this technical note, we demonstrate that the use of higher pH (>9) under reversed phase mode can dramatically improve the chiral separation of various amphetamine derivatives such as Methamphetamine, Ephedrine, Amphetamine, and 3,4-Methylenedioxymethamphetamine.*

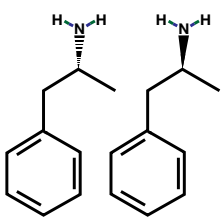
## Introduction
When it comes to most chemical and physical interactions of enantiomers, separation can be difficult due to the fact that both enantiomers share the same characteristics. Chiral compounds have historically been separated via derivatization followed by chromatography. More recently, enantioselective chromatographic techniques have been developed. Typical chiral chromatography is done via HPLC in normal or reversed phase conditions by a stationary phase functionalized with a chiral selector. These stationary phases range from chiral-functionalized silica, metal-ligand exchange, to polysaccharide phases. Recently, polysaccharide coated silica stationary phases have proven to be the most efficient in separating the widest variety of chiral compounds. They are versatile in their compatibility and are stable in acidic and basic conditions (pH 2-9) and can be run in normal, reversed, and polar organic solvents. While polysaccharide coated silica stationary phases are the most popular and the most widely successful columns in separating chiral compounds, there are still some compounds that prove difficult to separate, especially when limited to a specific pH range.

Historically, chiral amphetamines were separated from one another using chiral derivatizing agents to form diastereomers that could be separated by traditional means (reversed/normal phase HPLC, GC, etc.) However, by derivatizing the analytes, they become subject to a reaction yield, both for the diastereomer formation, and in the case of preparative studies, the reverse reaction as well. While this may not be as large of an issue on an analytical scale, analysis of the parent compound is preferable to that of the derivatized product. Specifically, for amphetamine analysis in toxicological assays, the parent compound, in its unaltered form, is the primary component for the qualitative and quantitative analysis for amphetamines in blood and urine. Additionally, stereochemistry can indicate illicit drug use. For example, (-) methamphetamine is non-psychoactive while (+) methamphetamine is a schedule II psychotropic substance. By eliminating a derivatization, analyzing the parent compound in its native form provides a more wholesome and precise means of analysis.

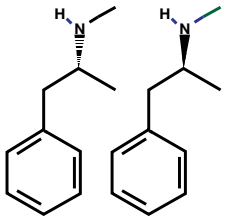
## Materials and Methods
Analytical standards for Methamphetamine, Ephedrine, Amphetamine, 3, 4-Methylenedioxymethamphetamine (MDMA), and Phentermine were purchased from Cerilliant® in 1.0 mg/mL concentrations and were further diluted to concentration in methanol. An Agilent® 1200 binary HPLC equipped with a multiple wavelength UV/Vis detector was used for the chromatographic separation and data acquisition. The HPLC column used in the successful separation of the components was an amphetamine selective phase, Lux 3 µm AMP 150 x 4.6 mm. Mobile phases used in this analysis were made using DI water generated from a Sartorius® arium® water system, and other solvents were purchased at HPLC grade (>99.5 %) from Honeywell®, all other reagents and additives were purchased at HPLC grade from Sigma-Aldrich®.

Ephedrine (+ and -), Amphetamine (-), and Phentermine were added in equal quantities to a 2 mL HPLC vial with a 200 µL glass insert. Methamphetamine (+ and -) was added in a 1:10 ratio relative to the other compounds in order to freely identify peaks of interest for method development purposes. Optimized separation was able to be achieved through manipulating a combination of high pH and temperature on a Lux 3 µm AMP stationary phase.

