

## **Thin-Layer and Column Chromatography: Identifying an Unknown Analgesic, Determining an Ideal Mobile Phase and Monitoring the Progress of the Mitsunobu Reaction.**

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Aspirin was compared to five standard analgesics using thin-layer chromatography (TLC). The ideal mobile phase solvent for the Mitsunobu reaction was determined as 35% ethyl acetate and 65% hexane. The progress of a Mitsunobu reaction was monitored using column chromatography and TLC.

Chromatography is an efficient and relatively simple technique for separating and identifying components of a mixture. The components are separated because they have different affinities to two distinct phases. One phase is stationary, while the other is mobile.

In most chromatography experiments, either silica, or alumina, serves as the stationary phase.<sup>1</sup> Silica and alumina are both polar and their polarity remains constant throughout the experiment. The term *absorbent* is commonly used when referring to the stationary phase. A mobile phase consists of a solvent that moves up a plate by capillary action or down a column due to gravity. Unlike the stationary phase, the polarity of the

mobile phase is easily controllable. Using pure solvents alone, or a combination of different solvents, allows for the possibility of an infinite range of polarities. Pure solvents alone range from polar to non-polar and mixtures of solvents will result in a wider variety of polarities. The choice of mobile phase is crucial for a clean separation. If the solvent chosen is too polar the components will move too close to the solvent front and likewise if the solvent is not polar enough the components will remain too close to the origin. In either scenario, separation is poor. The ideal mobile phase will move the components about half way between the origin and the solvent front.

Two common types of chromatography are thin-layer chromatography (TLC) and column chromatography. TLC is useful for separating and identifying unknown components of a mixture using only small amounts of the mixture. In TLC, the stationary phase consists of silica or alumina coated on a glass or plastic plate. For large separations or purifications where the product is needed for further experiments, column chromatography is advantageous. The stationary phase in a column chromatography experiment is also silica or alumina packed into a column with a constraint at one end or into a buret. Although 20-50 g of the silica or alumina per gram of sample will often suffice, some separations require ratios of 200:1 or higher.<sup>2</sup> It is important to select a column that will completely contain the absorbent with about 10-15 cm to spare and the height of the column should be at least ten times its diameter.<sup>3</sup>

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<sup>1</sup> Lehman J.W. *Operational Organic Chemistry. A Problem-Solving Approach to the Laboratory Course, Third ed*; Prentice Hall: Upper Saddle River, NJ, 1999; pp 618.

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<sup>2</sup> Lehman, pp 618.

<sup>3</sup> Lehman, pp 619.

In a previous experiment, a common analgesic was isolated from a prepared mixture by extraction. The common analgesic and five standard analgesics (salicylamide, ibuprofen, aspirin, acetaminophen and caffeine) were analyzed using TLC. The unknown and the five standards were all UV visible. Because the analgesics travel different distances up the plate they must have different affinities to the mobile phase. In this experiment the mobile phase was 200:1 ethyl acetate: acetic acid. The spot for the unknown was view under a UV lamp to be between the spots of aspirin and acetaminophen 1. We knew that the analgesic could not be acetaminophen because acetaminophen is too weak to react with HCl of the aqueous layer in the extraction mentioned previously.<sup>4</sup> We concluded, then, that the unknown was most likely aspirin.

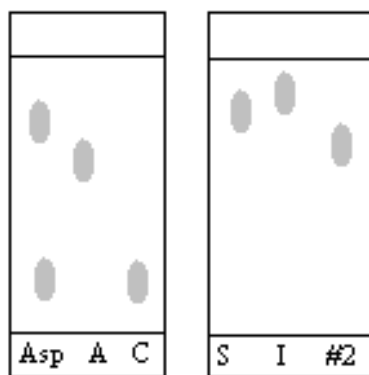


Figure 1

By simply looking at different TLC plates, it is often difficult to compare the movement of the spots. In order to compare the spots,  $R_f$  values are calculated. An  $R_f$  value is the ratio

<sup>4</sup> Knoerzer, T. *Fall Organic Chemistry Exp. #3. 2002.*

<http://www.pub.naz.edu:9000/~organic/fall/fallorganic/labstuff/fallexp3.htm>

between the movements of the spot to the movement of the solvent front 2. Because this ratio is the movement of a spot in relation to the distance the solvent front moved, the solvent front does not need to reach any specific point before it is removed from the developing chamber.  $R_f$  values ensure the ability to compare the spots from two or more different plate, assuming that the mobile phase used remained constant for all the plates.

