**BIOL 104 Forensic Biology**

**Lab 9 Analysis of Drugs and Poisons**

1. **Introduction**

Toxicology is the study of the adverse effects of chemicals on living organisms. In 1813 Mathieu Orfila wrote *Traite des poisons*, also called *Toxicologie generale*. However, Theophrastus von Hohenheim (1493-1541), also known as Paracelsus, is often called the “father of toxicology” for his statement, “All things are poison and nothing is without poison; only the dose makes a thing not a poison.” We will use various tests to identify chemicals whose properties vary from therapeutic to lethal, sometimes depending on the dose.

1. **Materials & Methods**

**Wipe down your lab bench and wash your hands. Be sure to wear your gloves and safety glasses.**

from Kemtec Analysis of Drugs & Poisons

1. **Identifying Over-The-Counter Drugs**

1. Use tweezers to place a few granules of aspirin into 3 depressions

in a horizontal row on a depression plate. Label this row “A” for aspirin.

2. Repeat step 1 with Alka-Seltzer (labeled “AS”), Sodium

bicarbonate (labeled “SB”), Tylenol (labeled “T”), Unknown A (labeled “UA”) and Unknown B (labeled “UB”).

3. Record the appearance of each powder and note its color and

appearance (fine, granular, crystalline) on the table.

4. Add 5 drops of distilled water (dH2O) to each powder in the first

column of the depression plate and record your observations on the table in the Results section. Do the powders bubble or effervesce? Do any of the powders dissolve? Use “NR” to indicate no reaction with dH2O.

5. Add 2 drops of Universal Indicator to the same depressions

where you added the dH2O. Let the reaction proceed for 3 minutes and then use a different toothpick to stir each one. Record the colors and indicate whether the pH, or concentration of hydrogen ions (H+) in the solution, is acidic, neutral, or basic using the chart provided.

6. Add 2 drops of Hydrochloric acid (HCl) to each powder in

the second column of the depression plate and record

your observations on the table in the Results section. Which powders effervesce, releasing carbon dioxide gas? Use “NR” to indicate no reaction with HCl.

7. Add 2 drops of Ferric (Fe+3) nitrate to each powder in the

third column of the depression plate and use a different toothpick to stir each one. Record the colors on the table in the Results section.

1. **Identifying Controlled Substances**
2. Be sure you are wearing your gloves and safety glasses.
3. Obtain a bag of simulated lysergic acid diethylamide (LSD), a hallucinogenic drug derived from ergot, a fungus that grows on rye and cereals, or prepared synthetically.
4. Shine an ultraviolet (UV) light directly at the sample. Record your observations in the Results section.
5. Shine the ultraviolet (UV) light onto the bags of over-the-counter substances tested in part A. Are any of them fluorescent? Record your observations in the Results section.
6. Use tweezers to place a few granules of simulated marijuana, dried portions of the *Cannabis sativa* plant, on a microscope slide. The active component of marijuana is tetrahydrocannabinol.
7. Observe the simulated marijuana using your compound light microscope. At the base of the leaf hairs, you should be able to observe small crystals of calcium carbonate.
8. Place a small drop of Hydrochloric acid (HCl) on top of the simulated marijuana. Can you see bubbles of carbon dioxide (CO2) gas? Record your observations in the Results section.
9. The Duquenois Test is also used to identify marijuana based on characteristic color changes when mixed with the Duquenois reagent (acetaldehyde, vanillin and ethanol).

**C. Identifying Heavy Metal Poisons**

Lead can be found in batteries, paint, gasoline and ceramic glazes. It affects the function of the blood, liver, brain and kidneys. Damage to the kidneys can be detected in urine as lead poisoning results in the excretion of large amounts of the amino acid alanine. Mercury can be found in batteries, thermometers, fungicides and industrial waste products. Certain fish are high in mercury. In humans, mercury accumulates in the brain and can lead to blindness, convulsions and even death. It also affects the kidneys, leading to increased amounts of the amino acid glycine in urine.

