



The Synthesis of Aspirin from Wintergreen Oil – Instructor Guide

Learning Objectives:

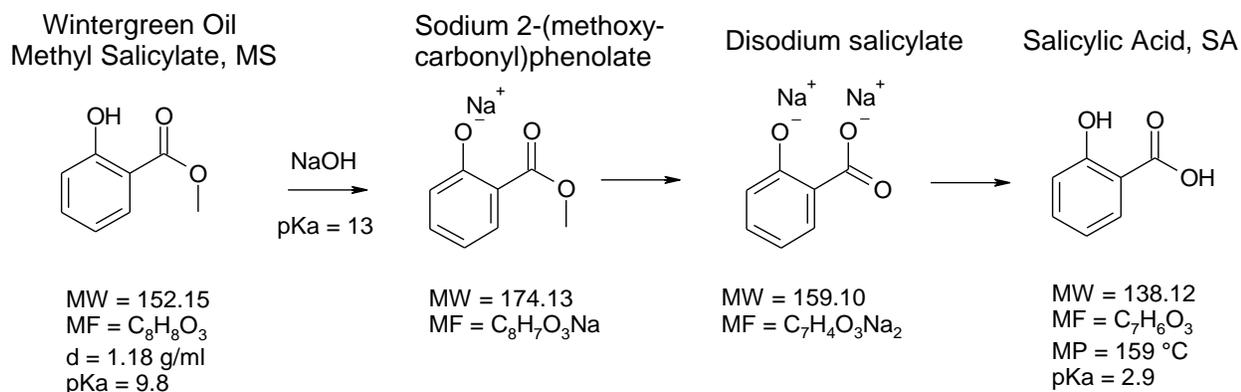
- Perform typical organic synthesis chemistry laboratory techniques
 - Equipment setup, Refluxing, Thermocouple controller use, Crystallization, Filtration, Drying,
- Perform Hydrolysis Reaction, Acetylation Reaction, Neutralization
- Perform qualitative and quantitative analyses using: UV, IR, HPLC, MP
- Calculate Moles, Grams, Stoichiometry, Yields and Purity

Background:

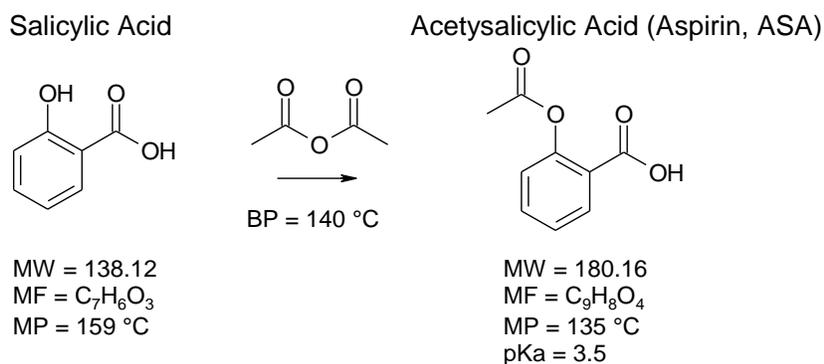
Our new company is preparing to manufacture aspirin by synthesis from wintergreen oil. There is a procedure developed intended for manufacturing on a large scale (100 kg) that requires testing for reproducibility as regards to yield and purity of the API. The two step synthesis encompasses aqueous base hydrolysis of wintergreen oil (methyl salicylate, MS) to salicylic acid (SA) followed by acetylation to acetylsalicylic acid (aspirin, ASA).

Scheme 1: Synthesis of Aspirin

STEP 1: Hydrolysis of methyl salicylate



STEP 2: Acetylation of Salicylic Acid

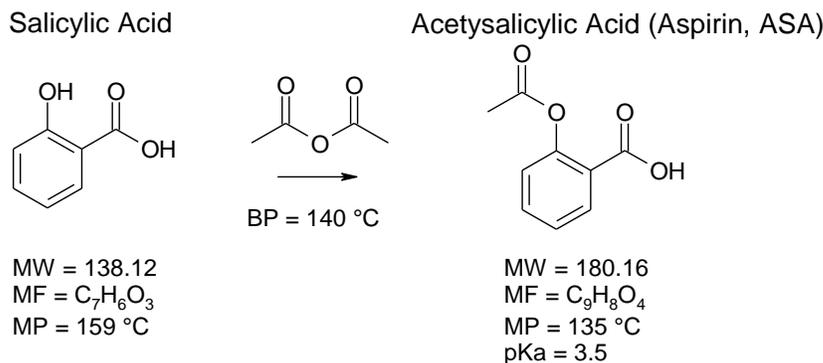




Experimental Procedures

Acetylation of Salicylic Acid to Acetylsalicylic Acid (ASA, Aspirin)

