

Gas Chromatography – Mass Spectrometry (GC–MS)*

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*Invited by Tom Bruno who is coordinating for publication in the ASM International Handbook,
Volume 10: Materials Characterization*

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Introduction

The previous article in this volume, “Gas Chromatography (GC),” discusses GC fundamentals as well as common GC detectors: the flame ionization detector (FID), thermal conductivity cell detector (TCD), electron capture detector (ECD), and sulphur chemiluminescence detector (SCD). Another common GC detector is the mass spectrometer. Mass spectrometry (MS) is a technique that is used to elucidate molecular mass and molecular structure for compound identification and/or quantification. While MS can be done without GC separation, interpretation of the data becomes increasingly more difficult when analyzing mixtures. Since virtually all materials we encounter in life are mixtures, not pure compounds, MS becomes a much more powerful technique when coupled with the separation capabilities of GC. GC-MS is a technique which is more complex than the other GC techniques and requires a detailed article of its own.

GC-MS is often used to qualitatively and quantitatively determine organic compound purity and stability and to identify components in a mixture. GC-MS is commonly used in many disparate fields, including environmental chemistry for atmospheric, soil, and water research; forensic science for detection of drugs of abuse (or metabolites) and in arson fire debris analysis; food science for determination of food or beverage quality and authenticity; and in developing renewable fuels.

This article will first explain the principles of mass spectrometry in terms of the most basic required instrumentation and the way a measurement is made, followed by an explanation of the mass spectrum, which is the data resulting from an MS measurement. Mass spectra contain a great deal of information that can be complicated to interpret. There are a variety of different types of mass spectrometers that are then briefly discussed, culminating in a section about recent advances in MS technology.

GC-MS Principles and Instrumentation

A GC-MS experiment begins with sample preparation, injection and separation on a GC column (as discussed in the previous article). Since the operation of a mass spectrometer requires a high-vacuum system, an interface is necessary to direct the molecules from the GC to the mass spectrometer. In the most common type of instrument, the molecules leaving the column enter an ionization chamber where they are bombarded with a stream of energetic electrons, which ionizes and fragments some of the molecules. The ions formed can include the molecular ion (unfragmented) and ions due to fragmentation or rearrangement reactions. The ions are accelerated and rapidly sorted according to the mass to charge ratio (m/z , where m is the mass and z is the charge) in a mass analyzer by use of a magnetic or electric field. The mass analyzer can sort thousands of different ion masses (m/z) per second. Ion abundance is then counted by a detector by measuring the current of electrons generated when the ions strike the detector for each m/z . MS serves as the detector for GC which generates a chromatogram indicating the quantity of each compound as a function of retention time. The underlying dimension of data specific to MS is called a mass spectrum, which is a histogram of the abundance of each ion as a function of m/z and serves as the fingerprint to identify the compound represented by a peak on the chromatogram.[1-3]

GC-MS Interface:

The vapor stream that exits a gas chromatograph is under pressure, sometimes over 5 times greater than atmospheric pressure. The mass spectrometer analyzer operates at a high vacuum to facilitate the ions traveling through the analyzer. High vacuum is achieved by use of a fore pump and a turbomolecular pump. For typical conditions (capillary column, 1-2 mL/min flow rate, and an internal diameter of 250-320 μm) the turbomolecular pump can handle the gas flow out of the GC. In this case the GC-MS interface is a heated metal tube. For larger columns or higher flow rates, or both, some of the vapor flow must be separated by another pump that draws most of the gas out of the interface tube prior to the sample and carrier gas entering the mass spectrometer. Care must be taken to ensure the analyte molecules do not condense or decompose in the GC-MS interface tube. The interface temperature is usually set to 10 $^{\circ}\text{C}$ above the final oven temperature.

GC-MS Ionization Techniques:

The gas exiting the GC enters an ionization chamber where the carrier gas and analytes are bombarded with high energy electrons. The most common method is electron impact or electron ionization (EI), a hard ionization technique (that is, there is an excess of energy applied to the analytes beyond what is required for ionization, thus causing ion fragmentation). In this technique the high-energy electron beam is created by applying a voltage to a heated filament. The neutral molecules that enter the ionization chamber are bombarded by the high-energy electron beam, which removes valence shell electrons, converting some of the molecules to molecular ions. Here, radical cations, $\text{M}^{\cdot+}$ (abbreviated hereafter as M^+), are formed, where M is the analyte molecule, the + sign indicates that the ions have a positive charge, and the dot symbolizes the unpaired electron. The ionized molecules may include the molecular ion; however, the molecular ion may also have sufficient energy to undergo rearrangement, bond cleavage, or fragmentation into mass fragment ions (product ions). Most of the sample, as well as the other products formed during electron ionization, is removed by the vacuum system.[4]

Typical operation of EI MS uses a beam of electrons with a potential of 70 eV. This has been established as the energy required to efficiently create ions in a reproducible way for organic molecules with sizes < 1000 atomic mass units (amu or Daltons). An example of the ions produced from EI MS of ethanol (CAS registry number 64-17-5) is presented in Table 1, which was adapted from Raphaelian's previously published edition of this article.[5] When universal EI GC-MS conditions are used, mass spectral databases can be utilized to identify the compound from inspection of the mass spectrum.[6, 7]

Table 1. The molecular or parent ion and characteristic fragment ions produced during electron ionization MS of ethanol; adapted from Raphaelian.[5]

Molecular/Parent Ion	$\text{CH}_3\text{CH}_2\text{OH}$	$\xrightarrow{-e}$	$(\text{CH}_3\text{CH}_2\text{OH})^+$	$m/z = 46$
Fragment Ion	$\text{CH}_3\text{CH}_2\text{OH}$	$\xrightarrow{-\text{H}}$	$(\text{CH}_3\text{CH}_2\text{O})^+$	$m/z = 45$

