Drug Testing and Analysis

Forensic laboratories perform drug testing and analysis on many different kinds of drugs or chemicals. Common samples which are tested for the presence of drugs include blood, urine, hair, and other bodily fluids. These samples may be recovered from a crime scene, be in an individual's possession, or be acquired in drug screening for school, sports, or employment. Drug screening differs from compound identification in that the expert is looking for a specific substance. In contrast to screening, in identification an unknown substance is identified through the running of multiple tests. These identifying tests determine if controlled or illegal substances are present.

In the United States, it is estimated that nearly 75% of evidence obtained by law enforcement and examined in forensic laboratories is drug-related. Many times a positive identification can be made on the visual appearance of the substance, such as marijuana or intact prescription pills, however, it is not possible to determine, with certainty, the identity of a plastic bag of unknown powder which could contain cocaine, methamphetamine, or a variety of other controlled or legal substances. The identification of the exact drug is important because an individual is often punished for the type of drug and the amount they possess based upon State and Federal guidelines. Each state closely mirrors the federal guidelines, but may vary in their penalties for possession. Criminal penalties also vary from substance to substance. Each offense is taken very seriously, and the fines or prison sentences increase dramatically with each offense.

At scene of an automobile accident where intoxication is in question, drug testing is extremely important. A sample of blood may be obtained for testing, and compounds within blood samples are often easily identified. A newer form of analysis involves testing hair samples for intoxication or other drug use. A hair follicle test will reveal intoxication immediately, and a strand of hair may retain evidence of drug or alcohol use for at least three months. Laboratories that perform these tests are operated by forensic scientists that can interpret the results of tests performed on controlled substances, clandestine samples, and pharmaceutical laboratory samples. Without interpretation these results have little value, and this interpretation is often written into a formal report that can be used as evidence in a court of law. Additionally, the forensic scientist is frequently asked to give expert testimony in court regarding these analyses.

The history of forensic drug testing and analysis dates back nearly 200 years. In the 1830's a Scottish chemist by the name of James Marsh was summoned to aid in a murder trial. The suspect, John Bodle, was believed to have used arsenic-laced coffee to kill his grandfather. James Marsh had been working to develop a chemical test to reveal whether or not a sample contained arsenic. His original test involved mixing the sample with hydrogen sulfide and hydrochloric acid, but, while able to detect arsenic in the sample, the results had deteriorated by the time he presented it to the jury. This left reasonable doubt in the minds of the jurors, and Bodle was acquitted of the murder. After this trial Marsh developed another chemical test that detected arsenic at a more sensitive level. In this test sulfuric acid and arsenic-free zinc combined to produce arsine gas. Marsh discovered that when this gas was ignited it decomposed leaving a metallic arsenic residue which would not deteriorate with the passage of time.

In 1901 the Nobel Prize was awarded to Karl Landsteiner for his work in identifying and classifying blood into various types. His work was a starting point for many scientists and physicians in the advancements of developing tests to use blood samples in criminal investigations. With these advancements, Edmond Locard of the University of Lyons in France determined that a special crime laboratory was necessary. Locard, thus, established this lab in 1910, and became known as the 'Sherlock Holmes of France'.

Following Locard's lead, August Vollmer set up an American Police crime laboratory in 1924. Following this, the Federal Bureau of Investigation, founded in 1908, set up a forensic crime laboratory in 1932. In the years since, few changes to the methods used in a chemistry laboratory to identify unknown substances have been made. One drastic change, however, has been influenced by the now widespread availability and convenience of testing methods. For instance, many companies now distribute drug screening kits to law enforcement agencies that allow them to take a number of different presumptive screen-
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ing tests out into the field for quicker results. These kits, relatively easy to use and widely available, allow for more decisions to be made in the field without delay and have reduced the number of unnecessary samples being sent to forensic chemistry laboratories for analysis. Also, the advancement of certain technologies, such as gas chromatography-mass spectrometry, now give results that are more accurate and specific than previous testing methods.

The work done by forensics experts varies from case-to-case, however there are two main categories of tests that must be used to properly identify an unknown substance: presumptive tests and confirmatory tests. When the exact identification of a particular substance or drug is in question, presumptive screening tests (sometimes referred to as preliminary tests or spot tests) are completed. This allows for a quick, initial identification or exclusion of the substance. To perform preliminary testing, drug experts must have an idea of what they are testing so that they may choose the correct presumptive test to identify the suspected substance. If the presumptive test generates a positive result, then a confirmatory test is performed to confirm the presumptive results. The presumptive test, however, can incorrectly return a positive result, which is called a false positive. A false positive test result means that the test has returned positive for the suspected substance, but is actually another substance.

