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A Review: Application and experimental designs of experiments for analysis of various organic compounds using GC-MS

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Abstract
Gas chromatography ("GC") and mass spectrometry ("MS") make an effective combination for chemical analysis. This article serves to demonstrate tools for an effective attack or defence of GC/MS evidence. The GC device is generally a reliable analytical instrument. The GC instrument is effective in separating compounds into their various components. However, the GC instrument cannot be used for reliable identification of specific substances. The MS instrument provides specific results but produces uncertain qualitative results. When an analyst uses the GC instrument to separate compounds before analysis with an MS instrument, a complementary relationship exists. The technician has access to both the retention times and mass spectral data. Many scientists consider GC/MS analysis as a tool for conclusive proof of identity. GC/MS analysis, where the effluent to the GC instrument is the feed to the MS instrument, is in wide use for confirmation testing of substances. Drug testing, manufacturing quality control and environmental testing are some typical uses.
Keywords: GC-MS, Gas Chromatography, Mass Spectroscopy.

1. Introduction

When gas chromatography coupled with the mass spectroscopy then it is synergistic combination for analysis of various compounds. In both techniques, the sample is in the vapour phase, and both techniques deal with about the same amount of sample (typically less than 1 ng. The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) 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. 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 increases certainty that the analyte of interest is in the sample. Gas chromatography separates components with in time and mass spectroscopy helps to determine the structural elucidation and identification of the each components present in it.[1,2,5,6]

By using GC-MS it is very easy to identify and quantify the volatile and semi-volatile organic compounds. Determination of molecular weight in combine mixture is also very easy by use of the GC-MS. Also it helps to compare the spectra of the compound with the reference compound for its structural determination. Other biological application of the GC-MS is that it is useful for the quantification of the drug concentration and its components in the human urine and blood. Also its important use in the determination of the drinking water pollutants by using the GC-MS. To know the impurities presents in the various drug components is also quantified easily. Identification of the unknown organic compounds in hazardous waste dumps. Identification of the reaction products by synthetic organic chemists. For analysis of the different compound by using the GC-MS it is necessary of sample must be present in the solution form for the injection into the gas chromatography and solvent must be volatile or organic nature. [3,7,9]

2. General principles and working

The primary goal of instrument analysis is to quantify an amount of substance. This is done by comparing the relative concentrations among the atomic masses in the generated spectrum. Two kinds of analysis are possible, comparative and original. Comparative analysis essentially compares the given spectrum to a spectrum library to see if its characteristics are present for some sample in the library. This is best performed by a computer because there are a myriad of visual distortions that can take place due to variations in scale. Computers can also simultaneously correlate more data (such as the retention times identified by GC), to more accurately relate certain data**.**

The pressure difference and incompatibility problem between GC and MS was solved by several ways. The earliest approach is simply split a small fraction of the gas chromatographic effluent into the mass spectrometer. Depends on the pump speed of the