STEP 2: Acetylation of Salicylic Acid



Important: Check off each step as you complete that step

Procedure

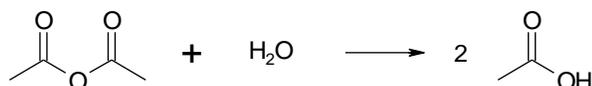
- Place a filled DI water squeeze bottle into a bucket of ice to cool.
- Place a weighing boat on top of a top load balance and press tare.
- Weigh ~3.0 g of salicylic acid into the weighing funnel using a spatula.
 - Record the mass to the nearest 0.01 g: _____
- Remove the center rubber septum from the 3-neck round bottom flask, add a magnetic stir bar to the reaction vessel.
- Place a powder funnel into the port and add the weighed salicylic acid to the 100 mL reaction vessel.
- Using the lab jack, raise the stirrer and heating mantle until the heating mantle touches the bottom of the reaction flask.
- Using a 6 mL pipette, transfer 6.0 mL of acetic anhydride into the reaction flask through the open port and replace the rubber septum stopper.

The instructor will demonstrate the correct handling of the pipette.
- Turn on the stirrer to make a slurry mixture.
- Using a re-pipette, add 100 μ L (0.100 mL) of 85% phosphoric acid through the stoppered port and re-stopper. Eject the pipette tip into the waste container.
- Ensure that the tip of the thermocouple probe is in contact with the slurry. See Picture1.

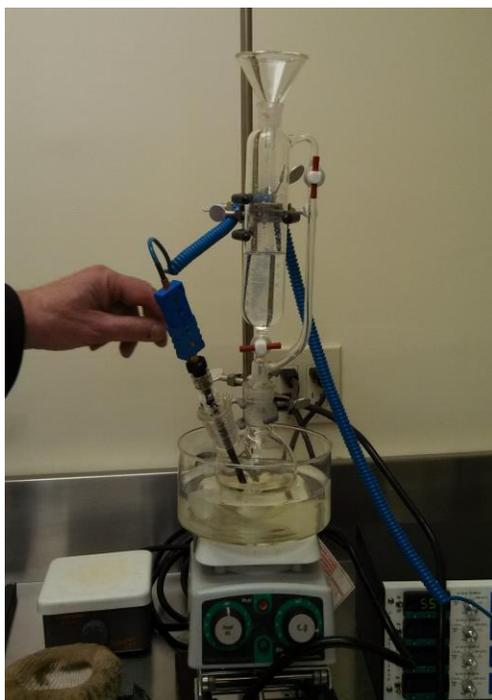
- Describe any changes to the reaction mixture that have occurred.

- When the reaction has cooled to 50 °C, using the re-pipette, add 1.0 mL DI water slowly in 100 μ L (0.100 mL) portions over 3-5 minutes through the stoppered port. Observe the temperature.

WARNING: The hydrolysis of acetic anhydride is exothermic and no more than 0.1 mL of water should be added at any time to control the amount of heat produced.



- Ensure that the addition funnel stopper is in the closed horizontal direction. Add 15 mL of DI water to the addition funnel. After you have added the first 1.0 mL, add the 15 mL of DI water to the reaction vessel all at once through the stoppered port. What happens to the temperature?
- Fill the crystallizing dish no more than half-way with tap water to make a water bath. See Picture 3.



Picture 3

- If no crystals appear as the flask cools, see the instructor for a few aspirin seed crystals. Add the seed crystals through the stoppered port to help initiate crystallization.
- Add lots of ice to the water bath and continue stirring the reaction mixture until it cools to 5°C or after 15 minutes, whichever comes first. Notify the instructor that the crystallization is complete.



