Idaho State Police
Forensic Laboratory Training Manual
Gas / Liquid Chromatography

1.0.0 Backround

In 1942, Martin and Synge developed a partition method of chromatography, n which the solute (material to be chromatographed) is partitioned between a stationary liquid phase absorbed on an inert support and a moving liquid, which is only partially miscible with the stationary phase. At that time, they pointed out that the moving liquid phase could be replaced with a gaseous one. Martin and James exploited this suggestion and, in 1952, published a paper that marks the birth of gas chromatography. Development of the technique was rapid, with the petroleum industry, in particular, playing a large part in its advance.

Gas-liquid chromatography (GLC) is a method of separating the components of a volatile mixture by distributing (partitioning) them between a stationary liquid phase and a moving gaseous phase. The stationary phase is loaded into a tubular column and gas is passed through the system. The sample is placed at the head of the column, vaporized, and passed down the column by the carrier gas. At the column exit is a device for detecting the solute as it is eluted from the column. The signal from this detector is amplified and displayed on a data system.

Any of the permanent gases, with the exception of oxygen, can be used as the mobile phase. From a theoretical standpoint, the lighter gases are more desirable. Hydrogen is to be avoided as a carrier gas because it is explosive and, in some instances, reactive with the solute. Helium is an excellent gas for GLC, but its use, because of cost, is restricted to situations involving certain types of detectors. Nitrogen is the carrier gas normally used in packed GC column chromatography, and helium is the preferred carrier gas in capillary GC column chromatography.

2.0.0 COLUMN SUPPORTS AND PHASES

The heart of any gas chromatograph is its column. The column tube can be made from a variety of materials including metals and glass. Glass is preferred for its chemical inertness. If the tube is filled with a liquid-coated solid support, it is called a "packed" column; if the inner wall of a small diameter tube is coated with the liquid, it is called an open tubular or capillary column.
In order to eliminate "active sites" on the various supports, great care is taken in their manufacture. Such sites are usually -Si-OH, onto which the solute can be adsorbed, leading to poor peak symmetry, possible irreversible adsorption, and even decomposition of the solute.

The rate at which the solute molecules pass through the column depends upon two factors:

1. Their affinity for the stationary phase
2. Their vapor pressure at the column temperature employed

The vapor pressure of solutes increases with increasing column temperature, thereby causing the solutes to elute faster because of less residual time in the stationary phase. A mixture of solutes of widely varying volatilities can thus all be eluted from the column in a singe run by starting at a low column temperature and then raising it. This technique is known as temperature programming.

When a solute is distributed between two phases under a given set of conditions (temperature and pressure), the ratio of the concentration of the solute on the stationary phase (C_L) to the concentration of the solute in the mobile phase (C_M) is referred to as the distribution or partition coefficient, K, and is expressed as $K = C_L/C_M$. Separation of two solutes can occur only when their K values are different. A separation that cannot be achieved on a nonpolar stationary phase may be possible on a polar phase if the two solutes are of different polarity. As a general rule, "like dissolves like." Hence, on a polar stationary phase, a solute of greater polarity will be more strongly retained (lower K value) than a solute of lower polarity. In addition to being a good solvent for the solutes, the stationary phase should be:

1. Thermally stable
2. Nonvolatile at the temperature used
3. Of low viscosity (in order to form thin films on the support)

For drug analysis, the most common liquid phases in use are the silicone gums and fluids. OV-1 and SE-30 are silicone gums and OV-101 and CD-200 are silicone fluids. All four liquid phases are methyl silicones whose active groups are 100% methyl groups. As a result, all four are nonpolar phases and all are considered chromatographically equivalent.[1] OV-17, which is a more polar phase, consists of 50:50 methyl silicone: phenyl silicone, which means that 50% of its active groups are phenyl and 50% are methyl groups. Generally, as the phenyl content increases, the polarity increases. Furthermore, substitution of more polar groups, such as $-C\equiv N$ or $-CH_2CH_2CF_3$, creates an even more polar liquid phase. Note that on a nonpolar column, the individual GLC peaks will emerge according to their boiling point, while on columns prepared with various polar phases, the individual components will be retarded according to the interaction between their polar groups and the active groups of the particular phase.

