PART I
A GC/MS PRIMER

1

INTRODUCTION

The combination of gas liquid chromatography (GC) for separation and mass spectrometry (MS) for detection and identification of the components of a mixture of compounds is rapidly becoming the definitive analytical tool in the research and commercial analytical laboratory. The GC/MS systems come in many varieties and sizes depending on the work they are designed to accomplish. Since the most common analyzer used in modern mass spectrometers is the quadrupole, we will focus on this means of separating ion fragments of different masses. Discussion of ion trap, time-of-flight, Fourier transform mass spectrometry (FTMS), and magnetic sector instruments will be reserved for latter sections in the book.

The quadrupole operational model is the same for bench top production units and for floor standing research instruments. The actual analyzer has changed little in the last 10−12 years except to grow smaller in size. High vacuum pumping has paralleled the changes in the analyzer, especially in the high efficiency turbo that have shrunk to the size of a large fist in some systems. Sampling and injection techniques have improved gradually over the last few years.

The most dramatic changes have been in the area of control and processing software and data storage capability. In the last 10 year, accelerating computer technology has reduced the computer hardware and software system shipped

GC/MS: A Practical User's Guide, *Second Edition*. By Marvin C. McMaster

with the original system to historical oddities. In the face of newer, more powerful, easier to use computer systems, the older DEC 10, RTE (a Hewlett-Packard minicomputer GC/MS control system) and Pascal-based control and data processing systems seem to many operators to be lumbering, antiquated monstrosities.

The two most common reasons given for replacing a GC/MS system is the slow processing time and the cost of operator training. This is followed by unavailability of replacement parts as manufacturers discontinue systems. The inability of software to interface with and control modern gas chromatographic and sample preparation systems is the final reason given for replacement.

Seldom, if ever, is the complaint that the older systems do not work, or that they give incorrect values. In many cases, the older systems appear better built and more stable in day-to-day operation than newer models. Many require less cleaning and maintenance. This has lead to a growing market for replacement data acquisition and processing systems. Where possible, the control system should also be updated, allowing access to modern auxiliary equipment and eliminating the necessity for coordinating dual computers of differing age and temperaments.

Replacement of older systems with the newest processing system on the market is not without its problems. Fear of loss of access to archived data stored in outdated, proprietary data formats is a common worry of laboratories doing commercial analysis.

1.1 WHY USE GC/MS?

Gas liquid chromatography is a popular, powerful, reasonably inexpensive, and easy-to-use analytical tool. Mixtures to be analyzed are injected into an inert gas stream and swept into a tube packed with a solid support coated with a resolving liquid phase. Absorptive interaction between the components in the gas stream and the coating leads to a differential separation of the components of the mixture, which are then swept in order through a detector flow cell. Gas chromatography suffers from a few weaknesses such as its requirement for volatile compounds, but its major problem is the lack of definitive proof of the nature of the detected compounds as they are separated. For most GC detectors, identification is based solely on retention time on the column. Since many compounds may possess the same retention time, we are left in doubt about the nature and purity of the compound(s) in the separated peak.

The mass spectrometer takes injected material, ionizes it in a high vacuum, propels and focuses these ions and their fragmentation products through a

magnetic mass analyzer, and then collects and measures the amounts of each selected ion in a detector. A mass spectrometer is an excellent tool for clearly identifying the structure of a single compound, but is less useful when presented with a mixture.

The combination of the two components into a single GC/MS system forms an instrument capable of separating mixtures into their individual components, identifying, and then providing quantitative and qualitative information on the amounts and chemical structure of each compound. It still possesses the weaknesses of both components. It requires volatile components, and because of this requirement, has some molecular weight limits. The mass spectrometer must be tuned and calibrated before meaningful data can be obtained. The data produced has time, intensity, and spectral components and requires a computer with a large storage system for processing and identifying components. A major drawback of the system is that it is very expensive compared to other analytical systems. With continual improvement, hopefully the cost will be lowered because this system and/or the liquid chromatograph/mass spectrometry system belong on every laboratory bench top used for organic or biochemical synthesis and analysis.

Determination of the molecular structure of a compound from its molecular weight and fragmentation spectra is a job for a highly trained specialist. It is beyond the scope and intent of this book to train you in the interpretation of compound structure. Anyone interested in pursuing that goal should work through Dr. McLafferty's book listed in Appendix E, then practice, practice, practice. Chapter 12 is included to provide tools to let you evaluate compound assignments in spectral databases. It uses many of the tools employed in interpretation, but its intent is to provide a quick check on the validity of an assignment.