Fragment Ion	CH ₃ CH ₂ OH	$\xrightarrow{-H}$	(CH ₂ CH ₂ OH) ⁺	m/z = 45
Fragment Ion	CH ₃ CH ₂ OH	$\xrightarrow{-CH_3}$	(CH ₂ OH) ⁺	m/z = 31
Fragment Ion	CH ₃ CH ₂ OH	$\xrightarrow{-OH}$	(CH ₃ CH ₂) ⁺	m/z = 29
Fragment Ion	CH ₃ CH ₂ OH	$\xrightarrow{-CH_2OH}$	(CH ₃) ⁺	m/z = 15

Chemical ionization (CI) is a common soft ionization technique.[2] This technique produces an adduct ion (MH⁺) where a proton (H⁺) is added to the molecule. The main difference between these ionization techniques is in the amount of energy that is transferred to the molecule and the type of ions generated. There is typically not enough energy to fragment the molecular ions, thus increasing the strength of signal produced by these ions. CI and EI are often complementary techniques. EI is the most commonly used ionization technique for GC-MS, thus, for the remainder of this article, only EI MS will be discussed.[2-4]

Mass Analyzers:

The direction and velocity of the positively charged ions are controlled by a repeller and accelerator plates that steer the ions out of the ionization chamber. Single focusing, magnetic-sector mass spectrometers utilize a magnetic field to focus the trajectory of ions of the same m/z value on the analyzer exit slit.[2] The velocity and trajectory of the ions is controlled by continuously varying the accelerating voltage and the magnetic field. The interested reader is referred to Ref. 2 for more details on magnetic sector mass analyzers. Unionized molecules are removed by vacuum pumps and molecules with negative charges are drawn to the repeller plate.[3]

The most commonly utilized mass analyzer is the quadrupole mass filter. The quadrupole consists of 4 solid, circular or hyperbolic in cross-section, rods aligned equidistant and parallel to the ion path. The quadrupole uses electric fields to separate the ions by their m/z instead of the magnetic fields used in the magnetic-sector mass spectrometer. Separation is achieved by application of a combination of radio frequency (RF) and direct-current (DC) voltages to each rod with opposite polarities on the pairs of opposing rods.[3, 5] The field strengths are varied to allow ions with stable oscillations to continue to the detector and ions with unstable oscillations to strike a rod, become neutral and be pumped out of the analyzer by the vacuum system.[2]

Mass analyzers have also been built based on separation of ions due to their time-of-flight (ToF) or flight time. Here, ions of similar initial kinetic energy travel down a long field-free flight tube. The smaller ions travel faster and reach the detector before the heavier (slower) ions. ToF is simple and fast (more than 100 spectra per second), and is theoretically boundless in mass range. [5, 8]

Mass analyzer performance is often characterized by mass resolution, Δm . The IUPAC definition states that this is the smallest mass difference between two equal magnitude peaks so that the valley between them is a specified fraction of the peak height. Two common specifications are for the 10 % valley definition or the 50 % full peak width at half maximum (FWHM) definition. Quadrupole mass analyzers are well-known for their unit mass resolution that is constant across the entire mass range. Magnetic sector and ToF mass analyzers are different than the previous mass analyzers. Their mass resolution is not constant across the entire mass range; it is smaller for smaller masses. To describe the performance of these mass analyzers, the resolving power, R , is defined as $m/\Delta m$.

Interpreting Mass Spectra

The Mass Spectrum:

The mass spectrometer acquires a mass spectrum and displays this data as a histogram of the abundance of the ions that reach the detector according to their mass to charge ratio (m/z); the spectrum is often plotted on a relative abundance scale. An example EI MS spectrum is presented in Figure 1. All peak intensities are normalized to the highest peak, in this case the $m/z = 31$ peak. Peaks for the molecular/parent ion ($m/z = 46$) and the fragmentation ions (also listed in Table 1) $m/z = 45, 31, 29$ and 15 are all present.[5, 9]

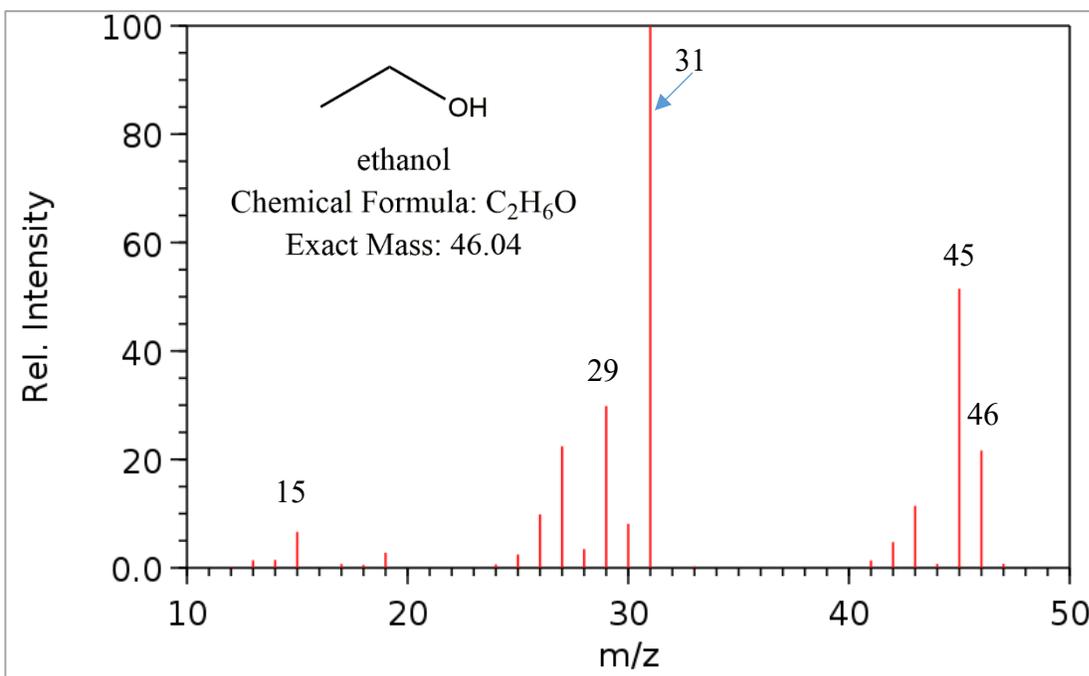


Figure 1: The EI MS spectrum for ethanol. Ions (m/z) are presented as a function of relative (Rel.) intensity normalized to the most abundant $m/z = 31$. The molecular ion at $m/z = 46$ is also observed.[9]

