GAS CHROMATOGRAPHY (GC)
ACCELERANT ANALYSIS

Objectives:

- To get acquainted with a general setup of a GC system.
- To develop a method for identifying accelerants

Introduction:

Gas chromatography (GC) is a separation-based analytical tool used extensively for analyses of volatile organic compounds like petrochemical products. Depending on the type of detector used, the analytical range varies from parts per million (ppm) to parts per trillion (ppt).

Instrumental parameters such as oven temperature, carrier gas flow rate, column length, and column stationary phase have an effect on the separation efficiency and analysis time. Each one of these parameters has to be optimized in order to obtain the best possible separation of analytes in sample mixtures in the least amount of time.

Flame ionization detectors (FID), electron capture detectors (ECD), and mass spectrometers (MS) are the most commonly used detectors for GC-based analyses. Each one has its own advantages and limitations in terms of sensitivity, versatility of use and associated costs. It is not always possible to have the best detector for a particular application, and often one has to use what is available to address the problem at hand.

In this experiment you are to separate the components of a number of accelerants to identify which one was a possible arson accelerant.

Study:

Extortions (protection money) cases have been reported to the police and subsequently the RCMP in Winnipeg. A number of businesses were suspected of being fire-bombed when extortion money was not paid. On April 30th police saw a suspicious vehicle at a bombed-out business and a chase ensued. A lone male abandoned the car in lot C at the University of Manitoba. On examination of the car an extortion note citing the address of a known burned business, was found. The police had another extortion note in their hands from a previous extortion complaint and wanted to know if the notes were written by the same pen, which could indicate the same extortionist in both cases. A brown beer bottle was also found in the vehicle which had a strong vapor emitted from it. There was a piece of cloth stuffed partially in the bottle. The cloth had a waxy substance on it. A smudged finger print was found on the bottle. Finger prints were also found on the steering wheel of the car. The police wanted to know if the prints on the bottle and steering wheel were the same and if they contained any of the substances associated with the cloth (thought to be a wick) or the contents of the bottle, which had mostly disappeared (leaked out or dumped).

Students will use the GC to determine if the identity of the “brown bottle contents” by comparing the GC chromatogram of the “brown bottle contents” with the chromatograms of known accelerants. Various accelerants can be separated on the GC to get a fingerprint of the

compounds present. These fingerprints may be useful to identify the unknown compound in the brown bottle.

Pre-Lab Questions:

Questions to be answered before doing the experiment. The answers are due at the beginning of the experiment.

1). What important parameters influence separation and retention times of compounds in the GC analyses?
2). What types of compounds is FID (flame ionization detector) is it generally good for?
3). Explain how oven temperature programming is useful for analysis of mixtures containing compounds widely ranging in their boiling points. Could similar separations be achieved at isothermal (constant oven temperature) conditions? Explain
4). Considering that there are many parameters for the instrument that can be changed through programming what are some other physical parameters that could be changed for the GC?

Instrument and Accessories:

The instrument is a Varian CP-3900 Gas Chromatograph. The 3900 is equipped with a flame ionization detector (FID). The GC column in the instrument has the following properties: The column is a Supelcowax ™ 10 column [based on polar phase carbowax (polyethylene glycol) 20M]. The temperature limits of column are 35° to 280°C.

Week 1:

Procedure:

Obtain the samples

1. Gasoline sample
2. Kerosene sample
3. Lighter fluid (butane) sample
4. Mineral spirits sample
5. Unknown accelerant (from brown bottle in abandoned vehicle (parking lot C).

Setting up a method:

Open Galaxie software from the Windows desktop. In the login window, enter "Group ID" under User Identification, "chem3590" under Group, and "GC3900" under Project. Click "OK" (password is GC3900). Go to File, New, and click on New Method. Select Varian 3900 system and click on Next. Name your method "Group X method", where X is your group number. At the bottom-left of the screen, click on the Systems tab. 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 back to the Data tab (bottom-left of the screen). 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. The autosampler does not need to be modified.

