Quality-Control Analytical Methods: Gas Chromatography

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Introduction

Chromatography is an analytical technique based on the separation of molecules due to differences in their structure and/or composition. Chromatography involves moving a sample through the system to be separated into its various components over a stationary phase. The molecules in the sample will have different interactions with the stationary support, leading to separation of similar molecules. Chromatographic separations can be divided into several categories based on the mobile and stationary phases used, including thin-layer chromatography, gas chromatography (GC), paper chromatography and high-performance liquid chromatography (HPLC).

GC is a physical separation technique in which components of a mixture are separated using a mobile phase of inert carrier gas and a solid or liquid stationary phase contained in a column. The separation is based on the interactions of the vaporized components in a mixture with the stationary phase as they are moved along by the mobile phase.

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GOALS AND OBJECTIVES

"Quality-Control Analytical Methods: Gas Chromatography"
Goal: The goal of this presentation is to provide compounding pharmacists with auxiliary information concerning the use of gas chromatography for quality control of compounded preparations.
Objectives: After reading and studying the article, the reader will be able to:

1. Review the basic theory behind chromatographic separation.
2. Discuss the basic instrumentation and components of gas chromatography.
3. Discuss the different analysis methods by gas chromatography.
4. Discuss the applications, strengths and limitations of gas chromatography.

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Since GC is a gas-based separation technique, it is limited to components that have sufficient volatility and thermal stability.

Practical Aspects of Gas Chromatography Theory

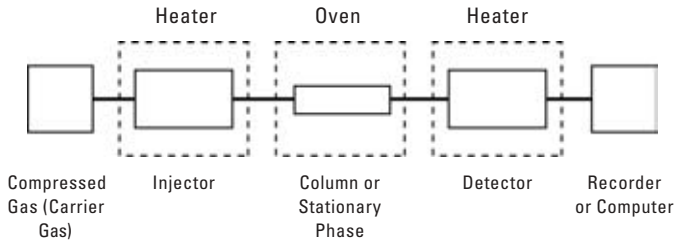
To understand GC and effectively use its practical applications, a grasp of some basic concepts of general chromatographic theory is necessary. Chromatographic principles, including retention, resolution, sensitivity and other factors, are important for all types of chromatographic separation, and were discussed in volume 8, issue 3 (May/June 2004) of the *International Journal of Pharmaceutical Compounding*.[1]

The GC section of *United States Pharmacopeia* (*USP*) *27* Chapter <621> outlines the basic theory and separation technique of GC. A compound is vaporized, introduced into the carrier gas and then carried onto the column. The sample is then partitioned between the gas and the stationary phase. The compounds in a sample are slowed down to varying degrees due to the sorption and desorption on the stationary phase. The elution of the compound is characterized by the partition ratio kD', which is a dimensionless quantity also called the *capacity factor*. The partition ratio can also be thought of as the ratio of the time required for the compound to flow through the column (the retention time) to the elution time of an unretained compound. The value of the capacity factor is dependent on several elements of the chromatographic system, including the chemical nature of the compound; the nature, amount and surface area of the stationary phase; the column temperature; and the gas flow rate. Capacity factor is essential for separation by GC because separation is only possible if the compounds in the sample have different capacity factors.

Samples and Sample Preparation

A variety of sample types can be successfully analyzed by GC. Unlike HPLC, which is used to separate larger molecules, GC is best suited for analysis of samples with smaller molecules. Another important characteristic of samples for GC analysis is that they must be volatile. If a compound is not volatile a technique called *derivatization* can be used to increase its solubility and add to its volatility. Derivatization of samples involves a chemical reaction that alters the molecular structure of the analyte of interest to improve detection. Different types of compounds called *derivatizing agents* can be used to increase the volatility of sample components. However, if possible it is best to avoid derivatization to keep the separation simpler.

Figure 1: Schematic of a Gas Chromatograph.



Hardware

The hardware components used in typical GC systems include an injector, a carrier gas, a column (stationary phase), an oven, a detector and a recorder or information processor (Figure 1). Several components have variable settings that can be used to optimize the analysis of different sample types.