Colorimetric tests are presumptive tests and include the tests that screen for illegal drugs. Samples for testing can be obtained straight from the substance or indirectly from blood, urine, saliva, or other bodily fluids. Color tests are highly sensitive and do not require expensive equipment or any special skills to perform. Most of these tests utilize high concentrations of sulfuric acid which serves as a reagent (a special substance used in a chemical reaction to detect the presence of an unknown compound or drug), which is then combined with the unknown sample and the color change is observed. The color change is then compared to a known reference color range and allows the unknown drug to be identified.

Another presumptive test is an odor test, often used for detecting the presence of cocaine. The process of this test is similar to the color tests but differs in that the result is not a production of a specific color but, rather, a specific odor that is produced. This odor can be either a fishy or minty smell.

Confirmatory tests are more specific, accurate, and expensive. A great advantage to these tests, however, is that they do not have the same risk of producing false positives. These tests are needed in order to accurately identify substances so that drug evidence may be admissible in a court of law. The equipment required for these tests is expensive, and a forensic drug chemist is required to analyze the information produced by these machines. The most important and widely used confirmatory test is Gas Chromatography-Mass Spectrometry (GC-MS).

Each unknown substance is made of many different molecules that, when combined create, a separate chemical compound (i.e. cocaine, LSD, HTH, etc.). A drug technician inserts a minute amount of the unknown substance into the GC-MS. The gas chromatograph then takes this chemical compound and reduces its chemical structure to individual molecules. The difference in the chemi-
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Cal properties of each molecule will separate the molecules as they travel the length of the column. The molecules take different amounts of time to exit the gas chromatograph, which enables the mass spectrometer to capture and detect the molecules individually. After the GC produces these fragments, the MS is used to classify the chemical compounds and create a ratio with its mass and electrical charge, called the mass-to-charge ratio. With this information about the unknown chemical substance, a forensic scientist is able to identify the chemical fragments and place them back in the proper order, thus restoring the whole chemical compound using their expertise and

Common Illegal Substances

Marijuana is the most widely used hallucinogen in the United States. The active compound, or psychoactive substance, contained in the marijuana plant is tetrahydrocannabinol, or THC. THC is produced by the cannabis plant through a natural process involving the plant’s own enzymes and chemicals. The active compound in marijuana can range from 1 to 20 percent, which is almost completely contained in the female marijuana flowers.

Cocaine is one of many illegal stimulants that are abused for the sense of euphoria that they give the user. Cocaine is made from the coca plant, grown only in the Amazon. To make one pound of cocaine a total of 500 pounds of coca plant is needed. Most cocaine is made into a powder to be snorted, or it is crystallized and heated to create fumes to be inhaled, known as “crack”. A stimulant is any highly addictive chemical that directly acts on the central nervous system. It creates increased alertness and energy, and decreased appetite and need for sleep. Other commonly known stimulants are caffeine, nicotine, amphetamines, and methamphetamine.

Methamphetamine is also abused for its feelings of euphoria, with similar side effects, and has a highly dangerous nature. It is widely know as “meth”, and can be injected, snorted, smoked, or ingested orally. Under low doses and controlled administrations, methamphetamines have many uses in medicine (i.e. ADHD, obesity, narcolepsy, etc.). Meth is made in foreign and domestic labs by combining commonly available drugs and chemicals, which is why these production labs are often found in communities and neighborhoods.

Opioids are very well known and widely abused because they can be prescribed by a licensed physician. These drugs act on neuron receptors in the brain and spinal cord which block pain signals, as well as stimulate euphoria similar to cocaine and methamphetamines. Opioids are contained in commonly prescribed drugs, such as Vicodin, Lortab, morphine and some cough medicines. They are alkaloids derived from compounds such as morphine, which come from the harvested opium plant seeds.
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knowledge of general and organic chemistry.

Ultraviolet Spectrophotometry is a confirmatory test similar to the GC-MS. In this analysis the unknown substance is identified through detection of the light being reflected by the main elements of the compound. This is analyzed and compared to a known sample range that helps identify the unknown drug.

Many real-life examples show the importance of forensic testing and how it can be used to clarify questionable cases. In 1984, FedEx employees examined a damaged package of a cardboard box wrapped in brown paper. Inside they found a tube that had a number of plastic bags placed inside one another. In the innermost bag they found a white powder substance, and they subsequently called the Drug Enforcement Administration (DEA) to investigate. When an officer arrived, he removed a small trace of the powder and performed a presumptive field test which indicated the presence of cocaine. A search warrant was issued for the address to which the package was to be delivered, and the recipients were arrested and eventually convicted of drug possession.