- The instructor will help you with the following steps:
 - Using the lab jack, lower the stirrer and ice bath from the reaction flask.
 - Remove the additional funnel and lay it aside carefully.
 - Remove the thermocouple and lay it aside.
 - Turn off the J-Chem Controller for your position.
 - Carefully remove the reaction flask from the apparatus.
 - One member of the team should hold the reaction flask upright so that it does not tip and spill your product.
- Place a 60 mL sintered glass filter funnel onto a cone rubber adapter set on a 250-mL filter flask that is securely clamped on a ring stand on the lab bench. Attach the side arm of the flask to a vacuum source and turn on the vacuum.
- **Gently** swirl the reaction flask and then pour the contents of the reaction flask into the filter funnel. See Picture 4.



Picture 4

- Using the chilled DI water squeeze bottle, rinse the reaction product off the sides of the reaction flask, swirl gently, and transfer any remaining solids to the filter. Repeat twice.
- Using a magnetic retriever, carefully remove the stir bar from the reaction product if it has dropped into the filter flask. Holding the magnetic retriever over the filter funnel, carefully rinse down the stir bar so that the rinse water deposits any residual product into the filter.
- Turn off the vacuum to the filter flask. Using the **ice cold** DI water bottle, wash the solid product with approximately 20 mL of **cold DI water**. Turn on the vacuum. Repeat once
 - What is being washed from the product? _____



- When no solution is observed dripping from the filter funnel, use a spatula to break up the product cake in the funnel and allow air to continue passing through the product for 5 min. Turn off the vacuum to the filter flask.
- Label a small crystallizing dish with your names. Weigh the labeled crystallizing dish and record its mass to the nearest 0.01 g. Tare mass: _____ g
- Using a spatula, carefully transfer the entire solid product to the weighed crystallizing dish. Reweigh the dish. Weight of dish and product: _____.
- Place a Kimwipe and rubber band on top of the crystallizing dish to prevent contamination.
- Place the covered crystallizing dish into the vacuum oven and dry at 90°C under vacuum for 30 minutes.
- Turn off the vacuum and carefully remove the crystallizing dish from the oven.

Warning: the crystallizing dish will be very hot!

- Allow the crystallizing dish to cool to room temperature. Remove the Kimwipe and rubber band, weigh the crystallizing dish to the nearest 0.01 g. Gross mass: _____ g
- Calculate the amount of product in the crystallizing dish. Net mass: _____ g Record the net mass in Table 1 on line (3).
 - Why did the weight change?
 - Can a yield be calculated? Why or why not? _____
 - Where is the rest of the yield? (Hint: Solubility of ASA in water is 2 g/L).
- Replace the kimwipe and rubber band to prevent spillage and contamination.
- Proceed to the analytical section for acetylsalicylic acid, then return to complete Table 1.

Table 1

	Product	Acetylsalicylic Acid
0	Molar Mass (g/mole)	180.16
1	Amount of salicylic acid (moles)	
2	Theoretical Yield of acetylsalicylic acid (g)	
3	Dried/Net Mass (g)	
4	% Purity (HPLC)	
5	Actual Yield of acetylsalicylic acid (g)	
6	Amount of acetylsalicylic acid (moles)	
7	Acetylation % Yield	



Analytical Procedures

IR (identity)

The infrared spectrum of each organic compound is unique to the types and number of bonds in the molecule. Obtain an IR spectrum for methyl salicylate, salicylic acid, and acetylsalicylic acid and compare to known identified spectra.

Method:

Sample preparation:

The instructor reviews the following steps before the students begin their work.

Running a Background IR Spectrum

1. Place one salt plate in the sample holder and lay the holder flat on the bench top.
2. Place a second salt plate over the first.
3. Place the cover over the plates and insert the holder into the IR spectrophotometer.
4. Record the background spectrum.
 - a. Select **“Instrument”** from the toolbar
 - b. Click **“Scan Background”** from the dropdown menu
 - c. Enter the sample ID: **“Background”**
 - d. Enter the Description: **“Air Background”**
 - e. Click **“OK”** to begin scan
 - f. Click **“Overwrite”**
 - g. Select **“File”**
 - h. Click **“Close”** from the drop down menu

Preparing a Salicylic Acid Sample in Acetone

- Using the spatula, transfer a very small amount of your sample to the agate mortar. Note each spatula is marked with line to provide guidance on the amount of sample required.
- Grind the sample to a very fine powder using the agate pestle.
- Add 4 drops of Acetone to dissolve the sample.
- Place one salt plate flat on the bench top.
- Place one drop of the Acetone / sample solution on the face of the salt plate.



