Separation of Excedrin by Column Chromatography

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FFR #2

Introduction

Many people take Excedrin® to relieve aches and pains, and in most cases it is taken to fight headaches. Excedrin® contains three key ingredients, which act as analgesic drugs and fight inflammation: aspirin, acetaminophen, and caffeine. Each of the components listed above has a unique effect while combating pain and inflammation.

Not only does aspirin fight inflammation, it also prevents certain types of cancer. When aspirin enters the body, it attacks cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). COX-1 and COX-2 are isoenzymes, but only the latter of the two has inflammatory effects. Aspirin reacts irreversibly with COX-2, which makes this enzyme an inactive inflammatory agent. By making COX-2 inactive, the body is also less receptive to cancer. Based on animal studies, the COX-2 enzyme is known to reduce cellular adhesion and preprogrammed natural cell death while at the same time increasing the rate at which new blood vessels form from old ones. By inhibiting the COX-2 enzyme, tumors will have a harder time forming and thus obstructing cancer. Aspirin is most effective at preventing esophageal cancer.[1]

One of the most used drugs for chronic pain is acetaminophen. Like aspirin, acetaminophen irreversibly reacts with COX-2, but unlike aspirin, it does not react with COX-1. This reduced reactivity is somewhat of a benefit, since deactivating COX-1 enzymes can be potentially harmful. Even though inhibited COX-2 enzymes allow for reduced inflammation and block tumor growth, reduced levels of COX-2 have led to increased blood pressure readings. The magnitude of increased blood pressure was found to be directly proportional to the amount of ingested acetaminophen. While acetaminophen is an excellent analgesic, it can be potentially dangerous to people with naturally elevated blood pressures.[2]

The final component of Excedrin® is caffeine. This component blocks adenosine receptors in the brain. When adenosine comes into contact with its receptors, fatigue, pain, drowsiness, widening of the

brain's blood vessels, and increased flow of oxygen to the brain are all side effects of this interaction. Also, adenosine causes pain sensors to become more active, and it causes more intense pain in torn and bruised muscles. By introducing caffeine into a person's system, adenosine receptors are blocked. While increased alertness is usually associated with increased pain and soreness, caffeine has the ability to increase alertness and reduce pain.[3]

Due to the various effects of the three components above, it is useful to have them in a single medication (Excedrin®), but it can also be extremely useful to use them separately. Below, the structures of aspirin, acetaminophen, and caffeine are shown, and the separation of these components is discussed.
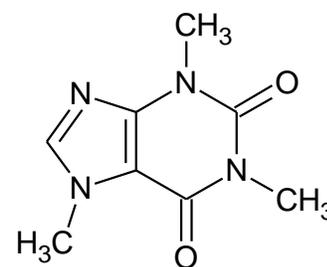
OH

O

O

H₃C O

Aspirin

A

NH CH₃

O

HO

Acetaminophen

B

CH₃

N O

N

N N CH₃

H₃C O
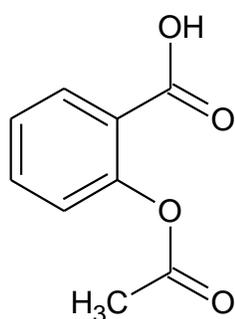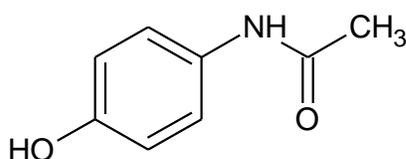
Caffeine

C

Figure I. Structure of Components separated by Column Chromatography

The components of Excedrin® are aspirin, acetaminophen, and caffeine as seen in figures IA through IC. Column chromatography can be used to separate the components listed above. When using a polar immobile phase and a less polar mobile phase, the elution of the compounds occurs based on the relative polarities of the components. Based on the polarities of the components above, aspirin, being the least polar, will elute first; acetaminophen will follow, and caffeine will shortly after acetaminophen.

Excedrin® is a very efficient at reducing pain and inflammation. It is also good at delaying cancer and making the brain alert, but it can also have vascular side-effects such as elevated blood pressure. Excedrin® is made up of aspirin, acetaminophen, and caffeine, and by using flash column chromatography, these three components can be separated from each other. Each component can then be analyzed and identified by ^{1}HNMR.

Experimental

Aspirin. A single Excedrin® tablet (250mg aspirin, 1.388mmol aspirin, 250mg acetaminophen, 1.654mmol acetaminophen, 65mg caffeine, 0.335mmol caffeine, unknown binders) was crushed and dissolved in dichloromethane (20mL). A TLC (33% Hexanes in Ethyl Acetate) standard of the solution was performed. Silica (0.5mL) was added to the dichloromethane/Excedrin® solution, and the solvent was evaporated to yield a white powder. Flash column chromatography (50% Hexanes in ethyl acetate) was performed to yield aspirin as white, flakey crystals (177mg 70.8%); ^{1}HNMR (60MHz, CDCl$_3$) δ (ppm) 10.2 (s, 1H), 8.0 (dd, 2H), 7.5 (m, 3H), 2.3 (s, 1H).

Acetaminophen. Further flash column chromatography (30% hexanes in ethyl acetate) was performed to yield acetaminophen as an off-white, chalky powder (240mg, 96.0%); ^{1}HNMR (60MHz, DMSO) δ (ppm) 19.6 (s, 1H), 9.1 (s, 1H), 7.3 (d, 2H), 6.6 (d, 2H), 1.9 (s, 3H).

Caffeine. Further flash column chromatography (100% acetone) was performed to yield caffeine with acetaminophen impurities (33%) as a bleach-white solid (132mg, 203.1%); ^{1}HNMR (400MHz, DMSO) δ (ppm) 8.0 (s, 1H), 3.8 (s, 3H), 3.4 (s, 3H), 3.2 (s, 3H).

