# TLC Analysis of Analgesics

Thin Layer Chromatography (TLC) is a very common and versatile analysis technique. TLC can be used to check the progress of a reaction, to determine the purity of a compound and even aid in the identification of a compound or mixture. In this experiment you will use TLC to identify the active ingredients in some commercial analgesics. The commercial analgesics will be composed of one or more of the following ingredients:

**Required Reading (Padias):**

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| **Topic** | **2nd Edition** | **3rd Edition** |
| Chromatography, general | pp. 162 – 163 | pp. 164 – 165 |
| Thin-Layer Chromatography | pp. 165 - 172 | pp. 167 – 174 |

**Special Safety Notes:**

Spotting capillaries are made of very fine glass and should be handled with care and disposed of in the glass bins.

**References**

Pavia, D. L.; Lampman, G. M.; Kriz, G. S.; Engel, R. G. *Introduction to Organic Laboratory Techniques, A Microscale Approach;* 3rd ed.; Brooks/Cole: Pacific Grove, CA, 1999; pp. 115 – 119.

**Procedure:**

Preparation of the TLC Plates

***(NOTE: Handle TLC plates by the edges only!)***

 Obtain two (2) 5 cm x 5 cm TLC plates; one will be your reference plate and one will be your sample plate. USE A PENCIL TO MAKE THE FOLLOWING MARKINGS ON EACH PLATE. Do not push down on the pencil; simply drag it across the plate to make a faint line. On each plate draw a line 0.5 cm from the bottom of the plate (this line is called the “origin”). Draw another line 0.5 cm from the top of the plate (this line will be the “solvent front”). On the bottom line of each plate (the “origin”), starting 1 cm from the side, make 4 tick marks at 1 cm intervals. On the reference plate, label four "lanes" as shown below to designate where each standard as well as the reference mixture will be spotted. On the sample plate, label four "lanes" as shown below to designate where each commercial analgesic as well as the reference mixture will be spotted.

Reference Plate Sample Plate

Spotting and Developing the Reference Plate

 Using a 1 L micropipet, spot 1 L each of the individual reference solutions and the reference mixture in the proper “lanes” at the origin on the reference plate. Using your tweezers, grasp the plate at the top and wave it back and forth in the air to assist in the evaporation of the spotting solvent. Prepare the TLC developing chamber by adding 4 mL of 1% acetic acid in ethyl acetate to a 400 mL beaker and cover with a watch glass. To place your reference plate in the developing solvent (eluent), remove the watch glass, and while grasping the plate at the top using tweezers, slowly lower the reference plate into the chamber so it stands more or less vertically in the chamber. Replace the watch glass to prevent solvent evaporation. Allow the eluent to rise up the plate by capillary attraction until the solvent reaches the “solvent front” line at the top of the plate. Remove the plate from the chamber using tweezers and wave it back and forth in the air to assist in the evaporation of the developing solvent from the plate. Examine the plate under UV light (254 nm) and GENTLY circle each spot with a pencil.

Analysis of the Commercial Analgesics

 Crush ¼ tablet of each commercial analgesic and place the crushed tablet into a **clean**, **dry** 3-mL or 5-mL conical vial. Add methanol until the level is slightly above the 2.0 mL mark on the vial (anywhere between the 2.0 and 3.0 mL marks should be fine). Cap the conical vials and shake each one (shake it hard!) for 1 min. Allow each mixture to settle for about a minute (the mixture may remain cloudy, even after this settling period). Using a 1 L micropipet, spot 1 L each of the individual analgesic solutions and the reference mixture in the proper “lanes” at the origin on the sample plate, allow the spotting solvent to evaporate, then elute the plate using 1% acetic acid in ethyl acetate as the developing solvent as described above. Allow the eluent to evaporate as described above and examine the plate under UV light (254 nm) and GENTLY circle each spot with a pencil.

Identification of Components

1. (a) Draw a picture of your reference plate. Include the Rf values for each spot and the identity of each spot. (b) Draw a picture of your sample plate. Include the Rf values for each spot. Show your calculations for the Rf values.

2. What can you conclude about the composition of each commercial analgesic? Explain your reasoning.

**Questions**

**(Fully explain how you arrived at each answer, including all calculations.)**

1. What would be the result of using a very polar solvent like methanol (instead of ethyl acetate) to develop your TLC plates?

2. A mixture of p-methylphenol and 4-methylcyclohexanol was run on a TLC plate using 3:1 hexane:ethyl acetate as the eluent. Only one spot was seen using a UV lamp. Does this mean that both spots have the same Rf or is there another explanation? How might you solve this problem?

3. A TLC analysis of a sample composed of *o*-xylene (1,2-dimethylbenzene), *o*-toluic acid (2-methylbenzoic acid), and *o*-cresol (2-methylphenol, or 1-hydroxy-2-methylbenzene) gave the plate below. Calculate the Rf values and match the three compounds to the three spots. Explain your reasoning.

