

Idaho State Police

Forensic laboratory Training Manual

Mass Spectrometry

1.0.0 Background

Mass Spectrometry (MS) is an important analytical technique for the identification of chemical compounds. In the mass spectrometer, the sample to be analyzed is vaporized and ionized yielding gaseous-charged particles with unique masses. These charged particles are separated, collected, and measured. The instrument produces a record known as the mass spectrum that records the abundance of the charged species at each mass to charge ratio (m/z). The position and abundance of the m/z values provide qualitative information about the compound.

A mass spectrometer, regardless of type or manufacturer, consists of a sample inlet system, an ionizing source, a mass filter to separate the ions by mass/charge ratio, and a detector.

2.0.0 MS Inlet Design

Chromatographic interfaces are applicable to a wide range of sample types. They are probably the most convenient and efficient means of introducing samples into the mass spectrometer. Mixed or impure samples can be purified and introduced into the analyzer by this means.

The use of a gas chromatograph as a sample introduction device for the mass spectrometer is widely accepted in the forensic science field. The only real limitation on sample types amenable to this system is that the sample components of interest must pass intact through the gas chromatograph. The gas chromatograph portion of the inlet system does not differ from the gas chromatograph used in analytical work in the laboratory except in the treatment of the column effluent; therefore, all the precautions applicable to gas chromatography in general also apply to this system.

Modern GC/MS instruments utilizing a capillary column in the GC allow the column effluent to be introduced directly into the ionization source of the mass spectrometer. Older models of GC/MS instruments, as well as those currently so designed, that use a packed column in the GC have column effluent flow rates far beyond the pumping capacity of mass spectrometers. These instruments used a variety of devices to enrich the effluent with sample, or remove excess carrier gas. The most popular of these devices are the fritted glass separator and the jet separator.

Several interfaces have been introduced in recent years that allow the effluent of an HPLC to be introduced into a mass spectrometer. Some of these interfaces produce a charged analyte with little or no fragmentation. The recent introduction of bench top MS systems with MS/MS capability allows the isolation of the charged analyte and subsequent fragmentation producing valuable structural information.

3.0.0 Ionization and Fragmentation Process

Upon entering the mass spectrometer, the samples must be ionized. Two methods widely in use today are electron impact ionization (EI) and chemical ionization (CI).

3.1.0 In electron impact ionization (EI), the sample molecules are ionized by bombardment with electrons produced by electrically heating a metal filament to the temperature at which it emits electrons. The energy of electrons generally determines the degree of fragmentation of the sample molecules. At low energy levels (approximately 10-15 eV), little fragmentation occurs in most molecules, thus leaving the molecular ion (M^+) abundant. At electron energies above 20 eV, extensive fragmentation generally occurs. Most EI/MS studies are conducted at 70 eV in order to avoid the effects of slight variations in electron energies. Low eV studies are generally employed only where the molecular ion is very unstable at 70 eV.

3.2.0 In chemical ionization (CI), an entirely different process occurs. Reagent gas molecules (e.g., methane, ammonia, isobutane) are introduced into the ionization chamber, wherein they are partially ionized by relatively high-energy electrons (approximately 100 - 500 eV). Sample molecules introduced into the ionization chamber are ionized by collision with the ionized reagent gas. This process generally results in the production of fewer fragment ions than in EI.

Fragmentation processes in CI are generally characterized by hydride abstractions, proton additions, or neutral losses of small molecules such as water, ammonia, etc., from the protonated parent molecule. These simpler fragmentation routes make the spectra much easier to interpret than those obtained by EI. Quite often in CI, quasi-molecular ions ($M+1$) are obtained, thereby easily identifying the molecular weight of the compound in question.

Thus, CI is a valuable tool in determining molecular weights, but is generally less effective than EI in elucidation of molecular structure. The two ionization processes often produce two completely different sets of positive ions from the same compound. This, therefore, gives two sets of physical parameters by which the compound can be characterized and this, with the addition of GC retention time data, is often sufficient to unambiguously establish the identity of a compound.