1.2 INTERPRETATION OF FRAGMENTATION DATA VERSUS SPECTRAL LIBRARY SEARCHING

How do we go about extracting meaningful information from a spectra and identify the compounds we have separated? A number of libraries of printed and computerized spectral databases are available to us. We can use these spectra to compare both masses of fragments and their intensities. Once a likely match is found, we can obtain and run the same compound on our instrument to confirm the identity both by GC retention time and mass spectra. This matching is complicated by the fact that the listed library spectra are run on a variety of types of mass spectrometers and under dissimilar

tuning conditions. However, with modern computer database searching techniques, large numbers of spectra can be searched and compared in a very short time. This allows an untrained spectroscopist to use a GC/MS for compound identification with some confidence. Using these spectra, target mass fragments characteristic of each compound can be selected, allowing its identification among similarly eluting compounds in the chromatogram.

Once compounds have been identified, they can be used as standards to carry out quantitative analysis of mixtures of compounds. Unknown compounds found in quantitative analysis mixtures can be flagged and identified by spectral comparison using library searching. Spectra from scans at chromatography peak fronts and tails can be used to confirm purity or identify the presences of impurities.

1.3 THE GAS CHROMATOGRAPH/MASS SPECTROMETER

From the point of view of the chromatographer, the gas chromatograph/mass spectrometer is simply a gas chromatograph with a very large and very expensive detector, but one that can give a definitive identification of the separated compounds. The sample injection and the chromatographic separation are handled in exactly the same way as in any other analysis. You still get a chromatogram of the separated components at the end. It is what can be done with the chromatographic data that distinguishes the mass spectral detector from an electron capture or a flame ionization detector.

The mass spectrometrist approaches the GC/MS from a different point of view. The mass spectrum is everything. The gas chromatograph exists only to aid somewhat in improving difficult separations of compounds with similar mass fragmentations. The only true art and science to him or her is in the interpretation of spectra and identification of molecular structure and molecular weight.

The truth, of course, lies somewhere in between. A good chromatographic separation based on correct selection of injector type and throat material, column support, carrier gas and oven temperature ramping, and a properly designed interface feeding into the ion source can make or break the mass spectrometric analysis. Without a properly operating vacuum system, ion focusing system, mass analyzer, and ion detector, the best chromatographic separation in the world is just a waste of the operator's time. It is important to understand the components that make up all parts of the GC/MS system in order to keep the system up, running, and performing in a reproducible manner.

1.3.1 A Model of the GC/MS System

There are a number of different possible GC/MS configurations, but all share common types of components. There must be some way of getting the sample into the chromatogram, an *injector.* This may or may not involve sample purification or preparation components. There must be a *gas chromatograph* with its carrier gas source and control valving, its temperature control oven and microprocessor programmer, and tubing to connect the injector to the column and out to the mass spectrometer interface. There must be a *column* packed with support and coated with a stationary phase in which the separation occurs. There must be an *interface* module in which the separated compounds are transferred to the mass spectrometer's ionization source without remixing. There must be the *mass spectrometer* system, made up of the ionization source, focusing lens, mass analyzer, ion detector, and multistage pumping. Finally, there must be a *data/control* system to provide mass selection, lens and detector control, and data processing and interfacing to the GC and injector (see Fig. 1.1).

The injector may be as simple as a septum port on top of the gas chromatograph through which a sample is injected using a graduated capillary syringe. In some cases, this injection port is equipped with a trigger that can start the oven temperature ramping program and/or send a signal to the data/control system to begin acquiring data. For more complex or routine analysis, injection can be made from an autosampler allowing multiple vial injections, standards injection, needle washing, and vial barcode identification. For crude samples that need preinjection processing, there are split/splitless injectors, throat liners with different surface geometry, purge and trap systems, headspace analyzers, and cartridge purification systems. All these systems provide sample extraction, cleanup, or volatilization prior to the introduction of analytical sample onto the gas chromatographic column.

