Drug Chemistry Section
Drug Chemistry Procedure Manual
Effective Date: September 1, 1996

Name of Procedure:

Gas Chromatography
Varian 3400 Gas Chromatograph and the Spectra-Physics 4270/4290 Integrator

Suggested Uses:

1. Qualitative analysis of a compound.

2. Quantitative analysis of a compound.

Apparatus Needed to Perform the Procedure:

Safety Glasses
Fume Hood
Gloves
Lab Coat
Varian 3400 Gas Chromatograph
Spectra-Physics 4270/4290 Integrator
High purity solvents (methanol, acetone, chloroform, methylene chloride and petroleum ether)
Sample vials (screw-top or septum seal)
10 μl syringe
Operation Manual for a Varian 3400 Gas Chromatograph
Operation Manual for a Spectra-Physics 4270/4290 Integrator
3% 0V- 17 or a Megabore Column
Nitrogen carrier Gas
Hydrogen Gas
Medical Quality Air
Known Drug Standards
Internal Standard

General Operational Procedures:

1. Switch main power to “ON”. (Rear toggle switch in the up position)

2. Open gas lines and allow column pressure to build to approximately 25 psi.

3. Set gas chromatograph parameters based on the type of material to be analyzed. (Refer to melting/boiling point data of material.)

General Operational Procedures (continued):

4. Program the integrator for the desired data output. (Refer to the Spectra-Physics 4270/4290 Users manual for specific details for choosing data output.)

5. Inject sample.

6. Collect data.

Operational Procedures:

Qualitative analysis involves the identification of specific components of a mixture based on comparison of retention times of unknown compounds and known standards. Quantitative analysis involves the measurement of a specific amount of a compound based on retention time, response factor, and peak area of a given component.

GC Conditions:

The GC conditions should be set according to the characteristics of the compound to be analyzed and the resultant chromatography. This may include changes in injector temperature, initial column temperature, final column temperature, and temperature program. Some general conditions include:

Parameter Set 1- a general set of parameters for isothermal analysis

Injector temperature- 280 degrees
Initial temperature- 230 degrees
Final temperature- 230 degrees
Temperature program- 0 degrees/minute (isothermal)

Parameter Set 2- a set used for a wide majority of drugs

Injector temperature- 260 degrees
Initial temperature- 120 degrees (hold for 1.0 minutes)
Final temperature- 260 degrees (hold for 10 minutes)
Temperature program- 15 degrees/minute

GC Conditions (continued):

Parameter Set 3- a set used for LSD, steroids and other high molecular weight compounds.

Injector temperature- 260 degrees
Initial temperature- 230 degrees (hold 2.0 minutes)
Final temperature- 280 degrees (hold 15 minutes)
Temperature program at 15 degrees/minute

Parameter Set 4- a set used for methamphetamine and other low boiling compounds.

Injector temperature- 200 degrees
Initial temperature- 80 degrees (hold for 3.0 minutes)
Final temperature- 250 degrees (hold for 1.0 minute)
Temperature program at 15 degrees/minute

Again, any of these sets may be changed based on the needs determined by the chemist.

Calibration:

The GC should be calibrated each time it is used with known standards of material of known concentrations.

1. Set up the desired GC parameters based on the type of sample.

2. Set up the proper file on the Integrator using the known standard of known concentration. Standard compounds will be selected by the chemist and prepared based on specific need. Refer to the Spectra-Physics SP4270/4290 Integrator User's Manual for assistance in setting up a method for calibration.

3. Inject 1 to 3 microliters of sample into the instrument .

4. Obtain the retention time(s) and response factor(s) for the compounds being analyzed.

Sample Preparation:

Almost all samples will be in the solid or liquid phase. Accordingly, they must be introduced into the GC in an acceptable liquid phase.

1. Solid samples are dissolved in the proper proportions (0.1-0.5 mg/ml) in the appropriate solvent.

2. Liquid samples are diluted to the proper concentrations (0.1-0.5 mg/ml) in the appropriate solvent.

Sample Analysis:

1. Solvent blank

   Before each case sample, a solvent blank is injected to ensure that no sample carry-over occurs and that the level of background noise is within acceptable limits. Injection of 1μl of solvent is sufficient for this task. The solvent selected should be selected for dissolution or dilution of the sample.

2. Sample injection

   After the proper dilutions, 1 to 3 microliters of solution are injected into the instrument using the same conditions as the blank run.

3. Qualitative Analysis

   There are two ways that the Spectra-Physics SP4270/4290 Integrator identifies a components of interest.

   a. Absolute Retention Time

   b. Relative Retention Time

   Refer to the Spectra-Physics SP4270/4290 Integrator Users Manual assistance in selecting the proper method of identification.

Sample Analysis (continued):

4. Quantitative Analysis

Once the compound has been "identified", the proper calculations can be applied using the integrated area of the peak. Set up a File on the Integrator using the known retention time(s) and response factor(s) calculated during Qualitative Analysis. Refer to the Spectra-Physics SP4270/4290 Integrator Users Manual for assistance in setting up the proper parameters and selecting the proper calculation method depending on the type of compound being analyzed and the type of data required from the analysis.

Documentation:

Copies of data generated from case samples become a part of the case file.

Safety Concerns:

Safety glasses, gloves, a lab coat, and a fume hood are essential when preparing solutions.

General maintenance should be conducted when the instrument is unplugged and cool to protect against burns and electrical hazards.

Literature References:

Spectra-Physics SP4270/4290 Users Guide, Spectra-Physics, 1986, San Jose, California.

Varian 3300/3400 Gas Chromatograph Operator's Manual, Varian Associates, Inc., Walnut Creek, California, 1988.

Skoog, Douglas A., **Instrumental Analysis**, Third Ed., Saunders College Publishing, New York, 1985, pp 727- 783.

Willet, John, **Analytical Chemistry by Open Learning - Gas Chromatography**, John Wiley & Sons, 1987.

Literature References (continued):

Bonelli, E. J. and McNair, H. M., **Basic Gas Chromatography**, Varian Aerograph, Walnut Creek, California, 1967.

Freeman, R. R., **High Resolution Gas Chromatography**, Hewlett- Packard Corporation, 1981.