4.0.0 Analyzer Design

Four basic types of mass spectrometers are known by the design of their mass filter:

1. Magnetic sector
2. Time of flight
3. Radio frequency (including quadrupole and ion trap)
4. Fourier transform: ion cyclotron (FTMS or ICR)

4.1.0 Magnetic Sector MS

Magnetic sector instruments separate the ions by extracting them from the ionizer, then accelerating them down a curved tube by means of a high potential (approximately 4 Kv) electrical field. Positioned along the curved tube is a powerful magnet designed to produce a wedge-shaped (sector) magnetic field. When the sample is extracted from the ionizer, it consists of a mixture of all the ions of various masses produced in the

ionization process. As these ions are accelerated toward the magnetic sector, the potential V , applied to the entrance slits, controls their velocities. Subsequently the ions are diverted into circular paths by a magnetic field parallel to the slits and perpendicular to the ion beam. The ion velocity in the magnetic field is given by the equation $zV=(1/2)mv^2$. A stable, controllable magnetic field separates the components of the total ion beams according to momentum. By this means, the individual ion beams are separated spatially and each has a unique radius of curvature (ion trajectory), r , according to its mass/charge (m/z) ratio. Only ions of a single m/z value will have the proper trajectory leading to the exit slit ahead of the detector.

By varying the accelerating potential and/or the strength of the magnetic field, each ion in turn can be focused on the detector, thereby enabling the recorder to produce a complete mass spectrum of a sample.

4.2.0 Time of Flight MS

Time-of-flight (TOF) mass spectrometers separate the ions by measuring the time required by each ion to travel the length of a straight tube 30 to 100 centimeters long. In the TOF mass spectrometer, sample ionization occurs for a short time interval, and then the ionizing voltage is turned off. The ions produced during this interval are then accelerated as an "ion packet" down the tube by means of an accelerating voltage (approximately 3 Kv). Since there is no further energy input to the ion packet after acceleration, the ion packet spreads out as it drifts down the length of the tube. This separation of the ions is a function of ion velocity derived from the kinetic energy imparted by the accelerating potential $zV=(1/2)mv^2$. Because all ions have essentially the same energy at this point, their velocities are inversely proportional to the square roots of their masses. As a result, ions with different m/z ratios spatially separate as they travel down the flight tube. Ions of high velocity (low m/z ratios) speed on ahead and arrive at the detector before the heavier ions of lower velocity (high m/z ratios). Thereby, groups of ions are formed which sequentially impact the ion detector.

4.3.0 Quadrupole MS

Quadrupole instruments are the most commonly used mass spectrometers for forensic drug analysis today.

Molecules from the inlet system, i.e., the gas chromatograph, enter the ion source where they are bombarded with electrons that are emitted from a hot filament. The neutral molecules are ionized to form a variety of products, including positive ions. The positive ions are generally used in routine forensic analysis because they predominate over the other species present by several orders of magnitude. While ionization can occur at any bond in the molecule, it does occur at certain preferred locations, giving rise to a distribution of ions which is reproducible and which constitutes a fingerprint of the original molecule.

The positive ions are electrically extracted from the ion source and injected into the quadrupole mass filter where they are separated according to their mass.

The governing principle of the quadrupole filter is based on alternating electric fields applied to four electrodes (rods) held in a square array. To each diagonally paired set of

rods a combination radio frequency (rf) and dc voltage of increasing amplitude is applied. One pair receives an RF voltage and a positive dc voltage, and the other pair receives an RF voltage with an 180° phase shift and a negative dc voltage. These voltages give rise to an electrostatic field that gives bounded oscillations to an ion fragment of the selected mass to charge (m/z) ratio and unbounded (collected and discharged on rods) oscillations to all ions of different m/z .

The mass of the ions transmitted is directly proportional to the voltage applied to the quadrupole filter. Since a linear ramp voltage is applied to the filter, the output of the mass numbers will also be displayed linearly. The separation between mass 19 and 20 is, for example, identical to that between masses 600 and 601.

Correct assignment of mass number for each mass peak displayed is far easier on a linear mass spectrum than a quasi-logarithmic mass spectrum as obtained with a sector type mass spectrometer. This advantage is particularly important in case of chemical ionization MS, where one is apt to find long stretches of blank space on the spectrum where no mass peaks appear. This advantage is of little consequence in modern instruments that employ digital data analysis systems.