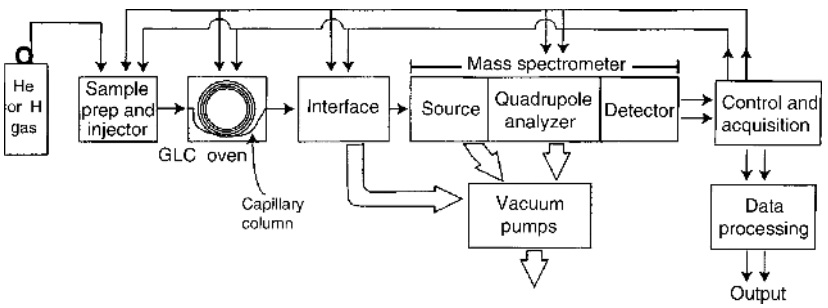

FIGURE 1.1 A typical GC/MS system diagram.

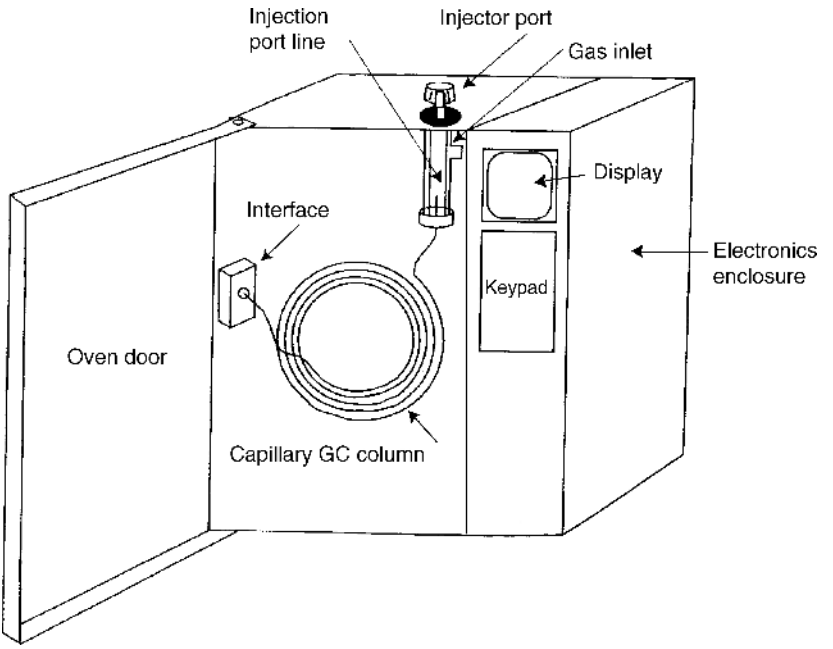

FIGURE 1.2 Gas chromatograph.

The gas chromatograph, Figure 1.2, is basically a temperature-controlled oven designed to hold and heat the GC column. Carrier gas, usually either nitrogen, helium, or hydrogen, is used to sweep the injected sample onto and down the column where the separation occurs and then out into the mass spectrometer interface.

The interface may serve only as a transfer line to carry the pressurized GC output into the evacuated ion source of the mass spectrometer. A jet separator interface can also serve as a sample concentrator by eliminating much of the carrier gas. It can permit carrier gas displacement by a second gas more compatible with the desired analysis, that is, carbon dioxide for chemically induced (CI) ionization for molecular weight analysis. It can be used to split the GC output into separate streams that can be sent to a secondary detector for simultaneous analysis by a completely different, complimentary method.

The mass spectrometer has three basic sections: an ionization chamber, the analyzer, and the ion detector (Fig. 1.3).

In the evacuated ionization chamber, the sample is bombarded with electrons or charged molecules to produce ionized sample molecules. These are swept into the high vacuum analyzer where they are focused electrically then selected in the quadrupole rods. The direct current (dc) signal charging