- Allow to evaporate and the sample to crystallize.
- Carefully place the salt plate sandwich into the sample holder.
- Carefully place the cover over the plates and insert the holder into the IR spectrophotometer.
- Record the sample spectrum.
 - Select **“Instrument”** from the toolbar
 - Click **“Scan Sample”** from the drop down menu
 - Enter the sample ID: S A
 - Enter Description: “Team 1, 2, 3 or 4”
 - Click **“OK”** to begin scan
 - Click **“Overwrite”**
 - Select **“View”**
 - Click **“Label Peaks”** from the drop down menu
 - Print a copy of the spectrum by clicking on the Instrument icon on the toolbar
 - Select **“File”**
 - Click **“Close”** from the drop down menu
- After obtaining the spectrum, disassemble the holder
- Place a few drops of isopropanol on each plate and wipe clean with a new Kimwipe.

Preparing a Acetyl Salicylic Acid Sample in Acetone

- Using the spatula, transfer a very small amount of your sample to the agate mortar. Note each spatula is marked with line to provide guidance on the amount of sample required.
- Grind the sample to a very fine powder using the agate pestle.
- Add 4 drops of Acetone to dissolve the sample.
- Place one salt plate flat on the bench top.
- Place one drop of the Acetone / sample solution on the face of the salt plate.
- Allow to evaporate and the sample to crystallize.
- Carefully place the salt plate sandwich into the sample holder.
- Carefully place the cover over the plates and insert the holder into the IR spectrophotometer.
- Record the sample spectrum.
 - Select **“Instrument”** from the toolbar
 - Click **“Scan Sample”** from the drop down menu
 - Enter the sample ID: ASA



- Enter Description: “Team 1, 2, 3 or 4”
 - Click “**OK**” to begin scan
 - Click “**Overwrite**”
 - Select “**View**”
 - Click “**Label Peaks**” from the drop down menu
 - Print a copy of the spectrum by clicking on the Instrument icon on the toolbar
 - Select “**File**”
 - Click “**Close**” from the drop down menu
-
- After obtaining the spectrum, disassemble the holder
 - Place a few drops of isopropanol on each plate and wipe clean with a new Kimwipe.
 - Replace salt plates into their container and dispose of used Kimwipes and micro pipette

Assistant Instructions

- Instruct students on basic operation of double beam IR spectrometer using the schematic over each instrument. FTIR spectrometer is too complex to describe.
- For background spectrum, ask students for the gases in air that could absorb IR light. Point out carbon dioxide as a green house gas and hence a strong global warming gas. Explain that computer saves the background spectrum and subtracts it from each of the sample spectra.
- Point out the loss of background peaks in the Acetone spectrum. Remind students that IR spectra reveal the type of bonds present in a molecule. Point out some bond type absorptions.

Reviewing the Sample IR Spectrum

Look at the IR absorbance bands which are present in the range of 2500 cm^{-1} to 3500 cm^{-1} . For the unknown spectrum, ask students to assess the difference between the methyl salicylate, salicylic acid and the unknown. Point out the loss of the ester carbonyl and the formation of the carboxylic acid peaks.

MP (identity and purity)

Each crystalline organic compound has a unique melting point related to the stability of its crystal lattice energy. Measure the melting point of each dried crystalline solid that was obtained and compare to known values.

Method:

- Dip the open end of a melting point capillary into the pile of crystals.
- Invert and tap the tube on the desk to collect the crystals at the bottom.



Instructor Note: Use long glass tubes if the samples don't go to the bottom of the capillaries. Hold the capillary at the top of the tube and drop causing the capillary to bounce up and down. Slide the glass tube up, remove the capillary and inspect the sample. Repeat as needed

- Place the capillary into the melting point apparatus.
- Turn the unit on and watch the sample through the sight glass.
- Record the temperature when the sample begins to melt and when fully melted
Record melting point range on the table of Assay results

Aspirin Analysis by HPLC during Synthesis

The Experiment: Your team will prepare samples of aspirin for qualitative and quantitative analysis by HPLC.

