Analysis of Two Over-the-counter Analgesics using High Performance Liquid Chromatography

Glenn Murray

Department of Chemistry

Georgia State University, Atlanta, GA

Fall 2023, CHEM 4010

Lab Report #4

Abstract

The purpose of this research was to identify and quantitate certain analgesics from over-the-counter pain relievers. Five aspirin standards of known concentration, and a standard mixture containing aspirin, salicylamide, acetaminophen, and caffeine were analyzed in an Agilent Series 1200 high performance liquid chromatograph via reverse phase HPLC. These results were used to help determine the identifications of compounds in a solution made from a headache relief product and the solution concentration of aspirin made from a headache relief powder, as well as a solution made from dissolving an aspirin tablet.

The compounds identified in the headache powder were acetaminophen, aspirin, and caffeine. The compounds were identified by comparing their retention time(s) to that of the standards. The aspirin concentrations were computed from the peak area (mAU.s), using a graph prepared from the peak area/concentrations of aspirin standards. Of the two unknowns, the aspirin unknown produced a calculated value of 3.7 mg/mL. Given the aspirin unknown was made by dissolving a tablet (325 mg) of Publix brand aspirin into a 100 mL solution, it should come to 3.25 mg/mL, assuming Publix is accurate about the amount of aspirin in their tablets. That is $12._6$% over advertised amount, per tablet.

The aspirin unknown was measured at 6.0 mg/mL. This was made from a Goodie's Headache powder, which is advertised to contain 520mg of aspirin. The powder was dissolved in 100 mLs of solvent, resulting in a theoretical concentration of 5.20 mg/mL. This comes out to $15._4$% higher than advertised amounts. While it's possible the companies have a higher than advertised amount of aspirin, counting on some decomposition, considering both were from 12 to 16% high it is more likely the calibration curve and process were in error.

1.0 Introduction

The purpose of this study was to analyze and identify the compounds in two analgesic unknown mixtures using reverse phase high performance liquid chromatography (HPLC).

High performance liquid chromatography is a category of liquid chromatography used for separating liquids and soluble solids via competition between the liquid of the mobile phase (MP) and the surface of the solid stationary phase (SP) for the analytes. It differs from gas chromatography in its MP is a liquid and, in its ability to analyze compounds that cannot be volatized or have very high boiling points. It also has the advantage of being able to use a wide variety of MP solvents, including the ability to change the solvent composition during elution.

In HPLC the MP is under high pressure, allowing for sufficient volume flow through the SP for practical chromatography. Two of the more common HPLC types are normal phase chromatography and reverse phase chromatography (RPC). NPC has a polar SP and uses a relatively non-polar MP.

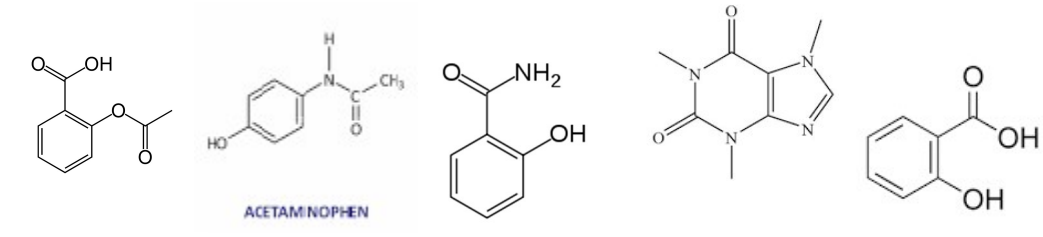
Reverse phase chromatography (RPC) is a one where the polar SP, such as silica gel, is replaced with a SP where the exposed surface is very non-polar. The silica gel silanol hydroxyls are chemically bonded to a non-polar molecule, for instance an eighteen-carbon alkane, which presents a strongly non-polar surface to the analytes and the MP.

Reverse phase chromatography (RPC) is a one where the polar SP, such as silica gel, is replaced with a SP where the exposed surface is very non-polar. The silica gel silanol hydroxyls are chemically bonded to a non-polar molecule, for instance an eighteen-carbon alkane, which presents a strongly non-polar surface to the analytes and the MP[1]. For RPC the solvent system is a more polar set of solvents than those used in NPC, often a base of water with another less solvent water miscible solvent mixed in. This study will use 60/40 methanol/water with 1% acetic acid as the MP. HPLC is suitable for analgesic analysis[2].