The ions passing through the quadrupole filter are quantitatively detected by an electron multiplier, amplified, and the resulting signal is fed to an appropriate data handling system.

4.4.0 Ion Trap MS

A new technique for mass analysis was introduced to mass spectrometers in the early 1980s. The quadrupole ion trap or Paul trap, named after its inventor Wolfgang Paul, is rapidly gaining wide acceptance in the field of chemistry, due largely to the intense research programs studying its function and applications.

Beam-type scanning mass spectrometers (e.g., quadrupole mass filter and magnetic sector instruments) operate on the principle of mass-selective stability. That is, ions that have a small range of m/z values maintain “stable” trajectories throughout the analyzer and can pass to an ion detector. The mass spectrum is collected by “scanning” the analyzer field so that the window of stable m/z values is sequentially swept across the entire m/z range of interest. The ratio of the width of the transmitted m/z window to the total width of the m/z range of interest determines the fraction of time during a scan that any given ion can be transmitted. This ratio can be considered the “duty cycle” of a scanning beam type instrument, and is typically 1%. Therefore, for a continuous ionization method >99% of all ions generated are lost simply because of the small magnitude of the duty cycle.

Ion traps utilize a principle that has been termed “mass selective instability.” The ion trap consists primarily of two hyperbolic shaped end cap electrodes bracketing a ring electrode. A sine-wave signal is applied to the ring electrode. When a potential exists between the ring electrode and the end caps, a quadrupole electric field is created within the electrodes because of their hyperbolic shape. As ions enter the trap the oscillating electrical field captures the ions in a “pseudo-potential well.” The ions begin to move toward one side of the trap (sliding down the side of the well) that, if allowed to continue for a sufficiently long period, the motion away from the center of the ion trap

would result in acceleration of the ion to one of the end-cap electrodes. However, if the potential is alternated at a sufficiently high frequency, an ion present at the center of the ion trap with a low initial kinetic energy cannot reach an electrode before it is repelled, and thus becomes “trapped.”

Under a fixed set of conditions ions of different m/z values have different trapping well depths. They also undergo motion with a unique set of frequencies. This characteristic allows the quadrupole ion trap to be used as a mass spectrometer rather than simply as an ion storage device. A variety of frequencies characterize the motion of a particular m/z ion in a quadrupole ion trap. By selectively adding energy to a specific m/z ion by manipulating the so called “fundamental z -dimensional secular frequency” that ion can be ejected through a hole in the end-cap electrode of the ion trap, thus giving rise to the term “mass selective instability.”

Current instrument designs have adopted a sequential approach to ion generation and filtering. The ion source generates the ions to be studied and the ion trap collects and stores the ions until an optimum number of ions have been collected (approximately 10^6 ions). The trap then ejects the ions sequentially for detection and analysis. When a strong ion source exists the duty cycle of the ion trap is only a few percent, in the same range as a scanning beam instrument. Conversely, with a weak ion source the accumulation time to fill the trap increases and hence the duty cycle increases. For example, if the ion trap can be filled in 1 ms and the mass analysis time is ~50 ms, the duty cycle is but a few percent. However, if the ion accumulation rate for an analyte ion of interest is sufficiently low to require an ion accumulation time of up to 1 s, a duty cycle as high as 95% is obtained. Because of this potential for a very high duty cycle, ion trap mass spectrometers can produce full scan spectra from much smaller samples than quadrupole or magnetic sector instruments.

In some ion trap instruments, sample molecules are fragmented inside of the ion trap instead of in a separate ion source. In these types of instruments, collisions between ionized fragments and neutral molecules occur. These collisions sometimes result in a form of chemical ionization where the neutral molecules are ionized by the charged ions instead of electrons. The resulting mass spectrum is an electron impact spectrum superimposed on a chemical ionization spectrum (which can include ions of higher molecular weights than the original molecule). Because this phenomenon is concentration dependent (i.e. the more molecules in the trap, the more collisions take place) the actual mass spectrum obtained with these instruments varies with the amount of sample introduced into the instrument.

5.0.0 Technical Concerns

Before attempting to interpret a mass spectrum, the operator should evaluate the data as to usability of the spectrum. The problem most frequently encountered is that of sample impurity, readily detected by observing the mass spectra at various points throughout the chromatogram. If one or more fragment ion peaks in the mass spectrum appear, disappear, or change intensities relative to the other ion fragments during production of the sample peak, the spectrum is not that of a pure compound.

