

# HPLC Method Development for the Separation and Quantification of Common Pain Relievers

Department of Chemistry, Lindenwood University St. Charles, MO  
Dylan Pritchett, Joshua Lewis, and Jennifer Firestine

## Introduction

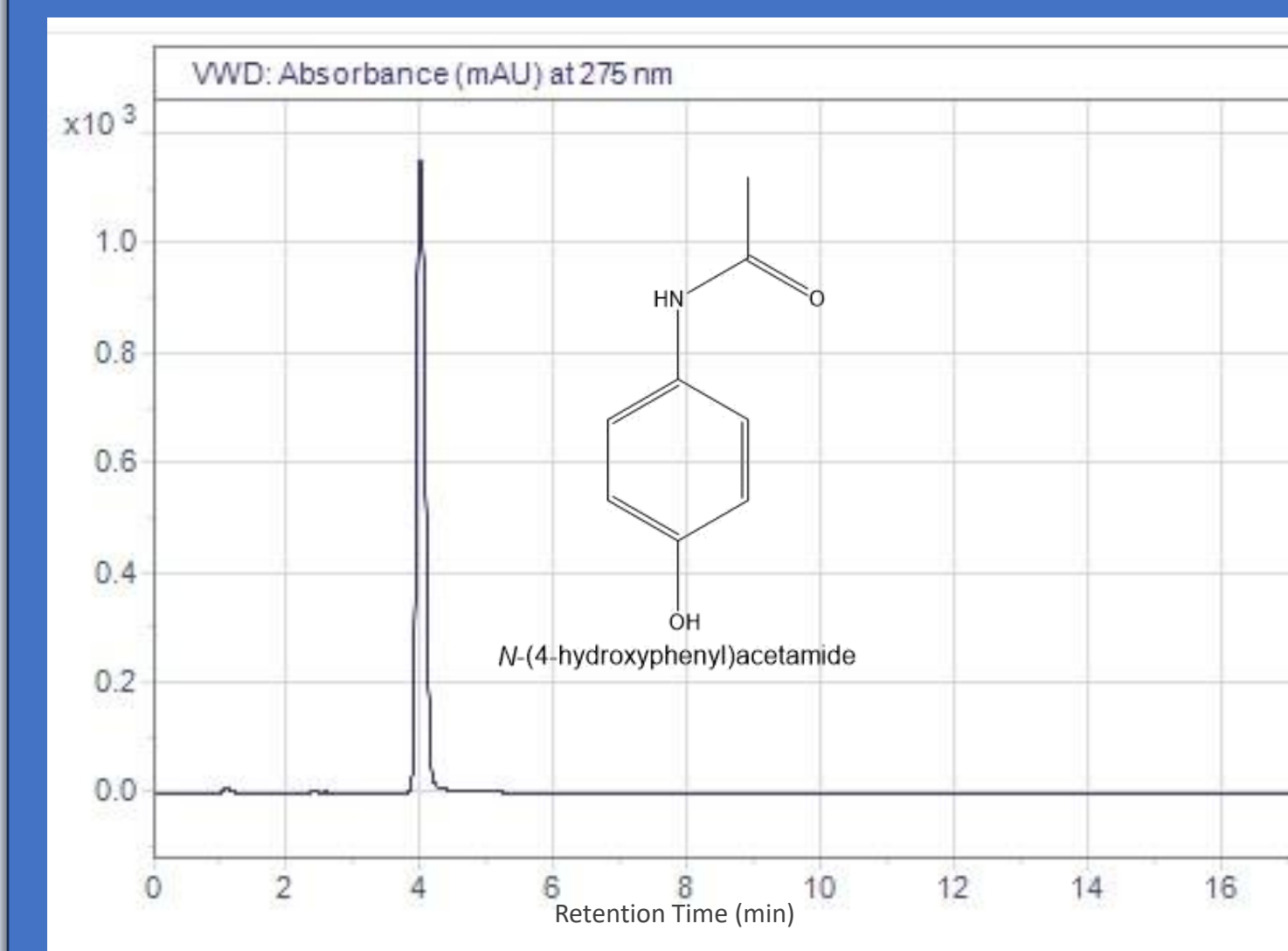
A variety of Excedrin-like over the counter pain relievers was used in method development for the separation and quantification of active ingredients via High-Performance Liquid Chromatography (HPLC). The developed method successfully separated acetaminophen, aspirin, and caffeine from tablets, allowing for the quantification of these ingredients in Excedrin brand pain relievers as well as generics from Walgreens, Walmart, and CVS. The quantified results were compared to analytically prepared standards and the packaging information, with the primary objective of creating a reproducible protocol that can be applied to similar HPLC instrumentation, using optimized instrument settings. The most effective HPLC settings were at 275nm  $\lambda$  and approximately room temperature. The mobile phase consisted of water, acetonitrile, triethylamine, and glacial acetic acid (94.1:5.5:0.2:0.2 v/v).