It is not uncommon to use HPLC reverse phase chromatography for identification and quantitation of analgesic compounds[3],[4]. Gas chromatography would be a poor candidate for this analysis due to the pyrolysis of these compounds at higher temperatures and their lack of volatility.

Acetaminophen, aspirin, salicylate, and caffeine were candidate compounds identified as potentially in the unknown headache powder. The second unknown was aspirin but of an unknown concentration.

The molecular structures of the standard and unknown constituents were - acetyl salicylic acid (aspirin), acetaminophen, salicylamide, and caffeine, with salicylic acid included, as it is a hydrolysis product of aspirin.



Acetyl salicylic acid | Salicylamide | Acetaminophen | Salicylic acid

Figure 1 Molecular structures of acetyl salicylic acid (aspirin), acetaminophen, salicylamide, and caffeine, respectively

The purpose of these experiments is to identify and partially quantitate the pain-relieving compounds in two over the counter medications, using standards to calibrate and get retention times for comparison.

2.0 Experimental

2.1 Chemicals:

The chemicals used were: Acetic acid, ACS reagent grade, >= 99.7%, from Sigma-Aldrich of St. Louis, MO, CAS# 758-12-3; HPLC grade methanol, made by Fisher Scientific of Fair Lawn, NJ. CAS# 67-56-1; 4'hydroxyacetanilide (acetaminophen) >98%, made by TCI, of Portland OR, CAS# 103-90-2; Lab grade caffeine, Ward's Science of Rochester, NY, CAS# 58-08-2; Acetylsalicylic acid (aspirin) 99%, Acros Organics, of New Jersey, CAAS# 50-78-2; Salicylamide, 98%, made by TCI of Portland, OR, CAS# -45-2; Headache Relief, BC On-the-Go stick packs, made by Medtech Products Inc., Tarrytown, NY.; Aspirin tablet, sold by Publix Supermarkets, Inc., of Lakeland, FL. Also acetone was used for cleaning the syringe.

The mobile phase (MP) will consist of a consistent mixture 40/60 H_2O/MeOH (v/v) w/1% acetic acid.

2.2 Equipment:

The HPLC instrument is an Agilent 1200 series HPLC with VWD detector made by Agilent Technologies, USA. The column is an Eclipse XDB-C18 4.6mm x150mm (packing particle size of 5µm), produced by Agilent Technologies. The syringe is a 100 µL gastight glass syringe #1710, Hamilton Co., Reno, NV. The software is an Agilent ChemStation made by Agilent Technologies.

2.3 Experimental Conditions:

The software was brought up and the method was checked to ensure it was isocratic 40/60 for this analysis. The syringe was cleaned with acetone and then air dried.

The initial sample name and run information was entered into the software and the software prepared to run. The sample was injected with the syringe twice to ensure flushing of any previous material. Then the valve was switched from load to run, causing the sample to enter the HPLC and the elution to begin. Once all peaks registered, the chromatogram recording was stopped, the data file opened, and the peaks that were not relevant (usually minor impurities or noise) were deleted from the dataset, and the data saved.

The above procedure was repeated for every trial run.

2.3.1 Procedures:

Each of the runs produced data that included retention time, in minutes, peak area in milli absorbance units-seconds, for each peak, along with other data not used here. All trial cycles followed the procedure

mentioned in the previous section.

The 5 different concentration aspirin standard trials were run twice, and the data collected. Then a trial for each of the aspirin/acetaminophen/caffeine/salicylamide standard mixtures were run, and the data collected.

Once all standards were run, two trials of the headache relief unknown sample and two trials of the aspirin unknown sample were run, data collected, and data analysis performed.

3.0 Results and Discussion

The only non-statistical equations used in this experiment is that of a line:

Equation 1 Equation of a line with regards to computing unknown concentrations

y = mx + c, where y is the area, measured in mAU.s, m is the slope of the line, x is the analyte concentration, and c is the y intercept.

