Separation of Excedrin by Extraction and Column Chromatography

Introduction

Analgesics are classes of drugs that help provide pain relief. Analgesics work by blocking the sensation of pain in the central and peripheral nervous system. These drugs commonly work by blocking sodium ion channels in sensory neurons that help propagate signals, enzymes that make or release neurotransmitters that reach the pain center of the brain, or stress receptors that initiate depolarizations as a result of pain. Analgesics have differing levels of intensity, some for mild, chronic pain, and others for severe, acute pain.

Excedrin is a common pain reliever that is made by Bristol-Myers Squibb. It is used to relieve headaches and has been one of the top-ten best-selling medicines in the United States. Over 180 million people take Excedrin worldwide, which highlights the importance of this drug. Excedrin contains the ingredients aspirin (250 mg), acetaminophen (250 mg), and caffeine (65 mg). Aspirin and acetaminophen are important analgesics, and caffeine is a key stimulant that prevents drowsiness caused by aspirin and acetaminophen. The three components of Excedrin were isolated in this experiment using extraction and column chromatography.

Acid-base extraction is a separation technique that utilizes the different levels of acidity or basicity of components in a mixture. Typically, components are strongly acidic, slightly acidic, neutral, slightly basic, or strongly basic. Components are isolated by being converted to their ionic form by being deprotonated (if the component is acidic) by a base or being protonated (if the component is basic) by an acid. After the components are ionized, they will migrate to the aqueous layer, which is separate from the other components in the organic layer. The two layers can then be separated using a separatory funnel, resulting in effective isolation of components.
Column chromatography is another separation technique that utilizes the different levels of polarity of components in a mixture\textsuperscript{1,4}. Components are isolated by eluting from the column at different times based on their level of polarity. For example, a more nonpolar component will elute first since it will adhere least to the polar stationary phase. In addition, the more nonpolar component can be eluted using a more nonpolar mobile phase because only it will be attracted to it, whereas the other more polar components will not be. The other polar components can later be eluted by switching to more polar mobile phases, resulting in effective isolation of components.

The purpose of this laboratory experiment was to separate the three components of Excedrin—aspirin, acetaminophen, and caffeine—using acid-base extraction and column chromatography. The final isolation products were characterized using 60 MHz 1H NMR, and the quality of separation for the two isolation techniques were compared based on purity and percent recovery.

**Experimental**

**Acid-Base Extraction**: One tablet of Excedrin was crushed and dissolved in ethyl acetate (20 mL). The mixture was filtered to give a clear solution. Aspirin was isolated first by extracting the organic solution with 10 mL, 1M aq. K$_2$HPO$_4$, and acetaminophen was isolated by extracting the organic solution with 10 mL, 1M KOH. Caffeine was isolated by washing (10 mL, sat. aq. NaCl) and drying (sodium sulfate) the remaining organic solution. Aspirin and acetaminophen were neutralized with 6 M HCl and the precipitate was collected using vacuum filtration.

Aspirin (0.0857 g, 34.3\%) 1H NMR (60 MHz, CDCl$_3$) $\delta$ (ppm) 11.709 (s, 1H), 7.056-8.174 (m, 4H), 2.337 (s, 3H). Acetaminophen (0.0626 g, 25\%). Caffeine (0.0186 g, 28.6\%).
**Column Chromatography:** One tablet of Excedrin was crushed and dissolved in ether (20 mL) and the mixture was filtered to give a clear solution. Silica (½ mL) was added to the three component solution (beaker, stir bar, warm water bath) until all the ether evaporated. A column was then slurry packed, with silica and 1:1 hexane/ethyl acetate. 1:1 hexane/ethyl acetate was used to elute aspirin, 1:2 hexane/ethyl acetate was used to elute acetaminophen, and acetone was used to elute caffeine. Fractions with the same components were combined and dried to give pure compound.

Aspirin (0.1528 g, 61.1%). Acetaminophen (0.095 g, 38%). Caffeine (0.0039 g, 6%).

**Results and Discussion**

The isolation of aspirin, acetaminophen, and caffeine from Excedrin utilized the differing acidities (extraction) and polarities (column chromatography) of the three compounds. Extraction involved separating the three components by reacting them with a base selectively, while column chromatography involved separating the components by passing them through a packed column. Aspirin was extracted first for both extraction and column chromatography (since it was the most acidic and least polar component); acetaminophen followed, and caffeine was extracted last since it was neutral and the most polar.

The isolation and purification was relatively successful based on the percent recovery. The percent recovery for extraction was 34.3% for aspirin, 25.0% for acetaminophen, and 28.6% for caffeine. The mediocre percent recovery can mostly be attributed to the technique and not to the nature of the reaction with the base. There are many ways product may have been lost in the extraction. First, when crushing the tablet, it is possible that some powder may have been lost or that there may still have been components with the solid binders. Also, it is possible that some
components may have been lost in the separatory funnel since they are only slightly soluble in water and some may have stayed in the organic layer. The culmination of these two reasons most likely explains why the percent recovery was not higher.

