GC/MS Evidence: *Attacking and Defending Chemical Compound Identification For Legal Practitioners*

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Introduction

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 defense of GC/MS evidence. To effectively use GC/MS evidence one must understand the process. First, the GC process will be considered, then the MS instrument will be presented. After a background in GC and MS is obtained, the reader will learn how to analyze the evidence produced by these instruments. The focus of this article lies in presenting the limitations to GC/MS analysis.

Gas Chromatography

GC analysis is a common confirmation test.[1] Among its uses are drug testing and environmental contaminant

identification. GC analysis separates all of the components in a sample and provides a representative spectral output.[2] The technician injects the sample into the injection port of the GC device.[3] The GC instrument vaporizes the sample and then separates and analyzes the various components.[4] Each component ideally produces a specific spectral peak that may be recorded on a paper chart or electronically.[5] The time elapsed between injection and elution is called the "retention time." The retention time can help to differentiate between some compounds. The size of the peaks is proportional to the quantity of the corresponding substances in the specimen analyzed. The peak is measured from the baseline to the tip of the peak.

Imagine a pile of different types of balls resting at the bottom of an inclined, paved driveway. This pile includes ball bearings, marbles, ping pong balls, golf balls, wiffle balls, handballs, tennis balls, hockey pucks, baseballs, soccer balls, volley balls, basketballs, footballs, and bowling balls. Attempt to move this motley collection of balls up the driveway with a normal leafblower. Some of the pile will quickly move to the top of the driveway immediately, some balls will migrate at varying speeds, and some balls may take an eternity to reach the end of the driveway.

The difference in the time that each type of ball takes to travel to the top depends upon the characteristics of each ball. Obviously, the lighter balls travel more quickly. Also, some balls may take longer due to their shape, like the hockey puck or the football. The different balls interact with each other as the air from the leaf blower acts on the pile. This interaction may hinder or accelerate the ball's travel as the balls strike each other. The surface characteristics of the ball may be important, as in the examples of the tennis ball and golf ball.

GC analysis depends on similar phenomena to separate chemical substances. A mixture of chemicals present in a specimen can be separated in the GC column. Some chemical and physical characteristics of the molecules cause them to travel through the column at different speeds. If the molecule has low mass it may travel more swiftly. Also, the molecule's shape may affect the time needed to exit the column. How the different substances relate to each other may cause the time needed to travel the column to increase or decrease. Interactions between the sample's molecule and the column surface may cause the molecule to be retained inside the column for a different amount of time than similar molecules that interact with the column differently.

Description of Process

The equipment used for gas chromatography generally consists of an injection port at one end of a metal column packed with substrate material and a detector at the other end of the column.[6]

A carrier gas propels the sample down the column.[7] The technician uses flow meters and pressure gauges to maintain a constant gas flow.[8] A gas that does not react with the sample or column is essential for reliable results.[9] For this reason, carrier gases are usually argon, helium, hydrogen, nitrogen, or hydrogen. Many analysts use helium because it does not react.[10] Hydrogen usually is a good carrier gas but it may react and convert the sample into another substance.[11] The ultimate choice for a carrier gas may depend on the type of detector used.[12]

To ensure proper separation, the sample must enter the column in a discreet, compact packet.[13] Normally the sample is injected into the injection port with a hypodermic needle and syringe capable of measuring the specimen amount.[14] The needle is stuck into a replaceable neoprene or silicone rubber septum that covers the injection port.[15] The injection port is maintained at a temperature at which the sample vaporizes immediately.[16] Ideally, the sample spreads evenly along the cross section of the column, forming a plug.[17]

The column is a metal tube, often packed with a sand-like material to promote maximum separation.[18] Columns are commonly obtained pre-packed by vendors.[19] As the sample moves through the column, the different molecular characteristics determine how each substance in the sample interacts with the column surface and packing. The column allows the various substances to partition themselves.[20]

Substances that do not like to stick to the column or packing move through the column rapidly.[21] Substances that do not like to stick to the column or packing are impeded but eventually elute from the column.[22] Ideally, the various components in the sample separate before eluting from the column end.[23]

The GC instrument uses a detector to measure the different compounds as they emerge from the column.[24] Among the available detectors are the argon ionization detector, flame ionization detector, flame emission detector, cross section detector, thermal conductivity detector, and the electron capture detector.[25] Choosing the proper detector depends upon the use.[26] Some considerations are that the flame detectors destroy the sample, the thermal conductivity detector is universally sensitive, and the argon ionization detector requires argon as a carrier gas.[27] The spectral output is usually stored electronically and displayed on a monitor. The technician can produce a hard copy record.

