**Aspirin, Acetaminophen, and Caffeine separation from Excedrin via Column Chromatography**

Introduction:

Excedrin is an over-the-counter pain reliever that contains three analgesic components: acetaminophen (250 mg), aspirin (250 mg), and caffeine (65 mg) that are widely used around the world. These three analgesic components and other non-steroidal anti-inflammatory drug mainly act on the peripheral nervous system and can work in combinations with other analgesics.[1] The main function of Excedrin is specific to targeting headaches.[2] Each of the components has different functions that treat pain caused by different factors. Aspirin is used to reduce certain substances in the body that produces pain, inflammation, and fever. Acetaminophen is also used to relieve pain and reduce fevers. Caffeine is used as a stimulant that works on the central nervous system, that helps improve blood flow by relaxing muscle contraction in blood vessels and helps counteract the effects of medication that causes drowsiness. Caffeine also increases the properties of acetaminophen by increasing the pain relieving effects of acetaminophen.[1] Aspirin and acetaminophen have different functions, but work simultaneously in order to relieve pain, relieve inflammation, and reduce fevers.

The purpose of this experiment was to separate the three analgesic components, Figure 1: Three Components of Excedrin, using column chromatography. Changing the solvents in the column elutes the three analgesic components based on the components polarity. The first component, aspirin, is the least polar and will be eluted first out of the column using 50% ethyl acetate/ 50% hexanes and monitored by TLC in order to observe whether or not the component has been completely eluted. Once the first component has been isolated the polarity is increased to 75% ethyl acetate/ 25% hexanes to elute the second component, acetaminophen and observed by TLC. The third component, caffeine, which is the most polar, is eluted last with acetone and observed by TLC. A percent recovery was calculated to see
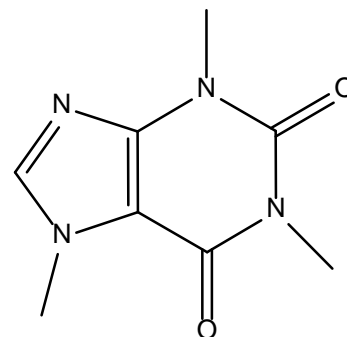
how much of the components were successfully separated. A ^{1}HNMR analysis was also ran to confirm the structures of the components.

Figure 1: Three Components of Excedrin
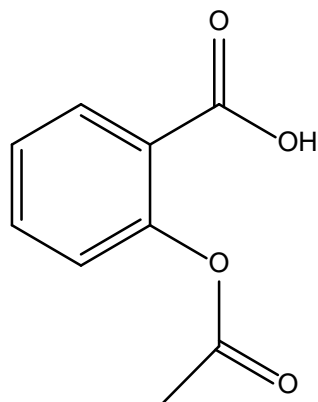
Experimental:
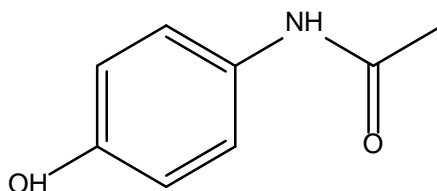
Aspirin. **Acetaminophen. Caffeine**. Excedrin (565 mg) and dichloromethane (20.1 mL) were added together and stirred. Added silica gel (0.50 mL) to mixture and heated via water bath and stirred. Excedrin is adhered to silica (stationary phase) and added to the packed column. Added 75% ethyl acetate and 25% hexanes to make the TLC solvent. The mixture was then separated via column chromatography. Monitored by TLC using 50% hexanes/50% ethyl acetate (100.5 mL) to elute aspirin (0.131 g, 0.727 mmol). Monitored by TLC using 25% hexanes/75% ethyl acetate (100 mL) to elute acetaminophen (0.002 g, 0.013 mmol). Monitored by TLC using acetone (101 mL) to elute caffeine (0.086 g, 0.443 mmol). ^{1}HNMR Aspirin (60 MHz, CDCl$_3$): δ (ppm) 2.339 (s, 3H), 7.193-7.44 (m, 4H), 8.075 (s, 1H). ^{1}HNMR Acetaminophen (60 MHz, DMSO): δ (ppm) 1.958 (s, 3H), 6.574-6.732 (d, 2H), 7.263-7.410 (d, 2H), 9.115 (s, 1H), 9.629 (s, 1H). ^{1}HNMR Caffeine (60 MHz, CDCl$_3$): δ (ppm) 3.415 (s, 3H), 3.590 (s, 3H), 3.992 (s, 3H), 7.4 (s, 1H).