Now move on to Injector. Here you can input the temperature of the injector port. Set the following parameters:

Injectors Method Controls:

- Heater: On
- Coolant: Off
- Temperature: 275 ºC
- Time: 0 min
- Split state: On
- Split ratio: 100

Next go to column oven. This is where you define the operating temperature for the oven or create a temperature program for the oven. Enter the following parameters:

Column Oven Method Controls:

- Coolant: Off
- Temperature: 70 ºC
- Time: 10.00 minutes
- Stabilization time: 0.50 min

Column pneumatics allows you to set the carrier gas flow rate for each column. Enter the following values:

Column Pneumatics Method Controls:

- Constant flow: enabled
- Column flow: 1 mL/min
- Pressure pulse: disabled

Next, you will set parameters for the detectors:

Detectors Method Controls:

- Heater: On
- Setpoint: 300 ºC
- Electronics: On
- Time constant: Fast
- Range: 12
- Autozero: Yes
- N_2 makeup flow: 25 mL/min
- H_2 makeup flow: 30 mL/min
- Air flow: 300 mL/min
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Injecting a sample:

Setting up a sequence for automated runs:

You need to set up a sequence of sample injections to be done automatically by the autosampler. To do so, go to File, New, and select New Sequence. Select Varian 3900 as your system, click Next, enter the number of lines needed (number of samples that you wish to run). Click next, name your sequence as "Group X sequence" and click on OK. You now have to fill in the cells of the sequence table. Select your optimal method for each line in the sequence. You can leave Method Properties blank; name your runs under Run Name, leave Description blank; enter 1 for Number of injections; enter vial number under Vial Number; Rack # is always 1; Injection Volume is 0.2 µL; select Unknown for Sample Type; leave Calibration, Level, Istd Values, and User Input blank; leave as 1 for Divisor and Multiplier; leave Dead Time at 0 min. Fill all the lines, making sure that you've correctly specified Run ID (suffix) and Vial # (these are the only entries which should vary in the sequence table).

You should have one extra line. For this line select "Overnight.METH" method. Enter 1 for Run ID; enter 1 under Run Time; enter 1 under Number of Injections, select vial 1; and set injection volume to 0. Have a demonstrator go over this sequence. Once you are satisfied that everything is ready to go, click on the green "Play" button located above the sequence table. If no error messages appear, then everything is in order.

Hit Start. The instrument will load your method, activate the changes, equilibrate, and finally will make the injection. You can observe the live chromatograms under the Systems tab. The run will stop at the conclusion of the run time and the data will be saved.

Viewing of the acquired chromatograms:

In order to view collected data, go to File, Open, and Open Chromatogram. Select an appropriate data folder (these are created automatically by date). Find your file; and open it. Your file name appears in the top-left portion of the screen (identified by a Vial icon), with three components: Data, Method, and Results. Clicking on Data will allow you to view your chromatogram. You can zoom in or out, and move it around by holding down the right mouse button and moving the cursor. You can open multiple chromatograms and overlay them or have them displayed in separate windows.

Identification of the compounds:

Open FID chromatograms of your samples. The retention times and integrated areas are listed for all analytes. Comparison between chromatograms may allow you to determine the identity of the unknown accelerant from the "brown" bottle.

Week 1 and 2: All of the analysis below probably cannot be done in the time allocated so the student should select the analysis to do based on availability of equipment and time available. You need to organize your time effectively.

Determination of Documents by Ink Analysis using UV-Visible Spectrophotometry and Reverse Phase Liquid Chromatography

Forensic significance of ink analysis:
Ink can be sampled for analysis from a variety of documents, including letters, envelopes, packages, calendars, diaries, currencies, ID cards, financial documents, contracts, wills and business records. Ink can also be directly sampled from pens, printer cartridges or photocopier toners.

Possible conclusions from chemical ink analysis:
More than one ink batch may be used to make a single product. Ink batches may be shared between manufacturers. Tens of thousands of pens may be made from a single ink batch. Thus it is unlikely that ink analysis could identify ***the*** pen which produced a particular document. But ink analysis may allow an investigator to:

1) Compare two (or more) inks to determine if the formulations are a match
2) Determine the composition of the ink extracted from a writing sample and provide information as to the possible writing implement which may have produced the sample

Background on ink composition:
Inks are complex mixtures of colorants and dyes, suspended in solvents and resins. The colorants supply the ink colour. Solvents carry colorants to the substrate (*ie*. the nib, ball-point or felt-tip). Resins may be added to the substrate to provide viscosity and help bond the ink to the substrate. Additives like anti-oxidants, preservatives, wetting agents, lubricants and trace elements may also be added to the inks (1).