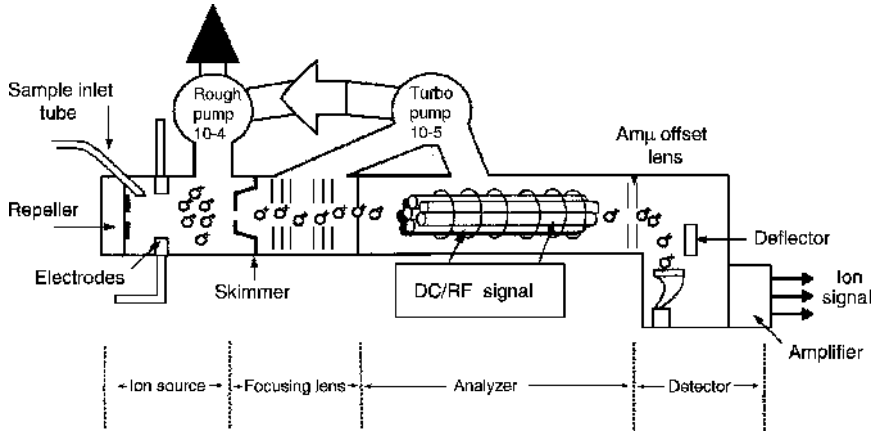

FIGURE 1.3 Quadrupole mass spectrometer.

apposing poles of the quadrupole rods creates a standing magnetic field in which the ions are aligned. Individual masses are selected from this field by sweeping it with a radio frequency (RF) signal. As different dc/RF frequencies are reached, different mass/charge ratio (m/z) ions are able to escape the analyzer and reach the ion detector. By sweeping from higher to lower frequency, the available range of m/z ions are released one at a time to the detector, producing a mass spectrum.

On entering the ion detector, the ions are deflected onto a cascade plate where the signal is multiplied and then sent to the data system as an ion current versus m/z versus time. The summed raw signal can be plotted against time as a total-ion chromatogram (TIC) or a single-ion m/z can be extracted and plotted against time as a single-ion chromatogram (SIC). At a single time point, the ion current strength for each detected ion fragment can be extracted and plotted over an m/z mass range, producing a mass spectrum. It is important always to remember that the data block produced is three dimensional: (m/z) versus signal strength versus time. In most other detectors, the output is simply signal strength versus time.

1.3.2 A Column Separation Model

Separation of individual compounds in the injected sample occurs in the chromatographic column. The typical gas chromatographic column used for GC/MS is a long, coiled capillary tube of silica with an internal coating of a either a viscous liquid such as carbowax or a wall-bonded organic phase.

The injected sample in the carrier gas interacts with this stationary organic phase and equilibrium is established between the concentration of each

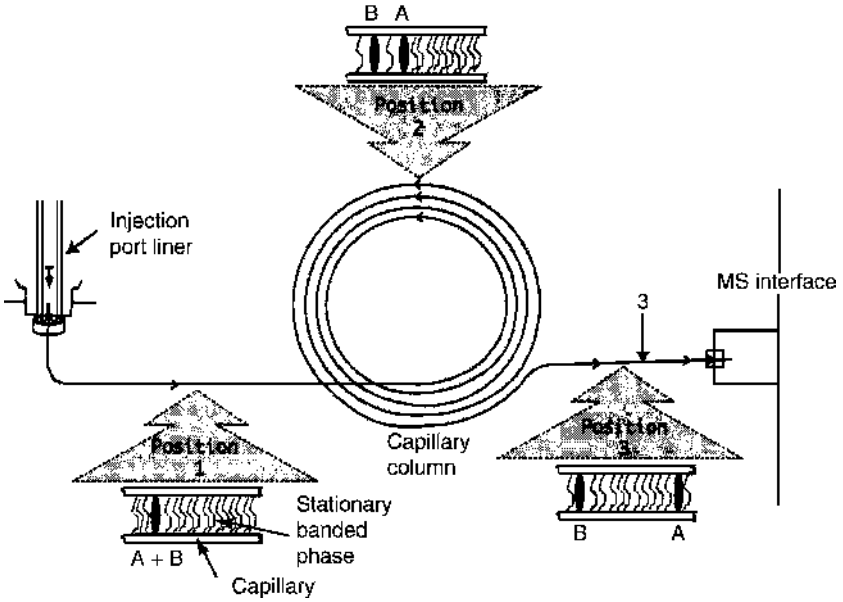

FIGURE 1.4 Chromatographic column separation model.

component in the gaseous and solid phases. As fresh carrier gas flushes down the column, each compound comes off the stationary phase at its own rate. Separations increase after many interactions down the length of the column; then each volatile component comes off the column end and into the interface (Fig. 1.4).

Both the injector and the column can be heated to aid in compound removal since not all components of the injected sample are volatile at room temperature. The column oven allows programmed gradient heating of the column. Temperatures above 400°C are avoided to prevent thermal degradation of the sample.