The argon ionization detector does not detect water, carbon tetrachloride, nitrogen, oxygen, carbon dioxide, carbon monoxide, ethane, or compounds containing fluorine.[28] The flame ionization detector does not respond to water, nitrogen, oxygen, carbon dioxide, carbon monoxide, helium, or argon.[29] If a specimen contains water, a flame ionization detector should be used.[30] The electron capture detector can not detect simple hydrocarbons but does detect compounds containing halides, nitrogen, or phosphorus.[31]

Retention Time

The amount of time that a compound is retained in the GC column is known as the retention time. The technician should measure retention time from the sample injection until the compound elutes from the column. The retention time can aid in differentiating between some compounds. However, retention time is not a reliable factor to determine the identity of a compound.[32] If two samples do not have equal retention times, those samples are not the same substance.[33] However, identical retention times for two samples only indicate a possibility that the samples are the same substance.[34] Potentially thousands of chemicals may have the same retention time, peak shape, and detector response.[35] For example, under certain conditions, DDT has the same retention time as PCBs (polychlorinated biphenyls).[36] Some believe that environmental testing

showed erroneously high amounts of DDT.[37] GC instruments showed only one peak for what is believed to be a mixture of DDT and PCBs.[38] This experimental data led to the banning of DDT in the U.S.[39] Bluntly, GC is "(O)ne of the quickest ways of getting the wrong answer in qualitative analysis."[40]

Quality Assurance/Quality Control Procedures

Before analyzing a sample, the technician should tune and calibrate the instrument.[41] Tuning can be accomplished using specific concentrations of Decafluorotriphenylphosphine and p-Bromofluorobenzene.[42] A technician can process a spiked sample (containing a known concentration of a substance) to check calibration and tuning.[43] If the GC/MS instrument does not detect the substance or shows a greater or lesser concentration than the known concentration, the technician must recalibrate the instrument.[44] Also, the technician can use a blank sample (containing no detectable compounds) to test the GC/MS instrument's data reporting accuracy.[45] If the device indicates the presence of a substance in the blank sample, the device may contain residue from prior analysis.[46] If this occurs, the technician must retune and recalibrate the GC/MS instrument.[47]

Proper scientific practice requires that the GC technician compare the spectral output with a known standard sample of the suspected substance.[48] The standard sample

must be analyzed with the same instrument, under the same conditions, immediately before and immediately after analyzing the unknown specimen.[49] If the resulting three spectral outputs do not agree, the technician can not make a reliable identification of the specimen based on the GC analysis.[50]

Analysis of Output

Less than ideal spectral peaks may indicate less than ideal analytical procedures or equipment. The technician can readily observe whether the output exhibits unsatisfactory results. Ideally, the spectral peaks should be symmetrical, narrow, separate (not overlapping), and made with smooth lines. GC evidence may be suspect if the peaks are broad, overlapping, or unevenly formed. If a poorly shaped peak contains a steep front and a long, drawn-out tail, this may indicate traces of water in the specimen.[51]

The GC technician should inject the specimen into the septum rapidly and smoothly to attain good separation of the components in a specimen. If the technician injects the specimen too slowly, the peak may be broad or overlap. A twin peak may result from the technician hesitating during the injection. A smoothly performed injection, without abrupt changes, should result in a smoothly formed peak. A twin peak may also indicate that the technician injected two specimens consecutively.

Limitations

Response Factor

The size of a spectral peak is proportional to the amount of the substance that reaches the detector in the GC instrument.[52] No detector responds equally to different compounds.[53] Results using one detector will probably differ from results obtained using another detector.[54] Therefore, comparing analytical results to tabulated experimental data using a different detector does not provide a reliable identification of the specimen.