Rearranged to form:

Equation 2 Equation for getting unknown concentration from HPLC peak area

y = x* m + c to yield a function to plug in area and return the concentration

3.1 Legend:

Symbols used in this paper appear in Table 6, with their definition.

3.2 Results

Silica gel consists of silica exposing silanol groups in the possible configurations below. The hydroxyl groups of the silanol groups allow for hydrogen bonding, dipole-dipole interactions, and form a polar, slightly

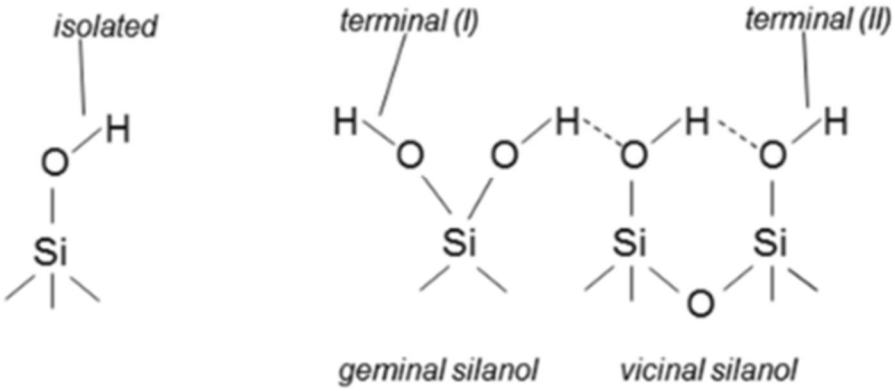


Figure 2 Silanol structure, in various configurations

acidic structure.

Above are the structures of silanol groups in silica gel[5].

For reverse phase chromatography, as used in these experiments, the silanol hydroxyls are chemically bound to C-18 ligands, masking the silanol hydroxyl and presenting a very non-polar SP surface. The following picture represents the structure of that stationary phase surface:

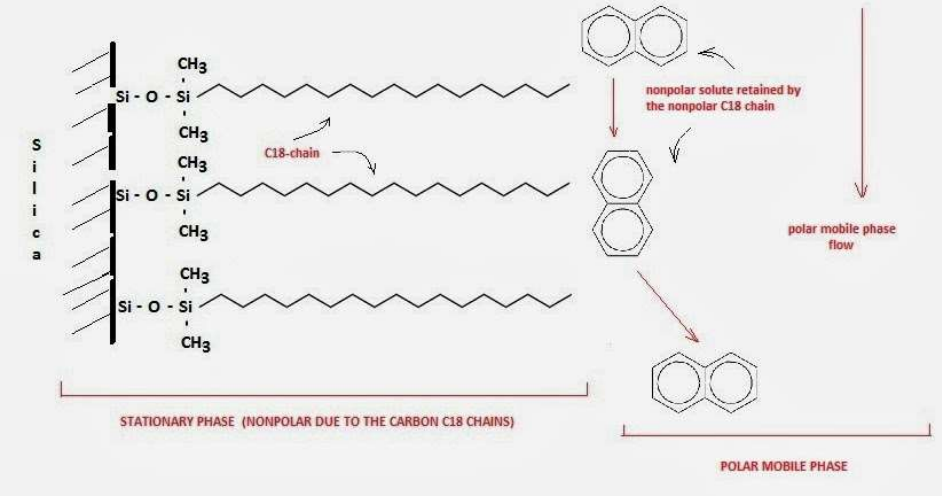


Figure 3 Reverse phase chromatography stationary phase structure

The analyte molecules shown are just examples.

Each of these molecules has a polar aspect that will allow it to compete between partitioning in the C18 portion of the SP and flowing with the more polar MP.

An example chromatogram, one of acetaminophen and one of aspirin, is shown in Figure 8and Figure 4, in the appendix.

The analgesics standards chromatogram was more typical for all the individual standards, except aspirin, which showed two peaks (Figure 4). One peak was for acetyl salicylic acid, and the other for salicylic acid, as a hydrolysis product of aspirin. Aspirin is not very stable, hydrolyzing into salicylic acid and acetic acid, which can be sensed as a vinegar-like smell in old bottles of aspirin.

