NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

A Charged Situation:
Capillary Zone Electrophoresis and Drug Screening

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Part I – The Big Day

Gazing into the mirror and psyching yourself up, "You've got this!" you say before you finish brushing your teeth. You can't believe that the day you've been waiting for is finally here! You've spent the last year training in a forensic chemistry and toxicology lab and today is your final performance test. If you pass, you will be allowed to work on cases without a more senior analyst by your side.

When you arrive at work, you are confident, but nervous. The analyst that you have been working alongside greets you with a smile. "Remember your training and you'll be fine," she says as she hands you a mock case file.

The case inside the file involves a 38-year-old male with no known major health conditions who was found deceased in his apartment by his girlfriend. Several bottles of pharmaceutical substances (Figure 1) were found in the kitchen and

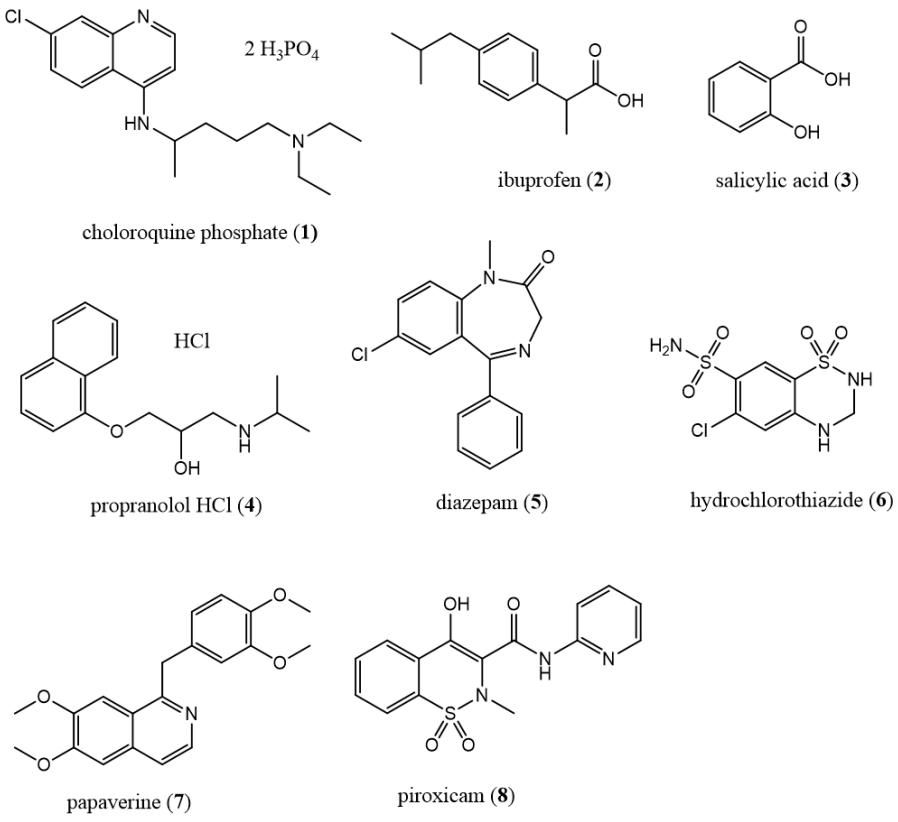

Figure 1. Structures of the pharmaceutical compounds found at the victim's apartment.

living room. It is your job to use liquid-liquid extraction (LLE) and capillary zone electrophoresis (CZE) to determine if any of these pharmaceuticals could have contributed to the death.

You know that you must first spike serum with pharmaceutical standards (1 μg/mL final concentration) so that you can identify if any of these are present in the victim's serum. After spiking, you have two different protocols for liquid-liquid extraction (LLE-1 and LLE-2).