In some cases, the structure of an unknown compound can be deduced from the molecular ion and fragmentation ions. In other cases, known analytical standards or comparisons of mass spectra with compounds found in library databases can be used. As of 2018, the National Institute of Standards and Technology/Environmental Protection Agency/National Institute of Health (NIST/EPA/NIH) Mass Spectral Library has EI MS spectra for 267376 chemical compounds, with new species constantly being added.[7]

Isotope Abundance:

In addition to the molecular ion (M^+) and the fragmentation ions, another useful tool for structural identification is the isotopic abundance of select ions. For example, in addition to the molecular ion, a small quantity of a species one mass unit larger, written as $(M+1)^+$ is often observed. In the case of the carbon, which is predominately ^{12}C , there is a small natural abundance of ^{13}C atoms. In fact, approximately 1.1 % of all carbon atoms in an organic molecule are ^{13}C . Figure 2 shows the EI MS spectrum for 1,2,3,4-tetrahydro naphthalene (CAS registry number 119-64-2) with the molecular formula $\text{C}_{10}\text{H}_{12}$. This molecule contains approximately 11 % ^{13}C atoms. The result of this in terms of the ion abundance is that the abundance of $[M+1]^+$ ($m/z = 133$) relative to $[M]^+$ ($m/z = 132$) should be approximately 11 %.

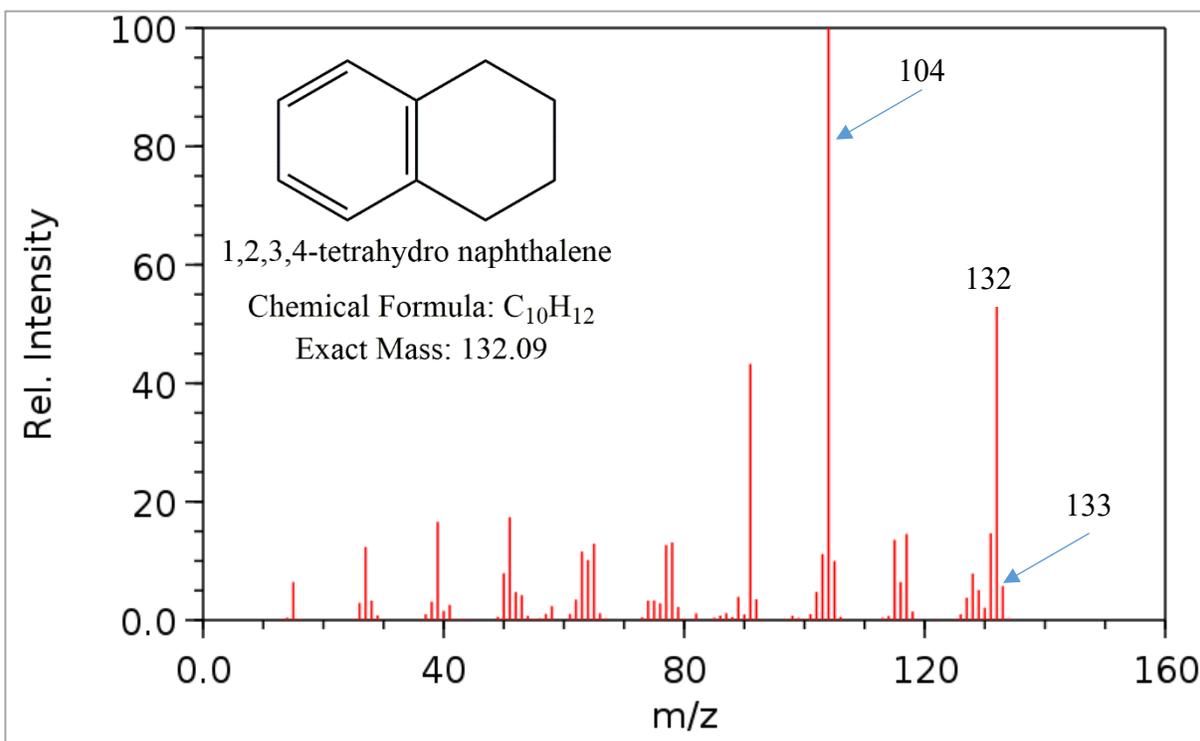


Figure 2: The EI MS spectrum for 1,2,3,4-tetrahydro naphthalene (tetralin). The ions (m/z) are presented as a function of relative (rel.) intensity normalized to the most abundant at $m/z = 104$. The molecular ion at $m/z = 132$ is observed and the molecular ion containing one ^{13}C atom is observed at $m/z = 133$. [9]

Carbon is only one of several elements with a significant natural abundance of multiple isotopes. Isotopic abundances of other common elements are presented in Table 2. Oxygen, for example, has an isotope two mass units larger than the predominate ^{16}O ; the presence of an ^{18}O isotope in a molecule makes it a (M+2) species. The isotopic contributions of the halogens chlorine and bromine (also (M+2) species) are also quite unique and the ratios can be used to determine the number of each element in an unknown. These patterns have historically been useful for identification of polychlorinated biphenyls (PCBs), pesticides, refrigerants, and other commonly halogenated species.

