International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Review Article **CODEN: IJPNL6**

A TYPICAL REVIEW ON PHARMACEUTICAL ANALYSIS OF GAS CHROMATOGRAPHY-MASS SPECTROPHOTOMETRY

Gattu Madhava Prathap*,B. Krishnamoorthy, M. Muthukumaran and Amreen Nishat

Montessori Siva Sivani Institute of Science & Technology College of Pharmacy-Mylavaram, Vijayawada, Andhrapradesh-521230, INDIA

***Corresponding author e-mail:** madhavaprathap@gmail.com

ABSTRACT

The present review article to discuss briefly about the construction and working mechanism of the hyphenated technique GC-MS. Here the importance of the hyphenated technique in the drug resolution was determined. GC with low resolution electron ionization (EI)-MS was used, together with confirmation with chemical standards, for identification work. The method was successfully applied in many fields like forensic department and environmental analysis. It is also used in the anti-doping analysis. The combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC - MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GC - MS has been widely heralded as a "gold standard" for forensic substance identification because it i s used to perform a specific test.

Keywords: GC-MS, chemical standard, forensic, anti-doping analysis.

INTRODUCTION

Gas chromatography–mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Gas chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one being a stationary bed of large surface area, and the other a gas that percolates through the stationary bed. When the stationary phase is a solid, the separation process is more precisely called gas solid chromatography.

This technique is generally used to separate gases in a gaseous solution. The more common technique (which will be used in this experiment) is gas liquid chromatography (GLC) in which the stationary phase is a porous solid covered with an absorbing liquid. GLC is used to separate a wide variety of organic compounds. The basic requirements for GLC are that the sample be volatile and that it not decomposes in the vaporization process. Since the vaporization occurs in an inert atmosphere, decomposition of the sample is generally not a problem [1].

Separation of a mixture into its components depends on the solubility differences of the sample vapor in a liquid (the stationary phase). The stationary phase is coated in a thin layer on solid particles (solid support) of large surface area and then packed uniformly into a column. A constant flow of the carrier gas passes through the column and transports solute molecules in the gas phase. The column is wound to fit inside an oven for precise temperature control.A sample of the analyte is introduced by syringe injection into the heated injector tube, where it is vaporized and mixed with carrier gas. As the sample vapor is carried through the column by the carrier gas, the analyte partitions between the gas and liquid phases according to the analyte components' solubility in the liquid at the column operating temperature. This equilibrium partitioning continues as the sample is moved through the column by the carrier gas. The rate at which the sample travels through the column is determined by the sample solubility in the

stationary phase, the carrier gas flow rate, and the temperature. Each component travels at a characteristic rate, and if the column has sufficient length and resolving power, the sample will be completely separated by the time it reaches the detector. The detector located at the column exit is the ITD mass spectrometer. It records the total number of ions entering the mass analyzer from the column. The chromatogram produced is called the total ion chromatogram. Each point in the chromatogram is a mass spectrum. Each component is identified by comparing its "retention time", the length of time that it remains in the column, to that of a standard. The retention time of a vapor depends on the column temperature limits and ramp rate, the column length, type of stationary phase, and carrier gas velocity. If these variables are kept constant, the retention time of a component may be tentatively identified by comparison to the retention time of a known standard run under identical operating conditions. If the response of the detector is linear, the area under a peak accurately represents the quantity of the component present.For a given gas chromatography column, the van Deemter theory is useful for determining the flow rate, which gives optimum efficiency at a given column temperature for a particular compound.

The van Deemter equation is

$$HETP = A + B / v + C v \quad (1)$$

HETP is the "height equivalent to a theoretical plate," and results from the treatment of gas chromatographic separations in terms of repeated equilibrations between a moving and a stationary phase.

The terms in the van Deemter equation represents
A=Eddy diffusion
B=Molecular diffusion
C=Non equilibrium effects due to flow of the mobile phase
v=carrier gas flow rate
Where A, B, C are constants.

HETP for a particular gas flow rate is calculated from the total number of theoretical plates
(N) and column length (L), i.e.

$$HETP = L / N \quad (2)$$

Where

$$N = 16(tR / W)^2 \quad (3)$$

Where
t_R = retention time of the component
W = width of the elution peak at its base

At optimum carrier gas flow rate HETP is a minimum. By measuring the HETP at several linear gas velocities (flow rates), the parameters A, B, and C in eq.(1) can be determined and the optimum velocity defined. If the sample contains materials with a wide range of boiling points, separation of all components isothermally is not practical. When the column is operated at low temperatures, the more volatile components will be distributed between the gas and liquid phases and will pass rapidly through the column, giving sharp, well-resolved peaks.