Moving down the column, the injection mixture interacts with the packing. Separation is countered by remixing due to diffusion and wall interactions. Finally, each compound emerges into the interface as a concentration disc, tenuous at first, then rising to a concentration maxima and then dropping rapidly as the last molecules comes off. If we were to run this effluent into an ultraviolet (UV) detector, we would see a rapidly rising peak reach its maximum height then fall again with a slight tail.

Ideally, each compound emerges as a disc separated from all other discs. In actual separations of real samples, perfect separation is rarely achieved. Compounds of similar chemical structure and physical solubility are only poorly resolved and coelute. In a chromatographic detector, they appear as

overlapping or unresolved peaks. Something else must be done to prove their presence, to identify their structure, and to quantitate the amounts of each compound.

1.3.3 GC/MS Data Models

The simplest data output from the mass spectrometer analyzer is a measurement of total-ion current strength versus time, a TIC (Fig. 1.5).

This is basically a chromatographic output representing a summation of the signal strength of all the ions produced by the mass spectrometer at a given time. The chromatogram produced is similar in appearance to a UV chromatogram with peaks representing the chromatographic retention of each component present. In a UV detector, however, you would see only the

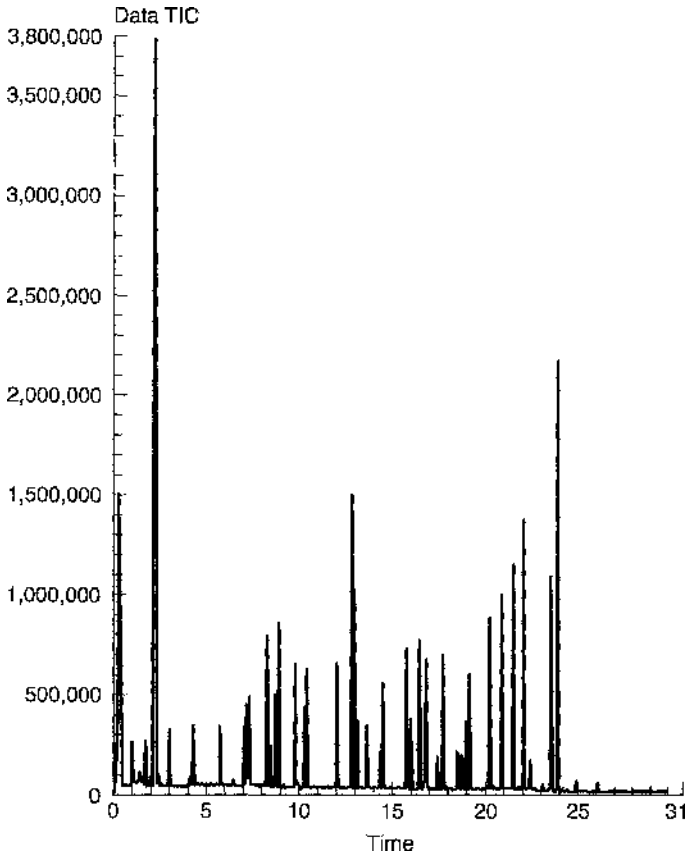

FIGURE 1.5 Total-ion chromatogram.

compounds that absorb UV light at the selected wavelength. In the mass spectrometer, any compound capable of being ionized and forming fragments would be detected. The mass spectrometer serves as a universal chromatographic detector.

The actual data output content is much more complex. If the mass spectrometer is in the scanning (SCAN) mode, the analyzer voltage is being changed continuously and repeatedly over a selected mass range. Different ion masses are reaching and being detected by the detector. Information is coming out each moment on the exact position of the analyzer. After calibration and combination with the ion concentration information, this provides the molecular mass and amounts of each ion formed. After these data are computer massaged, we receive a three-dimensional block of data whose coordinates are elapsed time, molecular mass (m/z), and ion concentration (Fig. 1.6).

Viewing this block of data on a two-dimensional display such as a integrator or a CRT screen while trying to extract meaningful information is nearly impossible. A three-dimensional projection on a screen can be made but is not particularly useful for extracting information. It does provide an overall topologically view of the data, which is useful for finding trends in the data set.