Colorants: are the portions of inks that allow them to be viewed in visible light (~380-780nm). The chemical compositions of the colorants will determine how visible light is absorbed and reflected, and hence how the colour of the ink is perceived.

Dyes(colorant): are found in fountain pens, ball-point pens, felt-tip pens and roller-ball pens. These are fine particles of soluble or insoluble material, suspended in solvent/resin. These typically are more vibrant than pigments, but tend to bleed more and to photo-decompose over time,

Examples of dyes:

A)Aryl methanes (Crystal Violet): hexamethyl pararosaniline chloride, derived from methane with hydrogens replaced by aryl rings. When Crystal Violet decomposes, there is a progressive loss of methyl groups. The colour of the dye is dependant on the pH of the solution.

B)Nigrosine dyes (Acid Black): a mixture of synthetic black dyes, made by heating a mixture of nitrobenzene, aniline, and aniline chloride in the presence of a copper or iron catalyst

Pigments: are found primarily in gel-pens. These are highly conjugated resonance structures which incorporate a metal. The pigments are water-insoluble, and more colour-fast (*ie*. more photo-stable) but less vibrant than dyes.

Solvents: Common solvents used in ink formulations include **glycols, alcohols and water**. Ball-points pens typically use glycol, but 2-phenoxyethanol (2-PE) may also be used.

Case information:
Two extortion notes were found in two vehicles abandoned on two different dates in different burned buildings. A writing pen was found in the pocket of a suspect.

Objectives:
The objective of the study is to determine if ink extracted from different notes used in various extortion/arson incidences were written by the same type of pen. *First*, the writing sample will be examined under a dissecting microscope to help determine which type of pen was used (*ie*. ball point, a gel pen or a felt tip pen), since different types of pens leave different impressions on the paper, and flow and absorb onto the paper differently. *Second*, the ink samples will be extracted with a variety of solvents to provide further information on the type(s) of pen used, since inks from ball point pens, gel pens and felt pens have different compositions and therefore different solvent solubilities. *Third*, ink samples will be extracted from the extortion notes using an appropriate solvent, and the samples scanned with a Cary spectrophotometer to obtain their absorption spectra. *Fourth*, (if time permits) the extracted inks will be separated by HPLC to obtain the dye composition.

Principal of spectrophotometry:
The basic principles of UV/Visible spectrophotometry are light is generated by a source lamp. This is normally a tungsten lamp for the visible region of the spectrum and deuterium for the ultra-violet range. In the case of the dual beam Cary 50 spectrophotometer in the MCAL the light source is a zenon lamp which covers the spectral range from 1200 to 200 nm. The light is dispersed into its constituent wavelengths in a monochromator which results in a narrow band of the dispersed spectrum passing from the exit slit of the monochromator. Optics are used to focus the monochromatic light, of a narrow wavelength band, to the sample to be measured. A UV/Visible chromophore within the sample absorbs a certain amount of light and the unabsorbed sample and reference beams are measured using Silicon detectors. Both the reference and sample signals are amplified and converted to digital signals.

Principle for HPLC analysis:
High precision liquid chromatography (HPLC) is an analytical separation technique which uses solvents at relatively high pressure to carry an analyte (the ink) through a chromatography separation column to separate the individual ink components. The individual components are visualized by their absorption of light by a chromophore in the sample. Different ink components would absorb light of different wavelengths. The detector is a diode array which measures the full spectrum of light. The diode array consists of a number of photosensitive diodes placed side by side and insulated from one another in the form of a multi-layer sandwich. Each diode may be only a few thousands of an inch thick and the output from each diode can be scanned, stored and subsequently processed by a computer. The polychromatic light from the tungsten and/or deuterium lamp is passed through the sample cell. It is dispersed by a quartz prism or a diffraction grating onto the surface of the diode array. Each diode receives light of a slightly different wavelength. Organic ink components have characteristic spectra that are

acquired and used to construct an absorption spectrum that can be compared with standard spectra for identification purposes. Alternatively, by selecting the appropriate diode, the wavelength of the light at which there is maximum absorption can be selectively monitored to provide maximum detector sensitivity for that substance.