Preparing the Sample

- In a 10 mL volumetric flask, add approximately 6 mg of the aspirin that you synthesized. Record the exact mass to the nearest 0.01 mg: _____ mg. Give this value to the instructor.
- Dissolve and dilute to the mark with HPLC solvent. Use a plastic transfer pipette to add the final amount.
- Label the flask.
- Seal the flask with a stopper. Place your thumb on the stopper, invert the flask and gently swirl. Repeat this 5 times.
- Filter through a 0.45 μ syringe filter into a labelled HPLC vial ($\frac{3}{4}$ full).
- Record the information for your sample in Table 2:

Table 2

Sample Name	Sample Amount (g)	Dilution Volume (flask size in mL)

Inputting Data into the HPLC Data System

- Put the HPLC vial into the autosampler, noting the vial location number: _____
- Input the sample name, sample amount, and dilution volume into the run sequence file.
- Once all the samples are in the autosampler and the sample information is added to the run sequence, start the HPLC and the samples will be analyzed.



Calculating the Amount of Aspirin in a Synthesized Sample

- As each sample is analyzed on the HPLC a report will be printed that provides the peak area, the mg/ mL of aspirin in your sample and its purity. Record purity in Table on line (4)
- Calculate the actual yield of acetylsalicylic acid produced (5), the amount of acetylsalicylic acid synthesized (6), and the % yield from your reaction (7) in Table 1. Remember:
 - moles of acetylsalicylic acid (7) = Actual Yield (5) / Molar Mass (0)
 - % Yield (7) = moles of product (6) / moles of starting material (1) × 100%

or

$$\% \text{ Yield (7)} = \text{Actual Yield (5)} / \text{Theoretical Yield (2)} \times 100\%$$



S2S Team Report

Step 2 Acetylation

Salicylic Acid identification

Tests	Team 1	Team 2	Team 3	Team 4
MP				

Step 2 Aspirin Assays

Tests	Team 1	Team 2	Team 3	Team 4
MP-ID				
HPLC Area% Purity				
Yield				
Mother Liquor assay				
Mass Balance of ASA				

Were the procedures clearly written?

What was the reproducibility of the reaction yields?

What difficulties or aberrations do you have to report?

How did the ID and quality of the methyl salicylate compare to the commercial grade?

What other assays might be of value in determining the quality of the products?

Are the procedures ready for scale-up?

How do we know if the samples were dry?

What recommendations does the synthesis team have to make?

Instructor Notes

Equipment and Materials:

Reagents – Acetylation of salicylic acid

- Isopropyl Alcohol
- Acetone for Cleaning

- Acetic Anhydride
- Phosphoric Acid (85%)
- Diionized water



Lab station Set-up - Acetylation of salicylic acid

- Magnetic stirrer controller (4)
- J-Kem reaction controller (2 Apollo models) and thermocouples assembly (4)
- Heating mantles (4 x 100 mL size)
- Rubber septum (4 x 24/40)
- Ring Stands (8) with assorted clamps
- 100 mL reaction flask (4 x 100 mL 2 or 3 neck)
- 10 ml re-pipette
- Dispenser bottles (1 -500 ml size for acetic anhydride)
- Pressure equalizing addition funnel (50 ml)
- Assorted beakers (4 x 250 4 x 1000 mL)
- Stir bar (8 x 1.5 in football shaped)
- Small Lab Jacks (4)
- Large lab Jacks(2)
- Air Displacement pipette with tips (100 - 1000 ul)
- Glass stoppers (4)
- Erlenmyer Flasks (4 x 250 ml)
- Crystalizing Dish (4 – 150 x 75)
- Crystalizing Dish (4 – 60 x 35)
- Spatulas (4)
- Scoopulas (4)

Filtering

- Sintered glass funnel (4 x 60 mL coarse)
- Filter flask (4 x 500 mL)
- Neoprene cone adapter for funnel/filter flask (4)
- Vacuum system (4 x aspirator vacuum system)
- Vacuum oven (1)
- Vacuum pump for oven (1)
- Rubber bands
- Tissues (4)
- Vacuum tubing for filter funnels and vacuum oven

Synthesis Notes - Acetylation of salicylic acid

- Turn on vacuum oven, under vacuum, before the class starts to heat up to 80.
- The students are in teams of two.
- Student should check each step as they complete the step
- **Add in the timing and logistics of the steps .**
-
- Any student with free time should seek his partner and exchange information.