1. Be sure you are wearing your gloves and safety glasses.

2. Obtain 3 strips of chromatography paper and draw a line in

pencil approximately 1 cm from the bottom of each strip.

3. Mark one strip “A” for alanine, the second strip “G” for

glycine and the third “U” for unknown.

4. Place 1 drop of simulated urine containing alanine on a

depression plate. Then use a capillary tube to transfer a small amount of this simulated urine to the center of the pencil line marked on the “A” strip of chromatography paper.

5. Repeat step 4 transferring simulated urine containing glycine

to the “G” strip and simulated urine from a poison victim to the “U” strip.

6. Place the chromatography strips in test tubes containing a

solvent of n-butanol, acetic acid and water. Allow the

solvent to travel at least half-way up the chromatography strips. Then remove the strips and mark how far the solvent traveled. This is called the solvent front.

7. Allow the strips to dry.

8. Use a Q-tip to dab the strips with a solution of ninhydrin.

9. Again, allow the strips to dry.

10. Draw a circle around each amino acid spot. Measure the

distance from the initial pencil line to the center of each spot. Also, measure the distance from the initial pencil line to the solvent front. Use the equation below to calculate the Rate of flow (Rf) for each amino acid:

**Rf = distance traveled by amino acid**

**distance traveled by solvent**

11. Record your Rf values on the table in the Results section.

1. Analysis of Unknown Powders Obtained from Crime Scene

Choose appropriate tests from parts A and B to analyze two unknown powders found at a crime scene: X and Y.

**Wash your depression plate and return all materials to the bins. Wipe down your lab bench and wash your hands.**

Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Score:

Date\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. **Results**
2. **Identifying Over-The-Counter Drugs**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Drug** | **Appearance** | **Reaction with dH2O** | **pH** | **Reaction with HCl** | **Reaction with Fe+3** |
| **Aspirin** |  |  |  |  |  |
| **Alka-Seltzer** |  |  |  |  |  |
| **Sodium bicarbonate** |  |  |  |  |  |
| **Tylenol** |  |  |  |  |  |
| **Unknown A** |  |  |  |  |  |
| **Unknown**  **B** |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

1. **Identifying Controlled Substances**
2. How did simulated lysergic acid diethylamide (LSD) appear under ultraviolet (UV) light?
3. Did any over-the-counter drugs also fluoresce under ultraviolet (UV) light? If yes, which ones?
4. Were you able to observe bubbles of carbon dioxide (CO2) gas when Hydrochloric acid (HCl) was added to the simulated marijuana?
5. **Identifying Heavy Metal Poisons**

|  |  |
| --- | --- |
| **Simulated Urine** | **Rf** |
| Alanine |  |
| Glycine |  |
| Unknown |  |

1. **Conclusions**
2. Which over-the-counter drugs reacted with distilled water (dH2O)?
3. What is pH? What pH levels are considered acidic? What pH level is considered neutral? What pH levels are considered basic?
4. Which over-the-counter drugs were acidic? Which were neutral? Which were basic?

4. Which over-the-counter drugs reacted with Hydrochloric acid (HCl)?

1. Which over-the-counter drugs reacted with Ferric (Fe+3) nitrate?
2. What is the identity of Unknown A? What test results support your conclusion?
3. What is the identity of Unknown B? What test results support your conclusion?
4. Did you find any “false positives” when using ultraviolet (UV) light to identify lysergic acid diethylamide (LSD)?
5. Why might the Duquenois Test be better than the method we used to identify marijuana?
6. Which reagent was used to detect amino acids in the simulated urine? In which other lab was this reagent used and why?
7. Which amino acid was detected in the unknown?
8. Which heavy metal poisoned our victim?
9. What is the identity of Unknown X? What test(s) did you choose to use? What test result(s) support your conclusion?
10. What is the identity of Unknown Y? What test(s) did you choose to use? What test result(s) support your conclusion?