Another example is the unexpected death of the famous actor, Heath Ledger. Ledger was an Australian and American film actor that played in well known films, such as “The Dark Knight” and “A Knight’s Tale”. On January 22, 2008, he was found dead by his housekeeper in his Manhattan apartment. A federal investigation into his death was pursued due to his young age and unexpected death. In his room were found numerous prescription pills, however, it was difficult to determine exactly what, or who, was responsible for his death. In the following weeks, Ledgers blood and tissue samples were analyzed and found to contain a lethal combination of prescription drugs. Without the ability to identify the compounds present in his blood at the time of death, the cause of death would have remained unknown.

A bachelor’s degree in a physical science is necessary for someone interested in pursuing a career in forensic drug chemistry. A solid background of general chemistry and organic chemistry should be obtained, as well as some coursework in pharmacology. There is additional training available up to the level of a PhD. Those working in pharmacology, pharmacokinetics, medicinal, clinical chemical, or drug chemistry may also transition into the field of toxicology. There are additional certifications required by the Forensic Toxicology Certification Board.

To prepare for this career, it is recommended that a high school student should pursue an education at a properly accredited college or university to receive a bachelor’s degree. Classes with an emphasis on sciences, particularly chemistry, should be heavily included. The American Academy of Forensic Sciences provides a website with a list of colleges and universities providing forensic degree programs. For more information visit this website: www.aafs.org.

Definitions

Presumptive Tests: If one of these tests has a negative result, the investigator can rule out the presence of an illegal substance. A positive result indicates the probable presence of a specific compound, however, due to false positives, a positive presumptive test result must be followed by a confirmatory test.

Confirmatory Tests: These tests give accurate and specific confirmatory results of the identity of a specific compound. Exact identification is only possible through a confirmatory test.

Colorimetric Tests: These tests are presumptive and indicate results through color changes when combined with certain substances.

Reagent: A special substance used in a chemical reaction to detect the presence of an unknown compound or drug.

GC-MS (Gas Chromatography-Mass Spectrometer): This instrument performs a confirmatory test by separating the molecules of a compound and identifying the exact substance.
In the abandoned truck in New Mexico, authorities found a large bag of an unknown, white, powdery substance. They immediately sent the bag to their drug testing laboratory. Upon receiving the bag of powder, forensic drug chemists decided that they must first determine what common cutting agent, if any, was utilized in the suspected drug sample. Because they did not know the identity of the powder, they wished to then analyze the sample by Gas Chromatography-Mass Spectrometry to determine if a controlled substance was present in the powder and, if so, the identity of the drug and the concentration of the drug in the evidentiary sample.
Persons of Interest

The Mondelos

Louise Ann Mondelo, the 38 year old wife of Lyle Mondelo and mother of Wally and Jan, is also one of the owners of Tumbling Water Land Development Company. Friends say that Louise was in an unhappy marriage and had recently filed for divorce.

Lyle Christopher Mondelo, the 40 year old husband of Louise Mondelo and father of Wally and Jan, is a part owner of Tumbling Water Land Development Company along with his wife.

John Wayne Gretzky

John Wayne Gretzky is 41 years old. He is a friend and business partner of the Mondelo’s in the Tumbling Water Land Development Company. According to rumors, John Wayne and Louise had a brief affair when Lyle and Louise first moved to Highland Park. He is known around town to be a greedy businessman, and has been suspected of shady deals in the past.

Larry Gretzky and Mitch Wilson

Larry Gretzky and Mitch Wilson were recently indicted on charges related to their apparent operation of a methamphetamine laboratory. Larry was bailed out by his brother, John Wayne, and Mitch was bailed out by his uncle, Lyle Mondelo. Larry and Mitch failed to appear in court and are currently missing. Police are interested in locating them for questioning.
1. Why must forensic technicians perform a confirmatory test after receiving a positive result on a presumptive test?

2. What is a false positive?

3. What is a drug that can usually be identified just on the visual appearance of the substance?

4. Why is it important to identify the exact drug and the quantity?

5. Describe the function of a GC-MS.
Lab Procedure

Lab 1:

1. In your group, label your two reaction plates. Label each vertical column of wells as follows: PP (Plaster of Paris), PS (Powdered Sugar), CS (Corn Starch), and S (Salt). On the second reaction plate, label the first two columns BA (Boric Acid), and TP (Talcum Powder). Label one more column with a question mark (for the unknown powder).