Results and Discussion:

The technique used to separate the three analgesics from Excedrin was column chromatography. Column chromatography is used to purify any mixture of liquid and solid with difference in polarity on a macro or micro scale. In this case, the separation of the analgesic from Excedrin is a solid mixture on a macro scale. The method of column chromatography that was used was the slurry method. In this method, the Excedrin was adhered to the silica (stationary phase) and then added to the packed column using different polarity of mobile phases depending on the polarity of the component being separated and monitored by TLC.

A standard TLC solvent was made to compare the R_f values of the components during the separation. Based on the standard TLC plate: aspirin had an R_f value about 0.57, acetaminophen had an R_f of 0.17, and caffeine had a R_f of 0.03. The TLC plate that contained the fractions was then observed under a UV light. For fractions 1-5, only fraction 2 had a spot with an R_f value of 0.50, indicating that the component was aspirin. Fractions 1, 3, 4, and 5 did not show most likely because of poor spotting and should have been re-spotted for better analysis. For fractions 6-10, the R_f value was 0.63 indicating that the component was aspirin. There was no spot for fraction 11 indicating that the tube contained pure solvent. The spots for fractions 12-15 did not leave the baseline indicating that there was bad spotting and possibly bad separation. Fractions 16-20 had an R_f value of 0.15 and fractions 21-25 had a R_f of 0.167, therefore fractions 16-25 can be identified as the acetaminophen component. There were no spots for fractions 26-28 indicating that there was pure solvent in the tube. The last fraction was at 30 and 31 that had an R_f value of 0.03 indicating that the component was caffeine. Therefore, based on the R_f values of the observed fractions on the TLC plate the first component to elute was aspirin, followed by acetaminophen, and then lastly caffeine. From the results of the column chromatography, the first component to elute out of the column was aspirin followed by acetaminophen and caffeine confirming the observed R_f values and therefore the experiment was successful. The percent recovery, however, was

rather poor. The percent recovery based on the amount of components in the tablet are as follows: aspirin percent recovery of 52.4%, acetaminophen of 0.8%, and caffeine 132%

To confirm the structures of the isolated components a ^{1}HNMR analysis was done for each of the component. For the first ^{1}HNMR (Figure 2, ^{1}HNMR Aspirin), the analysis shows that there is a singlet at 2.339 ppm integrated to 3 that indicates a methyl group. There are two peaks at 7.193 ppm and 7.440 ppm that are overlapping of doublets and triplets because of splitting patterns and is integrated to about 4, representing the four aromatic hydrogens and confirming the presence of an aromatic group. And lastly, a peak at 8.075 ppm that is integrated to about 1 and is shifted the furthest down field because of the hydrogen proton of the carboxylic acid group. There were some impurities that included acetone, $CDCl_3$ solvent and random junk impurities. The component that was identified was aspirin (Figure 3, Structure of Aspirin).
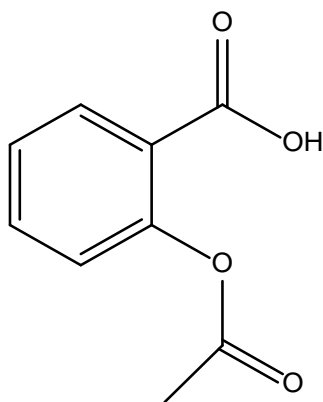