Injectors

Introduction of the sample into a GC system is a critical step in separation. The reproducibility of the amount of sample injected is important to ensure the reproducibility of results. A sample can be injected manually into the system or by using an auto sampler system. A major source of precision errors in GC is poor injection technique. Auto samplers are very effective and help ensure that precisely the same sample volume is injected every time, thereby eliminating injection errors. The injector temperature is also important for separation. The temperature of the injector is used to rapidly vaporize the liquid sample into a gaseous phase that can be carried to the column for separation. The temperature of the injector site can be varied to help optimize separation. Different sample components will dictate what temperature is necessary for vaporization.

Carrier Gas

In the early days of GC experiments, the carrier gas was seen merely as the mass transport system. However, it is becoming clearer that carrier gas is integral to the chromatographic process. Several inert gases can be used as the carrier gas or mobile phase of GC. Hydrogen, helium and nitrogen are all common carrier gases. Each carrier gas has its benefits and systems for which it is best suited. For example, helium is the most common gas used with GC/mass spectrometry systems.

Before the carrier gas can be used, it is important to ensure that it contains no oxygen because oxygen can have detrimental effects on the stationary phase of GC. Also, the chemical nature of the carrier gas has an effect on the efficiency of the GC column. The pressure at which the carrier gas is moving influences the retention time of samples on the column. Increasing the pressure decreases the retention time. Varying both the carrier gas and the pressure at which the gas is exerted on the column can ensure that the sample has ample time to interact with the stationary phase and improve the separation.

Column/Stationary Phase

The column and stationary phase are responsible for the majority of the separation of sample components. Interaction between the mobile phase, stationary phase and sample components determines how components are separated, so selection of columns and stationary packing material is critical. There is a great deal of variation in commercially available columns and packing material. Depending on the components to be separated, the mobile phase being used and the desired degree of separation, different combinations of column type, column length and packing material can be used to achieve optimal results.

Columns can be classified by column diameter and packing material. The three main types of GC columns are (1) conventional, (2) preparative and (3) capillary. Columns can be either packed or open. Packed columns can contain either a porous or nonporous stationary phase. A multitude of different materials are used to pack columns. Each material has its own properties, limitations and effective separation parameters. The capillary column is the most frequently used column for GC separations. Both conventional and capillary columns have advantages and disadvantages. For example, more packing materials are available for conventional columns, but capillary columns give improved sensitivity.

When selecting a column, a major choice is among nonpolar, moderately polar or polar columns. Special-phase columns such as chiral columns can also be utilized to separate isomers. Other important factors to consider are packing material, column length and column diameter.

Columns can be the most expensive component of a chromatographic system, so proper maintenance and use can help control cost. For proper column care it is best to consult the manufacturer's guidelines.

Oven

The column resides in an oven, and temperature, which greatly affects the effectiveness of the chromatographic separation, is an extremely important factor used in controlling GC. In many cases, isothermal (constant temperature) is not the most effective temperature mode for sample separation; in such cases, a temperature program can be used. Most GC temperature programs have an initial temperature, a ramp (degree increase per minute) and a final temperature.

Detector

The detector is used to sense the presence of a compound passing through and to provide an electronic signal to an integrator. A variety of detectors are commercially available to be used with GC, each having its own limitations and advantages:

- Electron capture (ECD). The ECD is used with organic compounds and has many environmental applications.
- Flame ionization (FID). The most commonly used detector in GC, FID is typically used with organic compounds and is widely used in quality-control analysis of pharmaceutical compounds.
- Mass spectrometry (MSD). The MSD can be coupled with GC as a powerful qualitative component for the identification of compounds.
- Nitrogen phosphorous (NID). This detector is used most commonly for drug analysis in tissues and bodily fluids.
- Thermal conductivity (TCD). This detector is considered a universal detector and is nondestructive to analytes.

Recorder

Just as it is with HPLC, the recorder in a GC system serves to convert the information collected by the detector into a format that is understandable. Since the detector signal is electronic, the use of modern data acquisition can aid in signal analysis. The most common data-acquisition technique is through use of a computer, which integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret. Other more advanced features can also be applied to a chromatographic system. These include computer-controlled automatic injectors, oven temperature programs and carrier gas pressure.