Table 1 Retention Times for Standards

				aspirin	
ALL STANDARDS	**acetaminophen**	**caffeine**	**salicylamide**	**peak 1**	**peak 2**
t_R (min)	1.134	1.274	1.483	1.649	2.53

In the above table, the two aspirin peak retention times can be seen.

The chromatogram of the aspirin unknown, trial #1, can be seen as Figure 6, in the appendix. Unlike many HPLC quantitation's, aspirin has two peaks, so the sum of both peaks is used for graph construction and in the computation of unknown concentrations.

3.3 Aspirin Concentration Calibration

Five standard solutions for aspirin were run: 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, and 10 mg/mL. The absorbances and averages can be found in Table 2, in the appendix.

Table 3 is constructed and used to produce the graph shown in Figure 7, all found in the appendix.

These produce the calibration equation:

Equation 3 Aspirin Calibration Equation

y = 0.0009x - 5.9307

This can calculate the aspirin concentration by the sum of aspirin peaks. For example, using Equation 2 with these values for m and c, an absorbance sum of 10000, it yields 0.0009 * 10000 – 5.9307 = 3.0_7 mg/mL.

The 0.9595 regression was only a moderate fit to a line, with an R^2 of 0.95_9. In this case, the lower the R^2, the less trustworthy the calibration equation is in calculating the aspirin concentration.

3.4 Identification and quantitation

When the retention times of the headache powder are compared to the standards, the four major peaks correspond to acetaminophen, caffeine, and aspirin. In the headache powder, caffeine and acetaminophen peaks elute first, followed by the two aspirin peaks. The acetaminophen, caffeine, and salicylamide standard's chromatograms can be found in Figure 8, Figure 9, and Figure 10, in the appendix. A typical aspirin chromatogram can be seen in Figure 6, in the appendix.

Aspirin, aka acetyl salicylic acid and its hydrolysis product, salicylic acid, are the least polar of the constituents being measured. Given this is an RP-HPLC elution, the least polar will show the greatest retention, with a low affinity for the more polar MP and a higher affinity for the non-polar SP. Salicylic acid is the least polar of them all and shows the highest retention time of about 2.55 minutes. Similarly, acetaminophen is the most polar, therefore is the least retained with a retention time of about 1.33 minutes. Caffeine and salicylamide fall in-between in polarity, so are eluted between those extremes.

The headache powder retention time and absorption area data are shown in Table 4. The total average absorbance areas of aspirin in the headache powder were 13227.7311 mAU.s, which calculated a concentration of 6.0 mg/mL, in conjunction with the calibration equation, Equation 2, derived from the plot in Figure 7.

The aspirin unknown gave a total average absorbance of 7555.37891 mAU.s, calculating to 3.7 mg/mL. The data is presented in Table 5. The aspirin concentrations exceeded the expectations, when taking into account the advertised aspirin amounts in the unknowns. Given the linear regression did not conform to the equation well, having an R^2 of about 0.95_9, the calibration plot and equation are suspects in this apparent quantitation error.

4.0 Conclusions

In this experiment, the compounds were identified as acetaminophen, aspirin, and caffeine, prepared from a Goodie's Headache powder. The compound retention times were compared to the standard's retention time to determine their identification. The aspirin concentrations were computed from the sum of both average peak areas (mAU.s), using an equation derived from a graph prepared from the total average peak areas vs concentrations of the aspirin standards. Both unknowns used one dose (one powder or one tablet) dissolved in 100 mL of solvent.

The aspirin unknown was calculated at 3.7 mg/mL. Being a tablet of Publix brand aspirin should be 325 mg, this computes to a 3.25 mg/mL aspirin solution if Publix is accurate about the aspirin amount in their tablets. This experiment calculated to 12.6% over advertised amount, per tablet.