Results and Discussion

Excedrin® components were separated using flash column chromatography. Ideal quantities of aspirin and acetaminophen were isolated, but the isolated caffeine was found to have a significant

amount of acetaminophen present. In order to completely purify the caffeine, a different mobile phase will need to be implemented, but first it is important to assess the implemented method of separation.

Based on the relative polarities of the three compounds in Excedrin®, the elution order can be easily predicted. Aspirin has an aromatic ester and an aromatic carboxylic acid functional group; therefore, it has the potential of being very polar. Since the two functional groups are ortho to each other, however, hydrogen bonding forces on the acidic proton cancel out with forces on the ester, and aspirin elutes first. Acetaminophen has a phenol and amide function group, but caffeine has multiple amide functional groups; therefore, acetaminophen is more polar than aspirin and less polar than caffeine. As a consequence, acetaminophen will elute second, and caffeine will elute last.

The order of elution predicted above was, in fact, observed during the experiment and identified by ^{1}HNMR. Beaker 1 contained all of the fractions with the pure first eluted compound. Figure 1 shows the analysis of this compound. The peak at 10.1ppm represents the acidic proton, and the peaks between 8.0ppm and 7.0ppm represent the aromatic protons. At 2.3ppm, that peak is representative of the methyl group on the ester. By quick ^{1}HNMR analysis, the first eluted compound was aspirin as predicted.

Beaker 2 contained all of the fractions with the pure second eluted compound. Figure 2 shows the analysis of this compound. The peak at 9.6ppm represents the proton on the nitrogen, and the peak at 9.1ppm represents the proton on the phenol group. The peaks at 7.3ppm and 6.6ppm represent the aromatic protons while the peak at 1.9 ppm represents the protons on the carbonyl. By quick ^{1}HNMR analysis, the second eluted compound was acetaminophen as predicted This sample was not entirely pure, since it contained a trace amount of water and a lot of acetone.

Beaker 4 contained all of fractions with caffeine and acetaminophen impurities. Due to the recognized impurities, the sample was identified by 400 MHz ^{1}HNMR. The peaks integrated in figure 3b correspond to those identified for acetaminophen above. The peaks integrated in figure 3c correspond

to caffeine. The peak at 8.0ppm represents the proton on the five membered ring, and the peaks at 3.8ppm, 3.4ppm, and 3.2ppm represent the protons on the alkane groups bonded to the nitrogens. In this sample there were also other impurities, such as a trace amount of acetone and a lot of water.

In both beakers 2 and 4 there were traces of water and acetone, and both instances are justifiable. Acetone was used as a cleaning agent during the collection of the fractions in beaker 2. Since the mobile phase at that time was 33% hexanes in ethyl acetate, acetone was the most polar and thus the final compound to dissolve during the evaporation of solvent. The acetone could have easily been locked inside the solid acetaminophen at the time. Also, the compound was left open to the atmosphere for a couple of weeks. Trace amounts of water from the atmosphere could have entered the sample during this time. As for beaker 4, acetone was the sole solvent used, so when evaporating, it would have done so more efficiently since the polarity of the solvent was constant throughout. Again, this compound was left open to the atmosphere like the acetaminophen, so water from the atmosphere could have entered the sample. Before the samples from beaker 2 and beaker 4 were placed in the NMR tubes, the NMR tubes themselves were blown with nitrogen gas, but the rubber tubing could have also had water in it, in which the nitrogen gas deposited the water on the walls of the NMR tube. The aspirin sample contained no impurities because its NMR tube was not blown out with nitrogen gas, and the NMR sample was made only a few days after being exposed to the atmosphere.

Mentioned above, the separation of caffeine from the acetaminophen failed, and no pure caffeine was obtained. The separation of these last two components needs to be done using different mobile phases in order to obtain better separation. In the experiment, the first twelve fractions were obtained and identified by TLC (33% hexanes in ethyl acetate). Fractions 3-6 contained aspirin and 11-12 contained acetaminophen. The mobile phase at the time (50% hexanes in ethyl acetate) eluted the aspirin and also started to elute the acetaminophen. Then, fractions 13-37 were collected using a higher polarity mobile phase (33% hexanes in ethyl acetate), but the fractions mixed with caffeine in fractions 31-34.

Instead of using that mobile phase, the original mobile phase should have been used. This would have pulled out the acetaminophen before caffeine started to elute even though many more fractions would have needed to be collected. Then, acetone was used to elute the rest of the acetaminophen and caffeine, but there was no pure caffeine because the acetaminophen had not eluted completely out.

Overall, 177mg of aspirin was collected. Since there were no impurities in the ^{1}HNMR, it can be assumed the entire collected sample was, in fact, aspirin. The percent recovery was 70.8 percent. Of the acetaminophen, there was 240mg collected from both the pure and impure samples. Since the acetaminophen and caffeine/acetaminophen samples had water and acetone in them, the actual acetaminophen recovered was most likely less than 240mg, giving a recovery of less than 96 percent. Of the caffeine sample, caffeine made up approximately 66 percent of the sample with respect to the acetaminophen. Therefore, the mass of caffeine recovered was calculated to be 132mg, but there was only 65 mg of caffeine in the Excedrin® tablet to begin with. It can be concluded there was much less than 66 percent caffeine in that sample, most of the mass belonging to water as per the ^{1}HNMR spectrum results.

In conclusion, Excedrin® can be separated into aspirin, acetaminophen, and caffeine provided the correct mobile phases are used and care is taken to keep water and acetone out of the first two elutions. Combined, these three compound create an excellent analgesic with cancer preventative and increased alertness side effects. Care must be taken, however, while giving acetaminophen to people with naturally high blood pressure.

References

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