Another problem that reduces the value of a mass spectrum is that of molecular ion (M^+) instability. If M^+ is very small or absent, it may be overlooked, causing the wrong molecular weight to be assigned to the sample compound and making spectrum interpretation impossible. Several methods of identifying M^+ are available e.g.; lowering the ionizing voltage to increase the relative abundance of M^+ , examination of the molecular ion cluster for the presence of $M^+ +1$, $M^+ +23$, $M^+ -1$, etc., or checking for irrational losses (3 to 13 amu) from the peaks at highest amu. Use of chemical ionization rather than electron impact ionization simplifies the assignment of molecular weight.

Careful plans should be formulated before running a sample on the mass spectrometer. All available sample information should be studied (e.g., microscopic, chemical, GC retention data, etc.). Samples should be at least partially “cleaned up” to prevent contamination of the ion source.

6.0.0 Reading

MS Fundamentals (HP Multimedia Course), Hewlett Packard Company

7.0.0 Exercises

1. Watch an autotune and try to identify what part of the instrument is being adjusted for each screen shown.
2. Compare the mass spectra of heroin, morphine, and codeine. Prepare a narrative explaining the differences and/or similarities.

8.0.0 Questions

1. What type of inlet(s) are the Mass Spectrometers in your department equipped with?
2. Briefly explain the following terms:
LC/MS -
GC/MS
MS/MS -
3. Does the addition of chromatographic capabilities increase or decrease the utility of the MS for routine analysis? Explain.

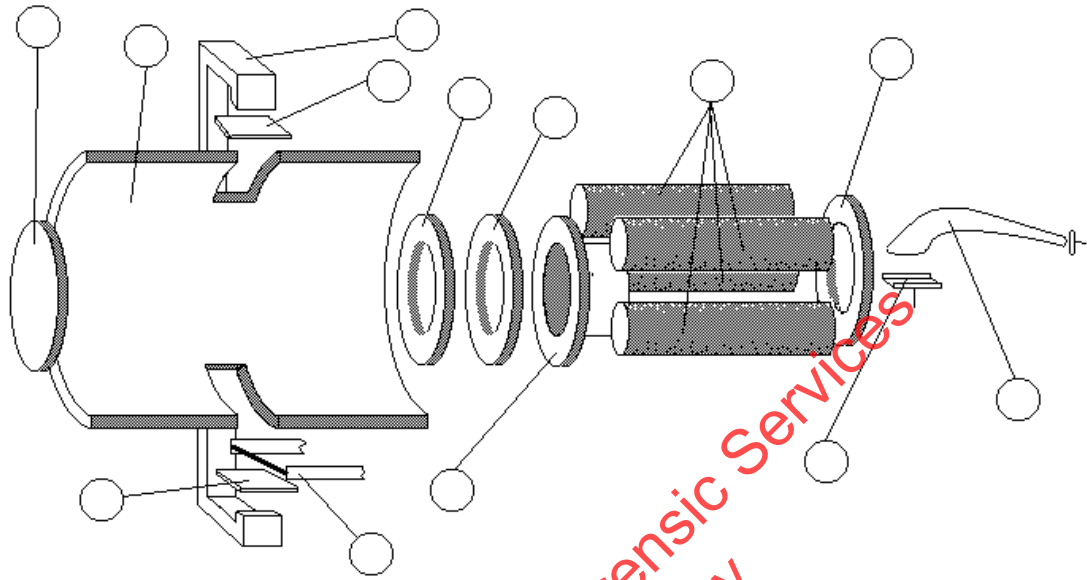


Figure 1

4. Describe the following mass spectrometer parts and their function or purpose. Place a letter in each circle of Figure 1 that corresponds with the letter next to each part's name given below.

- A. Ionization chamber
- B. Filament -
- C. Magnet(s) (two functions)
- D. Electron focus -
- E. Target -
- F. Repeller -
- G. Drawout plate and Drawout plate aperture -
- H. Ion focus -
- I. Entrance lens -
- J. Electron Multiplier -
- K. Deflector -
- L. Iris -
- M. Mass Filter -

5. What is meant when it said that the molecules were ionized at 70 eV?

6. Why does a dirty ion source affect the quality of mass spectra?

7. Why do high mass ions typically have better resolution and lower sensitivity than low mass ions at a given repeller voltage in quadrupole systems?