Table 2: Relative natural isotopic abundances of common elements; adapted from Raphaelian and Hubschmann.[5, 8]

Element	$[\text{M}]^+$		$[\text{M}+1]^+$		$[\text{M}+2]^+$	
	Mass	%	Mass	%	Mass	%
H	1	99.98	2	0.015		
B	10	19.7	11	80.22		
C	12	98.89	13	1.11		
N	14	99.63	15	0.37		
O	16	99.76	17	0.04	18	0.2
F	19	100				
Mg	24	78.7	25	10.13	26	11.17
Si	28	92.21	29	4.7	30	3.09
P	31	100				
S	32	95.02	33	0.76	34	4.22
Cl	35	75.53			37	24.47
Br	79	50.5			81	49.5
I	127	100				

A prevalent chemical identification need at the current time of this article that relies on accurate measurement of isotopic ratios is the proper identification of pesticides. There are many pesticide compounds that are banned from use under all or select applications. To determine if a compound has been illegally applied, analysis of soil samples by GC-MS (and GC-MS/MS, which is discussed later) is common practice, in addition to identification with known standards with an electron capture detector. See the previous article GC for more info. For example, the banned pesticide chlordane (CAS registry number 57-74-9) has the formula $\text{C}_{10}\text{H}_6\text{Cl}_8$, and its mass spectrum is shown in Figure 3. For many banned substances, their ion fragmentation patterns have been previously documented in searchable databases. For more information on

how to identify halogenated species by their isotopic ratios, the reader is referred to a more comprehensive analysis by McLafferty.[2]

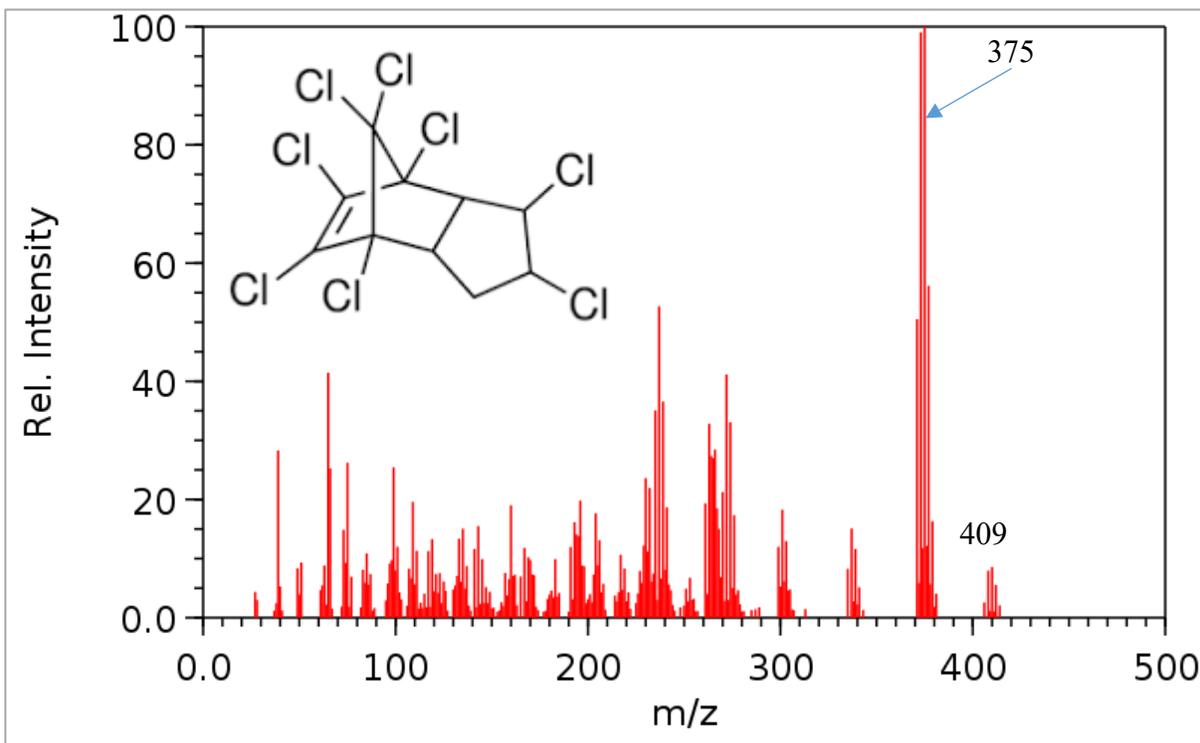


Figure 3: The EI MS spectrum for chlordane. The ions (m/z) are presented as a function of relative (rel.) intensity normalized to the most abundant at $m/z = 375$. The molecular ion at $m/z = 409$ is observed.[9]

GC-MS Methodology

Sample Preparation:

Sample preparation is necessary to optimize analyte concentration to remove compounds that can negatively affect the GC-MS instrument or interfere with analyte detection, and. Sample preparation depends on the parameters selected for the GC-MS instrument, column phase and dimensions, sample composition, and detector limitations. Some sample preparations are done in-line, such as headspace or thermal desorption. Samples can also be diluted in an appropriate solvent. The solvent purity is important, and the solvent must dissolve and not co-elute with the analytes of interest. Hubschmann provides an excellent review and detailed discussion of sample preparation techniques.[8]

Operational Considerations:

For injection of the prepared sample onto the column, the injector can be cooled or heated to optimize the vaporization of the entire sample. For a GC-MS instrument equipped with a

split/splitless injector, the vapor volume injected onto the capillary column is controlled by operating the inlet in either splitless or split mode. High-purity GC carrier gas, free from oxygen, moisture and hydrocarbons is necessary to avoid fouling the detector. The GC flow rate can be operated with either constant head pressure or constant flow, with the flow rate through the GC column optimized for the detector. For a typical MS detector in high vacuum, less than 2.0 mL/min flow out of the GC is necessary. A low-bleed GC column must be used to avoid high background noise. More detail about the chromatography can be found in the previous article of this handbook.

Data Acquisition:

Mass spectra can be collected for each peak from $m/z = 1.6$ to $m/z = 800$ and up to $m/z = 1050$ for the newest mass spectrometers. Data can be collected continuously in scan mode, selected ion monitoring (SIM) mode, or scan/SIM mode can be used simultaneously

In scan mode, a range of ions is selected (e.g., $m/z = 15$ to 500) and the voltages on the quadrupole are ramped continuously to scan over this predetermined range. In GC-MS analyses, a total ion chromatogram (TIC) is constructed, which is a plot of the sum of the intensity for all ions in a predetermined range as a function of time. The TIC has two dimensions of data, with each point on the TIC containing a distribution of the relative intensity of each ion scanned. For example, Figure 4 shows a TIC for the jet fuel, JP-8. Examining specifically the peak at 4.66 minutes, Figure 5 shows the mass spectrum with a molecular ion at $m/z = 142$ and fragmentation ions of those typically observed for EI MS analysis of n-decane. By comparing the mass spectra across a peak observed in the TIC, the peak purity (resolution of separation) can be assessed. Additionally, an average of the mass spectra collected across a peak is often useful for identifying an unknown analyte. Background ions can also be subtracted to improve compound identification with library searching.

