GC–Forensic Analysis Experiment

Introduction:

Gas Chromatography (GC) is an instrument commonly used in forensic analysis of samples from victims or crime scenes, particularly in the area of toxicology. If someone is suspected to have been poisoned, samples of their blood, urine as well as evidence from the scene can be used to confirm this suspicion and possibly to identify who may have been responsible. In GC analysis you identify samples by their retention time compared to known standards. If a Mass Chromatography instrument is the detector for the GC the identification of unknown compounds can be detected by their fragmentation pattern.

Objectives:

1) To become familiar with the operation of GC instrument with a Flame Ionization detector (FID).
2) To introduce the idea of forensic analysis.
3) To contemplate the handling of sensitive samples in different lab settings.
4) To determine time lines and flow charting that affects the results of the analysis.

Pre-lab questions:

1) What analytical problems could occur in handling samples from crime scenes?
2) What would be the advantages and disadvantages of having a mobile lab for analysis?
3) Why are **time lines** important in measuring samples from a crime scene?
4) Is the GC a good instrument for measuring constituents in blood or would HPLC be better? Explain.

Background:

The authorities of a small town are called to what appears as a drug party gone bad. A teenager is found, who is not breathing, at the scene. The teenager is rushed to hospital and could not be revived. At the scene a jar with a liquid is found close to where the teen was located.

In the apartment of one person who also attended the party (a suspect) 20 menthol cigarettes were found in a liquid substance as if the suspect was trying to extract something from the cigarettes. It is believed that the suspect may have extracted nicotine from the cigarettes and made an alcohol/water solution to make an amateur drug (poison). This may have been fed to the unsuspecting teen as a drug mixture to obtain a “high”. The partially empty jar held approximately 250 ml. and only about 10 ml. was left in the jar. A sample of the drink (highball) 10 ml. was collected and saved for analysis at the local crime laboratory. The suspected contents were to be analysed using GLC for identification and quantitation.

Urine and blood samples were obtained from the teen at the hospital approximately 0.5 hours after been transported in the ambulance and 0.5 hours passed before they were analysed at the lab. The compounds suspected to be present in the "highball" drink and analysed were nicotine (a very toxic substance), and menthol. If nicotine and menthol are found in the "highball" the source of the poisoning would be confirmed If nicotine plus cotinine (a metabolite of nicotine) are found in the blood and urine of the victim, this would indicate nicotine poisoning presumably from ingestion of the "highball" drink.

The instrument you will be using is a Varian CP-3900 Gas Chromatograph. The 3900 is equipped with a FID detector. The instrument is fitted with a Chrompack capillary GC column having the following properties: coating: CP-Sil 8 CB; column length: 50 m; outer diameter: 0.2 mm; support thickness: 0.33 µm. Samples are automatically injected using a Varian CP-8400 autosampler, however manual injections are possible.

Setting up a method.

The software that runs the instrument is called **Compass**. The password is 3900GC. In the login window, enter "student" under User Identification, "4590" under Group, and "GC3900" under Project. At the bottom-left of the screen, click on the Systems tab and then check off Varian 3900 option at the top-left of the screen. A system status schematic should appear in the right-hand portion of the screen displaying current system conditions. Go to File, and click on New Method. Select Varian 3900 system. Name your method based on group and date. Go back to the Data tab (bottom-left of the screen). Under data click on "control". Click on the Instrument Icon (found in the top region of the screen below the "Overview" button). You should now be in the "Varian CP 3900 – Control Method" screen. This is where you make changes to various parameters of the instrument components.

Experimental Validation of suspected compounds:

Students must prepare a method, and run standards and samples. The **handling of samples** including **flow charts** with **time lines** should be recorded and a description included in "Materials and Methods".

The material will be analysed on the Varian 3900 GLC using the specified method
The identity of the compounds in the unknown solution and the urine and blood will be determined by their retention times compared to standards.

Materials and Methods:

Materials: Nicotine stock (100 mg/100 ml in ethanol), menthol (100 mg/100 ml in ethanol), cotinine stock (100 mg/100 ml in ethanol).