Equipment and reagents:
Cary 50 (Varian, [Agilent]) UV-Vis spectrophotometer
Varian (Agilent) Prostar HPLC system with Diode Array detector
Ink pens, acetonitrile (HPLC grade), acetic acid

Method:

Microscopic examination:
Examination of the ink traces using a microscope should shed light on the type of pen used to make the extortion notes. Ball point pens leave a pressure line on the paper and there are gaps in the ink line with frayed ink edges. The ink flows more evenly from gel pens but there is a white (empty) line down the middle of the ink line where the ink does not penetrate. The ink from a felt tip pen is absorbed into the paper more than a ball point pen.

The student can draw lines with a felt tip pen, a ballpoint and a gel pen and then compare the lines, under a microscope, to lines from the extortion notes.

Separation of inks by solubility:
Felt tip pen inks, gel inks and ballpoint pen inks are made from different formulations and the inks are therefore soluble in different extraction solvents. The second step in the analysis of the inks is to determine their solubility. This should determine the category of the pen (s) used in the extortion. The ball point pen "black" inks are soluble in 40% acetonitrile (or pyridine), the gel inks using dyes (versus pigments) as color are soluble in water, whereas pigment based gel ink is virtually insoluble, and the felt tip inks are soluble in 50:50 ethanol : water. Ball point pen ink may also be soluble in ethanol : water. Mark a piece of filter paper with ball point, felt, and gel inks. Add a drop of solvent onto the ink on the filter paper. The inks that are soluble in the solvent will dissolve and diffuse into the paper. The insoluble inks will not diffuse and will remain as a line on the filter paper. The ink on the extortion notes can be treated in the same manner to determine in what solvents they are soluble. The extractability of the ink in the particular solvent will give you an idea if the inks from the extortion notes are from a felt, gel or ball point pen.

Absorption spectrum using Cary 50 spectrophotometer:
The solubility test will give a good idea of the type of ink that you have. This combined with the microscopic examination should lead you in the direction of which type of pen was used to make the extortion notes.

Determine the absorption spectrum of the ink samples from the various extortion notes using the Cary 50 spectrophotometer. If the notes were written by the same pen or have the same ink composition the spectrums should be the same. The spectrums of other black ink pens will also

be compared. This will demonstrate the variability in the spectra of different ink formulations from different pen manufacturers.

Operation of the Varian Cary 50 UV-VIS Spectrophotometer:

1. Start-up

1.1 Turn on the computer and monitor if they are not already on. Note that the Cary 50 spectrophotometer is powered from the computer.

1.2 From the desktop click on the icon **Cary WinUV** to open the Cary WinUV window.

3.2 Click on the icon "**Scan**" to open the scan application.

2. Method Set-up – Scan Mode

2.1 Click the "zero" button to zero the system. The program is ready to load the blank.

2.2 Load your blank (extraction solvent). Click on "OK" to complete the zeroing of the instrument.

2.3 With the blank solution in the cell holder, click the "zero" button several times until the absorbance is near 0.0000.

Instrument set-up:

3.1 Set up the instrument parameters by clicking on the icon "**setup**". This will display the Setup Dialog/Cary tab: dual beamStart: 200 Stop: 800 Mode: Abs

3.2 Once you are satisfied with your method setup click on "**OK**" to confirm any changes your have made and close the Setup dialog.

3.4 Take a 1 cm quartz cuvette and rinse it well with milli-Q water. Carefully wipe the transparent sides with Kimwipes. Do not scratch the cuvette or touch the surfaces with your fingers. Dust free gloves are good to use.

3.5 Fill the cuvette with your sample solution, no more than ¾ full. Check to see whether there are air bubbles on the optical surfaces. Refill the cuvette if necessary.

3.6 Carefully install the cuvette in the cell holder (the holder is quite stiff, so be careful). It should slide in with the clear faces parallel to the light path.

3.7 Click on "**Start**" to start the scanning.

4. Data Storage

4.2 Spectra can be saved by "saving data as"

4.3 You can print out spectra or reports as a pdf to take with you

5. Shutting Down

5.1 Remove the cuvette from the cell holder by pulling up the black tab on holder carefully (it is quite stiff), empty the solution, rinse it with DI water, gently rinse out all liquid remaining from the outside, and place it back into the storage case.