The aspirin unknown from the Goodie's Headache powder was measured at 6.0 mg/mL. Goodie's Headache powder is advertised as containing 520 mg of aspirin. This should result in a theoretical concentration of 5.2 mg/mL. This experiment's result came out 15.4% higher than advertised amounts. While it's possible the companies have a higher than advertised aspirin content, counting on some decomposition, considering both were between 12 and 16% high, it is more likely the calibration curve and process were in error, for the region measured.

5.0 Reference

[1] Vitha, M, *Chromatography, Principles and Instrumentation*, John Wiley & Sons, inc., 2017, pp 165

[2] Ferguson, G., Quantitative HPLC Analysis of an Analgesic/Caffeine Formulation: Determination of Caffeine, Journal of Chemical Education, Vol. 75, No. 4, April 1998, https://doi.org/10.1021/ed075p467

[3] Mamolo, M., Vio, L., Marich, V., Simultaneous quantitation of paracetamol, caffeine and propyphenazone by high-pressure liquid chromatography, Journal of Pharmaceutical and Biomedical Analysis, Vol 3, issue 2, 1985, pp 157-164, https://doi.org/10.1016/0731-7085(85)80019-4

[4] Ferguson, G., Quantitative HPLC Analysis of an Analgesic/Caffeine Formulation: Determination of Caffeine, J. Chem. Educ., 75, 4, 467, April 1998, https://doi.org/10.1021/ed075p467

[5] Hattori, Hideshi & Arudra, Palani & Abdalla, Amr & Al-Khattaf, Sulaiman. (2020). Infrared Study of Silanol Groups on Dealuminated High Silica MFI Zeolite to Correlate Different Types of Silanol Groups with Activity for Conversion of 1-Butene to Propene. Catalysis Letters. 150. 10.1007/s10562-019-02972-8.

6.0 Appendix

6.1 Run Details

Agilent 1200 series HPLC with VWD detector, using an Eclipse XDB-C18 RP 15.0 cm column, with a particle size of 5 μm.

This was run at room temperature (approx. 15° C) and eluted with 60% methanol in water mobile phase, containing 1% acetic acid. The detector was operating at a λ of 254 nm.

6.2 Formulas

Equation of a line – this was only used to derive the equation following

y = mx + c

Substituting values of m=0.0009 and c=-5.9307 into the equation of a line yeilds:

$y = 0.0009 * x - 5.9307$

or

$C_{aspirin} = 0.0009 * mAU.s - 5.9307$

Example Computation

$15127.7_9 * 0.0009 - 5.9307 = 7.6_8$ mg/mL

6.3 Significant Figures

Significant figures used were the significant figures of the raw data plus one, for all calculation results.