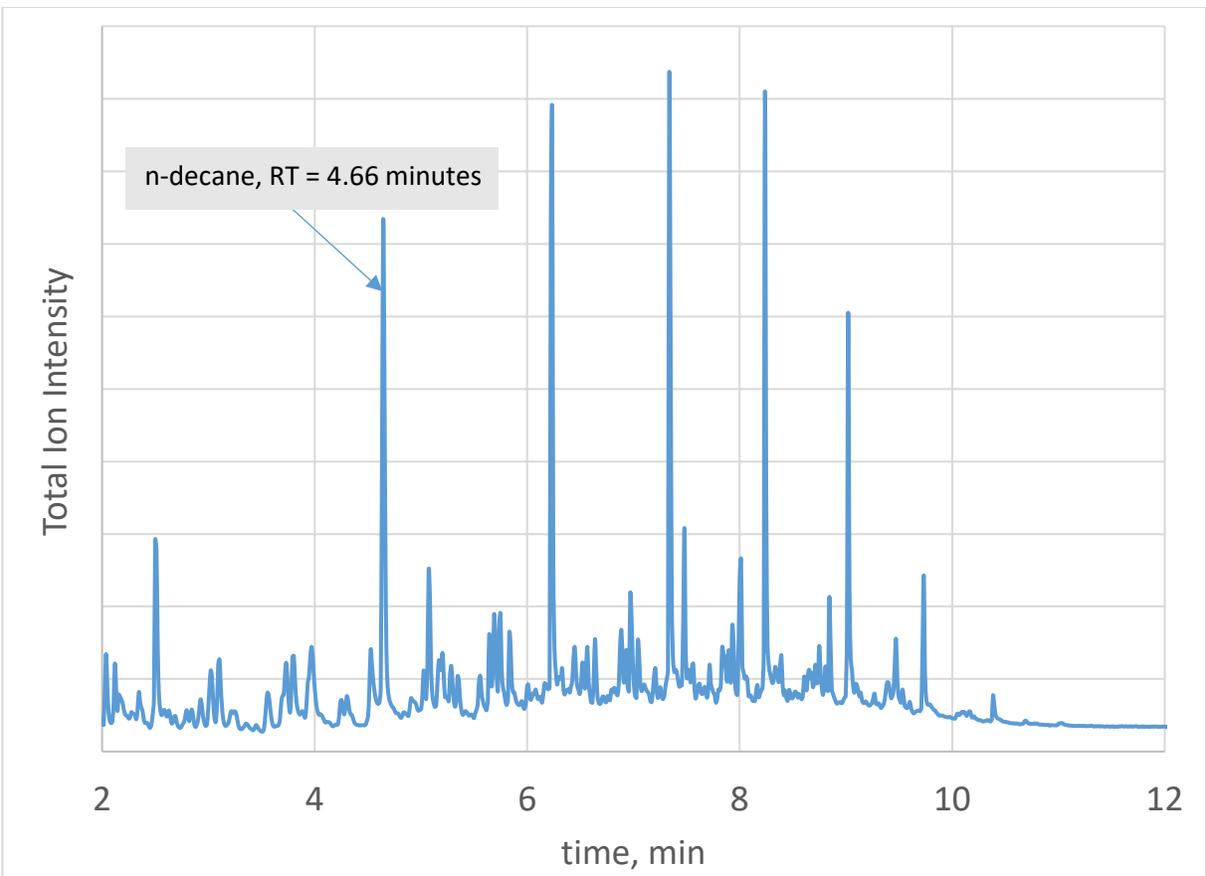


Figure 4. A total ion chromatogram (TIC, a plot of the total ion intensity versus time) obtained with EI GC-MS for the kerosene-based jet fuel, JP-8.