Figure 3: Structure of Aspirin

For the second ^{1}HNMR (Figure 4, ^{1}HNMR Acetaminophen), the analysis shows that there is a singlet peak at 1.958 ppm that is integrated to 3 indicating the presence of a methyl group. There are four different peaks at 6.574 ppm, 6.732 ppm, 7.263 ppm, and 7.410 ppm that are overlapping because of splitting pattern and indicates the four aromatic hydrogens that confirm the presence of an aromatic group. There is another peak at 9.115 ppm that is shifted downfield that indicates the hydrogen proton of the hydroxyl group. The last peak that is shown is at 9.629 ppm, which is the furthest shift downfield

and indicates the hydrogen proton of the amide group. There were some impurities that included

acetone, water, and DMSO solvent. The component that was identified was acetaminophen (Figure 5,
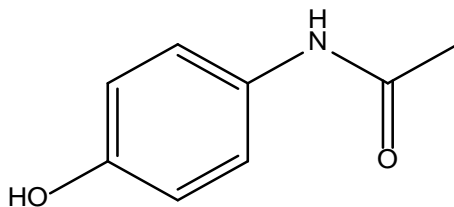
Structure of Acetaminophen).

Figure 5: Structure of Acetaminophen

For the third ^{1}HNMR (Figure 6, ^{1}HNMR Caffeine), the analysis shows three different peaks at

3.415 ppm, 3.590 ppm, and 3.992 ppm each singlets integrated to about 3. These three peaks represent

the 3 methyl groups of the compound. The last peak of interest was not indicated on the ^{1}HNMR but it

should be around 7.4 ppm, this represent the hydrogen proton off the five-member ring. There was a

slight impurity with the $CDCl_3$ solvent. The component that was identified was caffeine (Figure 7,
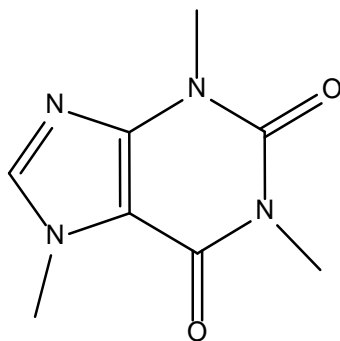
Structure of Caffeine).

Figure 7: Structure of Caffeine

In conclusion, the R_f values calculated was correct based on the elution order during the column

chromatography. There was a low percent recovery of acetaminophen because acetaminophen forms a

fine solid that is difficult to filter.[3] There was a percent recovery of over 132% for caffeine that possibly

contained other impurities such as water and possibly the other components. According to Kevin Revell,

typical results for percent recoveries for aspirin is around 60-100%, acetaminophen 10-20%, and

caffeine 40% when using chromatography.[3] Possible sources of error are throwing out possible fractions remaining in solvent, melting the crude product during evaporation of solvent via warm water bath and nitrogen, etc. Future improvements that should be made in future experiments is to improve the percent recovery of the components by practice setting up a column chromatography that contains no cracks or bubbles for better separation, better spotting on TLC plate to see the process of the components eluting and using different mobile phase.

References:

(1) Mccaffery, Margo. "Analgesics." *Nursing* **1996**, *26.1*, 41-42.

(2) Wenzel, Richard G., Carrie A. Sarvis, and Michelle L. Krause. "Over-the-Counter Drugs for Acute Migraine Attacks: Literature Review and Recommendations." *Pharmacotherapy* **2003**, *23.4*, 494-505.

(3) Revell, Kevin D. "Separation of the Components of a Commercial Analgesic Tablet: A Two-Week Sequence Comparing Purification by Two-Base Extraction and Column Chromatography." *Journal of Chemical Education* **2011**, *88.10*, 1413-415.

Supplemental Information:

Figure 1: Three Component of Excedrin

Figure 2: ^{1}HNMR of Aspirin

Figure 3: Structure of Aspirin

Figure 4: ^{1}HNMR of Acetaminophen

Figure 5: Structure of Acetaminophen

Figure 6: ^{1}HNMR of Caffeine

Figure 7: Structure of Caffeine