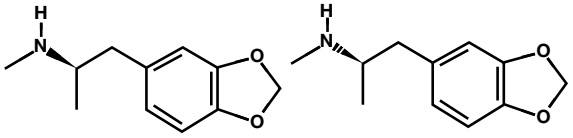
## Chemical Structures of Analytes



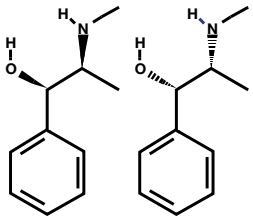
Amphetamine (-, +)
p$K_a$ = 9.90



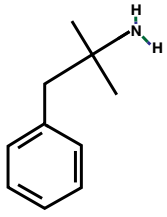
Methamphetamine (-, +)
p$K_a$ = 10.28



MDMA (-, +)
p$K_a$ = 10.14



Ephedrine (- , +)
p$K_a$ = 9.52, 13.89



Phentermine
p$K_a$ = 10.25

# APPLICATIONS

**Figure 1.**
Low pH and High pH Comparison of Methamphetamine Enantiomers

**Column:** Lux 3 µm AMP
**Dimensions:** 150 x 4.6 mm
**Part No.:** 00F-4751-E0
**Mobile Phase:** 5 mM Ammonium Bicarbonate pH (3.0 or 11.0)/Methanol (45:55)
**Flow Rate:** 1.0 mL/min
**Detection:** UV/Vis @ 218 nm (ambient)
**Temperature:** 45 °C

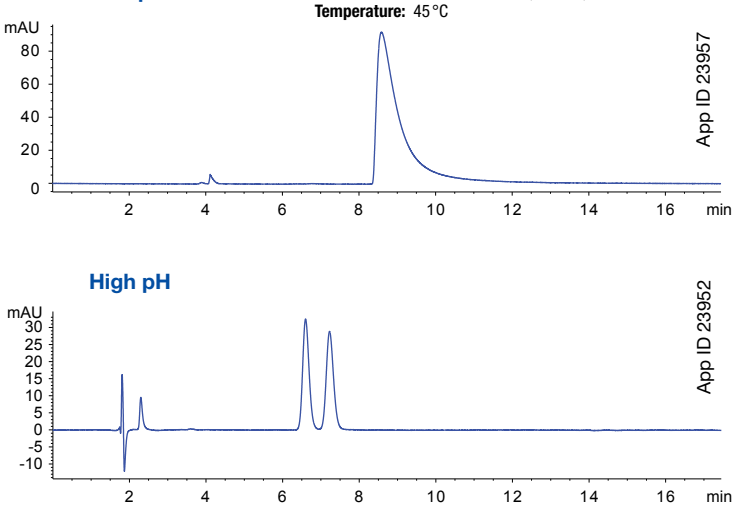



**Figure 2.**
High pH Separation of Chiral Amphetamines

**Column:** Lux 3 µm AMP
**Dimensions:** 150 x 4.6 mm
**Part No.:** 00F-4751-E0
**Mobile Phase:** 5 mM Ammonium Bicarbonate pH 11.0 adjusted with $NH_4OH$/Methanol (45:55)
**Flow Rate:** 1.0 mL/min
**Detection:** UV/Vis @ 218 nm (ambient)
**Temperature:** 45 °C
**Sample:** 1. (+) Ephedrine
2. (-) Amphetamine
3. (-) Ephedrine
4. (+) Methamphetamine
5. (-) Methamphetamine
6. Phentermine