Likewise, for column chromatography, the percent recoveries were relatively mediocre. The percent recovery was 61.1% for aspirin, 38.0% for acetaminophen, and 6.0% for caffeine. Because there was no reaction involved in this isolation, the percent recovery can entirely be attributed to the technique. It is possible that, again, some product may have been lost when crushing the tablet of Excedrin. However, the main reason for the mediocre percent recovery (and especially the poor recovery of caffeine) is that some components were trapped in the column. Caffeine was likely to be trapped in the polar stationary phase since it is the most polar, resulting in the lowest percent recovery. Aspirin, however, had the highest percent recovery since it is likely that aspirin was not trapped as much in the polar stationary phase (since aspirin is nonpolar). The culmination of these two reasons most likely explains the overall mediocre percent recoveries and the relative trend of percent recoveries and polarities.

The spectral data for the acid-base extraction aspirin matches the structure of aspirin, suggesting that the isolation via acid-base extraction was successful. Unfortunately, NMRs of the other components could not be obtained, so their characterization will be discussed later in the TLC section. The 1H NMR for aspirin has peaks at 11.709 (s, 1H), 7.056-8.174 (m, 4H), 2.337 (s, 3H). These match the theoretical peaks for aspirin since peaks from 7-12 suggest a phenol, 6.5-8 suggest an aromatic ring, 3-5 suggest an N-H bond, and 2-2.5 suggest methyl ketone C-H bond. The integration values also match, and there are no unusual peaks in the spectrum. This confirms the identity of aspirin and also suggests that the purity is high.
The TLCs suggest that the isolation of the three components for both isolation techniques was successful. Because aspirin is the least polar component, it is expected that it will travel the highest on the TLC plate since it is least attracted to the polar stationary phase. The order of the components on the TLC plate should be aspirin, acetaminophen, and caffeine. For the extraction, the TLC plate showed that aspirin’s spot traveled the furthest, acetaminophen’s spot traveled the next furthest, and caffeine’s spot traveled the least. This is consistent with the expected order of distances traveled, suggesting that the components were all successfully isolated. The Rf value was 0.304 for aspirin, 0.190 for acetaminophen, and 0.076 for caffeine. There were no additional spots in each lane either, which suggests that the purity of the components is high as well.

Likewise, for column chromatography, the order of spots on the TLC plate matched the expected order based on the components’ polarities. Also, the Rf values were very similar to the ones obtained in extraction: the Rf value was 0.289 for aspirin, 0.169 for acetaminophen, and 0.048 for caffeine. Because the Rf values were so close to the ones obtained in extraction, it can be concluded that the identities of the isolation products were, in fact, aspirin, acetaminophen, and caffeine. Despite the possibility that other compounds can share the same Rf values as the three components, it is very unlikely that they would be present and appear in both the extraction and column chromatography TLCs. Therefore, it is safe to conclude that the isolation was successful and the three components were successfully characterized.

The TLCs also showed that the purity of the components from column chromatography was quite high. There were very large gaps between when the first, second, and third products eluted. About 70 mL of pure mobile phase eluted between aspirin and acetaminophen, and about 30 mL of pure mobile phase eluted between acetaminophen and caffeine. In addition, there were
no irregular spots on any of the TLC plates, which confirm that the three components from
column chromatography were isolated with relatively high purities.

Because the percent recoveries for polar compounds were lower for CC compared to
acid-base extraction, it can be concluded that acid-base extraction is a better separation technique
to yield better percent recoveries. Also, the NMR for aspirin from the acid-base extraction was
the only NMR that worked, which also supports the conclusion that acid-base extraction is a
better isolation technique. While both methods resulted in the intended products, the aspirin from
the acid-base extraction may have worked better for the NMR because it was more crystallized
and pure, allowing it to dissolve more easily in CDCl$_3$ to give the proper characterization.

Overall, this isolation was successful since the three components were effectively isolated
and had a high purity based on the TLC results. However, the percent recoveries were all quite
mediocre, which can mostly be attributed to the limitations of both techniques. In addition, the
characterization of aspirin by 1H NMR was consistent with the structure of aspirin, suggesting
that isolation was successful. While the other components were unable to be characterized by 1H
NMR, the fact that the 1H NMR for aspirin matched so well to its structure serves as a testament
to how successful the isolation truly was.

References


(3) D’Orazio, G. *J Chromatogr A.* 2013, 1285, 118-23

(4) Williamson, K. *Macroscale and Microscale Organic Experiments*; Brooks/Cole:
Belmont, 2007; 150-163.