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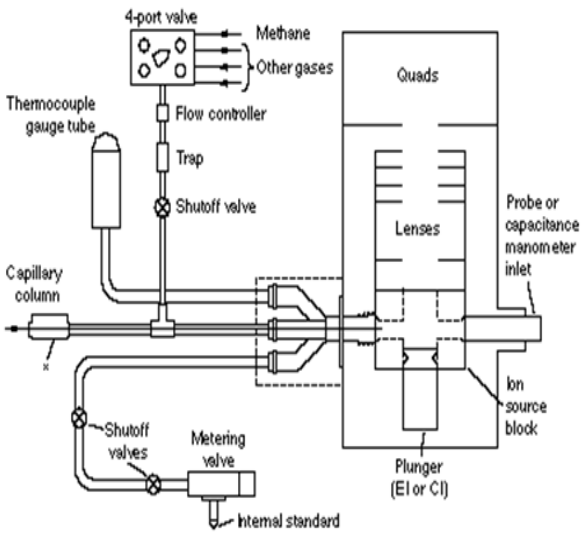
mass spectrometer, about 1 to 5% of the GC effluent or analytic was splitting off into the mass spectrometer, venting or drain out the remaining 95 to 99% of the analytics into the air or atmosphere. It is not best way to use the two methods in combinations so need to improve it. These interfaces reduces the pressure of the GC effluent from about 760 torr to l0Ü to 10 Ö torr, but at the same time they passed all (or most) of the analytic molecules from the GC into the mass spectrometer. These interfaces no longer remain as GC carrier gas splitter but act as carrier gas separator; that is, they separates the carrier gas from the organic analytic and actually increases the concentration of the organic compound in the carrier gas. The most important and useful GC carrier gas separator is called the jet separator. So, it is advantage for this device to takes of the differences in diffusibility between the carrier gas and the organic compound. The carrier gases generally a small molecule such as helium or hydrogen with a high diffusion coefficient, whereas the other organic molecules have much lower diffusion coefficients. In operation, the GC effluent is sprayed through a small nozzle indicated because of its high diffusion coefficient, the helium is sprayed over a wide solid angle, whereas the heavier other organic molecules are sprayed over a much narrower angle and tend to go straight across the vacuum region. By collecting the middle section of this solid angle with a skimmer and passing it to the mass spectrometer, the higher-molecular-weight organic compounds are separated from the carrier gas, which is removed by the vacuum pump. Most jet separators are made from glass by drawing down a glass capillary, sealing it into a vacuum envelope, and cutting out the middle spacing.

It is very important that the spray orifice and the skimmer be perfectly aligned. [1, 3, 5] These jet separators work on high flow rates used for packed GC columns (10 to 40 mL/min); but having some disadvantages. Packed GC column is almost infinite source of small particles upstream of jet separator. If particles escape from the column, it can become lodged in the spray orifice and stop the gas flow out of the GC column and into the mass spectrometer. Some Part of this problem can be eliminated with a filter between the GC column and the jet separator, but eventually a particle will plug up the orifice. In fact, sometimes it is not a particle at all, but rather tar which is use as pyrolyzed GC stationary phase that has accumulated in the spray orifice over time. Clearly, these devices require continues maintenance.

Recently, the most common strategy, which is ideally suited for capillary GC columns, is to pass all of the carrier gas flow into the ion source of the mass spectrometer. It works only when the GC gas flow is small and mass spectrometers pumping speed is high to handle effectively gas flow. For most capillary GC columns, the gas flow is 1 to 2 mL/min, and for most modern mass spectrometers, the pump speed is at least 300 L/sec. The development of flexible, fused silica capillary columns has made this approach routine. In fact, the only time a jet separator is now used is for a few applications that require packed or thick stationary phase GC columns.

In practice, most GC-MS interfacing is now done by simply inserting the capillary column directly into the ion source. The fused silica column runs through a 1/16-in.-diameter tube directly into the ion source. Other gases, such as methane for chemical ionization, are brought into the ion source by a T joint around the capillary column. One of the other two lines into the ion source is used for a thermocouple vacuum gauge tube so that the pressure in the ion source can be roughly measured. The remaining line into the ion source is for the delivery of the mass spectrometer calibration standard, perfluorotri butylamine. Most joints are welded together to avoid leaks when this inlet system is thermally cycled or vented. The only removable fitting is at the junction of the GC column and the far end of the inlet tube. Once the ferrules are on the GC column and it is in the ion source, it is desirable to cut off a few centimeters of the column, if possible. This eliminates the possibility of fine particles partially occluding the end of the column. If the end of the column cannot be placed directly in the ion source, the material in the GC-MS interface becomes important. The interface is held at 250 to 280 °C; thus, it should not include a reactive metal (such as copper). In some interfaces, glass-lined stainless steel tubing has been used, even though this tubing is difficult to bend properly. Following figure give detail idea about the GC-MS interface for fused silica capillary GC column.[7,8,9]

A typical GC-MS interface for fused silica capillary GC columns. The end of the GC column enters the ion source of the mass spectrometer



For capillary GC-MS, the best interface is no interface at all; run the flexible, fused silica GC column directly into the ion source. Using a column that is 25 to 30 m long by 220 to 250 µm inner diameter gives an ion source pressure of 10Ü to 10Ö torr, a more than acceptable pressure at which to obtain electron impact spectra. This gives helium or hydrogen GC carrier gas velocity of 25 to 35 cm/sec

or a flow of about 1 to 2 mL/min. The GC columns most widely used for GC-MS are those in which the stationary phase has been chemically bonded to the fused silica; DB-5 is a common trade name.