8. How does an electron multiplier work?
9. What adjustments can be made to the electron multiplier to increase the signal?
10. Why do electron multipliers “wear out”? What indicates that the EM is wearing out?
11. What is the maximum typical electron multiplier operating voltage? What are the current multiplier voltages on the mass spectrometers in your department?
12. Describe how chemical ionization differs from electron ionization.
13. Why is chemical ionization used?
14. Why must the ion source, mass filter and detector be in a vacuum?
15. Why are two vacuum pumps used together on a mass spectrometer?
16. How fast do the rotor blades in a turbo molecular pump spin?
17. In closed systems, even a small vacuum pump can achieve pressures in the 1×10^{-7} Torr range. Check the ion gauge’s of the mass spectrometers in your section and see what pressures are actually being achieved and if the pressures are lower or higher, explain.
18. What does PFTBA stand for, what is it used for and what is another name for it?
19. What are the major PFTBA ions?
20. What happens when a tune is performed?
21. What should the ion peak shapes on an autotune report look like?
22. Describe Scan and SIM modes of data acquisition and the reason(s) for their use.

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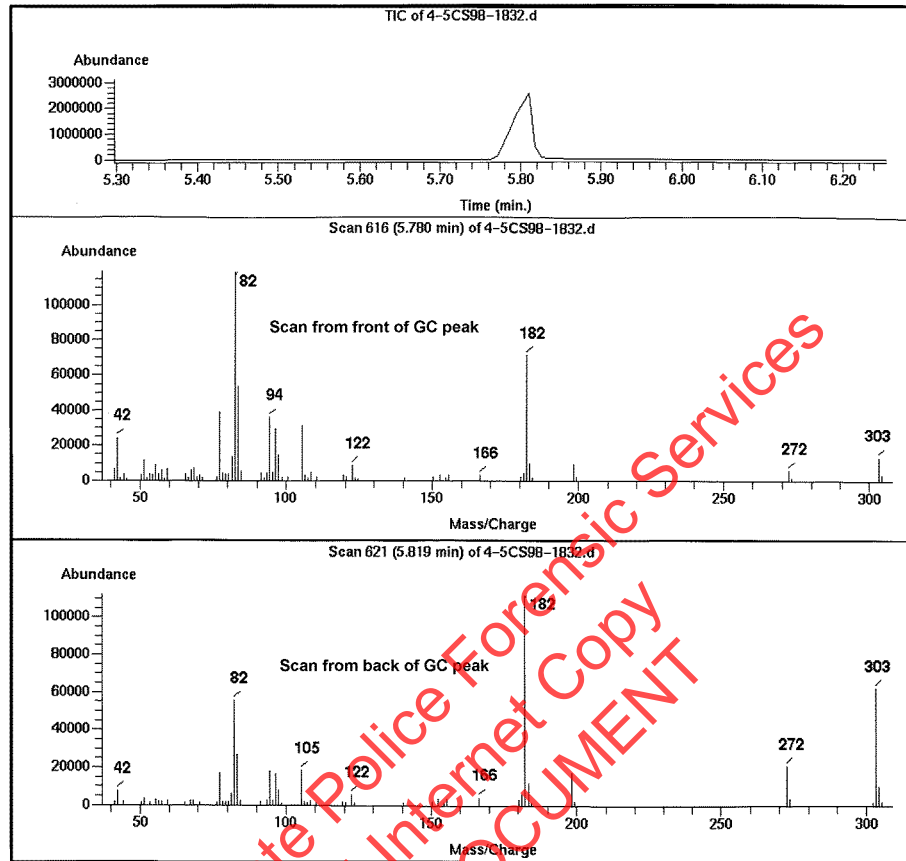