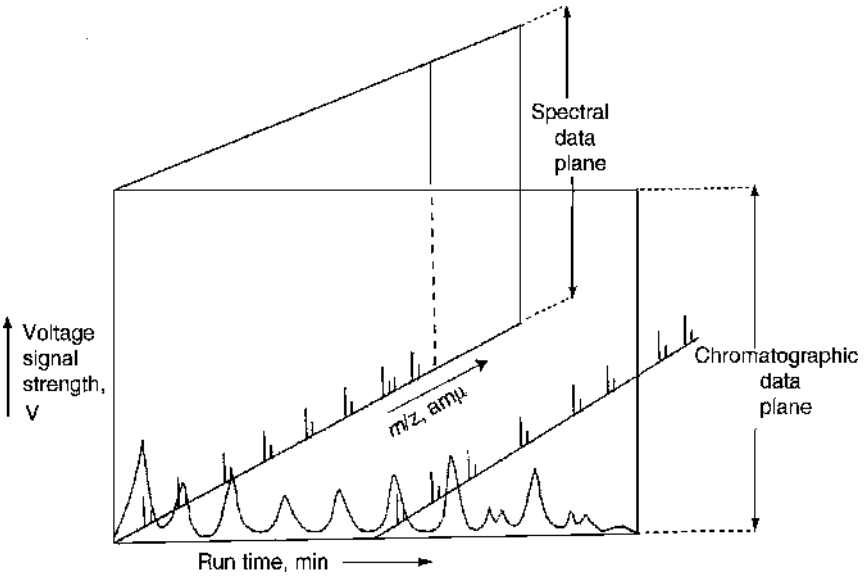

FIGURE 1.6 Three-dimensional GC/MS data block.

If we select a data cut at a single molecular mass, we can produce a SIC similar to that produced by a UV detector tracing at a single wavelength (Fig. 1.7).

The series of peaks produced represent the concentration of ions of the selected molecular mass present throughout the chromatographic run. Compounds that do not form an ion with this mass will not be present in the single-ion chromatogram. Comparison with the TIC shows a much simplified chromatogram, but all peaks in the SIC are present in the TIC.

An SIC can also be produced by running the GC/MS in a fixed-mass mode in which the analyzer is parked at a given molecular mass position through out the chromatographic run. This single-ion monitoring (SIM) mode has an additional advantage. Because the analyzer is continuously analyzing for only a single ion, the summed ion yield is much higher and detection limits for this

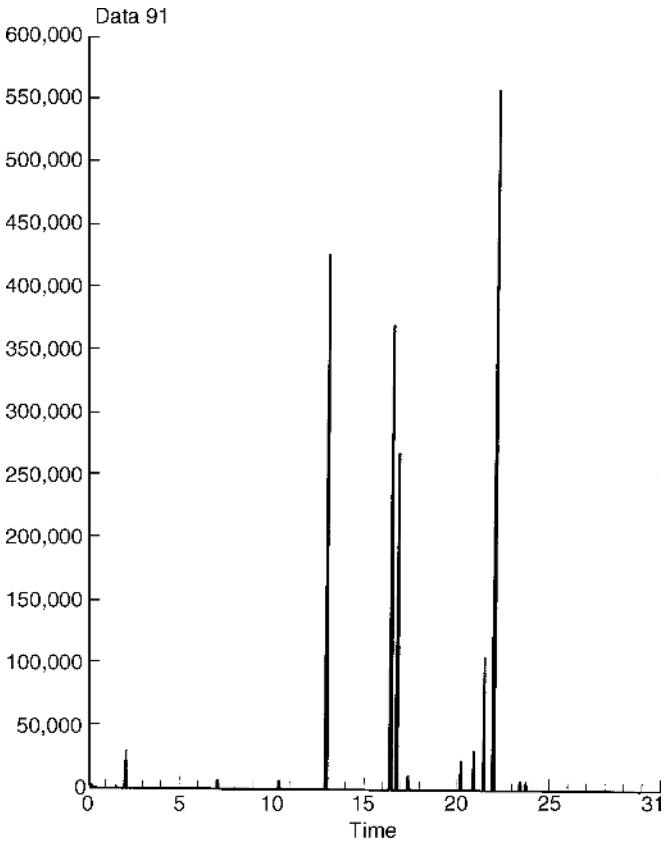

FIGURE 1.7 Single-ion chromatogram.

ion are much lower. The mass spectrometer becomes a much more sensitive detector, but only for compounds producing this mass fragment. Other compounds lacking this fragment ion will be missed. A good detector for trees instead of forests—for trace analysis of minor contaminants.