The high-boiling components, however, will remain dissolved in the stationary phase and will be eluted very slowly, if at all. Since the vapour pressure of the latter solutes is low, partitioning will occur over broad bands of stationary phase, resulting in broad, poorly resolved peaks. If the column is operated at a temperature which gives well-defined peaks for the less volatile components, the low boiling fraction will pass through the column with very little partitioning into the liquid phase. As a result, it will appear as one or two sharp, poorly resolved peaks, often with retention volumes approaching the dead space of the column. By utilizing temperature programming, all the compounds can be eluted at temperatures approximating the ideal temperature for separation from adjacent solutes. By employing a low initial temperature, the low boiling components will be distributed between both phases in the column and will appear at the detector as sharp, well resolved bands. The higher boiling fractions will remain 'frozen' at the injection point. As the column temperature is raised, the vapor pressure of the less volatile components increases and they distribute themselves between the two phases. As a result, they move down as well defined bands, eluting at characteristic temperatures. By careful choice of the temperature ramp rate and carrier gas flow rate, each component can be eluted at a temperature approximating the optimum for separation from adjacent solutes. Although the resolution of closely spaced peaks cannot be improved over that at a single optimum temperature, the resolution of widely spaced peaks can be improved considerably.[2]

INSTRUMENTATION

Gas chromatography-mass spectrometry (GC-MS) is a hyphenated technique; consisting of two analytical procedures in sequence, namely a Gas Chromatography (GC) separation followed by Mass Spectroscopy (MS) detection. The purpose of the GC step is to separate multiple compounds in a sample so that they reach the MS detector one at the time.[Figure No:1].

The GC-MS is composed of two major building blocks:

- the gas chromatograph
- the mass spectrometer.

The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off of) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio. These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame ionization detector) cannot differentiate between multiple molecules that happen to take the same amount of time to travel through the column (*i.e.* have the same retention time), which results in two or more molecules that co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore, when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically lends to increased certainty that the analyte of interest is in the sample.[3]

The purpose of the GC step is to separate multiple compounds in a sample so that they reach the MS detector one at the time. The GC uses a high-resolution fused silica capillary column housed in a temperature-controlled oven. The capillary column contains a stationary phase; a fine solid support coated with a nonvolatile liquid. The solid can itself be the stationary phase. When a sample solution is introduced into the injection port it is vaporized immediately because of the high temperature (up to ~300 ^{0}C) and low pressure (~10-4-10-7 torr). The sample is conveyed through the length of the tubing by a carrier gas or mobile phase. As the components of the sample travel through the column, they interact to varying degrees with the stationary phase depending on their affinity for this material. As a result, different compounds will travel with different speeds through the capillary tubing and will exit from the column after a distinct retention time. The temperature of the oven containing the capillary column can be controlled to optimize the separation. When the mobile phase passes through the detector, a signal is produced related to the concentration of a particular compound. A plot of this signal as a function of time generates a series of symmetrical peaks in a chromatogram which provides some information on the sample composition. The retention time of the peaks may help identify the sample components by comparing them to the retention time of some standard, while the heights of the peaks or the area under the peaks provide a quantitative measure of the amount of each component. In theory, sample components would exit the column one by one and each peak represents a specific compound. But in reality, compounds often travel with similar speeds. This deviation from ideality will produce overlapping peaks. Analytes are typically neutral and must be ionized for detection purposes. Methods of ion production include electron impact, which uses electrons to ionize the compounds as they elute from the GC. Here, molecular ions are formed by collision with the electron beam. This also imparts excess energy, and the ions fragment in characteristic and predictable ways. The individual fragments are detected based on their mass:charge ratio, which is usually equivalent to the molecular weight of the compound for small molecules. Another alternative is chemical ionization. Here, a reagent gas (say methane or isobutane) is introduced into the MS at a pressure of about one torr. This moderates the energy transfer, and minimizes fragmentation. That way most if not all of the ions present in many the spectra are protonated molecular ions, and adducts with the ionization gas, where the dot tells a free radical apart from a standard ion. This is useful in determining molecular weights, but frequently gives little or no structural information.[4]

EQUIPMENT PERFORMANCE AND RELATED VARIABLES

Distribution Constants or relative rates at which the species are eluted are determined by the degree the solutes distribute themselves between the mobile and stationary phases. Distribution equilibria in chromatography defines the transfer of an analyte between the mobile and stationary phases,

$K = [\Phi]S / [\Phi]M$

where [Φ]S is the molar concentration of the analytes in the stationary phase and [Φ]M it molar concentration in the mobile phase. If K is constant over a wide range of analyte concentrations, then [Φ]S is directly proportional to [Φ]M. When this holds true, chromatographic peaks are symmetrical, Gaussian distributions and retention times are independent of the amount of analyte injected.