3.0.0 COLUMN EFFICIENCY

The efficiency of a particular GC column is dependent upon the substance being chromatographed, the molecular diffusion of the substance in the mobile phase and

[1]Liquid phases termed equivalent or identical may sometimes reflect dissimilarities due to different polymerization grades or the presence of impurities.

mass transfer in the stationary phase, the operating conditions of the GC (such as flow rate and temperature), the quality of the packing material, the uniformity of the packing within the column and column and system geometry.

A measure of the efficiency of a column is obtained by calculating the number of theoretical plates, *n*, in the column with the equation:

$$n = 16\left(\frac{t_R}{W_b}\right)^2 = 5.54\left(\frac{t_R}{W_h}\right)^2$$

t_R = retention time of the substance (seconds)
W_b = the width of the base of the peak obtained by extrapolating the relatively straight sides of the peak to the baseline (seconds)
W_h = peak width at half height (seconds)

Note that *n* obviously will vary with the length of the column. To compare the efficiency of columns of different lengths, one can determine the height equivalent to one theoretical plate by ratioing the column length (L) to the number of theoretical plates *n* as follows:

HETP = h = L/*n*

4.0.0 GC DETECTORS

In principle, it is possible to make use of any physical difference between the pure carrier gas and carrier gas plus solute as a method of detection of the solute. About 40 different detectors have been described in the literature. One of the most commonly used detectors in forensic laboratories is the flame ionization detector (FID). This detector contains a hydrogen flame, the resistance of which is monitored by means of polarizing electrodes. The introduction of the solute into the flame increases the ion concentration in the flame, thus resulting in a decrease in the resistance of the flame with a corresponding increase in ion current. This current is then amplified and recorded. Others that are sometimes used include the nitrogen-phosphorus detector (NPD) for its specificity, and the electron-capture detector (ECD) for its high sensitivity to halogen atoms.

5.0.0 SOLVENT EFFECTS

Ideally, a nonpolar solvent should be used to dissolve the sample; however, ideal solvents are rare in GLC because of the differences in solubility and polarity between sample and solvent. Almost any solvent can be used as a vehicle to introduce a compound or mixture of compounds onto a gas chromatographic column. The only limiting criteria is that the desired compound(s) be soluble in that solvent and that the solvent selected be compatible with the GC detector. This requirement, for example, eliminates the use of halogenated solvents with electron capture detectors. With the use of packed columns and flame ionization detectors, gas chromatography is fairly immune to the ill effects of small amounts of moisture or particulate matter injected into the instrument. Over long periods of time, however, these effects may

accumulate. Continual injection of particulate matter, such as insoluble sugars, creates a buildup of material at the very beginning of the column that serves to promote the breakdown of sensitive compounds. The combustion of halogenated solvents at the FID detector forms mineral acids (e.g., hydrochloric acid) that are corrosive to the hardware of the detector. The use of certain solvents will gradually "strip" the column of its stationary phase, reducing the level of coating and its effectiveness as a chromatographic medium. Although these problems may not be evident during the short period of time that a gas chromatograph is used during an analysis, they constitute maintenance problems that must eventually be attended to in order to ensure continued efficient use of the instrument.

6.0.0 INTERPRETATION

Chromatographic techniques alone are sometimes sufficient for an analyst to identify the components in a mixture with a high degree of certainty, or at least to narrow down the range of possibilities. Such identifications are based on the fact that for a given column under fixed operating conditions a particular solute is eluted by a definite volume of carrier gas. When the carrier gas is flowing at a constant rate, the retention of the solute can be expressed in terms of the retention time. The retention time of the sample solute is then compared to the retention time, under the same chromatographic conditions, of a solute of known identity. Since the use of one stationary phase is not sufficient to state with a high degree of certainty that a given component is identical to the compound of know identity, it is necessary to repeat the analysis on a stationary phase of different polarity in order to be reasonably certain of the identification. Supplementary methods such as infrared, ultraviolet, nuclear magnetic resonance, and mass spectroscopy may be combined with GLC to provide a more positive identification.