## Determining Mobile Phase

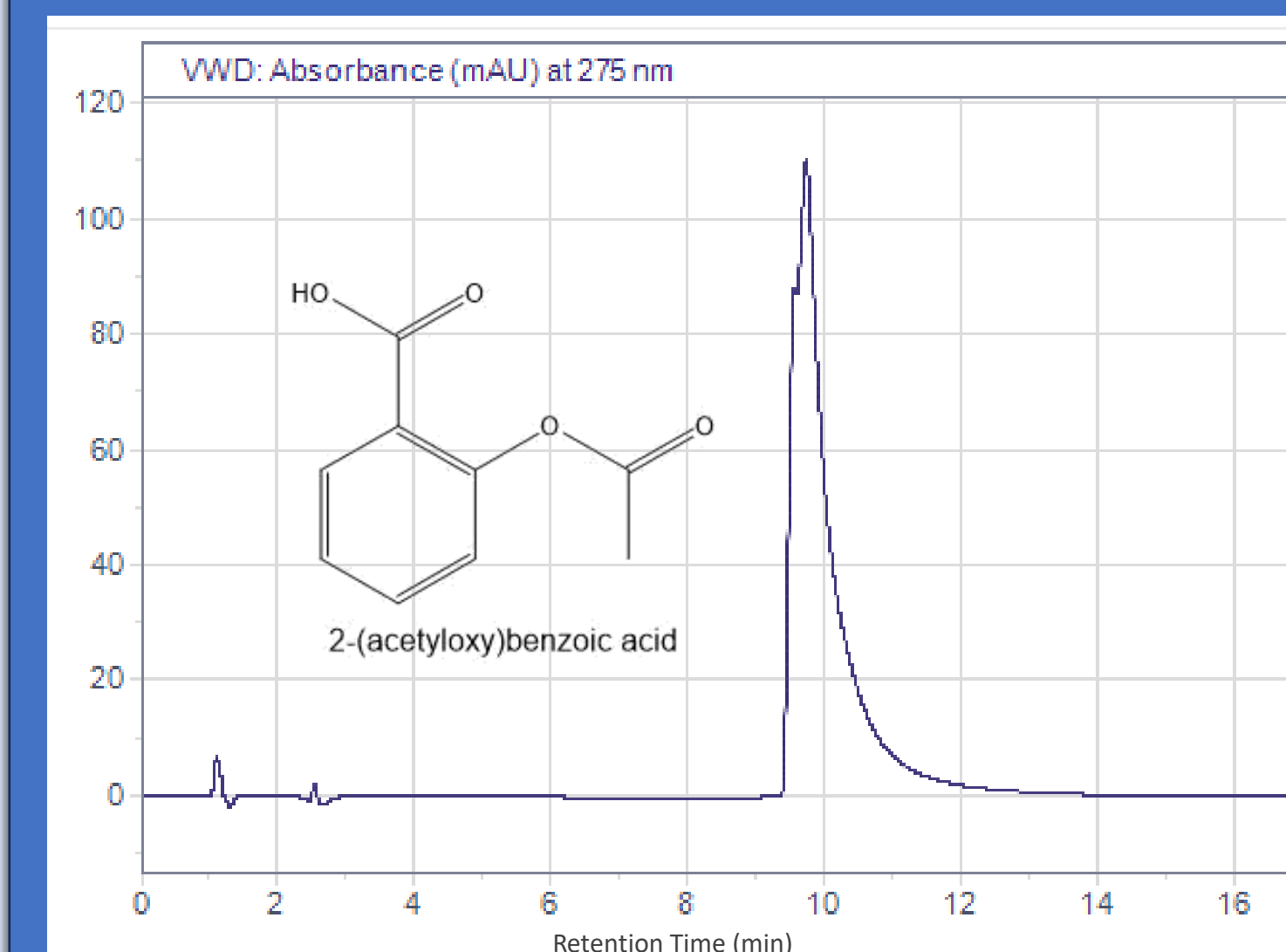
The initial mobile phase was based on a method sourced from Merck Millipore, which employed an isocratic mixture of water, methanol, and glacial acetic acid (69:28:3 v/v). However, this mobile phase did not provide sufficient separation (Figure 1), and subsequent HPLC runs revealed that aspirin and caffeine were co-eluting. To address this, a more polar mobile phase was chosen, consisting of water, acetonitrile, triethylamine, and glacial acetic acid (94.1:5.5:0.2:0.2 v/v). This adjustment resulted in improved separation (Figure 2) and acceptable run times.