***Retention time (tR)*:** Retention Time of an analyte is defined as the time it takes after sample injection for the analyte to elute and reach the detector.
tR= Retention volume/Flow rate

Dead time: The time for unretained species to reach thedetector is defined as the dead time.
The efficiency of the column is described by the height equivalent to theoretical plates,
HETP = L / N
where N is the number of theoretical plates. the number of theoretical plates is,
$N = 16(tR / W)^2 = 5.55(tR / W_{1/2})^2$
where W is the peak width measured in the same units as tR and W1/2 is the peak width measured at half the peak height. You can use either form of the equation. The resolution of a column is defined as its ability to separate a mixture of compounds.

WORKING MECHANISM OF GC-MS

The power of the GC/MS technique comes from the fact that not only are components of a mixture separated and detected quantitatively, but the detector (the ITD) also provides information concerning the structure of each of the components. Therefore, compounds can be identified not only by comparing the retention time to a standard, as in conventional GC, but also by its mass spectrum. An unknown can also be identified in most cases based solely on its mass spectrum, eliminating the need to run standards for retention time data. Therefore, it is not necessary to know what you are looking for, as in the case of GC.[5] The chromatography for GC and GC/MS are identical in theory. However, the column used in the GC/MS experiment is a capillary column as opposed to the packed column used in the GC experiment done in Chemistry 105. A capillary column is simply a long tube made of glass with a small internal diameter. A 30 cm column with an internal diameter of 0.25 mm is used. The stationary phase is actually bonded to the interior of the glass capillary, eliminating the need for packing a solid support in the column. Different columns may have bonded phases of different characteristics depending on the type of separation to be carried out. After the components of a mixture are separated in the column, they reach the ion trap detector as pure compounds (if the separation was successful). The compounds are ionized by electron impact (EI) by passing the stream of gas over a beam of electrons accelerated to energy of 70 eV. This energy is used to form ions by stripping away an electron, and may break some of the bonds of the compound. Differing populations of the ions will have differing amounts of internal energy. Some of the molecules will become ionized but will not fragment, forming a "parent ion". A parent ion, or molecular ion, has the same mass in atomic mass units as the neutral molecule (it differs by only the mass of an electron). It is the highest mass peak in the spectrum. Many of the ions formed may have sufficient internal energy to fragment, forming a smaller mass ion and a neutral. The neutrals formed are not detected. Only ions are detected. By using the same energy electrons to ionize the compounds, the resulting mass spectra are highly reproducible, not only on a given instrument, but on other instruments using 70 eV electron impact ionization. In this way, libraries of mass spectral data have been generated, so that an unknown can be identified by searching through and matching the mass spectra. Different classes of compounds have some fragmentation characteristics that can be used to help identify unknown compounds. For example, compounds with many strong bonds, such as aromatic compounds, may be less likely to fragment. These compounds are characterized by mass spectra which are dominated by a single peak, the molecular ion. Straight chain hydrocarbons, however, fragment much more easily, and may show little or no abundance of the molecular ion in their mass spectra.

APPLICATIONS

Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage or on human beings.

Environmental monitoring and cleanup: GC-MS is becoming the tool of choice for tracking organic pollutants in the environment. The cost of GC-MS equipment has decreased significantly, and the reliability has increased at the same time, which has contributed to its increased adoption in environmental studies. There are some compounds for which GC-MS is not sufficiently sensitive, including certain pesticides and herbicides, but for most organic analysis of environmental samples, including many major classes of pesticides, it is very sensitive and effective.

Criminal forensics: GC-MS can analyze the particles from a human body in order to help link a criminal to a crime. The analysis of fire debris using GC-MS is well established, and there is even an established American Society for Testing Materials (ASTM) standard for fire debris analysis. GCMS/MS is especially useful here as samples often contain very complex matrices and results, used in court, need to be highly accurate.

Law enforcement: GC-MS is increasingly used for detection of illegal narcotics, and may eventually supplant drug-sniffing dogs. It is also commonly used in forensic toxicology to find drugs and/or poisons in biological specimens of suspects, victims, or the deceased.[6]

Sports anti-doping analysis: GC-MS is the main tool used in sports anti-doping laboratories to test athletes' urine samples for prohibited performance-enhancing drugs, for example anabolic steroids.[7]

Food, beverage and perfume analysis: Foods and beverages contain numerous aromatic compounds, some naturally present in the raw materials and some forming during processing. GC-MS is extensively used for the analysis of these compounds which include esters, fatty acids, alcohols, aldehydes, terpenes etc. It is also used to detect and measure contaminants from spoilage or adulteration which may be harmful and which are often controlled by governmental agencies, for example pesticides.