A "response factor" must be calculated for each substance with a particular detector.[55] A response factor is obtained experimentally by analyzing a known quantity of the substance into the GC instrument and measuring the area of the relevant peak.[56] The experimental conditions (temperature, pressure, carrier gas flow rate) must be identical to those used to analyze the specimen.[57] The response factor equals the area of the spectral peak divided by the weight or volume of the substance injected.[58] If the technician applies the proper technique, of running a standard sample before and after running the specimen, determining a response factor is not necessary.

Worn Septum

An injection port septum should last between 100 and 200 injections.[59] Higher injection port temperatures shorten the septum's lifespan.[60] A leaking septum adversely affects the GC instrument's sensitivity.[61]

If a portion of the specimen leaks back out of the septum, the amount of the specimen is not recorded. This event makes any eventual quantitative result erroneous. If air should leak into the injection port through a worn septum, the oxygen and water contained in air may skew the results. Any oxygen may react with the specimen components. If this happens, the GC instrument will provide results indicating the presence of this unintended reaction product, instead of the original compounds present in the specimen vial. Any water in the column adversely affects the GC instrument's ability to separate components.[62]

Injection Port Temperature

The temperature of the GC injection port must be high enough to vaporize a liquid specimen instantaneously.[63] If the temperature is too low, separation is poor and broad spectral peaks should result or no peak develops at all.[64] If the injection temperature is too high, the specimen may decompose or change its structure. If this occurs, the GC results will indicate the presence of compounds that were not in the original specimen.

Residual Impurities

Ideally, all components of a specimen elute completely from the GC column. If any substance remains inside the column, the substance may elute during subsequent analyses with other specimens. This may result in an unexpected peak in the output. The peak produced should be broad.

Carrier Gas

If the GC instrument uses hydrogen for the carrier gas, the technician must consider whether the hydrogen may react with any of the compounds present in the specimen. If the hydrogen does react, a broad peak will result. When using a thermal conductivity detector, care should be taken as a false peak may occur if the carrier gas's thermal conductivity is in the range of the thermal conductivity of any compound in the specimen. An unstable carrier gas flow rate may produce a drifting baseline and false broad peaks. A carrier gas should be pure. Regular changing of the gas filter should prevent significant impurities.

Crucial Factors

GC analysis is highly reliable if the instrument is properly maintained, the technician follows proper procedures, and the interpretation of the results is competent. While some factors rarely affect GC analysis, some factors are absolutely essential for the use of reliable GC evidence. In all cases a technician must

process a standard sample containing a verified composition identical to the presumed contents of the collected specimen. This standard sample must be processed before and after the collected specimen under identical conditions. Any output from the collected specimen that does not match the standard sample is inconclusive. If tabulated reference data exists for the relevant conditions, the specimen data must match the reference data.

If advance notice of GC testing is available, an adverse party should observe the procedure. If a retained consultant or the knowledgeable attorney observes the technician's use of the GC instrument, important information can be recorded. The technician's preparation of the specimen and the subsequent injection can be observed for errors or malfunctioning equipment. The observer should record the instrument's make, model, serial number, injection temperature, column temperature, carrier gas flow rates and pressure, identify the type of detector used, and observe any manipulation of the data by use of a computer.

Ensure that the technician properly starts measuring the time at injection and records the time of elution. Any discrepancy in the time will produce an erroneous retention time. If the procedure can not be observed, the adverse party should seek all pertinent information (experimental

conditions, measurements, instrument identification) and hard copy output.

Mass Spectrometry (MS)

MS analysis is commonly used in arson investigations, engine exhaust analysis, petroleum product analysis, and for blood monitoring in surgery. MS identifies substances by electrically charging the specimen molecules, accelerating them through a magnetic field, breaking the molecules into charged fragments and detecting the different charges. A spectral plot displays the mass of each fragment. A technician can use a compound's mass spectrum for qualitative identification.[65] The technician uses these fragment masses as puzzle pieces to piece together the mass of the original molecule, the "parent mass."

The parent mass is analogous to the picture on top of a puzzle box, a guide to the end result obtained by putting together the fragment masses, or puzzle pieces. From the molecular mass and the mass of the fragments, reference data is compared to determine the identity of the specimen. Each substance's mass spectrum is unique. Providing that the interpretation of the output correctly determines the parent mass, MS identification is conclusive.