- After Student 2 places the isolated aspirin in the vacuum oven, prepares samples for the mother liquor HPLC assay, which is started and all team members can then have lunch.
- Student 1 finishes assays and begins to compile data on the salicylic acid.



- Student 2 recovers the dried aspirin, gets weight of product and prepares samples to run HPLC of the solids. While those assays are in progress Student 2 runs IR (if there is time) and MP.
- Student 2 compiles data on aspirin.
- Teams prepare final report and team discussion with instructors/mentors, comparing yield, IR, MP, HPLC, and Spectrophotometer data. They should assess the quality of the tech transfer package (purity, yields, ID). Make recommendations-does further work need to be performed, gap analysis, should other assays be performed, or is the process ready to test scale-up on 1 kilogram scale?

Melting Point

- Melting Point Apparatus (2)
- Melting Point Capillary tubes
- Ring Stand and clamp
- Glass tube

FT IR

- NaCl salt plates (2)
- Salt plate holders (2)
- Hyman Spatula (2)
- Agate Mortar and pestle (2)
- Acetone in a Dropping Bottle
- Micro pipettes
- Isopropanol in a wash bottle for cleaning
- Kimwipes
- Infrared spectrometer – 2 units

HPLC Equipment and Materials for Instructor:

Reagents:

- **Aspirin (USP Standard)**
- **Salicylic Acid**
- **Methanol (HPLC grade only)**
- **Acetic acid**
- **DI water**

Supplies:

- 3 appropriate volumetric flasks and stoppers
- Minimum of 5 HPLC autosample vials and tops (in drawer #61 opposite LCs)
- Plastic transfer pipettes, minimum 10
- Waste container
- Pencil/marker (for labeling HPLC vial)
- Freshly prepared Aspirin / Salicylic acid standard (~600 ppm ASA, ~10 ppm SA)

Prepared Reagents

HPLC Aspirin / Salicylic Acid Standard:



1. In a 25 mL volumetric flask add approximately 150 mg of aspirin and 5 mg salicylic acid.
2. Dissolve and dilute to volume with Stock Standard Diluent.
3. Mix well
4. (Store in the freezer between school visits. Will keep 1-2 weeks)
5. Transfer 1.0 mL to a 10 mL volumetric flask and dilute to volume with Sample Diluent
6. This solution must be prepared fresh daily.

HPLC Diluents

Stock Standard Diluent

1. Combine 190 mL of methanol and 10 mL acetic acid.
2. Mix well
3. Store in freezer

Sample Diluent

1. Combine 300 mL of methanol and 700 mL water.
2. Mix well

HPLC Mobile Phase

1. Combine 400 mL of methanol, 600 mL water, and 5 mL of acetic acid.
2. Mix well by shaking in a closed container, vent, and stir for ~ 30 min to remove dissolved gases.

Setting up the HPLC System:

The following are the HPLC conditions using HPLC 4:

- Detector (switch on left rear of unit) at 254 nm, 0.800 Range, 0.05 RSP
- HPLC pump (switch on right side front) using Method #07 (1.20 mL/min, 100% mobile phase A). If not on Method #07, press F6 key (DIR) and arrow up or down to Method #07. Press F4 key (RCL) and recall Method #07.
- Autosampler using Method #07 (vials 1-5, 15 uL, 4.0 min run time).
- Vacuum degasser (switch on ride side – the rear of the unit)
- Mobile phase A = 40/60 methanol plus 0.5% acetic acid
- Syringe wash = 30/70 methanol/water
- Aspirin standard (position 1 in autosampler)

Before starting up the pump, open the front left door on the pump, place a syringe on the fitting, open the valve by turning CCW. Use the Purge key and set flow rate to 3 mL/min. Purge for 3 min. Press STOP. Close valve, remove and empty syringe and press key F8 (STRT). Watch to see that the pressure only varies by ~50 – 70 psi after a minute or so. If the fluctuation is higher, purge the pump again to remove trapped air. This start up procedure only needs to be done at the beginning of the day.