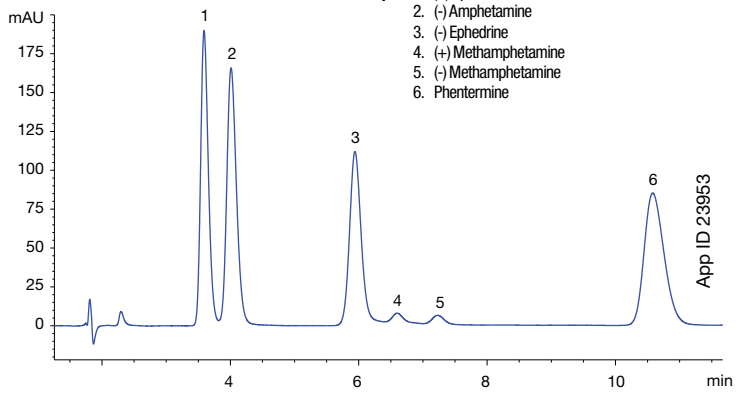



**Figure 3.**
Temperature Effects on the Retention and Peak Shape of Chiral Amphetamines

**Column:** Lux 3 µm AMP
**Dimensions:** 150 x 4.6 mm
**Part No.:** 00F-4751-E0
**Mobile Phase:** 5 mM Ammonium Bicarbonate pH 11.0 adjusted with $NH_4OH$/Methanol (45:55)
**Flow Rate:** 1.0 mL/min
**Detection:** UV/Vis @ 218 nm (ambient)
**Temperature:** Top to Bottom (20 °C, 35 °C, 50 °C)

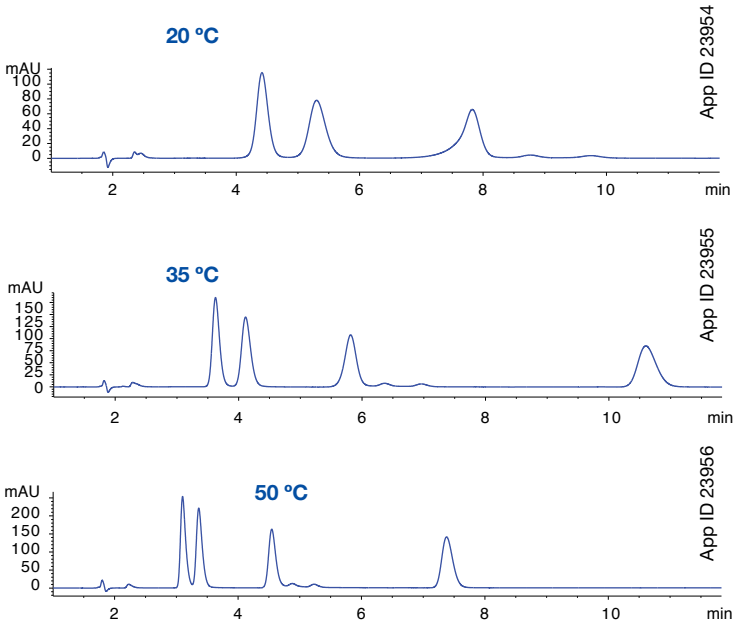
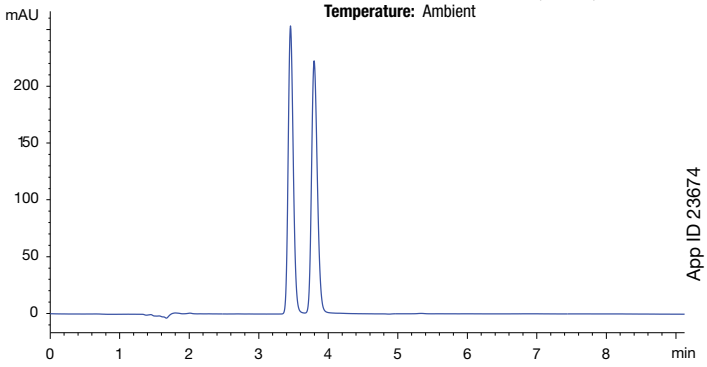



**Figure 4.**
Separation of MDMA Enantiomers

**Column:** Lux 3 µm AMP
**Dimensions:** 150 x 4.6 mm
**Part No.:** 00F-4751-E0
**Mobile Phase:** 5 mM Ammonium Bicarbonate pH 11.0 adjusted with $NH_4OH$/Acetonitrile (40:60)
**Flow Rate:** 1.0 mL/min
**Detection:** UV/Vis @ 218 nm (ambient)
**Temperature:** Ambient



## Results and Discussion

The advantages of high pH modes of separation are demonstrated in **Figure 1** where a low pH separation is compared to that of an appropriately adjusted high pH separation. The low pH mode lacks the selectivity to separate the enantiomers of methamphetamine. Typically, when dealing with chiral separations on polysaccharide phases, it is advised to limit analysis to compounds that exhibit two of the following traits; aromaticity, charged groups, polar groups, hydrophobic groups, or conjugation. Although in many cases, a molecule that displays more than two of these characteristics simultaneously in solution may cause additional modes of interaction that may inhibit the chiral aspect of separation.