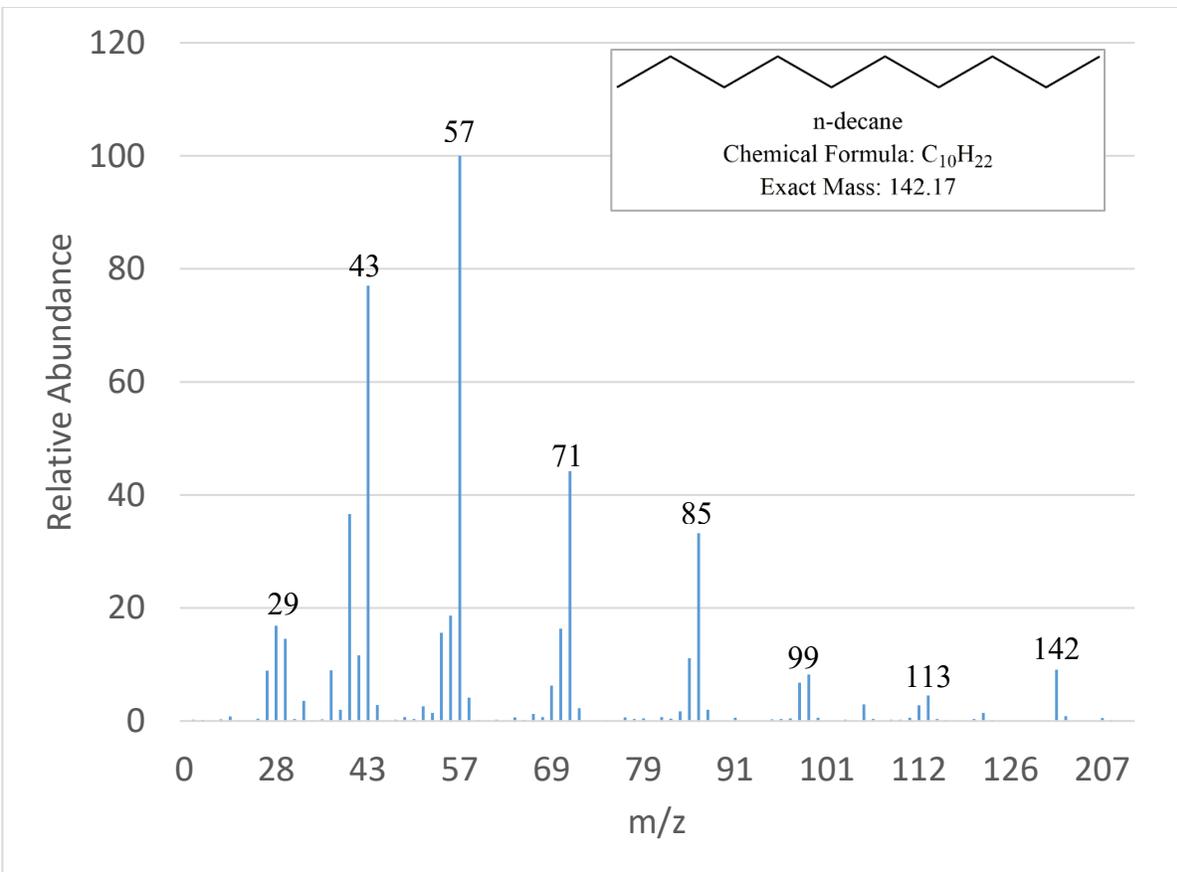


Figure 5. The EI MS spectrum of the peak at 4.66 min from the TIC displayed in Figure 4. This spectrum displays the molecular ion at $m/z = 142$ and the fragmentation ions typically observed for n-decane.

SIM mode enhances the sensitivity for predefined ions that the analyst selects. This is done by setting the analyzer voltages to scan for only a single ion or a group of ions. SIM mode results in improved signal to noise because the analyzer can spend more time collecting specific ions and noise is reduced. Selecting an appropriate ion to analyze for with SIM mode requires *a priori* knowledge of the mass spectrum for that compound. This can be obtained by analyzing an analytical standard of the analyte of interest in scan mode. Figure 6 shows the mass spectrum for cannabidiol (CBD), the non-psychoactive component of cannabis that is of interest due to its potential for medical applications.

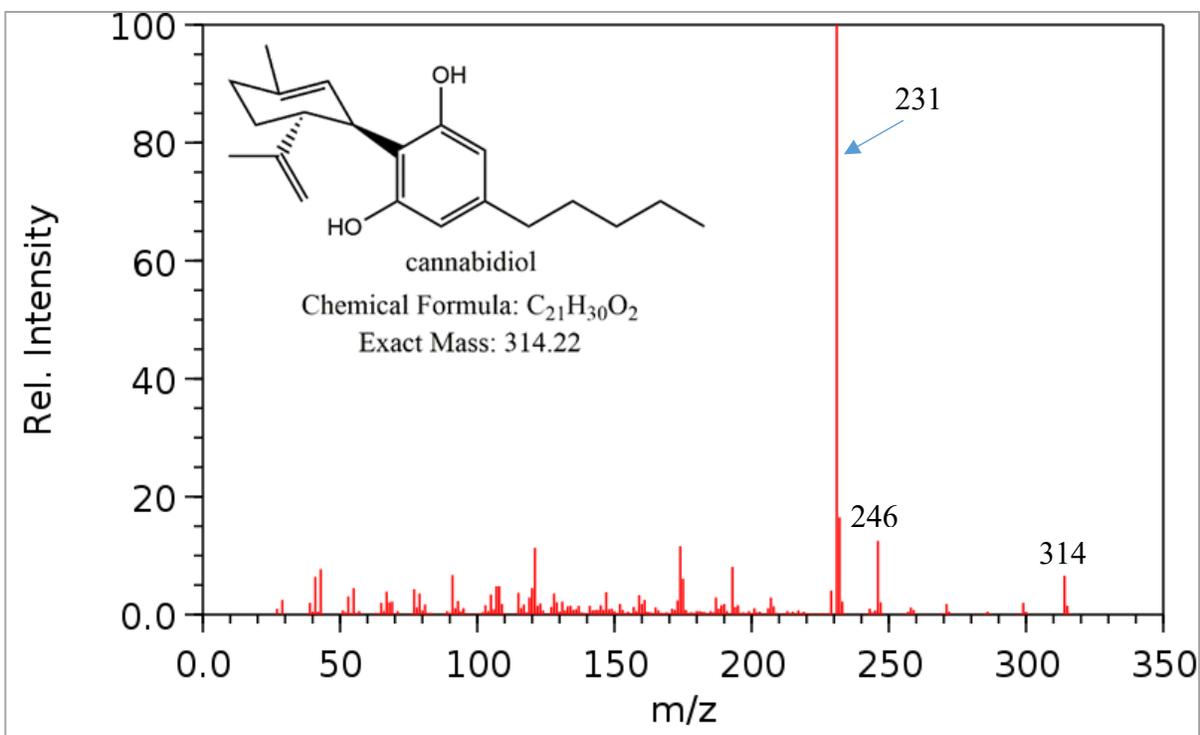


Figure 6. The EI MS for cannabidiol (CBD). The ions (m/z) are presented as a function of relative (rel.) intensity normalized to the most abundant at $m/z = 231$. The molecular ion at $m/z = 314$ is observed.[9]

Inspection of Figure 6 shows that the molecular ion for CBD is observed ($m/z = 314$), additionally the most abundant ions observed are $m/z = 231$ and 246. Selecting these three ions for SIM mode analysis would be a good starting point to develop a method for detecting trace quantities of CBD.