Going back to our original three-dimensional block of data in Figure 1.6, we can select a data cut at a given time point which will provide us with a display of molecular mass versus ion concentration called a mass fragment spectra or simply a mass spectra (Fig. 1.8).

Generally, these data are not displayed as an ion continuum. The ion mass around a unitary mass is summed within a window and displayed as a bar graph with 1-amu increments on the *m/z* mass axis, as shown in Figure 1.8.

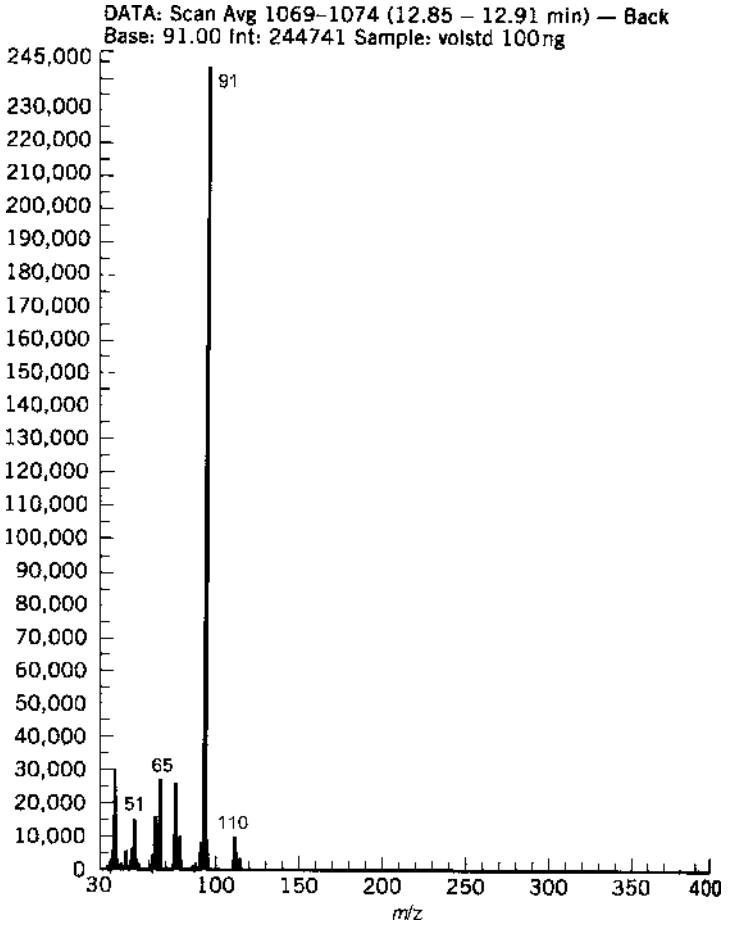

FIGURE 1.8 Mass fragment spectra (mass spectra).

The mass spectrum of a resolved compound is a record of the fragmentation pattern of this compound under a given set of experimental conditions. It is characteristic of that compound and can be used to definitively identify the chemical nature of that compound. In the same or a similar instrument under the same tuning conditions, this compound will always give the same fragments in the same ion concentration ratios. Libraries of compound fragmentation patterns can be created and searched to identify compounds by comparison with known fragmentations. Further decomposition of isolated fragments can be studied in triple quadrupole GC/MS/MS systems to identify fragmentation pathways useful in determining structures of unknown compounds.

There is a lot of arm waving involved with the statement "under a given set of experimental conditions." Different ionization methods and voltages will affect the fragmentation ions produced. Under certain conditions, only a single major ion is produced, the molecular ion. It is formed by the original molecule losing an electron to form this ion radical, whose mass is equal to the molecular weight of the compound, a very useful number to have in identifying compounds.

Changes in the geometry, calibration, cleanliness, and ion detector age of the mass spectrometer can all produce variations in the fragmentation pattern and especially in the ion concentration ratios. Variations in the chromatographic conditions can lead to overlapping peaks and change the relative heights in the fragmentation pattern. Learning and controlling these is what converts GC/MS from a science to an art. All of this has lead to a proliferation of instrument types and calibration standards attempting to tame these variables.