Astrochemistry: Several GC-MS have left earth. Two were brought to Mars by the Viking program.[8)]Venera 11 and 12 and Pioneer Venus analysed the atmosphere of Venus with GC-MS.[9] The Huygens probe of the Cassini-Huygens mission landed one GC-MS on Saturn's largest moon, Titan. The material in the comet 67P/Churyumov-Gerasimenko will be analysed by the Rosetta mission with a chiral GC-MS in 2014. [10]

Medicine: Dozens of congenital metabolic diseases also known as inborn error of metabolism are now detectable by newborn screening tests, especially the testing using gas chromatography–mass spectrometry. GC-MS can determine compounds in urine even in minor concentration. These compounds are normally not present but appear in individuals suffering with metabolic disorders. This is increasingly becoming a common way to diagnose IEM for earlier diagnosis and institution of treatment eventually leading to a better outcome. It is now possible to test a newborn for over 100 genetic metabolic disorders by a urine test at birth based on GC-MS. In combination with isotopic labeling of metabolic compounds, the GC-MS is used for determining metabolic activity. Most applications are based on the use of 13C as the labeling and the measurement of 13C-12C ratios with an isotope ratio mass spectrometer (IRMS); an MS with a detector designed to measure a few select ions and return values as ratios.

CONCLUSION

GC and MS are useful tools for chemical analysis, especially when used together. An attorney can present an effective attack or defense of GC/MS evidence with a basic knowledge of the analysis process and an insistence on documentation of important indicators that may affect GC/MS results. At the minimum, a technician must process standard samples before and after analyzing a specimen in question. In litigation an adverse party should seek hard copy output, including system conditions. Finally, no analytical technique produces results that are completely without doubt. An effective advocate should always seek corroboration of GC/MS results.

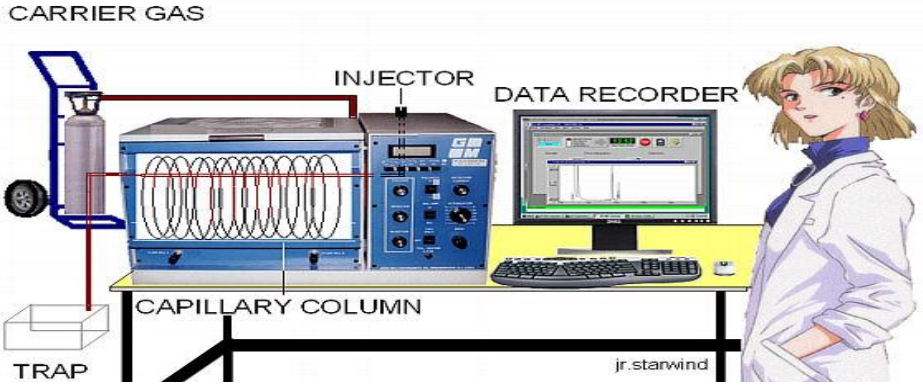

Figure 1: Schematic diagram of the GC-MS instrument

REFERENCES

1. Silverstein RM, Bassle GC, Morrill TC. Spectrometric Interpretation of Organic Compounds. 4th ed., New York, NY. Wiley: 1981.
2. Mc Lafferty. F. Interpretation of Mass Spectra. 3rd ed.,University Science Books: Mill Valley CA:1980.
3. http://www.nlm.nih.gov/cgi/mesh/2011/MB_cgi=mode=&term=Gas+chromatography mass + spectrophotometry.
4. McEwen, Charles N, Kitson, Fulton G, Larsen, Barbara Seliger. Gas chromatography and mass spectrometry: a practical guide,; Boston: Academic Press:1996, ISBN 0-12-483385-3.
5. McMaster, Christopher, McMaster, Marvin C. GC/MS: a practical user's guide;New York; Wiley:1998,ISBN 0-471-24826-6.
6. JamesAT, Martin. AJPBiochem J, 1952; Gas-liquid partition chromatography: the separation and microestimation of volatile fatty acids from formic acid to dodecanoic acid,Biochem J;50: 679–680.
7. Tsivou M, Kioukia-Fougia N, Lyris E, Aggelis Y, Fragkaki A, Kiousi X, Simitsek Ph, Dimopoulou H, Leontiou I-P, Stamou M, Spyridaki M-H, Georgakopoulos C, 2006;"An overview of the doping control analysis during the Olympic Games of 2004 in Athens, Greece.".Analytica Chimica Acta 555:1–13doi:10.1016/j.aca.2005.08.068
8. http://appel.nasa.gov/items/Viking_GCMS_case_07%2025%2006.pdf
9. VA Krasnopolsky, VA Parshev. 1981; "Chemical composition of the atmosphere of Venus". Nature 292(5824): 610–613. Bibcode 1981Natur.292..610K
10. Goesmann F, Rosenbauer H, Roll R, Bohnhardt H. 2005; "COSAC onboard Rosetta: A bioastronomy experiment for the short-period comet 67P/Churyumov-Gerasimenko *Astrobiology* 5 (5): 622–631. Bibcode 2005AsBio.5..622G