6.4 Raw Data, Charts, and Graphs

6.41 Unknown's and Calibration Data

Data File C:\CHEM32\1\DATA\LGT\ASPIRIN4MG1.D
Sample Name: aspirin 4mg trial 1

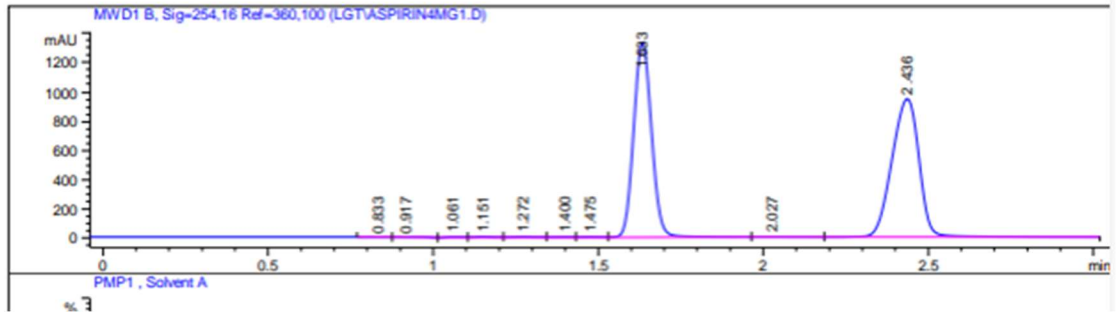


Figure 4 Aspirin chromatogram, 4 mg/mL std

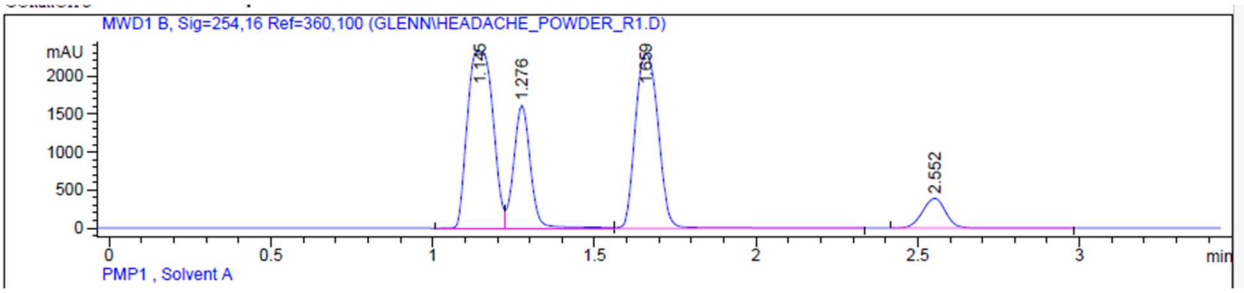


Figure 5 Goody's headache relief unknown, trial 1 chromatogram

Column Description : BDS Hypersil C18
Product# : 28105-254630 Batch#:
Serial# :
Diameter : 4.6 mm Length : 250.0 mm
Particle size : 5.0 µm Void volume : 60.0 %
Maximum Pressure : 400 bar Maximum pH : 9
Maximum Temperature: 25 °C
Comment :

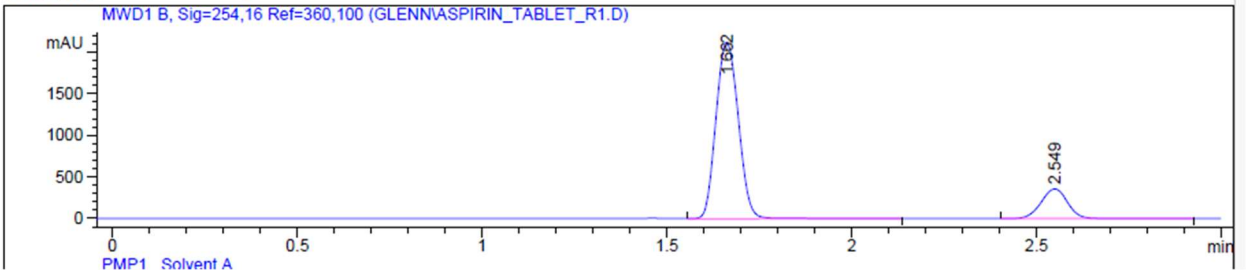


Figure 6 Aspirin chromatogram, Unknown #1, Trial 1

Table 2 Aspirin at conc of 2, 4, 6, 8, and 10 mg/mL

ASPIRIN STANDARD		aspirin 2 mg/mL		aspirin 4 mg/mL		aspirin 6 mg/mL		aspirin 8 mg/mL		aspirin 10 mg/mL
	peak 1	peak 2	peak 1	peak 2	peak 1	peak 2	peak 1	peak 2	peak 1	peak 2
t_R (min) trial 1	1.635	2.483	1.652	2.522	1.654	2.531	1.656	2.431	1.654	2.555
t_R (min) trial 2	1.638	2.492	1.655	2.527	1.655	2.542	1.656	2.426	1.652	2.557
tR (min) Avg	**1.6365**	**2.4875**	**1.6535**	**2.437**	**1.6545**	**2.5365**	**1.656**	**2.429**	**1.653**	**2.556**
A (mAU.s) trial 1	7212.76074	712.59790	10402.10000	1156.94568	11764.80000	1683.09290	12881.40000	7440.82080	13833.00000	2602.40186
A (mAU.s) trial 2	7139.36865	711.59473	10293.80000	1149.36670	11715.80000	1696.69666	12945.00000	7882.63086	13878.60000	2601.51270
A (mAU.s) Avg	7176.06470	712.09632	10347.95000	1153.15619	11740.30000	1689.89478	12913.20000	7661.72583	13855.80000	2601.95728
total A (mAU.s)	7888.16101		11501.10619		13430.19478		15028.76543		16457.75728	

Table 3 Concentration and associated peak areas.