Description of Process

Today many different types of MS instruments exist, each one using a different apparatus and process for producing mass spectra.[66] This article's description of the MS process will limit itself to a basic description of a conventional large magnet mass spectrometer. Such a MS instrument contains a sample inlet, an ionization source, a molecule accelerator, and a detector.[67]

MS analysis requires a pure gaseous sample.[68] The sample inlet is maintained at a high temperature, up to 400° C (752° F), to ensure that the sample stays a gas.[69] Next the specimen enters the ionization chamber.[70] A beam of electrons is accelerated with a high voltage.[71] The specimen molecules are shattered into well-defined fragments upon collision with the high voltage electrons.[72] Each fragment is charged and travels to the accelerator as an individual particle.[73]

In the acceleration chamber the charged particle's velocity increases due to the influence of an accelerating voltage.[74] For one value of voltage only one mass accelerates sufficiently to reach the detector.[75] The accelerating voltage varies to cover a range of masses so that all fragments reach the detector.[76]

The charged particles travel in a curved path towards the detector.[77] When an individual charged particle collides with the detector surface, several electrons (also

charged particles) emit from the detector surface.[78] Next, these electrons accelerate towards a second surface, generating more electrons, which bombard another surface. Each electron carries a charge.[79] Eventually, multiple collisions with multiple surfaces generate thousands of electrons which emit from the last surface.[80] The result is an amplification of the original charge through a cascade of electrons arriving at the collector.[81] At this point the instrument measures the charge and records the fragment mass as the mass is proportional to the detected charge.[82]

The MS instrument produces the output by drawing a array of peaks on a chart, the "mass spectrum."[83] Each peak represents a value for a fragment mass.[84] A peak's height increases with the number of fragments detected with one particular mass.[85] As in the case of the GC detectors, a peak may differ in height with the sensitivity of the detector used.[86]

Analysis of Output

Each substance has a characteristic mass spectrum under particular controlled conditions.[87] A technician can identify a specimen by comparing the specimen's mass spectrum with known compounds.[88] Quantitative analysis is possible by measuring the relative intensities of the mass spectra.[89]

Usually a mass spectrum will display a peak for the unfragmented molecule of the specimen.[90] This is commonly the greatest mass detected, called the "parent mass."[91] Like the picture on a puzzle box, the parent mass is used to fit the pieces together from the other peaks in the mass spectrum. The parent mass reveals the mass of the molecule while the other peaks indicate the molecule's structure.[92]

Determining the parent peak and consequently the molecular mass of the specimen is the most difficult part of MS analysis.[93] Identifying the parent mass is outside the scope of this article.[94] Assuming that a technician can correctly determine the molecular mass, the technician makes an educated guess of the specimen's identity and compares the mass spectrum to reference spectra for confirmation.[95] The mass spectra for larger molecules containing carbon are complicated and require tedious calculations that are subject to error.[96] Computers are commonly used for spectral analysis.[97]

Limitations

Resolution

The "resolution" is a value that represents the instrument's ability to distinguish two particles of

different masses.[98] The greater the MS instrument's resolution, the greater its usefulness for analysis.[99] An MS instrument provides more accurate results for larger molecules when the instrument has a high resolution.[100] A high resolution MS instrument is advisable for analyzing body fluids because they have high molecular masses. A low resolution MS instrument may not sufficiently characterize a large mass substance.

Pressure

If the interior pressure in an MS instrument is too high, erroneous results may occur. As the specimen molecule breaks up, the fragments accelerate. If a fragment collides with another fragment, then these two fragments may combine to make a new particle.[101] In this event, the detector will register the mass of this new particle on the mass spectrum. The reference spectra for comparison are produced under low pressure conditions which minimize collisions between fragments. A technician would find a spectral peak where one is not expected. In the puzzle analogy, this is similar to finding pieces from a different puzzle in your box and trying to make these extraneous pieces fit. As this is impossible, any MS analysis under high pressure conditions would depend greatly on guesswork by the technician.