2. On the horizontal rows, label the top with DW (Distilled Water), 1A (Isopropyl Alcohol), and L1 (Lugol’s Iodine). Repeat this process for the second plate.

3. Open the first powder, Plaster of Paris. Using a clean wooden spatula, place a small scoopful of the powder in each well in the PP column.

4. Close the first powder, then open the second powder, Powdered Sugar. Using a new wooden spatula, place a small scoopful of the powder in each well in the PS column.

5. Repeat these steps for each powder, including the unknown evidence powder. Be careful to use a new spatula for each new powder to prevent cross contamination. Ensure the lid for each powder is closed before scooping the next powder. Avoid placing too much powder in each well, as only a small amount is necessary for analysis.

6. Record physical observations of each powder on the Data Collection Sheet. Note the physical characteristics, such as the color of the substance and whether it is a powder or crystal.

7. After recording your observations, place several drops of distilled water in each well of the first row (D1) of powders. Record the reactions you observe on your Collection Sheet.

8. Follow the same procedure for each of the remaining two liquids, recording the reactions you observe after dropping each liquid into the wells of powder.

9. Examine your data for the known substances, and then compare it to the unknown powder. Decide the cutting agent, if any, in the powder found in the truck.
Lab Procedure

Lab 2. Part A

Your instructor has provided you with a set of data generated on a GC-Mass Spec. Forensic chemists use GC-Mass Spectrometry to test for illegal drugs in the same way you will be analyzing this data set.

The first set of data (Knowns) is generated by analyzing several known drugs to produce their mass spectra for comparison purposes. Forensic chemists can also rely on tables of mass values, if they are available, for reference.

The second data sheet (Unknown) is the analysis of the drug sample itself.

You will compare the mass spectrum produced from the crime scene with the spectra of known drugs. By comparing the peaks on the spectra, which represent ions of various masses, you can identify what drugs, if any, are present in the white powder from the crime scene.

1. Analyze the known spectrum for Oxycodone. Two graphs of the same sample are included. Analyzing the first graph:
   • What measurement is represented on the X axis? 
   • What measurement is represented on the Y axis?

2. Analyzing the second Oxycodone graph:
   • What measurement is represented on the X axis?
   • What measurement is represented on the Y axis?
   • Which graph is associated with the GC portion of the analysis?
   • Which graph is associated with the Mass Spec portion of the analysis?
   • What is the significance of the different peaks or lines observed on each graph?
   • The five different included knowns are:

3. Analyze each known GC/MS graph individually and, using a metric ruler, record the major peak heights in mm. Fill in the table on your data collection sheet.

4. The crime scene sample will now be analyzed. Measure the major peaks of this sample in mm and record this data in your data collection sheet.
   • Which of the five known drugs does the unknown drug resemble most?
   • Do significant differences exist between the unknown drug from the crime scene and the known drug it resembles the most? If so, what?
   • What is the source of additional peaks present in the crime scene sample that are not present in the known sample?

Lab 2. Part B

Your instructor has provided you with a second set of data generated on a GC-Mass Spec. Forensic chemists use GC-Mass Spectrometry to test for the quantity of an illegal drug in the same way you will be analyzing this data set.

The first set of data (Knowns) is generated by analyzing a very accurate series standards of known amounts of methamphetamine to produce their mass spectra for comparison purposes.

1. Review the known data set.
   • What four concentrations of drugs were used in this analysis?
   • What does 1.0 mg/ml signify?

2. Measure the height of the primary peaks of these four concentrations of methamphetamine data in mm using a ruler and record these values in your data sheet. This data will be used to:
   • Enter and format data in an Excel spreadsheet
Lab Procedure

in a form appropriate for graphing

• Create a scatter plot from spreadsheet data
• Insert a linear regression line (trendline) into the scatter plot
• Use the slope/intercept formula for the regression line to calculate a concentration (x value) for a known peak height (y value).

3. Open Excel and title the spreadsheet page in cell A1
4. Label Cell A2 as the concentration of the known solutions.
5. Label Column B2 as the peak height in mm for each of the four concentrations.
6. Enter your data for each of the four concentrations into the first two columns in the spreadsheet.

Creating the Initial Scatter Plot

1. On the insert tab, click the scatter chart button and select scatter with only markers.
2. When the chart appears, select the chart, go to the design tab, and click the select data button.
3. Add a data series in the select data source pop-up window.
4. Choose the series name (pick cell A1)
5. Choose the series x values (pick cells A3: A6)
6. Choose the series y values (pick cells B3: B6)
7. Click okay on the edit series popup and the data source popup windows.