Although GLC can provide quantitative information about a sample, a number of sources of error can occur, and these must be minimized in order to increase the accuracy of this technique. Such error sources include:

1. Adsorption problems
2. Measurement of the amount of solute injected
3. Measurement of the instrument responses to a solute

Adsorption of the solute can occur on "active sites" of the inert support. Preferential adsorption of one of the sample components will render the quantitative analysis meaningless. Adsorption can be detected by plotting detector response against sample size for diminishing sample sizes. If adsorption effects are present, the plot will cross the abscissa at a point representative of the amount of the solute lost. Adsorption effects can be minimized by using a large sample size and "saturating" the column with the solute prior to beginning the quantitative analysis.

7.0.0 QUANTITATIVE ANALYSIS

In GLC, comparative analysis is the basis for single and multiple component quantitative analysis. Three GLC quantitative techniques used in forensic laboratories are presented below.

7.1.1 PEAK AREA MEASUREMENT

Peak area (as well as peak height) is proportional to the amount of solute present. Today's modern gas chromatographs are equipped with on-line computing integrators that will electronically determine peak area as well as compute the potency for each individual peak desired. Furthermore, computing integrators monitor and control the gas chromatographs, even correcting for drifting baseline and overlapping and/or asymmetrical peaks.

7.1.2 DIRECT COMPARISON OF PEAK AREA

This method is sometimes referred to as the "dilute and shoot" method. To determine the concentration of a component in a sample, quantitative solutions of the sample and its corresponding authentic standard are prepared. A specific volume, usually 1-3 µL, of each solution is injected into the GLC and the resulting area for the sample component peak is compared directly to the ratio of the area for the authentic standard peak versus its solution concentration. The concentration of the component (comp) in the sample is then derived proportionately as follows:

$$\frac{\text{Peak Area}_{comp}}{\text{Concentration}_{comp}} = \frac{\text{Peak Area}_{std}}{\text{Concentration}_{std}} \qquad \text{therefore}$$

$$\%\ \text{Comp. in Sample} = \left(\frac{\text{Peak Area}_{comp}}{\text{Peak Area}_{std}}\right)\left(\text{Concentration}_{std}\right)\left(\text{Dilution}\right)\left(\frac{100}{\text{Wt. of Sam.}}\right)$$

Obviously, sample size is a major factor that affects peak area (as well as peak height). Consequently, the method of sample injection is critical in this quantitative method since the exact size or volume of injection must be measured. To minimize this major source of human error, all peak area measurements are performed in duplicate in order to check reproducibility of injection size with chromatographic response (i.e., peak area).

In addition to sample size, however, there are instrumental factors that will affect peak area (as well as peak height) if not precisely controlled. Factors that affect chromatographic response are fluctuations in carrier gas flow rate, fluctuations in detector or column temperatures, lack of uniformity of column packing material, dirty detectors, etc. The true advantage of the GLC quantitative method is that it is simple and rapid.

7.2.0 THE INTERNAL STANDARD METHOD

7.2.1 SELECTING THE INTERNAL STANDARD

The term "internal standard" refers to a measured amount of a pure constituent that is equally transferred to the quantitative solutions of the sample and the corresponding authentic standard. Ideally, the structure of the internal standard should closely resemble the molecular structure of the component being quantitated or at least be in the same chemical class, so that it can "mimic" the detector response of the component and precisely compensate for minor column or other instrumental variations. Unfortunately, ideal internal standards are rare. As an alternative, a number of solid organic hydrocarbons have been successfully used as internal standards for the quantitative GLC analysis of various drugs. Some of the hydrocarbons used are:

1. n-Tetradecane $C_{14}H_{30}$
2. n-Docosane $C_{22}H_{46}$
3. n-Tetracosane $C_{24}H_{50}$
4. Octacosane $C_{28}H_{58}$
5. Triacontane $C_{30}H_{62}$

The use of solid hydrocarbons as internal standards has become quite popular because of their availability, high purity, and relative ease in handling. However, if one was to search the literature, there are other compounds used as internal standards, such as Benzopinocolone, Squalene, Cholesterol, etc.