Parent Mass

Finding the correct parent peak in the mass spectra may be difficult. Finding the parent peak helps to determine the parent mass, which should lead to determining the specimen's molecular mass.[102] For high molecular mass compounds, like drugs and body fluids, a parent peak is often not observed. This makes qualitative identification difficult. A special type of MS, chemical ionization MS, reduces the likelihood of missing the parent mass.[103]

High Speed Scanning

High speed scanning MS instruments are able to rapidly analyze specimens.[104] However, the increased speed is a tradeoff for decreased resolution.[105] Quantitative measurements are unreliable with high speed scanning.[106]

Technician's Skills

As in the puzzle analogy, knowing the shape of a piece of the molecule helps to join the pieces together. To determine the specimen's molecular structure before fragmentation, the technician needs to employ skill and art to determine the molecular structure from mass spectra patterns.[107] Computers and databases can assist, but a human expert is necessary to distinguish between likely and unlikely answers.[108] Alone, a computer can not determine molecular structures as well as a competent human.[109] This causes the weight of MS evidence to depend greatly on the

technician's qualifications and proficiency with MS spectrum analysis.

Crucial Factors

MS analysis is highly reliable if the instrument is of sufficient resolution and the technician's interpretation of the results is competent. While some factors rarely affect MS analysis, some factors are absolutely essential for the use of reliable MS evidence. In all cases a technician must process a standard sample containing a verified composition identical to the presumed contents of the collected specimen. This standard sample must be processed under identical conditions, both before and after processing the collected specimen . Any identification based on output from the collected specimen that does not match the standard sample is inconclusive.

Because MS is highly sensitive, care should be taken that not even the slightest trace of a previous sample remain within the MS instrument.[110] The technician should run a "background spectrum," an analysis without a specimen, before analyzing the specimen in question.[111] This practice is the only way that an independent analyst can definitely interpret MS output.[112]

If tabulated reference data exists for the relevant conditions, the specimen data must match the reference data. Despite the use of sophisticated instruments, computers, and

proficient personnel, there is always some doubt in conclusions based on interpretation of mass spectra.[113] One example is the pair of narcotic compounds, N-methyl-3-piperidylbenzilate and N-methyl-4-piperidylbenzilate.[114] The compounds have the same molecular mass but differ in the position of one molecular group.[115] In some instances, two molecules that only differ in structure may be separated with the proper instrument and technique.[116]

If advance notice of MS testing is available, an adverse party should observe the procedure. If a retained consultant or the knowledgeable attorney observes the technician's use of the MS instrument, important information can be recorded. The observer should record the instrument's make, model, serial number, resolution, pressure, and identify the type of detector used.

It is important to observe and record which possible compounds the computerized database produced. As the technician uses personal judgment to rule out these other compounds, an adverse attorney should consider the likelihood that one of the other contending identifications may be the proper choice. If the procedure can not be observed, the adverse party should seek all pertinent information (experimental conditions, measurements, instrument identification) and hard copy output.

In all instances, hard copy data is essential. The mass spectra should include the scale of mass units reported.[117] This enables an independent analyst to check whether the specimen in question contains major molecular fragments reported in the literature.[118] The mass spectra should also include the MS pressure and the accelerating voltage.[119] An independent analyst can use the operating conditions to resolve whether discrepancies in the mass spectrum arise from misidentification or from instrumental malfunction.[120] Providing a properly labeled printing of the mass spectra is easy, not time-consuming, and of minimal cost.[121]

GC/MS Combination

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 can not 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.[122]

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.

Limitations

Although many consider GC/MS to be the "gold standard" in scientific analysis, GC/MS does have some limitations. Because great faith is maintained in GC/MS analysis, erroneous results are not expected and hard to dispute. However, false positives and false negatives are possible.

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.[123] 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.[124] For the 5000-spectra memory bank, the typical computer result is limited to as many as six possible identifications.[125]

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.[126] Mr. Brown's diminished capacity defense to capital murder relied on the assertion that Mr. Brown was under the influence of narcotics at the time of the shooting.[127] The prosecution introduced GC/MS evidence that showed Mr. Brown's blood to be free of narcotics.[128] 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.[129] Obviously, an example like this demonstrates that analytical evidence, including GC/MS, should always be confirmed with another reliable technique.

A more advanced analytical method is MS/MS, a tandem series of instruments, which has the advantage of increased sensitivity.[130] One court states that MS/MS analysis has never produced a false positive in the FBI laboratory.[131] However, MS/MS is not widely used yet as the instrument's cost is prohibitive.[132]

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.

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