The initial scatter plot will now appear on the same spreadsheet page as your original data.

• Your data should look as though it falls along a linear path
• Your chart is highlighted with square ‘handles’ on the corners. With your graph highlighted, you can click and drag the chart to where you would like it located on the spreadsheet page. Grabbing one of the four corner handles allows you to resize the graph.

Creating a Linear Regression Line (Trendline)

8. Right click any data point on the chart and select the add trendline menu option.
9. Choose a linear trendline and check the “display equation on chart” and “display r-squared value on chart” checkboxes. The r-squared value is a measure of the linearity of the data. A value of 1 means that the average of all points would fall an equal distance from each side of a line and we say that the data has low variance. A value of 0 means that almost all points fall to one side of the line, and thus the data has very high variance.

10. You can move the equation around the chart by dragging it.

11. Label the horizontal axis by clicking the layout tab, then the axis titles button, primary horizontal axis title, title below axis. You can edit the axis title by double clicking and typing in it. Label the axis “concentration of known solution (mg/ml)”

12. Label the vertical axis by clicking the layout tab, then the axis titles button, primary vertical axis title, rotated title. You can edit the axis title by double clicking and typing in it. Label the axis “peak height (mm)”
Using the Regression Equation to Calculate Drug Concentration

The linear equation shown on the chart represents the relationship between concentration (x) and peak height (y) for the compound in solution. The regression line can be considered an acceptable estimation of the true relationship between concentration and peak height.

You have been given the GC Mass Spec graphs for one solution of unknown concentration.

1. Using the linear equation, calculate the concentration of the unknown solution. As the value of y (peak height) is known, you will solve for x (concentration). A sample calculation of this is shown below:

   \[ y = 2071.9x + 0.111 \]
   \[ y - 0.0111 = 2071.9x \]
   \[ (y - 0.0111) / 2071.9 = x \]

2. Write your equations below.

   
   
   

Calculating the Amount of Drug in the Crime Scene Sample

After calculating the concentration of drug in the unknown sample from the crime scene, you must determine what percentage of the white powder is actually drug. It is critical to know how much drug was actually present in the sample, as this affects what level of crime the suspect can be charged with. In the federal system, different drug quantities can result in different minimum sentences necessary for sentencing. In the crime scene sample, 2.0 mg of white powder was dissolved in 1.0 ml.

3. Using the graph and the solution, record the number of milligrams of drug present in the sample below.

    

4. To calculate the percentage of drug, divide this concentration by the original 2 mg/ml, then multiply this value by 100. Record what percentage of the sample is drug below.

    

The New Mexico State Police weighed the material recovered from the crime scene and determined that they had recovered 13 g of white powder.

5. Determine the grams of drug recovered by multiplying the percentage of drug in the sample by the number of grams of powder recovered. Record your answer below.

   
   
   

   

   

   

## Data Collection and Calculations

<table>
<thead>
<tr>
<th>White Powder Name</th>
<th>Physical Observations</th>
<th>Reaction</th>
<th>Speed of Reaction</th>
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<tbody>
<tr>
<td></td>
<td>DW:</td>
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<td>DW:</td>
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<tr>
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</table>
# Data Collection and Calculations

**Lab 2. Part A: Major Peak Heights for Known Drugs and Unknown Substance.**

<table>
<thead>
<tr>
<th>Oxycodone</th>
<th>Cocaine</th>
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<tbody>
<tr>
<td>Peak time (in minutes)</td>
<td>Peak time (in minutes)</td>
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<tr>
<td>Peak height (in mm)</td>
<td>Peak height (in mm)</td>
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<th>Ketamine</th>
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<td>Peak time (in minutes)</td>
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<tr>
<td>Peak height (in mm)</td>
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<table>
<thead>
<tr>
<th>Methamphetamine</th>
<th>Unknown Substance</th>
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<tbody>
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<td>Peak time (in minutes)</td>
</tr>
<tr>
<td>Peak height (in mm)</td>
<td>Peak height (in mm)</td>
</tr>
</tbody>
</table>
Post-Lab Questions

1. Why is it important to do a presumptive test before performing a confirmatory test?

2. Which powders had the strongest reactions during the white powder test?

3. Which two powders reacted the most similarly to each other?

4. According to your white powder test, what is the identity of the cutting agent in the powder found at the scene of the crime?

5. What data do you get from a GC-Mass Spec?

6. In measuring the peaks of the unknown and comparing it to the known samples, what drug was found at the scene of the crime?

7. What was the concentration of the drug that was found? Why is it important to know the concentration and amount?