5.2 Close the Cary WinUV menu, but you can leave the computer on.

Preparation of the samples:

Most inks from black ball-point pens should be soluble in an extraction solvent with the composition 40% acetonitrile plus60 % 0.1M aetic acid). Four pieces (1 cm x 1cm) of the extortion notes with equal ink content are cut and extracted with 1 ml. of acetonitrile plus 0.1M acetic acid : water (40:60). The extract is sonicated in a water bath for 15 minutes. Determine the absorption spectrum of the ink using the Cary 50 spectrophotometer scan method. A library

of the spectrum and HPLC separations of other similar inks will be made to determine if there are discernable differences between black inks.

Preparation of standards:
A major constituent of black inks are crystal violet and methyl violet B. Pure samples (Sigma-Aldrich) of these dyes will be dissolved in 40% acetonitrile plus 0.1M aetic acid : 60% aqueous acetic acid (0.1M) and separated on the reverse phase C_{18} column to identify and semi-quantitate the amount of these dyes in the pen ink. Comparing the retention time of the dyes with the black ink components will determine if these dyes are components of black pen inks. Standard solution of the two dyes will be supplied and the student can determine the approximate dye concentrations.

Method: HPLC separation
A reverse phase C_{18} column will be used for the HPLC separation with a diode array (Varian model 335) UV-Vis detector. The HPLC system is operated by *Galaxie* software. To open the software click on the *galaxie* icon. Insert Id as *student*, Group as *3590*, and Project as *PDA*. Click on *systems* icon and click in *PDA* box. This opens the set up screen for manual operation of pumps and detectors. In the *212 screen* (pumps) set % B at 40% and pump A at 60% and flow rate at 0.50 ml/min. In *335 screen* click on yellow UV and Vis icon to turn on the ultra violet (deuterium) and visible (tungsten) lamps. This will start the pumps so the column will be equilibrated, and the lamps stabilizing.

To set up a method for the analysis open *file: new: new method*. This will open a new method screen. Under *data* (bottom left) open *contro*l screen to set major method parameters. In *autosampler* screen use default values. Copy values for future reference. Open *212 screen* and set solvent program. The solvent program consists of solvent B at 40% (acetonitrile) and solvent A (aqueous acetic acid 0.1M) at 60%. The elution flow-rate is set at 0.50 mL/min. A gradient will be used starting at 40% acetonitrile (pump B) and increasing to 65% acetonitrile over 5 min, then increasing to 90% acetonitrile over 1min (total of 6 min) and staying at 90% acetonitrile for 6 min (total of 12 min). Save this method as "Ink and your name and date". *When you run a sequence of multiple samples you need to re-equilibrate the HPLC column after each run. To do this in your method (in the 212 screen) after the 12 min run time add another line and set %B to 40% for 2 min (total of 14 min), then add another line and set %B to 40% for 10 min (total of 24 min).* **See instructor on how to have instrument shut down automatically.**
Open the *335 screen*. Set the analogue wavelengths for detection in channel 1 and 2 at two wavelengths chosen from the scan of the ink from the spectrophotometer or do a pre-run and use the major peaks from the diode array channel. Check the *auto zero* box. This will zero the instrument before each run. *Save as* your method under your group and date.

Sequential injection:
In the tool bar under *file* open *new sequence*. The name of your system is displayed. Press *next* and then enter the number of samples you are going to run. Press *next* and give your sequence a name. Press *O.K.* In sequence table choose *method to run sequence (must change autosampler in your method to partial loop from none)*, add run name and ID (numbers only), run time, vial #'s, injection volume, sample type as *unknowns*, multiplier and divisor should be 1. When

sequence table is complete press *green arrow* and **run will start**. At end of run all data can be accessed from *file, open chromatogram* and find month day and *sample name*.

Data:

The HPLC data that is run overnight will be taken from the computer the following day. Comparisons of the chromatograms from the various ink samples will determine the similarity of the inks from the different extortion notes. The areas of the peaks from the inks and the standard methyl violet 2B and crystal violet will determine the approximate concentration and ratio of these dyes in the various inks. Hopefully this data will give you enough information to decide if the notes were written by pen(s) from the same manufacturer. This should also tell you if the pen found on the suspect contained the same ink composition as the ink on the extortion notes.

References:

1) *Industrial Dyes: Chemistry, Property, Applications 2003*