Turning Over a New Leaf-
The search for a New Caffeinated Tea

Learning Objectives:

- Introduction to organic chemistry
- Isolation and purification of an organic compound from a natural product using chemical and physical properties
- Identification of extracted substance
- Liquid-liquid extraction technique
- Crystallization
- Melting Point (238^0C)
- HPLC

The Problem

The objective is to find tea that has the highest concentration of caffeine. Before this is used for human consumption, we need to determine the caffeine content. We also need to confirm that what we think is caffeine is indeed caffeine. Therefore, you must isolate the caffeine from this new tea leaf. Purification will be by liquid-liquid extraction followed by recrystallization. Methods used for the determination and characterization of the amount of caffeine will be HPLC and melting point.

Caffeine
1,3,7-trimethyl-1H-purine-2,6(3H,7H)-dione

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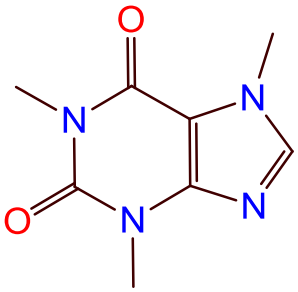

Chemical Formula: $C_8H_{10}N_4O_2$
M.P.= 238 ^{O}C
Molecular Weight: 194.19
Elemental Analysis: C, 49.48%; H, 5.19%; N, 28.85%; O, 16.48%

Experimental Procedure:
Isolation/ Purification:

1. Turn on rotory evaporator heating bath to 55^{o} C and start chiller circulating.
2. Measure 100 mL of tea extract in graduated cylinder and transfer to 125 mL Erlenmeyer flask. Add stir bar to flask.
3. Use pipettor to transfer 1 mL of tea extract to a labeled (**sample 1**) 10 mL volumetric flask
4. Spot tlc plate with tea extract
5. Weigh 1.6 grams of sodium carbonate (Na_2CO_3) on balance. Add to tea extract and stir for 3 minutes. (Note: the mixture becomes clear)
6. Transfer the mixture to a 250 mL separatory funnel
7. Add 30 mL of isopropyl acetate (iPrOAc) from dispenser, stopper the funnel and rock the mixture 20 times. (do not shake violently as this will result in an intractable emulsion)
8. Let the layers separate (~ 5 min). There will be some emulsion. Open the stopcock to let the bottom water layer separate out until the point of the emulsion is at the bottom. Add the upper layer to a 100 mL erlenmyer flask. It's OK if the upper layer contains some emulsion. (Note: label the lower layer Erlenmeyer and upper layer Erlenmeyer so as not to confuse them.)
9. Repeat steps 7 and 8 two more times collecting all the isopropyl acetate layers in the same Erlenmeyer flask.
10. Return the isopropyl acetate fraction to the seperatory funnel and add 50 mL of saturated NaCl and shake.
11. Drain the lower aqueous layer and add the upper layer to the 100 mL Erlenmeyer flask.

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12. Add dry sodium sulfate with a spatula to the combined isopropyl acetate layers and swirl. If the solid clumps up, add a little more sodium sulfate to dry the mixture. Filter through a funnel with fluted filter paper into a 100 mL Erlenmeyer flask. Rinse the funnel with filter paper with ~10 mL of isopropyl acetate.
13. Weigh your 100 mL round bottom flask and record the weight.
14. Add ~ 50 mL of the filtrate to your 100 mL round bottom flask and evaporate the isopropyl acetate on a rotory evaporator with water bath set to 55° C to obtain a solid film on the flask.
15. Spot crude material on tlc plate.
16. Repeat step 14 until the entire iPrOAc layer is evaporated.
17. Dry further under vacuum.
18. Re-Weigh the flask.
19. Subtract the weight of flask from step 13 to obtain the weight of the crude caffeine.
20. Weigh out a 2-3 mg sample of the solid for HPLC analysis and set aside **(sample 2)**
21. You will now purify the sample further by recrystallization
22. Add 5mL isopropanol to round bottom flask containing crude caffeine. Place in warm bath if sample does not dissolve. Place flask in ice water bath and leave over lunch. If sample does not crystalize, add seed crystal of caffeine.
23. Use plastic pipet to transfer material to glass filter funnel attached to vacuum flask and filter crystalized caffeine.
24. Take sample of purified caffeine for HPLC analysis **(sample 3)**
25. Spot purified caffeine on tlc plate

Identification and Characterization:

1. Run an HPLC analysis of the crude and purified caffeine products.
2. Take a melting point of reference caffeine, the isopropyl acetate extract solid and the final sublimed/ recrystallized product.
3. Run TLC of three collected samples

Thin Layer Chromatography

1. Add a few drops of isopropanol to caffeine in a vial. This will not dissolve all of it, but enough will dissolve to allow you to do TLC on the solution.
2. Spot the TLC plate with the tea extract, isolated caffeine and with authentic caffeine. Label the samples on your plate.
3. Develop the chromatogram using a mixture of 1:1 isopropyl acetate: acetone (5 mL of each solvent) as mobile phase.
4. After the development, air dry the plate and examine the plate under UV light to observe the spots. Outline the spots with a pencil and confirm the purity of caffeine

from Rf value (It is the ratio of distance traveled by sample spot from the origin to the solvent front from the origin) of both the spots.

Melting Point

1. Scrape crystal into a small pile
2. Place the crystals into capillary by crushing tube (open end down) on top of pile of crystals
3. Place samples in the Melting Point Apparatus
4. Record melting range

HPLC

1. Prepare Caffeine standard - prepare a standard of approximately 0.04 mg/mL.
    a. Weigh ~100 mg caffeine into a 100 mL vol flask
    b. Add ~5 mL of methanol to wet and ~50 mL 30/70 methanol/water
    c. Place in an ultrasonic bath and sonicate until dissolved
    d. Dilute to volume with 30/70 methanol/water and mix well
    e. Transfer 2.0 mL of this solution to a 50 mL vol flask
    f. Dilute to volume with 30/70 methanol/water and mix well
    g. Filter into HPLC vial with syringe filter **NOTE: Autosampler vials can only be filled between ½ and ¾ of total volume**
2. Dilute **sample 1** 10 fold
    a. Dilute 1 mL of tea extract to 10 mL in volumetric flask with HPLC solvent
    b. filter into HPLC vial **NOTE: Autosampler vials can only be filled between ½ and ¾ of total volume**
3. **Samples 2 and 3**: prepare ~ 0.04 mg/ mL solutions and filter
    a. Weigh out ~4 mg and dilute to 100 mL
    b. Filter into HPLC vials **NOTE: Autosampler vials can only be filled between ½ and ¾ of total volume**
4. Run your 3 samples on HPLC

Record amount caffeine isolated and purity of compound

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