- LLE-1: To 1 mL of spiked serum is added 0.1 mL 2 M NaOH. Then 6 mL dichloromethane is added, and the solution is mixed on a rocker for 10 minutes. After centrifugation (3000 rpm) for 5 minutes and separation of the layers, the dichloromethane layer is collected and evaporated in a vacuum evaporator at a temperature of 350 °C.
- LLE-2: To 1 mL of spiked serum is added 0.1 mL 2 M HCl. Then 6 mL dichloromethane is added, and the solution is mixed on a rocker for 10 minutes. After centrifugation (3000 rpm) for 5 minutes and separation of the layers, the dichloromethane layer is collected and evaporated in a vacuum evaporator at a temperature of 350 °C.

Questions

1. For LLE-1, which of the pharmaceutical standards would end up in the dichloromethane layer? (Note: you may wish to start by determining if each compound is acidic, basic, or neutral and noting any relevant pK_a values.)

2. For LLE-2, which of the pharmaceutical standards would end up in the dichloromethane layer?

After the extractions, the dichloromethane layer is evaporated and reconstituted in a suitable buffer (see below). You have two different capillary zone electrophoresis (CZE) methods to separate and identify peaks for each of the compounds. Both protocols use an uncoated fused-silica capillary of 57 cm length and 50 cm to the detector. The internal diameter of the capillary is 50 mm, and a voltage of 30 kV is applied at a constant temperature of 250 °C. Peaks are detected using UV at 214 nm. Formamide is also added as a EOF marker. Your choice of buffer compositions are:

- CZE1: 50 mM sodium phosphate buffer, pH 2.5
- CZE2: 90 mM sodium borate buffer, pH 8.4

Questions

3. Draw a simple schematic of a CZE capillary, noting the positions of the anode and cathode, the diffuse ion layer, and the relative order of elution for different sizes and charges of ions.

4. Explain the apparent mobility of anions, cations, and neutral compounds in CZE. Include the effect of electroosmotic flow (EOF) and electrophoretic mobility.

5. Which combination(s) of LLE and CZE methods should you use to analyze the standards and the victim's blood? Explain your choice(s).

Part II – The Analysis

When you extract the spiked serum sample with LLE-1 and run the samples using CZE-1, you obtain the results shown in Figure 2.

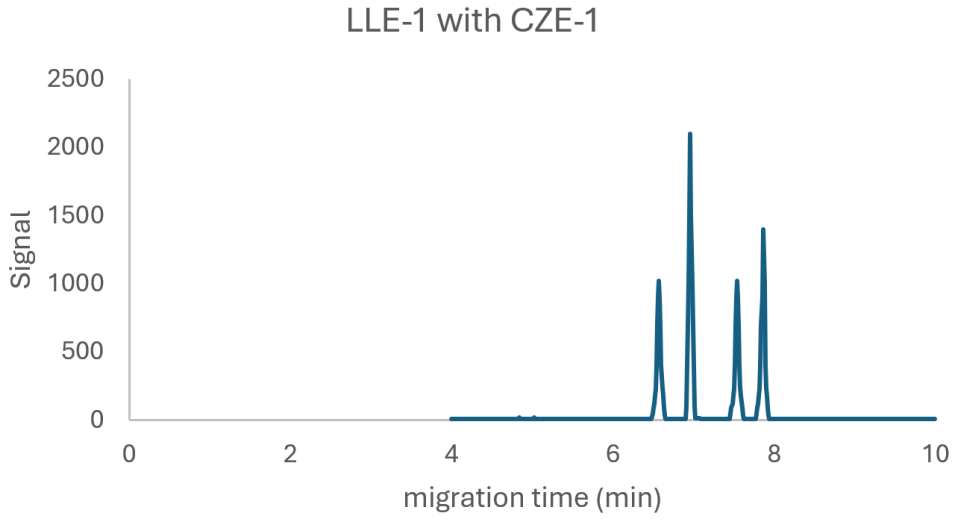

Figure 2. Standards in serum extracted with LLE-1 and analyzed with CZE-1.