Figure 2

23. What differences can be observed between the two spectrums in Figure 2. Explain the reason for these differences.

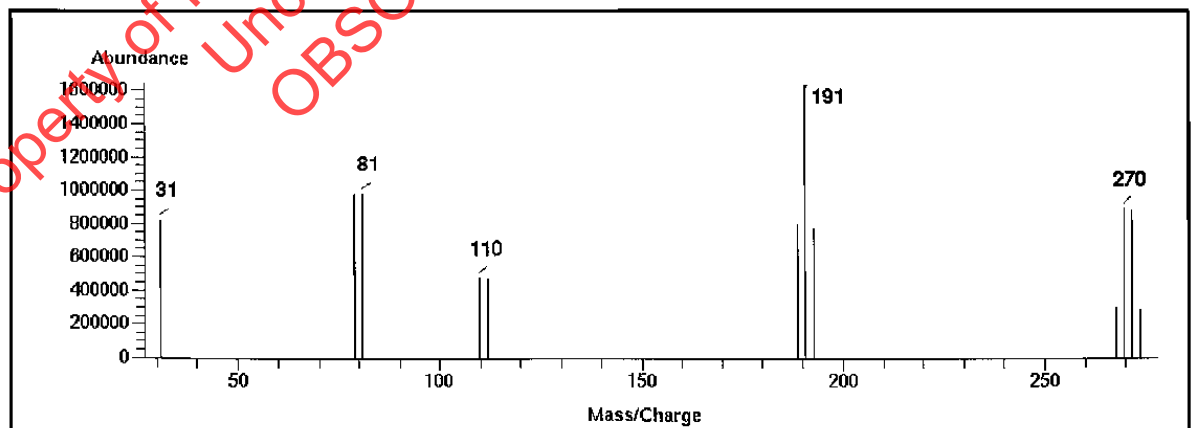


Figure 3

24. What compound would give the mass spectra in Figure 3.

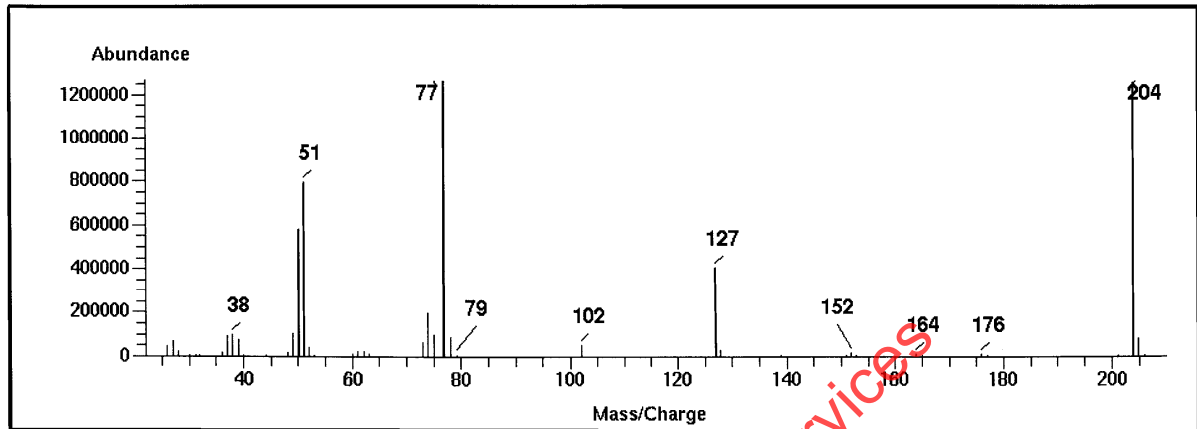


Figure 4

25. What compound gives the spectra shown in Figure 4?
26. The following mass ions are usually indicative of what structural features:
 m/z 91 -
 m/z 77 -
 m/z 58 -
27. Are the mass spectra usually collected at the ISP positive or negative ion spectra?
28. What are potential consequences of improper shutdown and venting?
29. What information should be on all hardcopy from the GC/MS?
30. What is required to use GC/MS as part of the identification of a drug?
31. When is a Performance Verification check performed on each GC/MS?
32. What is the molecular ion of chloromethane (CH₃Cl)?
33. What major difference is there between the mass spectra of straight chain and branched chain alkanes?

9.0.0 HISTORY

Prior to revision 3 modules in the training manual did not have individual history pages.

Revision #	Issue or review date	History	Author or Reviewer
3	7/08/11	Added 9.0.0	David Sincerbeaux