$C_{aspirin}$ (mg/mL)	Peak A (mAU.s)
2.0	7888.16101
4.0	11501.10619
6.0	13430.19478
8.0	15028.76543
10.0	16457.75728

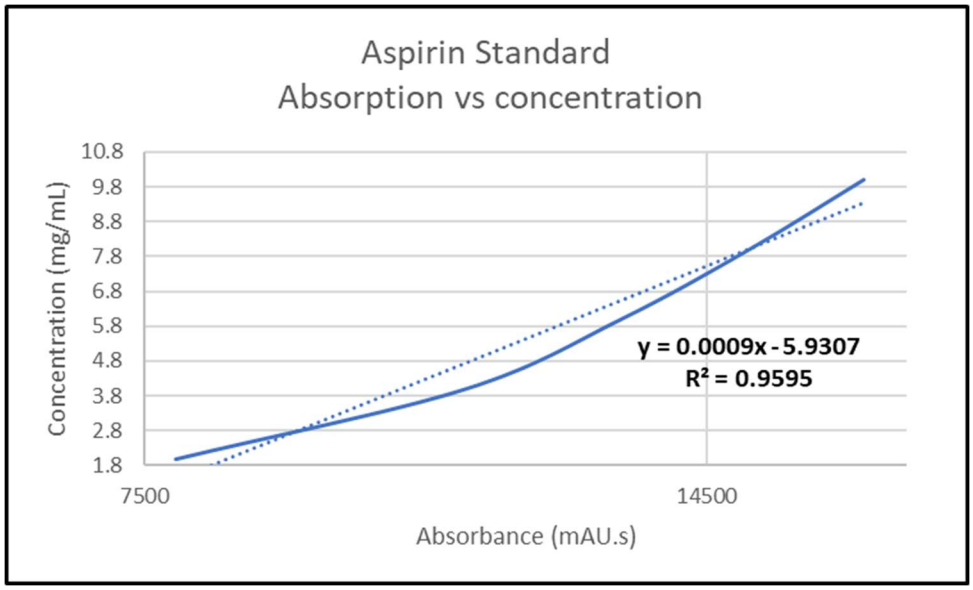


Figure 7 Graph of concentration vs total peak area.

Table 4 Headache powder data with ID

Headache Powder	**peak 1**	**peak 2**	**peak 3**	**peak 4**
t_R (min) trial 1	1.145	1.276	1.659	2.552
t_R (min) trial 2	1.146	1.276	1.659	2.552
t_R (min) Avg	1.146	1.276	1.659	2.552
IDENTIFICATION	acetaminophen	caffeine	aspirin	aspirin
A (mAU.s) trial 1	12754.90000	5814.85791	11277.5	1950.23108
A (mAU.s) trial 2	12754.90000	5814.84791	11277.5	1950.23108
A (mAU.s) Avg	12754.90000	5814.85291	11277.5	1950.23108
total A (mAU.s)			13227.73108	

Table 5 Aspirin unknown data

Aspirin Unknown	**peak 1**	**peak 2**
t_R (min) trial 1	1.634	2.433
t_R (min) trial 2	1.633	2.431
t_R (min) Avg	**1.6335**	**2.432**
A (mAU.s) trial 1	2056.02075	5568.05371
A (mAU.s) trial 2	2017.99146	5468.69189
A (mAU.s) Avg	2037.00611	5518.37280
total A (mAU.s)	7555.37891	

Table 6 Symbol table

t_R	Retention Time (minutes)
A	Peak Area (mAU.s)
t'_R	Adjusted retention time (minutes)
t_0	Dead time – time for unretained solute (minutes)
C	Concentration (mg/mL)
mAU.s	Milli absorbance unit-seconds (units of peak area)