## Retention Times of Standard Solutions

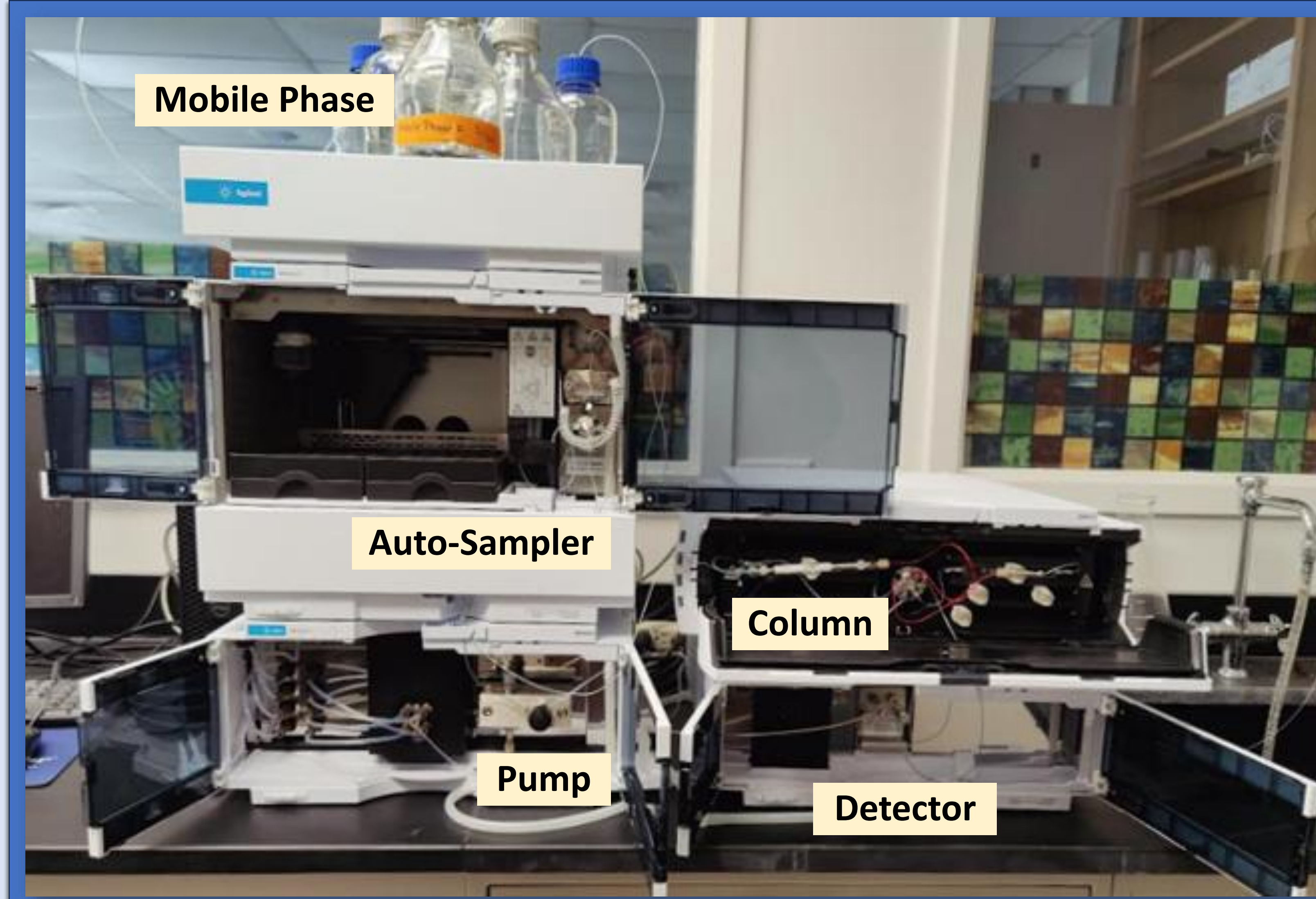