NOTE: if the screen becomes unfamiliar, the QUIT key will bring it back to the beginning condition.



At the beginning of the day, press the FLUSH key on the autosampler to remove air from the syringes.

Setting up the TurboChrom Data Acquisition:

The computer should be set up and running. If so, pressing the admin key gets you to the TurboChrom program screen. If the computer has shut off, follow the procedure below:

- Turn on computer
- Go to the TCNav icon
- Login with a “admin” logon and “admin” password
- Status may start with “Port Comm Error” in red, will become “No Method” in red after 2 to 5 min. If not, shut off computer and start again

The data acquisition system uses “Sequences” and “Methods”. The naming convention for sequences is as follows:

“Today’s date” as in Mar08

The data files in each of these sequences will also follow the same convention of date.

To find the sequences (and to save them correctly), open the Sequence Editor (Build Sequence) and go up one directory level. It is in the folder marked “Aspirin”. The entire hierarchy from the C-drive is as follows:

C:\PenExe\TcCS\Ver6.3.2\Aspirin

Copies of all the aspirin files (aspirin.seq, aspirin.mth, and aspirin.rpt) are found in the Templates directory:

C:\PenExe\TcCS\Ver6.3.2\Templates

All the working aspirin files must reside in the Aspirin Data directory.

Running the HPLC Experiment:

The prepared sample will be loaded into an HPLC autosampler vial (only fill 3/4 of the way) and then taken to the HPLC to input the sample information (sample name, sample volume, flask volume) into the sequence. Once this is done the vial will be placed into the autosampler in the appropriate position.

When all of the samples have been entered into the sequence file, the data should be saved and the Sequence Editor MUST be closed prior to initiating Instrument Setup on the screen. (This also goes if either the Method Editor or the Report Editor is opened. They must be closed for the data system to initiate setup.)

The autosampler is set for running 5 injections. If you are running less, then the autosampler must be reset for the lower value. Press the F2 key (METH). Using the arrow keys, move to the “LAST” column and enter the number for the last injection. Press the yellow ENTER key. Once the value is accepted, press the F6 key (STOR) and the unit will give you a new method number. Input 1. When the unit asks if you want to overwrite the method press yes (the #1 key) and when it asks for a new name press no (the #0 key).



Click Instrument Setup and browse the Aspirin directory for the correct date and team color sequence. Load it and be sure that lines 1 through 5 are listed for loading (or 1 through the number of rows filled out in the sequence). Approximately 30 sec. or less after the Instrument Setup is closed the status screen will change color to green and say SYSTEM READY. If this does not happen, confirm that the sequence, method, and report editors are closed. Once closed, the Instrument Setup must be re-initiated as above.

Once the system is ready, press START on the autosampler to begin the run.

Click on Real Time Plot to open a screen that allows viewing of the chromatogram as it is generated.

Open the Sequence Editor and the current file as “Read Only” to be able to see what the upcoming sample is.

NOTE: If you miss inputting the correct number of injections during the Instrument Setup, after the last autosampler injection, go to “Run” on the toolbar and “Clear Setup”. Go to the instrument “PE-LC” under “Lab” to clear and return to the red No Method status.

Discussing the HPLC Experiment:

Once the run is ongoing, the following points can be discussed:

- What the void volume is
- How the blank is the water used to dilute the samples and the need to prove it is free of aspirin
- How chromatography is needed to separate all the impurities of the aspirin sample
- How the student’s results compare to a real aspirin sample
- How the aspirin samples have other peaks that are due to the impurities they contain
- How this is similar to drug testing by pharmaceutical companies
- Anything else that seems appropriate...

Clean –up

Reaction Vessel:

- Rinse the addition funnel, plastic funnel, ice beaker, crystallizing dish, NaOH beaker and HCl beaker with di-ionized water
- Rinse the reaction vessel, stirring bar, spatulas, temperature probe, filtering flask and weighing dishes with acetone for cleaning.
- Reassemble the reaction vessel

Miscellaneous:

- Empty ice from the ice coolers and vacuum traps
- Empty vacuum traps and rinse with water

NOTE: Make sure the night-light is on over the sink. If not, reset the GFI outlet so the sink drainage pump functions properly.





Discussion points

Helpful hints

Power point slides

Wrap-Up:

Applying Your Knowledge