6.4.2 Standard's Chromatograms

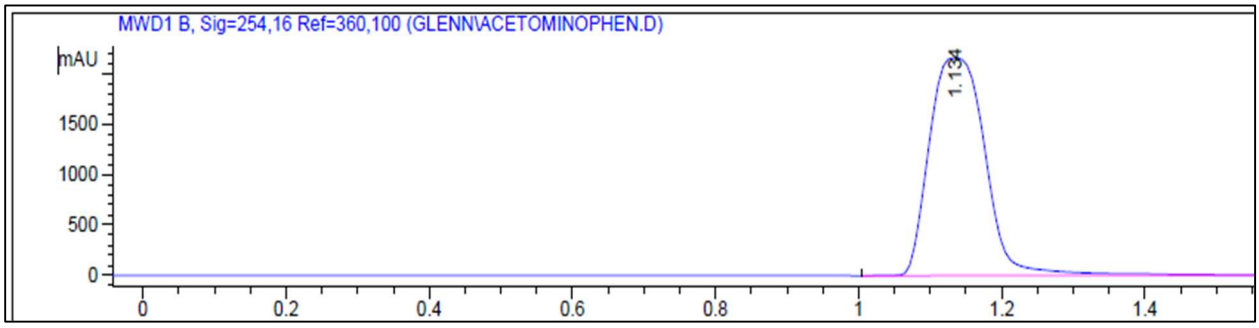


Figure 8 Acetaminophen Standard Chromatogram

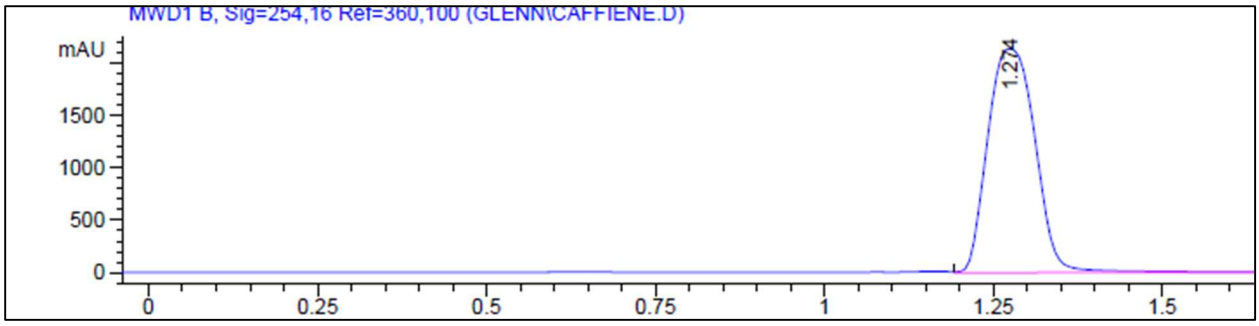


Figure 9 Caffeine Standard's Chromatogram

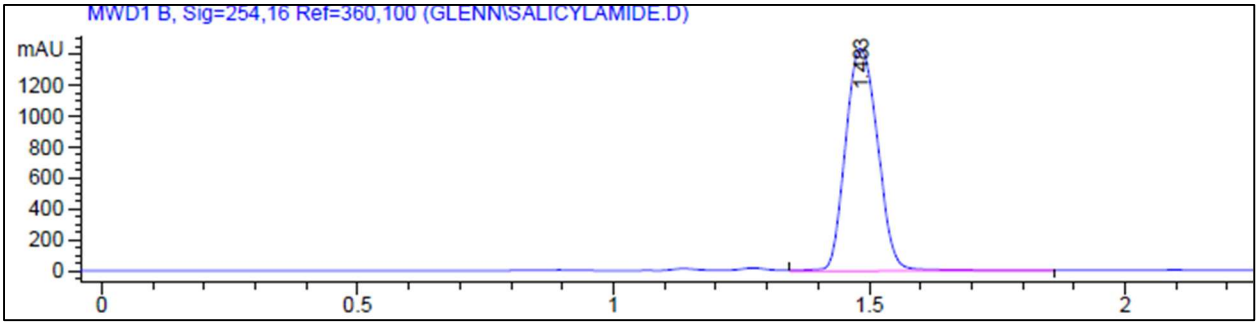


Figure 10 Salicylamide Standard's Chromatogram