Figure 7 displays the resulting TICs when scan (Figure 7a) and SIM (Figure 7b) modes are used simultaneously to detect trace quantities of CBD. Here, scan mode analyzed $m/z = 33$ to 550 and SIM mode analyzed only the ions $m/z = 231, 246, 299,$ and 314. Figure 8 displays the average of mass spectra collected from 5.387 to 5.537 min.

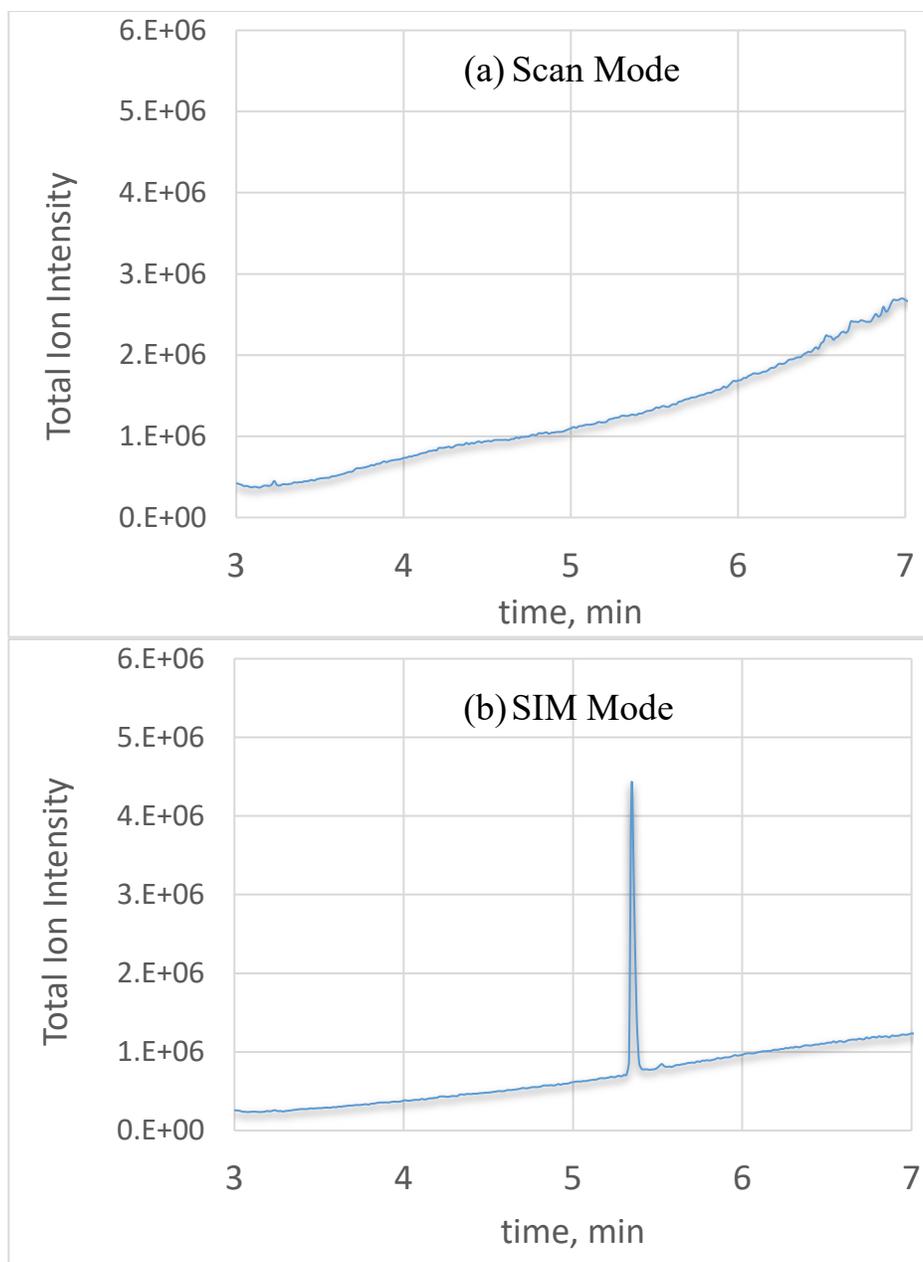


Figure 7. The TICs for the molecule cannabidiol, acquired simultaneously with EI GC-MS operated in (a) scan mode or (b) SIM mode.