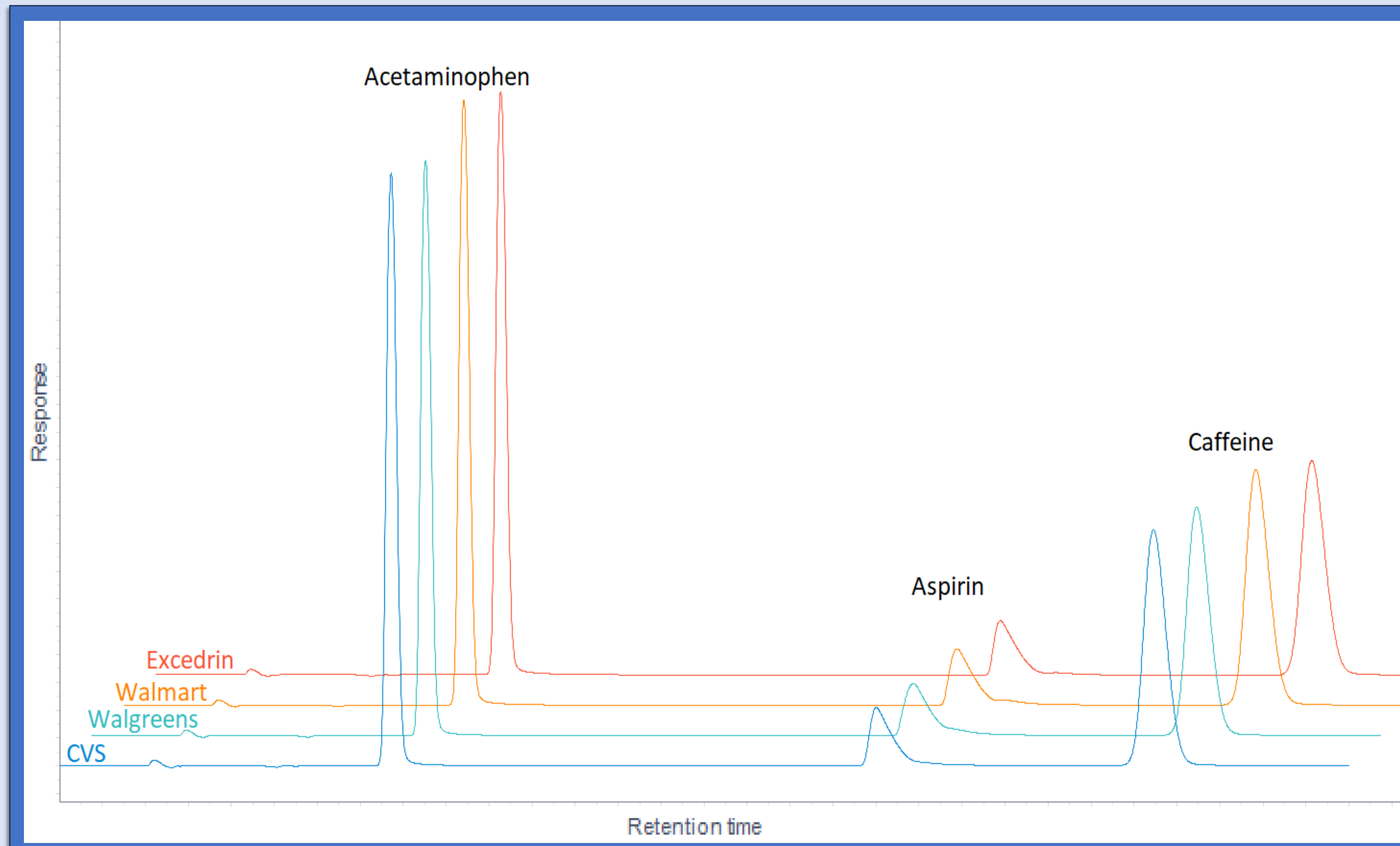
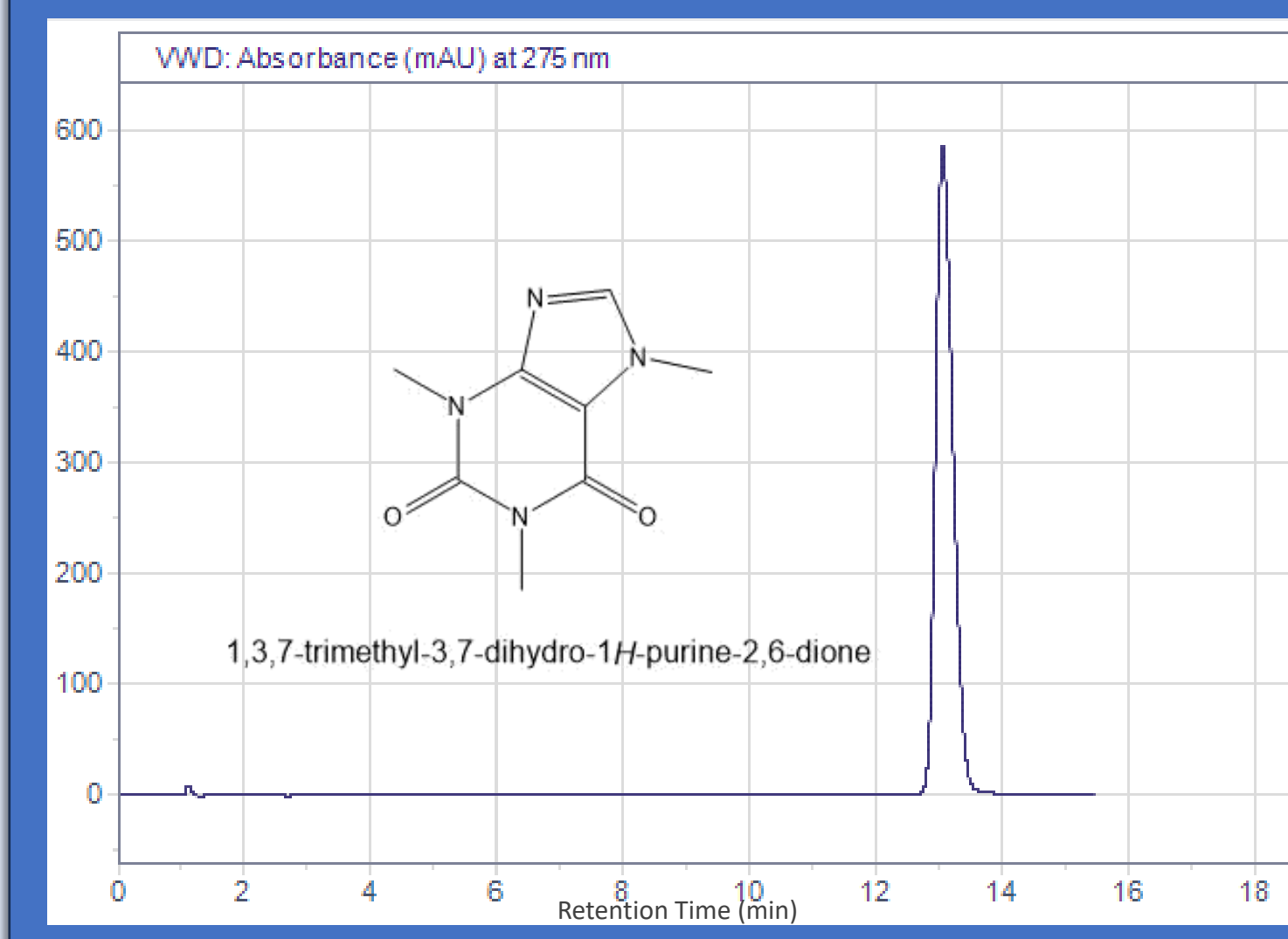
### Acetaminophen