The selection of an internal standard should meet the following requirements:

1. The compound selected must not be present in the sample.
2. Its GLC chromatographic peak should be completely resolved.
3. It should elute close to the resolved component peak being quantitated.
4. The ratio of its peak area to the area of the component peak should be close to unity.

7.2.2 The major advantage of this quantitative method is that the exact size of injection need not be known which eliminates a major human error. In addition, this method nullifies the effects of instrumental variations since the internal standard will be influenced by these variations to the same extent as the component of interest.

In short, this quantitative procedure involves the addition of equivalent amounts of an internal standard to the sample and authentic standard solutions. After chromatographing and measuring the peak area, the ratio of the peak area of the sample (i.e., component of interest) to the peak area of its internal standard is compared to the ratio of the peak area of the authentic standard to its internal standard. This can be expressed as

$$\frac{A_{smpl}}{A_{is/smpl} \times C_{smpl}} = \frac{A_{std}}{A_{is/std} \times C_{std}} \qquad \text{therefore}$$

$$\%spl = \left(\frac{A_{spl}}{A_{std}}\right)\left(\frac{A_{is/std}}{A_{is/spl}}\right)(C_{std})(\text{Dil fac})\left(\frac{100}{\text{Spl Wt}}\right)$$

A_{spl} = peak area of sample component

A_{std} = peak area of authentic standard
$A_{is/std}$ = peak area of internal standard in the authentic standard solution
$A_{is/spl}$ = peak area of internal standard in the sample solution
C_{std} = concentration of the standard solution
Dil fac = dilution factor
% spl = percentage of the component in the sample

Obviously, the internal standard method will take a little longer to perform than the direct comparison method described above. However, its overwhelming advantages make it the method of choice in GLC.

8.0.0 CAPILLARY COLUMN CHROMATOGRAPHY

Since 1957, open tubular columns (OTC) coated with a thin film of a liquid phase were found to provide enhanced resolution and greater sensitivity in comparison to packed GC columns. The adaptation and development of these columns led to what is now known as capillary column chromatography or more precisely "wall coated open tubular (WCOT) column chromatography."

WCOT columns provide two major advantages:

1. Increased column efficiency
2. A decrease in pressure drop per unit length of column (in comparison to packed columns)

The significance of these two advantages is that it has led to the production of WCOT columns having a very high number of theoretical plates that account for the enhanced resolution and increased sensitivity achieved. The fused silica WCOT columns with bonded (or polymerized) stationary phases of various film thickness have been known to provide over a 500-fold increase in sensitivity in comparison to conventionally packed columns, and they provide high quality chromatograms that are characterized by sharp narrow peaks with baseline resolution.

Because of the small amount of stationary phase in WCOT columns, the size of the injection must also be kept small.

8.1.0 SPLIT MODE

In this mode, only a small portion of the vaporized, mixed injection is allowed to enter into the column and the remainder is vented through a vent port (a design feature on the injector). This split ratio is controlled by balancing the carrier gas flow rate to the vent flow rate (which is controlled by a needle valve). If the vent flow rates are adjusted within a range of 25 mL/min to a corresponding column flow rate of 1 mL/min, the useful vent: column split ratio will be 25:1. This means that 25 parts of the injection will be vented and only 1 part (which corresponds roughly to 4% of the injection) is allowed to pass on to the column. A vent: column ratio of 100:1 means that only 1 part of the injection (roughly 1%) is allowed to pass on to the column.

Split injection can provide acceptable and reproducible results. However, it should be recognized that for mixtures containing components of widely varying volatility,

discrimination in favor of the more volatile components must be considered as a possibility.