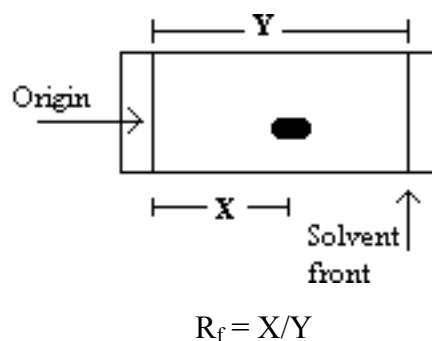


Figure 3

The  $R_f$  values for the analgesic TLC plates are given in **Table 1**. There is a difference of 0.066 between the  $R_f$  value for the unknown and the  $R_f$  value for acetaminophen, and there is a -0.077 difference between the  $R_f$  values of the unknown and aspirin.

Table 1

Analgesic	$R_f$ Value
Acetaminophen (A)	0.476
Salicylamide (S)	0.738
Aspirin (Asp)	0.362
Ibuprofen (I)	0.619
Caffeine (C)	0.785
unknown (#2)	0.362
	0.542

The differences between the unknown spot and the two standard spots are very close which poses difficulties

for identification, even though we know the unknown is not acetaminophen.

Ideally, the unknown, aspirin and acetaminophen should be spotted on another TLC plate using a solvent that is not as polar as the mobile phase. A slightly less polar solvent would move the spots towards the middle of the plate, away from the solvent front and hopefully yield more distinct  $R_f$  values.

The method of chromatography is also used for purification. In a synthesis of  $\alpha$ -Acyl-Functionalized Azacycles, part of a reaction was found to contain 77% of the desired product by "chromatographic purification."<sup>5</sup>