Occasionally, there have been problems with the plastic cladding on the outside of the GC column. This cladding is usually hot (typically 250 °C) and under vacuum. Thus, it may decompose, giving background ions in the mass spectrum or weakening the fused silica itself.[1,3,5]

3. Application of GC-MS in various drug compounds and other biological compounds analysis

When attempting to discover the important factors and then optimise by these factors, experimental design gives a powerful suite of statistical methodology. Design of experiment identify significant factors and then optimise a response with respect to them in method development. In a head space-solid-phase micro-extraction (HS-SPME) combined with gas chromatography tandem mass spectrometry (GC–MS) methodology for the simultaneous determination of six important organotin compounds namely monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT), monophenyltin (MPhT), diphenyltin (DPhT), triphenyltin (TPhT) has been optimized using a statistical design of experiments. The analytical method is based on the ethylation with NaBEt4 and simultaneous head space-solid-phase micro-extraction of the derivative compounds followed by GC–MS analysis. The main experimental parameters influencing the extraction efficiency selected for optimization were pre-incubation time, incubation temperature ,agitator speed, extraction time, desorption temperature, buffer (pH, concentration and volume), headspace volume, sample salinity, preparation of standards, ultrasonic time and desorption time in the injector. The main factors (excitation voltage, excitation time, ion source temperature, isolation time and electron energy) affecting the GC-IT-MS response were also optimized using the same statistical design of experiments. [10, 11]

Essential oils are produced by plants for many reasons including protection against various bacterial, fungal and viral infections. Numerous essential oils and their major constituents are known to exhibit promising antimicrobial activity and can therefore be a good source of biologically active molecules and or fractions. It is generally accepted that a crude phytomedicine needs to be evaluated holistically and there search method best suited for this approach is metabolomics. In this study an on targeted metabolomics approach was followed to explore the antimicrobial activity and chemistry of various commercial essential oils. The antimicrobial activity of the essential oils was determined against three Gram-positive and two Gram-negative bacterial organisms as well as two yeasts. The essential oil composition was determined by gas chromatography coupled with mass spectrometry (GC–MS) analyses.[14,15]

Gas chromatography coupled to single quadruple mass spectrometers (GC-SQ/MS) was used for the analysis of 35 multiclass pesticides. Pesticide contamination of foodstuffs has become a worldwide concern, prompting various levels of regulation and monitoring. Traditionally, pesticides are quantified with gas chromatography (GC) combined with selective detectors (ECD, FID, etc.). Selective GC detectors are great tools to quantify one or two pesticide classes at a time. However, screening for a number of different pesticides re- quires multiple runs utilizing various GC configurations. Chromatographic run times are often long because of the need to achieve sufficient chromatographic resolution for unambiguous quantification. Gas chromatography with mass spectrometry (GC-MS) provides positive confirmation of various pesticides in a single analytical run; its superior selectivity allows interference-free quantification even with peak co elution. As a result, GC-MS has become a preferred technique for pesticide analysis because of its single-run capability.[21]