### Aspirin



### Caffeine



## Sample Preparation

The tablets were crushed and dissolved in a mixture of methanol and glacial acetic acid (95:5 v/v). The resulting solution was gravity-filtered into 100 mL volumetric flasks, then diluted to 100 mL with deionized (DI) water. The prepared samples were transferred to 1.5 mL vials for analysis using the HPLC instrument. For the stock solutions, lab-grade reagents were prepared by accurately weighing the components of the medication according to the packaging specifications: 250 mg of acetaminophen, 250 mg of aspirin, and 65 mg of caffeine. These ingredients were added to 100 mL volumetric flasks, diluted with DI water, and transferred to 1.5 mL vials, before being analyzed with the HPLC instrument.

## Optimization

The goal of reducing the run time to under ten minutes was explored, with the method incorporating column heating to accelerate the process. As a result, the run time was successfully shortened to less than 9 minutes (Figure 3), down from approximately 12.5 minutes. However, this increase in temperature led to the degradation of acetylsalicylic acid (aspirin) into salicylic acid, which was confirmed by spiking samples with salicylic acid (Figure 4). Based on these observations, heat was decided as a poor method for decreasing run times.

Figure 3: Increasing temperature resulted in degradation of 2-(acetyloxy)benzoic acid peak.

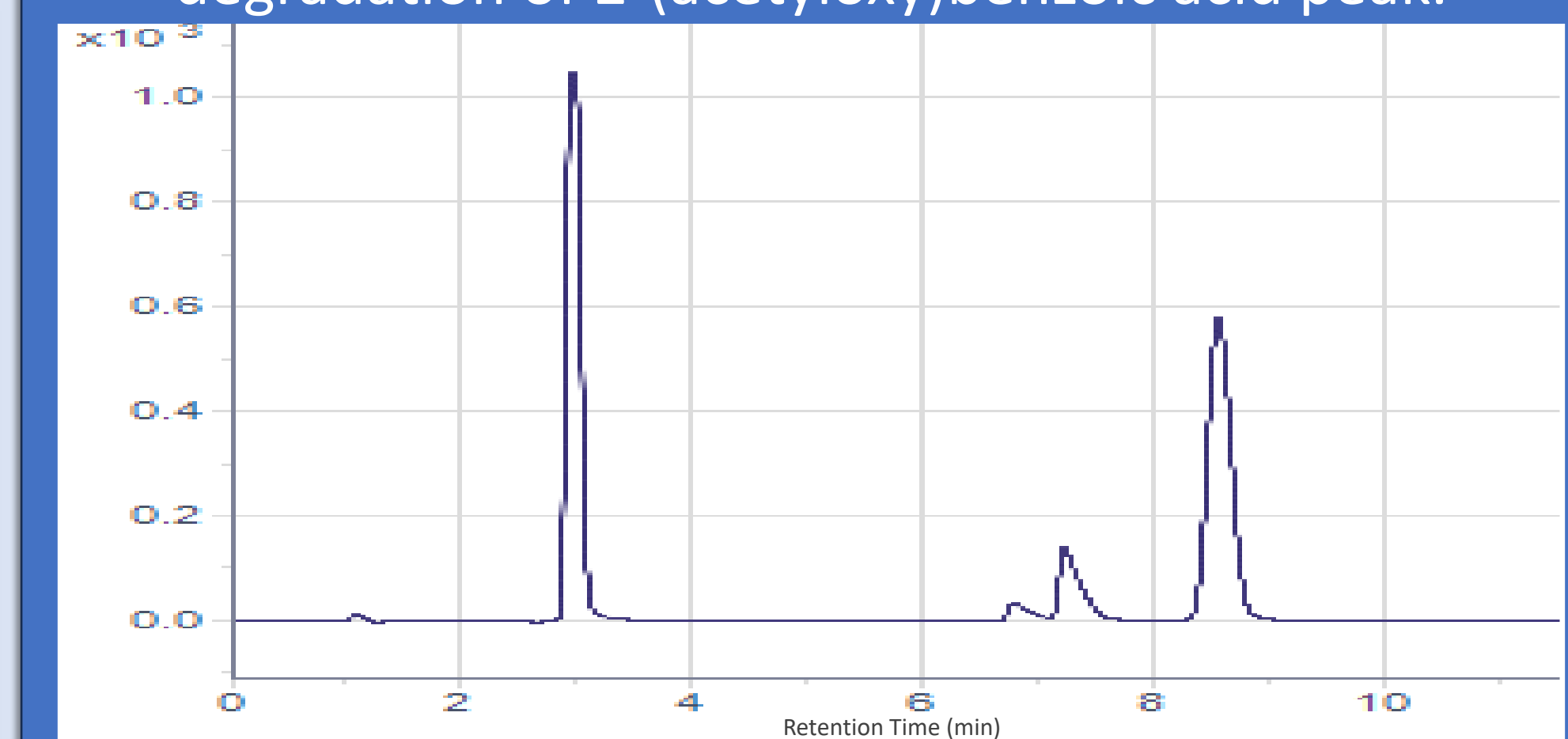
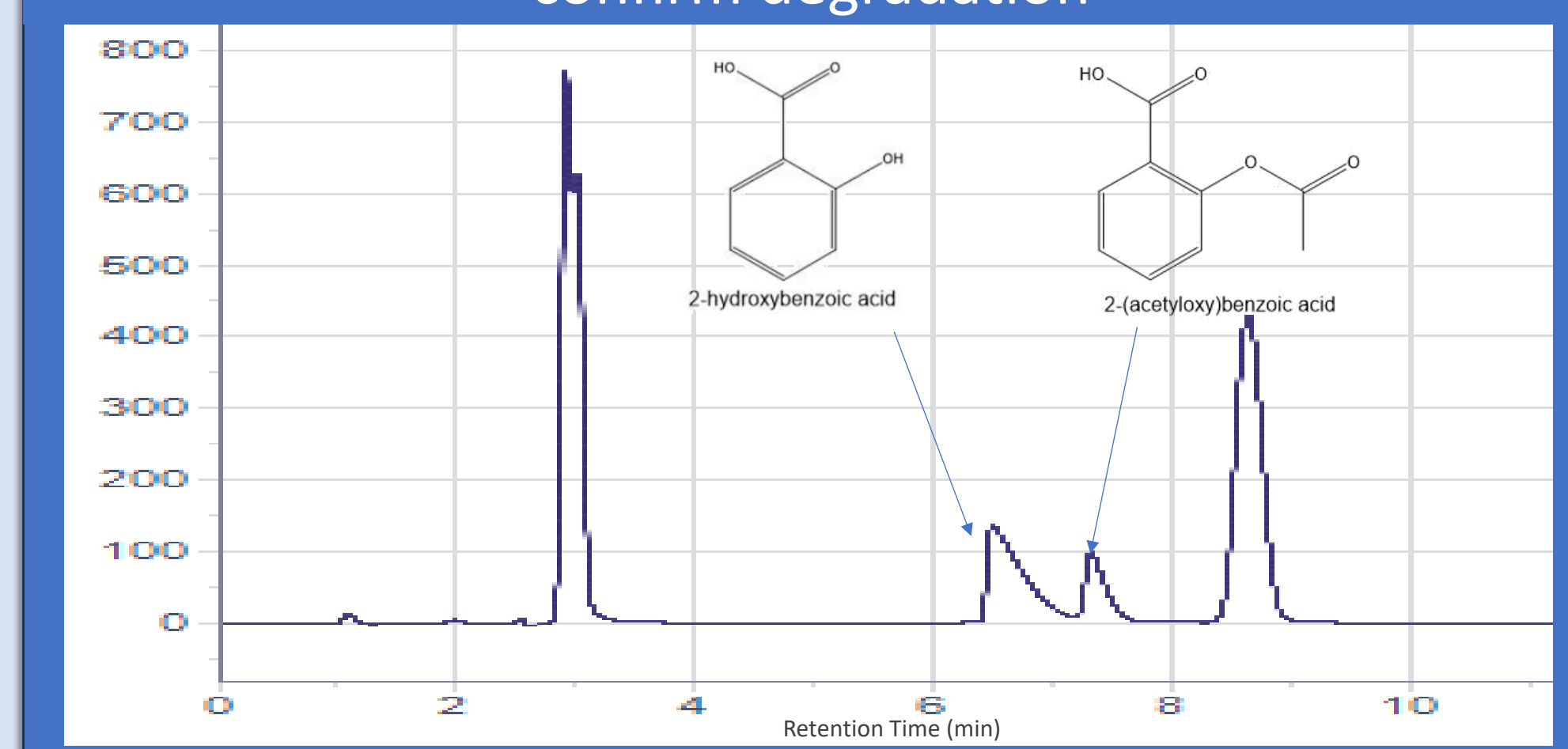