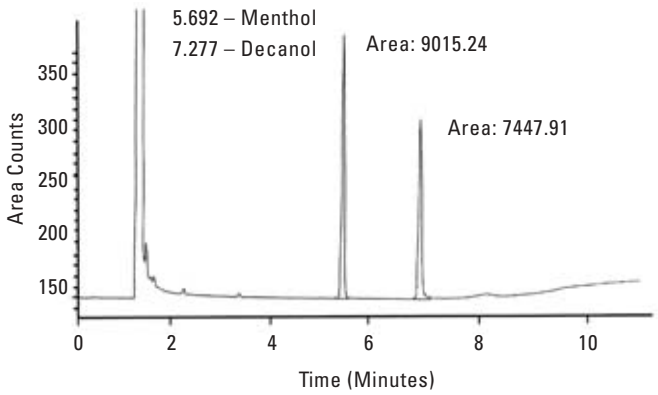
Quantitative Analysis

The basic theory for quantitation of sample components involves the measurement of peak height or peak area. For peaks that are well resolved, both peak height and area are proportional to the concentration. Three different calibration methods, each with its own benefits and limitations, can be used in quantitative analysis: (1) the external standard (std), (2) the internal standard (IS) and (3) the standard addition method. For GC, the most commonly used quantitative methods are the IS and standard addition methods. For our present purpose, only the IS method is discussed.

The IS is an effective method because it tends to yield the most accurate and precise results of all the quantitative methods. With this method, an equal amount of an IS, a component that is not present in the sample, is added to both the sample and calibrator solutions. The IS selected should be chemically similar to the analyte and have a similar retention time and similar derivatization. Additionally, it is important to ensure that the IS is stable and does not interfere with any of the sample components. The IS should be added before any preparation of the sample so that extraction efficiency can be evaluated. Quantitation is achieved by using ratios of peak height or area of the component to the internal standard, as shown in the following formula:

$$\text{Conc.}_{\text{sample}} = \left(\frac{\text{Area}_{\text{IS}_{\text{calibrator}}}}{\text{Area}_{\text{IS}_{\text{sample}}}}\right) \times \left(\frac{\text{Area}_{\text{sample}}}{\text{Area}_{\text{calibrator}}}\right) \times (\text{Conc.}_{\text{calibrator}})$$

Figure 2: Example Chromatogram Illustrating Gas Chromatography Analysis of Menthol with Decanol Used as an Internal Standard.



Note: The area ratio of menthol/decanol was used in determining the concentration of the sample. The sample was a 110 ppm (µg/mL) solution.

mL = milliliter ppm = parts per million µg = micrograms

Figure 2 is an example chromatogram produced in the GC analysis of menthol, with decanol employed as an IS.

Alternatively, a calibration curve is established by plotting the response ratios of calibrator/IS versus concentration of the calibrators. Sample concentration is then extrapolated from the regression equation of the calibration curve based on the response ratio of sample/IS.

Validation

It is important to use a validated GC method when performing analysis. Typical analytical characteristics evaluated in a GC method are similar to those evaluated in a liquid chromatographic validation and may include precision, accuracy, specificity, limit of detection, limit of quantitation, linearity and range, among other factors. It is important to consider the US Food and Drug Administration (FDA) and *USP* guidelines when validating GC methods used for pharmaceutical samples. *USP 24* Chapter <1225> provides guidance on validation of compendial methods, including definitions and determination. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines provide suggestions concerning validation of pharmaceuticals. Valuable sources of information

providing regulatory guidance may be found on the FDA website at http://www.fda.gov/cder/guidance.

System suitability tests provide an evaluation for the function of the overall GC system. This includes all pieces that make up a system, such as the instrument, reagents, packing material, details of the procedure and even the analyst. These tests imply that all the components of a system constitute a single system for which the overall function can be tested. These tests are very valuable and have been accepted in general application since reliable and reproducible chromatographic results are based on a wide range of specific parameters.

In most laboratories a standard operating procedure outlines the specifications of running a system suitability test. To evaluate system suitability, at least five replicate injections should be made of a single solution that contains 100% of the expected active and excipient ingredients levels. The peak response is measured, and the coefficient of variation for five replicates should not exceed the limit set by the testing monograph, or 2%. Using the *USP* method, the tailing factors of the analytes should not be greater than 2. Peak-to-peak resolutions (R) are also determined by using the *USP* calculations and should be at least 1.5. The system test should be used to ensure the quality of the data and of the analysis.

Summary

Both GC and HPLC are widely used methods for the quality control of both commercially manufactured and compounded pharmaceuticals.

Resources

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