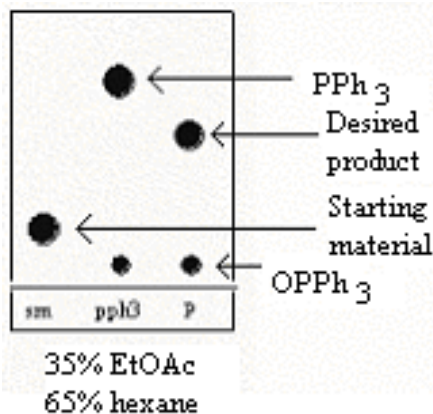


Figure 3

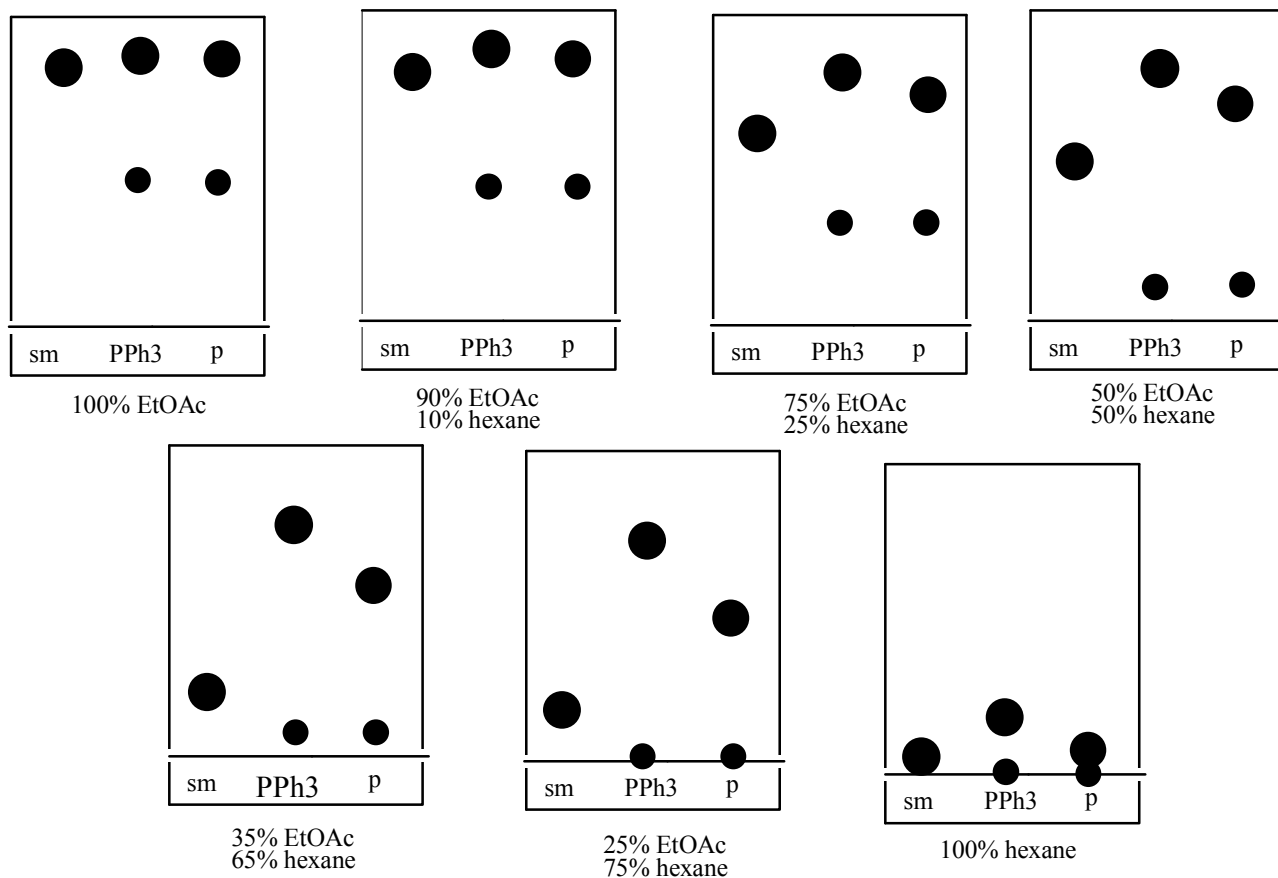


Figure 4

<sup>5</sup> Occiato, E.G.; Prandi, C.; Ferrali, A.; Guarna, A.; Deagostino, A.; Venturello, P. *J. Org. Chem.* **2002**, Vol. 67, No. 20, 7144-7145.

Monitoring the progress of a reaction is a third application of chromatographic techniques.

In order to monitor the progress of the Mitsunobu reaction, we needed to determine the ideal mobile phase for separation using TLC. Seven mobile phases consisting of different quantities of ethyl acetate (EtOAc) and hexane were tested using TLC. Because the reactants and products were UV visible, the plates were viewed under a UV lamp. The ideal mobile phase is somewhere in the range of 50% (EtOAc): 50% hexane to 35% EtOAc: 65% hexane. Both the 50:50 and 35:65 mixtures of ethyl acetate: hexane moved the spots halfway up the plate. The lower spots in the PPh<sub>3</sub> and P lanes are the same material, a byproduct, meaning that the reactant mixture is not pure **3**. However, because there is no starting material or triphenylphosphine (PPh<sub>3</sub>) left in the product (P) lane, the reaction went to completion.

As for the other mobile phase solvents, 100% EtOAc solvent was too polar and the spots moved too far up the plate. Likewise, the 100% hexane solvent was very non-polar and the spots stayed too close to the origin.

Once the ideal mobile phase was determined, column chromatography was used to monitor the progress of the Mitsunobu reaction mixture. Collecting fractions from column chromatography is valuable for large separations and purifications, especially when the components are needed for further testing or reactions. The desired product of the Mitsunobu reaction, was separated using a 1:9 ethyl acetate: hexane mobile phase. The ideal mobile phase was not used at the beginning of the chromatography because the 35% EtOAc 65% hexane would be too polar

and move more components down the column than were desired. After collecting ten fractions, the first, fifth and tenth fractions were analyzed by TLC in order to find out if the samples contained pure components. The first fraction contained a faint spot while the fifth fraction was pure. The tenth fraction contained the desired product along with a faint impurity spot. The ten fractions were combined together because we were more concerned with separation than of complete purity. The combined ten fractions will be used for further experiments.

## Experimental Section

**Identification of aspirin.** To separate solutions of ethanol were added the unknown, salicyimide, aspirin, caffeine, acetaminophen and ibuprofen. The mobile phase used was 200:1 ethyl acetate: acetic acid. Spots were viewed under a UV lamp.

**Determination of the ideal mobile phase in order to monitor the progress of the Mitsunobu reaction.** The mobile phases tested were 100% ethyl acetate (EtOAc), 90% EtOAc and 10% hexane, 75% EtOAc and 25% hexane, 50% EtOAc and 50% hexane, 35% EtOAc and 65% hexane, 25% EtOAc and 75% hexane, and 100% hexane. Spots were viewed under a UV lamp.

**Column chromatography.** To a buret column of 36 cm in length and 2.5 cm in diameter was added 1.5 cm of sand, followed by 15 cm of dry 70-30 mesh silica, followed by 1.5 cm of sand. The column was packed with a 1:9 hexane: EtOAc mobile phase. The column was flashed with nitrogen. Ten fractions were collected and the first, fifth and tenth

fractions were tested using TLC with a 35% ethyl acetate, 65% hexane mobile phase.

**Supporting Information Available:**

Lehman, John. *Operational Organic Chemistry: A Problem-Solving Approach to the Laboratory Course, Third edition*. Prentice Hall: Upper Saddle River, NJ, 1999; pp 116, 617 – 630.