Figure 4: Spiked sample with 2-hydroxybenzoic acid to confirm degradation



## Conclusion

The developed HPLC method successfully separated and quantified the active ingredients with both branded and generic OTC pain relievers. Method optimization included adjustments to the mobile phase composition and exploring column heating, which resulted in a reproducible protocol with reliable separation in a run time appropriate for an undergraduate lab.

Figure 1: Initially 2 peaks presented when standards were combined into a single run.

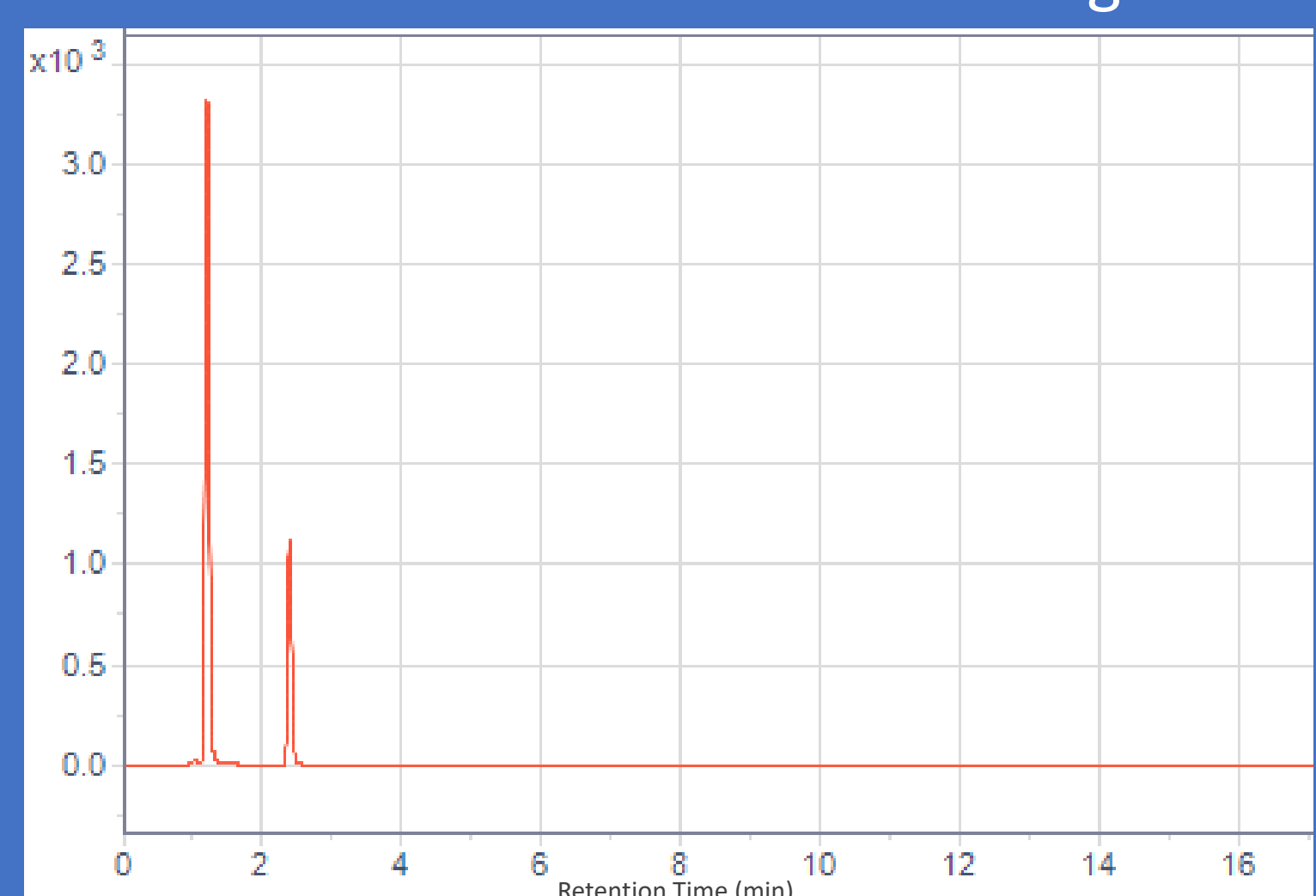
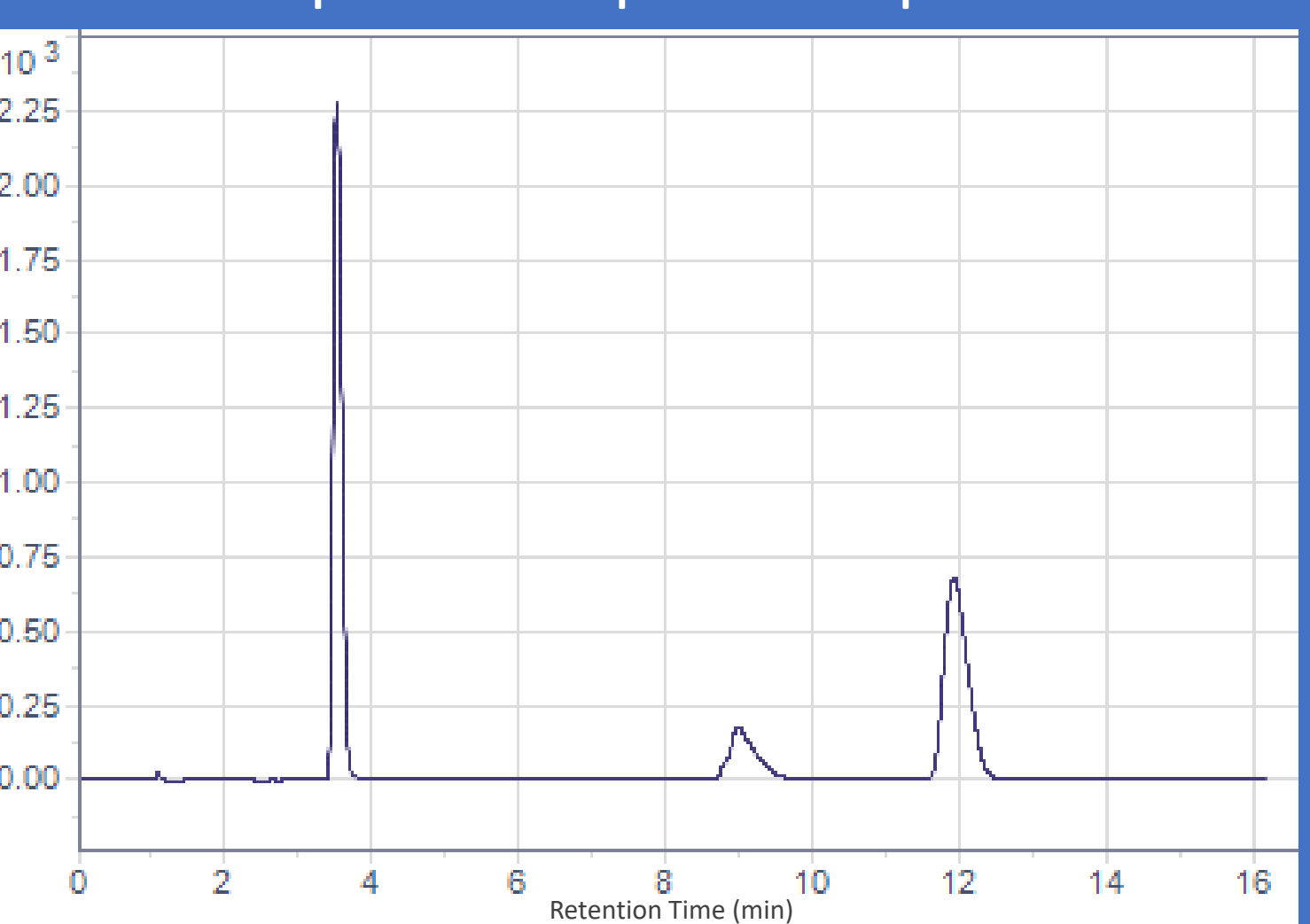


Figure 2: Improved mobile phase yielded adequate compound separation.



References: Millipore Sigma. *Acetaminophen, Aspirin and Caffeine (USP) Excedrin Tablet Dissolution*. [www.sigmaaldrich.com](http://www.sigmaaldrich.com)