Method: The GC parameters were determined to give good separation of the standards (nicotine, menthol, cotinine). The starting general parameters are injector temperature 275^{o}C with split off initially and then after 1 min a split of 20. The split can be adjusted to improve peak shape and/or affect sensitivity. The column start temperature use 70^{o}C and final temperature of 265 ^{o} C based on the boiling temperatures of the suspected compounds to be analysed. The ramp is set at 20 ^{o} C per minute. The constant flow rate is 1 ml/min. s. The FID detector temperatures are set at 300 ^{o} C and the electronics are on. The gas flow rate should be 1 ml/min. The gases should be nitrogen 25, hydrogen 30 and air 300 for the FID detector.

A mixture of nicotine, menthol, and cotinine are to be analysed on the GC. The above compounds are to be mixed (a combination sample) and made up to 2 ml. with ethanol and used to confirm the separation method and determine retention times using the 3900 GC. The final concentration of each of above compounds should be approximately 0.05 mg/ml. This procedure can be used to determine if the above toxic compounds are in the "high ball", blood and urine samples from the crime scene.

The identity of the compounds in the "highball" and the urine and blood samples are to be determined by comparing the retention times of nicotine, menthol and cotinine run separately (samples are in tray). The samples will be identified by retention times and the concentrations by the integrated area of the peaks compared to standards with the same retention times.

Obtain 2 mls. each of urine, blood and highball extracts from the lab supervisor. These samples have been extracted from the highball, blood and urine on a 1 to 1 basis. The samples are in ethanol.

Extracted samples:

Standards: The concentration of the compounds in the "highball", blood and urine will be measured by use of external standards. The "highball" should be diluted by a factor of 10 with ethanol to fit in the standard curve (below). It is the student's responsibility to make sue the unknowns fall within the standard curves.

Prepare a standard curve consisting of menthol, nicotine, and cotinine from the stock solutions of each of these standards diluted in ethanol. The concentration range should be 5 ug/ml and 100 ug/ml. We will use a 2 point curve due to limited time to run samples on GC. If your sample does not fall in standard curve adjust your standards or your samples. The samples have been extracted from the highball, blood and urine on a 1 to 1 basis. The samples are in ethanol.

Setting up a sequence for automated runs. You can set up a sequence of sample injections to be done automatically by the autosampler. To do this, go to File, and select New Sequence. Select Varian 3900 as your system, click Next, enter the number of lines (samples), click next, name your sequence and click on OK. You now have to fill in the cells of the sequence table. You can leave Method Properties blank; name your runs

under Run Name (prefix) enter any number under Run ID (suffix); You can leave Description blank; enter the Number of injections; enter autosampler vial number under Vial Number. Rack # is not used; Injection Volume is 1.00 µL; select Unknown for Sample Type; leave all other columns as default values.

Name all standards and samples in sequence table as unknowns. After the run you can identify samples under *peak identification.*

Viewing of the acquired chromatograms. In order to view collected data, go to File, Open Chromatogram. Select an appropriate data folder (these are created automatically by date). Your file name appears in the top-left portion of the screen (identified by a Vial icon), with three components: Data, Method, and Results. You should be able to view your chromatogram. You can open multiple chromatograms and overlay them or have them displayed in separate windows.

Data: All methods and data will be collected in a hard cover note book. If any changes in the data or notes are made they must be initialed. The notes should be self explanatory and could be copied and given to a jury or lawyer and must be clear and understandable and must flow in a coherent manner. This report should be very definitive in the results demonstrating what has happened in the crime scenario.

A report must be written in the format of a lab report with all data, discussions and conclusions as indicated by the data. If poisonous substances are found the LD_{50} should be reported and any further testing recommended. Time lines for analysis should be included in "Materials and Methods" section to determine the validity of the analysis do to degradation of samples, etc.

Discussion: The discussion should cover all the results and also include validity of results due to time lines and suggested conclusions from the data. If poisons are determined, are they strong enough to cause problems? What are LD_{50} of poisons, etc.

Questions:

1) Why does a GC work well for the compounds studied in this experiment?
2) What chemical technique could be used to expand the number of compounds that can be measured by GC?
3) Could the LC-MS be used to measure the suspect compounds? Explain.
4) How is chemical toxicity (LD_{50}) determined?