When looking at the low pH example, the nitrogenous base of the methamphetamine molecule is protonated and charged, thus adding an additional mechanism under which the molecule can interact with the stationary phase and is likely the cause for peak tailing and decreased selectivity. For the high pH example (pH 11), the predominant species is deprotonated, and therefore relieves the charge from the nitrogenous base and greatly reduces peak tailing, resulting in successful enantiomeric separation. This correlation holds true for many of the compounds in this analysis.

After defining the effective pH range for the analysis of these compounds, a mixture of amphetamines was created to develop a method for the selective separation of amphetamines and their enantiomers (**Figure 2**). This separation demonstrates how operating at an optimized pH range, and with an optimized temperature setting, can be beneficial to the speed and efficiency of a separation, although, an optimized temperature setting would not have been intuitive if not for the temperature comparison study shown in **Figure 3**. This three chromatogram overlay shows the separation of the amphetamine mixture at varying temperatures. At around 20 °C the mixture does show selectivity for the compounds of interest, though, the peak tailing and broadening is substantial and the late eluting peaks are heavily retained, thus leading to a longer analysis time. As the temperature increases, each peak's retention time decreases along with a substantial increase in peak efficiency and symmetry. As the temperature approaches 50 °C, the peak shape continues to improve and run time continues to decrease, though the threshold for resolution becomes compromised. After further method development, the optimal temperature for maximizing separation, analysis time, and peak shape was found to be 45 °C. Investigation of more complex substituted amphetamines was performed in the high pH medium shown in **Figure 4**. The enantiomers of MDMA were able to be quickly and efficiently separated from one another on this phase, thus further reinforcing the sentiment that this phase, when analyzed under appropriate pH conditions, can demonstrate selectivity across numerous different amphetamines and amphetamine-like compounds, and their enantiomers. Maximizing the positive effects of pH and temperature can facilitate a fast, wholesome separation of amphetamines and their enantiomers on this Lux 3 µm AMP stationary phase.

## Conclusion

Temperature and pH are important factors when optimizing a chiral separation. High pH conditions are especially useful when analyzing basic compounds like amphetamines. At low pH, amphetamines are positively charged and in turn may interact readily with any exposed silanol sites and can instigate peak tailing and possibly overshadow any chiral selectivity the stationary phase may otherwise demonstrate. Traditional polysaccharide HPLC columns do not typically facilitate a high pH mode of separation due to their decreased stability in highly basic conditions. The Lux 3 µm AMP stationary phase does not suffer from the same instability as other polysaccharide phases. Temperature can be a useful tool in speeding up analysis time, increasing peak sharpness, reducing tailing, and in turn, increasing peak symmetry. When used in combination with one another, pH and temperature have demonstrated to be useful tools in optimizing the separation of amphetamines and substituted amphetamines on a robust polysaccharide stationary phase.

## Ordering Information

| Lux® 3 µm Analytical Columns | | | SecurityGuard™ Cartridges‡ | |
|---|---|---|---|---|
| Phase | 150 x 3.0 | 150 x 4.6 | 10/pk | 10/pk |
| AMP | 00F-4751-Y0 | 00F-4751-E0 | AJ0-8475 | AJ0-8476 |
| | | | for 2.0-3.0 mm ID | for 3.2-8 mm ID |

‡ SecurityGuard Cartridges require holder, Part No.: KJ0-4282

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