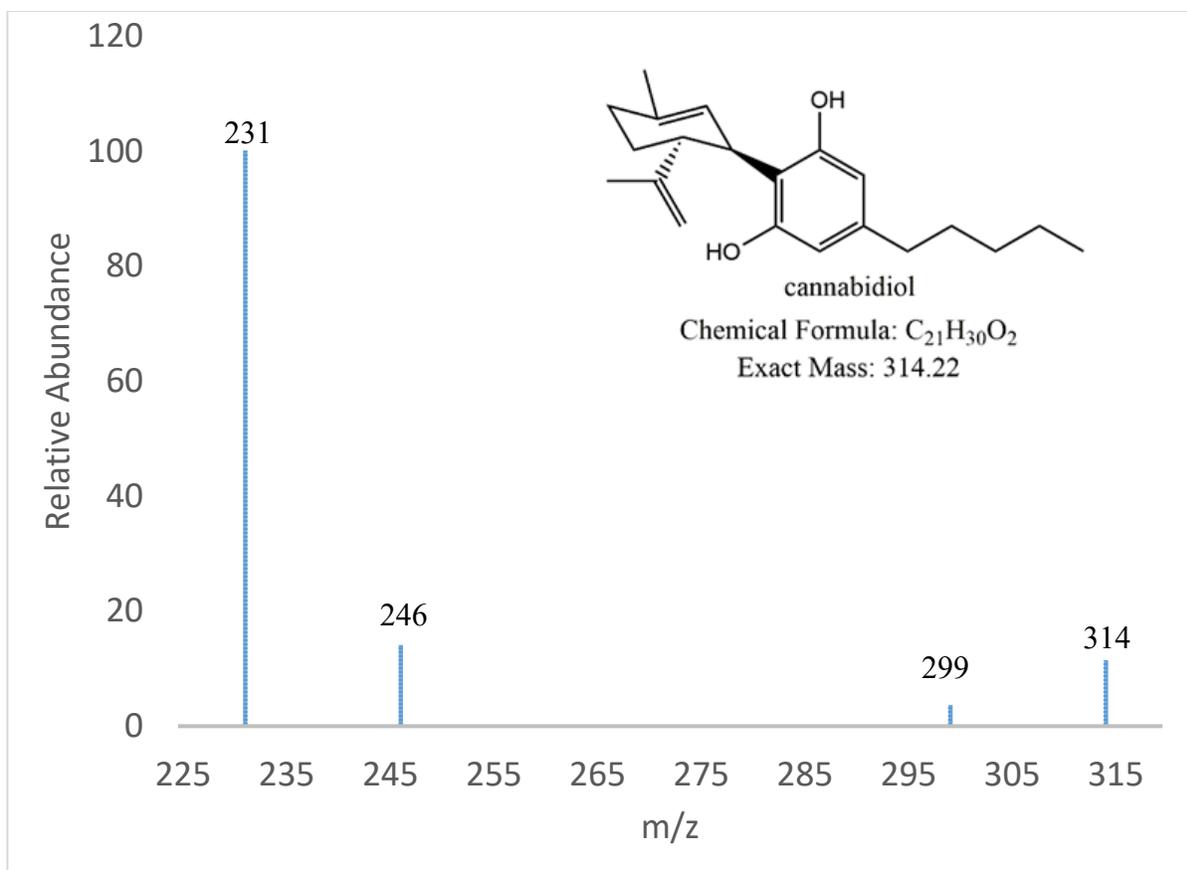


Figure 8. The average of mass spectra acquired in SIM mode for cannabidiol (CBD) obtained with EI GC-MS with the ion peaks as a function of relative intensity.

Advances in MS

Exact Mass Measurements:

Low-resolution analyzers (e.g., rounded quadrupoles) are limited to producing data with an integer m/z value. This does not allow for unambiguous ion identification. In many cases mixtures can still be analyzed with the use of known analytical standards or comparisons of mass spectra with compounds found in library databases.[7] Certain analyzers (e.g., ToF, Fourier-transform (FT) orbitrap, magnetic sector, hyperbolic triple quadrupole, and FT ion cyclotron resonance), provide data with accurate mass of 5 ppm or less.[8]

Recent advances in optical instrumentation, high-speed electronic recording, and signal processing have made it possible for ToF analyzers to be a viable addition to the GC-MS system, providing high-resolution detection of compounds without *a priori* knowledge of chromatographic retention time. The maximum resolving power (FWHM) of the ToF is up to 50000 ($m/\Delta m$), which equates to a mass accuracy of up to 5 ppm. For example, both perhydrofluorene (C₁₃H₂₂) and anthracene (C₁₄H₁₀) have the same nominal molecular mass of 178 Da, however their exact masses differ by 0.094 Da, as shown in Figure 9. These two species could therefore easily be resolved by Q-TOF-MS. Exact mass, however, cannot be used to

distinguish between isomers.[10] It is possible that MS-MS could be used to distinguish these isomers. MS-MS will be discussed next.

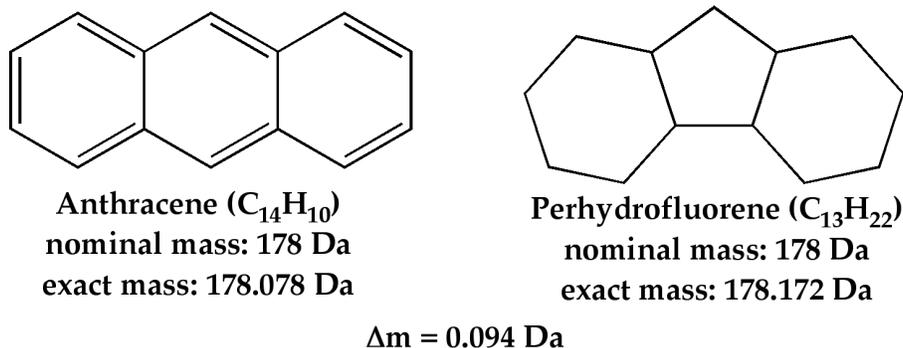


Figure 9: Example of two hydrocarbons with same nominal mass, but different exact masses that can be distinguished using the time-of-flight analytical technique.

Tandem Mass Spectrometry

In addition to high-resolution mass spectra, structural information and trace analysis of complex mixtures can be deciphered with tandem mass spectrometry (MS/MS). Tandem MS is a technique in which two mass analyzers are separated by a collision chamber. Tandem MS can be used for determining the exact mass of organic molecules, to determine complex structures (e.g., protein sequencing) or for confirmation of a drug identification.[8]

MS/MS uses the first mass analyzer to select only the precursor ion of interest (in the example considered above, m/z 178) to enter a separate cell where it is fragmented by collision-induced dissociation (CID) with an inert gas that is used to quench or fragment the parent (or precursor) ion into additional product ions called daughter ions. The product ions are then analyzed in the second mass analyzer, which produces a mass spectrum.[6]

The resulting product ions correlate to a molecule, making it possible to differentiate between isomers and provide an additional identification tool.

The analytical capabilities of high-resolution MS and MS/MS are even further enhanced by the option to perform two-dimensional gas chromatography. This technique serves the purpose of separating complex mixtures into families of polarity prior to detection by time-of-flight mass spectrometry. Cryogenic modulation is used to improve the separation by taking the output of the first GC column (for example a polar material) and injecting it into a column of differing material (non-polar).

Conclusions

This article began with an explanation of the principles of mass spectrometry and a discussion of the instrumentation and how a measurement is made. The basic information for interpreting a mass spectrum and a total ion chromatogram is then presented. There are a variety of different types of data acquisition modes, for example, full scan mode and SIM mode. The uses for these

different modes is discussed. Different types of mass spectrometers that are commonly coupled with GC separation are also discussed, culminating in a section about the recent advances in GC-MS technology. This is only an introduction to the topic which is explored in more depth in the references.

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