8.2.0 SPLITLESS MODE

In this mode, approximately 90% of the sample injection passes onto the column. With only the carrier gas flowing at a rate of about 1 mL/min, the vaporized injection is allowed to pass into the column. The solute concentrates at the head of the column because of "solvent effects;" meanwhile, solvent vapor passes through the column (what also permits concentration of the solute band at the head of the column is condensation which occurs due to the low temperature at which the column is maintained prior to commencing temperature programming). At a preselected time (about 1 minute) after injection, a valve on the injector is turned on which alters the injection flow to permit purging of the injector system of any residual vapor.

The splitless mode requires care in the selection of the solvent medium and chromatographic parameters and is more difficult to optimize and control than the split mode; however, it permits far greater sensitivity to be achieved.

Considering that WCOT column chromatography is used to detect quantities of components at trace levels, the forensic chemist should be acquainted with the following terms relating to specific levels of sensitivity and detection:

Milligram	mg	10^{-3}g
Microgram	mcg (µg)	10^{-6}g
Nanogram	ng	10^{-9}g
Picogram	pg	10^{-12}g
Femtogram	ftg	10^{-15}g
Attogram	atg	10^{-18}g

Note that the level of detection at the attogram level, fentogram level, and mid-picogram level can only be achieved with capillary column gas chromatographs equipped with an electron capture detector (ECD). The flame ionization detector (FID) can detect components at the high picogram level and the microgram level.

9.0.0 READINGS

Clark, Isolation and Identification of Drugs, 2nd Edition, pp. 178-200

10.0.0 QUESTIONS

1. What are capillary columns made of?
2. What is a bonded phase and what are the advantages of bonded phases?

3. What are cross-linked phases and what are the advantages of cross-linked phases?
4. Describe the difference between a DB-1 type column, a HP-5 type column, and a DB-17 type column.
5. Rank in order from low to high polarity the following phases. HP-1, HP-5, HP-50, HP-35.
6. The upper temperature limits for capillary columns are usually given as two numbers (such as 325/350°C). What do these numbers mean.
7. What is a theoretical plate and how many does a typical capillary column have?
8. How much does the length of a typical capillary column have to be increased to double its resolution? How much would this increase in length add to the isothermal retention time?
9. How is column diameter related to resolution?
10. How is retention time related to column diameter?
11. In general terms, how hot should the injector temperature be?
12. Why are split injectors used?
13. Give one way to calculate the split ratio for a column.
14. Why is glass wool placed in an injection port liner?
15. Why are splitless injectors used?
16. Describe cold trapping.
17. What is the difference between split and splitless injection liners?
18. What are the benefits of pressure programmable injectors?
19. Describe the proper way to make an injection onto a GC.
20. Should split and splitless injections be made at the same speed?
21. What is septa bleed and how does it affect GC data?
22. List some common symptoms of a dirty injector.
23. Why should glass injector liners be silated
24. If nitrogen carrier gas gives the best optimum efficiency and is less expensive, why are most G.C's set up to use Helium?
25. What do the following GC detector abbreviations stand for?
    a. FID
    b. NPD
    c. TCD
    d. ECD
    e. MSD
26. Define the Limit of Detection (LOD) of a GC detector.
27. Define the Linear Dynamic Range (LDR) of a GC detector.
28. How does a FID detector work?
29. Describe in technical terms the theory of a GC separation.
30. What is the purpose of the internal standard in quantitative procedures using gas chromatography?
31. What are the possible error sources in GC?
32. What is the difference between calculations using internal standards and those using external standards for GC quantitations?

33. What is the cause of a high background on a GC/MS with a principle ion of 44 am? How can this be remedied?
34. What are some potential causes of tailing GC peaks?
35. What are some potential causes of fronting GC peaks?
36. What are some potential causes of a broad solvent peak?

The answers to the most of the following questions are to be found in the ISP SOP's

37. How often should a blank be run on a GC?
38. How often should a standard be run on a GC?
39. How often is GC performance verified?
40. How do the answers to the previous 3 questions compare with the answers to the same questions for GC/MS?
41. By definition in the GC/MS SOP, what is a matching retention time?
42. Prepare an explanation of gas chromatography suitable for use in court.