Question

6. Assign each of the peaks in Figure 2 to one of the compounds in Figure 1.

When you extract the spiked serum sample with LLE-2 and run the samples using CZE-2, you obtain the results shown in Figure 3.

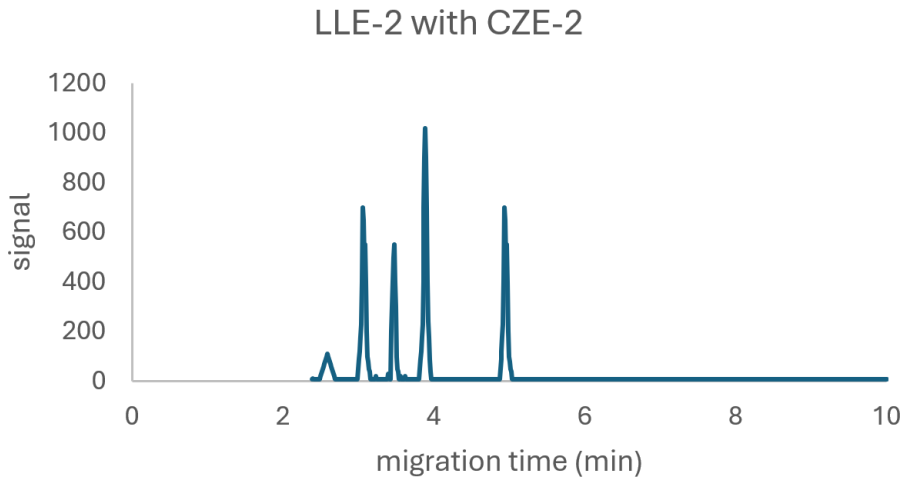

Figure 3. Standards in serum extracted with LLE-2 and analyzed with CZE-2.

Question

7. Assign each of the peaks in Figure 3 to one of the compounds in Figure 1.

You then analyze the victim's serum using both methods and the results are shown in Figure 4.

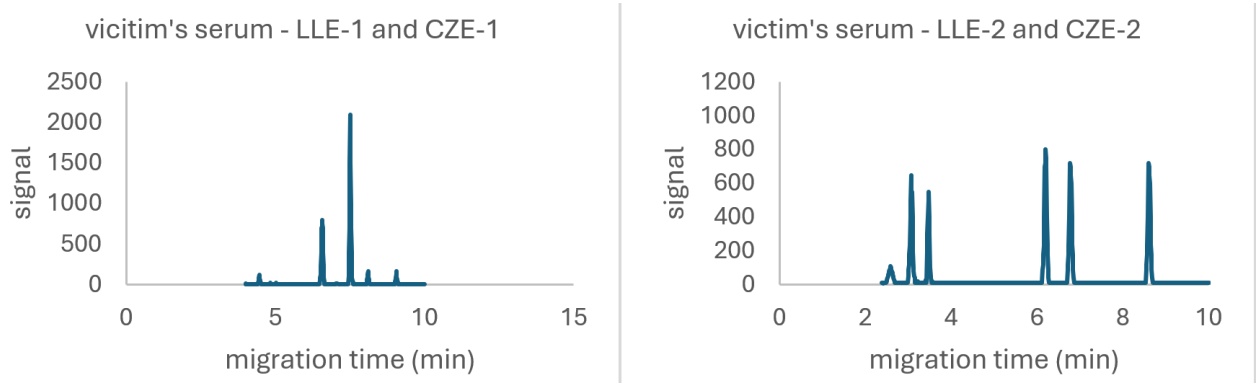

Figure 4. Analysis of victim's serum. The left-hand panel is for LLE-1/CZE-1. Analysis using LLE-2/CZE-2 is shown in the right panel.

Questions

8. Using Figure 4, were any of the pharmaceutical compounds from Figure 1 found in the victim's serum? Explain how you determined this.

9. What other information would you need to determine if any of these compounds contributed to the victim's death?