A rapid and sensitive analytical method using gas chromatography-mass spectrometry (GC-MS) was developed for the measurement of neonicotinoid (NEO) metabolites 6-chloronicotinic acid (6CN), 2-chloro-1, 3-thiazole-5-carboxylic acid (2CTCA) and 3-furoic acid (3FA) from human urine. After acid hydrolysis, the metabolites were extracted using solid phase extraction (SPE) column (Bond Elute Plexa PCX) and eluted with methanol.N,O-bis (trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosi-lane (BSTFA-TMCS, 99:1) was used for the derivatization of metabolites and analyzed by GC-MS with the electron ionization mode. The elution solvent, derivatization reagent and its conditions were mainly optimized for improved detection and quantitation of the metabolites based on signal-to-noise ratio, recoveries and reproducibility**.** Also determination of the free organic acid content in human urine is also easy by using GC-MS to be used as a tool for the quantitative detection of metabolic or other health disorders.[18,19]

3.1 Environmental monitoring and clean-up

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.

3.2 Criminal forensics

GC-MS can analyse 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.

3.3 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.

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.

3.4 Security

A post–September 11 development, explosive detection systems have become a part of all US airports. These systems run on a host of technologies, many of them based on GC-MS. There are only three manufacturers certified by the FAA to provide these systems, [citation needed] one of which is Thermo Detection which produces the EGIS, a GC-MS-based line of explosives detectors. The other two manufacturers are Barringer Technologies, now owned by Smith's Detection Systems, and Ion Track Instruments, part of General Electric Infrastructure Security Systems.

3.5 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 contaminant from spoilage or adulteration which may be harmful and which is often controlled by governmental agencies, for example pesticides. Several GC-MS have left earth. Two were brought to Mars by the Viking program. Venera 11 and 12 and Pioneer Venus analysed the atmosphere of Venus with GC-MS.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.

3.6 Medicine

Dozens of congenital metabolic diseases also known as Inborn error of metabolism are now detectable by new born 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 new born for over 100 genetic metabolic disorders by a urine test at birth based on GC-MS.

In combination with isotopic labelling of metabolic compounds, the GC-MS is used for determining metabolic activity. Most applications are based on the use of 13C as the labelling 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.[1,7]

4. Limitations

Some problems with GC/MS originate in improper conditions in the GC portion of the analysis. If the GC instrument does not separate the specimen's compounds completely, the MS feed is impure. This usually results in background "noise" in the mass spectrum. If the carrier gas in the GC process is not correctly deflected from entering the MS instrument, similar contamination may occur.

Also, the MS portion suffers from the inexact practice of interpreting mass spectra. An analyst must correlate computer calculations with system conditions. The typical memory bank for MS identification contains about 5000 spectra for a particular group of compounds. Even if a competent analyst could find conclusive results pointing to one substance out of 5000 substances, this does not rule out the remaining over 200,000 known existing chemicals. For the 5000-spectra memory bank, the typical computer result is limited to as many as six possible identifications. In one instance, erroneous GC/MS results may have been responsible for a criminal defendant receiving a death sentence. John Brown killed a police officer and wounded two bar patrons in a shoot-out on June 7, 1980 in Garden Grove, California. Mr. Brown's diminished capacity defence to capital murder relied on the assertion that Mr. Brown was under the influence of narcotics at the time of the shooting. The prosecution introduced GC/MS evidence that showed Mr. Brown's blood to be free of narcotics. The California Supreme Court overturned the jury's death sentence because the prosecution never introduced evidence from a radioactive immunoassay ("RIA") test that detected phencyclidine (PCP) in Mr. Brown's blood. Obviously, an example like this demonstrates that analytical evidence, including GC/MS, should always be confirmed with another reliable technique. Another limitation of GC-MS is only compounds which having vapour pressures more than 10 torr can be analysed by gas chromatography - mass spectrometry (GC-MS). If compounds having lower pressures then it can be analysed by chemically derivatization (for example, as trimethylsilyl ethers). In some cases determining positional substitution on aromatic rings is often difficult. Also certain isomeric compounds cannot be distinguished by mass spectrometry (for example, naphthalene versus azulene), but they can often be separated chromatographically. Quantitatively accuracy of the instrumentation is needed to be maintained by calibration. [7]

5. Conclusion

GC and MS are useful tools for chemical analysis, especially when used together. An attorney can present an effective attack or defence 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.

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