GC–Forensic Analysis Experiment

Introduction:

Gas Chromatography 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 is collected. These samples can be analysed and the results used to confirm if the victim has been poisoned and possibly to identify who may have been responsible.

Objectives:

1) To become familiar with the operation of the Varian (Agilent) 3800 GLC instrument with an FID detector for the analysis of forensic samples.
2) To contemplate the handling of sensitive samples in different lab settings.
3) To determine time lines and flow charting that affects the results of the analysis.

Pre-lab questions:

1) What analytical problems could occur from handling samples from crime scenes?
2) What would be the advantages of having a mobile lab for analysis?
3) What would be the advantage, and possible disadvantage of using the GC-MS to analyse the samples compared to the GC-FID?
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. A package of mothballs were also found in the vicinity. It is believed that the suspect may have extracted nicotine from the cigarettes and mixed the nicotine and the mothballs into 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 approximately 0.5 hours after arrival at the hospital and 1 hours passed before they arrived at the lab. The compounds
suggested to be present in the “highball” drink and analysed were nicotine (a very toxic substance), menthol, and naphalene (from the moth balls). If nicotine and menthol and naphalene are found in the “highball” the source of the poisoning would be confirmed. If nicotine plus cotinine are found in the blood and urine of the victim, this would indicate nicotine poisoning presumably from ingestion of the “highball” drink.

**Experimental Validation of suspected compounds:**

Students must prepare methodology to show how the analysis should be done and how the data is produced. The handling of samples including flow charts with time lines should be recorded and a description included in “Materials and Methods”.

The” best method” for analysis of the material will be determined by experimentation. The material will be analysed on the Varian 3800 GLC using a determined method comparing two different columns. A broad temperature program will be used to determine if compounds in the solution can be separated, and how many compounds can be isolated. The program will be modified to get the optimum separation.

The identity of the compounds in the unknown solution and the urine and blood will be determined by their retention times compared to standards plus the sample should be spiked with the suspected compounds to confirm their identification.

**Materials and Methods:**

**Materials:**

Nicotine stock (10 mg/ml in ethanol), menthol (10 mg/ml in ethanol), cotinine stock (2 mg/ml in ethanol), napthalein stock (10 mg/ml in ethanol), ethanol.

**Week 1: Method**

The GC parameters are determined to give good separation of the standards (nicotine, menthol, cotinine, napthalein). The starting general parameters are injector temperature 275°C with a split of 20. The split can be adjusted to improve peak shape and/or affect sensitivity. The column start temperature should be 80°C and final temperature of 285°C based on the boiling temperatures of the suspected compounds to be analysed. The gradient should be set at 20 °C /min. Hold times should be 1 min. at the start of the run and 3 min. at the end of gradient. Delay time can be 0.5 min. The Silicone based and wax column can be compared for best separation. The FID detector temperatures are set at 285°C and the electronics are on. The gas flow rate should be 1 for both columns. The gases should be nitrogen 25, hydrogen 30 and air 300 for the FID detector.

*Note: The ECD detector should have a flow of 1 ml/min and the heater on at 200 C and electronics off. This detector is not being used and there is no need to mention it in the write up. The front injector is not checked and is kept at 100C with 0.1 ml of flow.*
A standard mixture of nicotine, menthol, cotinine, naphalein are to be run on the GC. The above compounds are to be mixed (a combination standard sample) and made up to 2 ml. with ethanol and used to determine the separation method using the 3800 GC. The final concentration of each standard should be approximately 0.1 mg/ml. The sample should be run using both columns to determine the best separation technique. Modifications to the method can be made to determine an optimal method. The mixture run will give you the retention times of the components that should be in the “high ball”, the urine, and blood of the victim. This procedure than can be used to determine if the above toxic compounds are in the “high ball”, blood and urine samples from the crime scene and possibly their concentrations.

The identity of the compounds in the “highball” and the urine and blood samples are to be determined. The compounds will be identified by retention times. Each of the known standards (nicotine, naphalene, menthol and cotinine) will have to be run individually to determine their individual retention times. These retention times will be compared with the compounds in the highball, blood and urine, to identify if these compounds are present.

The integrated areas of the peaks of unknown samples will be compared to integrated area of standards of known concentration with the same retention times to determine concentrations of the unknown samples.

Standards and samples should be run using a sequence table.

Setting up a sequence table.

You can inject samples one at a time or you can set up a sequential run to inject samples one after another automatically. It is best to set up a sequence to run your samples since you can easily keep track of the samples you ran. You can inject samples using your selected method and the injections will be done automatically by the auto sampler. To make a sequence click on file, new, sequence. Fill in method, run name (prefix), run ID (suffix), run time, no. of injections, vial #, injection vol., sample type (unknown). Upon clicking on Start the instrument will load your method, activate the changes, equilibrate, and will make the injection.

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.

Week 2: Extracted samples

Standards:

Prepare a standard curve consisting of menthol, naphalein, nicotine, and cotinine from the stock solutions of each of these standards diluted in ethanol. The concentration range should be 5 ug/ml to approximately 100 ug/ml. You will need to make sure that your dilutions of standards are in the range of the unknowns. You may need to add additional concentrations of standards, depending on your results.
Obtain 2 mls. each of urine, blood and highball crime scene extracts from the lab supervisor. These samples have been extracted from the highball, blood and urine on a 1 to 1 basis. The samples were extracted into ethanol. Any dilutions therefore should be done using ethanol. Run the standards (first in sequence table) and then the samples from the “high ball”, urine and blood.

When run open the chromatograms and determine the areas of the standards and unknowns. Make graphs of standard concentration verse area and use curve to determine the concentration of the unknowns in the “high ball”, blood and urine.

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 if 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 LD50 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 due 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 LD50?

Questions:

1) Why does a GLC 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 GLC?
3) Could the LC-MS be used to measure the suspect compounds? Explain.
4) How is chemical toxicity (LD 50) determined?