1.4 SYSTEMS AND COSTS

Instrument system costs are not widely advertised by manufacturers unless you work for the federal government and are buying off a Government Service Administration price list. To come up with even ballpark figures, I have talked to former customers who have recently purchased systems and have talked to manufacturers at technical meeting. The numbers in Table 1.1 represent an educated guess at 2005 system pricing.

In the past, systems could be divided in two basic types, floor standing research systems designed for the mass spectrometry research laboratory and desktop systems designed for both commercial analytical laboratories and the university analytical chemistry laboratory. A new product niche has opened in the last 10 years. These systems are simpler, easier to maintain and calibrate,

TABLE 1.1 Estimated GC/MS System Prices

MS type	GC system	MS only	GC/MS	AS/GC/MS data
Quadrupole	Production	N/A	N/A	$36,800[a]
	Benchtop	$72,000	$8000	$90,000
	Research	$90,000	$96,000	$111,000
	Triple quadrupoles	N/A	N/A	$250,000–$500,000
	Used quadrupole	>$3000	>$5000	$3000–$50,000
Ion trap	Ion detector	$65,000	$72,000	$82,000
	Research MS/MS	N/A	N/A	$450,000

[a]No autosampler.

and aimed at the quality control and analytical testing laboratories. They are advertised at a third of the price of desktop system of 12 years ago. The jury is still out on these, but some of their manufacturers have good pedigrees and track records.

I have included pricing on GC/MS/MS systems and on research and desktop ion trap GC/MS systems for comparison with the quadrupoles because many users consider these the analytical systems of the future. The three-dimensional and linear ion traps seem to be simpler, more sensitive, ideal systems for MS/MS studies. If the future is truly toward smaller, more compact systems, the linear ion trap GC/MS system may lead the way because of its versatility and increased sensitivity for trace component studies.

Overall, there definitely is a trend toward lower pricing and ease of operation. This will make systems more available to the average research investigator and commercial laboratory.

There is a growing market for older GC/MS systems because of price and the availability of upgraded data systems, both from GC/MS manufacturer and from third-party sources. It is true that the old data system is usually the worst part of the older system; computer technological advances having left them in the dust. They are difficult to learn, hard to use, and very difficult to connect into modern data networks, since their data formats are obsolete or on the verge of becoming obsolete.

Pumping and analyzer section almost always work. Ion detectors and data systems can generally be replaced if necessary. Once retrofitted, these systems usually perform like champs.

However, be aware that there are some real old dogs out there. Systems that were never very good and no amount of retrofitting will improve them. Systems without butterfly valves in the oil pump that dump pump oil into the analyzer in case of power failures, systems whose manufacturers have disappeared into the night, or-one-of-a-kind systems in which no two systems

have the same control inputs or detector outputs. I know because I have demonstrated replacement data systems on all of these. Let the buyer beware!

When retrofits work, they are often great buys. I had a customer who purchased a hardly used GC/MS from a hospital for $25,000, added a modern data/control system for $22,500, and had a state-of-the-art system for under $50,000. I know a production facility just getting started that bought 12-year-old systems for $3000 each, modernized the data system, networked them, and ran them day and night until they could afford to replace them with 20 newer systems. They purchased bare systems, without a processing and control computer, and moved the existing data/control systems to each new instrument as they purchased them. Operator retraining was negligible as well as system switchover time.

The key to buying older system is to buy one made by a company that was successful when the system was sold and is still successful. Talk to someone who has used or is still using the same type of instrument. Find out what he thinks about it—its strengths and limitations.

1.5 COMPETITIVE ANALYTICAL SYSTEMS

What other analytical systems do you need to consider when selecting an instrument to use in your research? Table 1.2 gives us an idea of the size of the analytical systems market in 2006.

If you need the definitive identification provided by a GC/MS system, there are few competitive system and none at the same relatively mature state of development. On the horizon are a few contenders for the crown. LC/MS has a fairly broad application potential, others fit better in specific analytical niches.

1.5.1 Liquid Chromatography/Mass Spectrometry (LC/MS)

The high performance liquid chromatograph (HPLC) connected to the mass spectrometer in my opinion offers the best potential as a general MS

TABLE 1.2 2006 North American Chromatography System Sales

GC/MS	226 million	4.9% growth
LC/MS	154 million	5.0% growth
HPLC	1206 million	5.8% growth
GC	326 million	2.0% growth
SPE	112 million	6.0% growth
IC	106 million	5.7% growth

LC/GC Magazine 2007 Media Planner online.

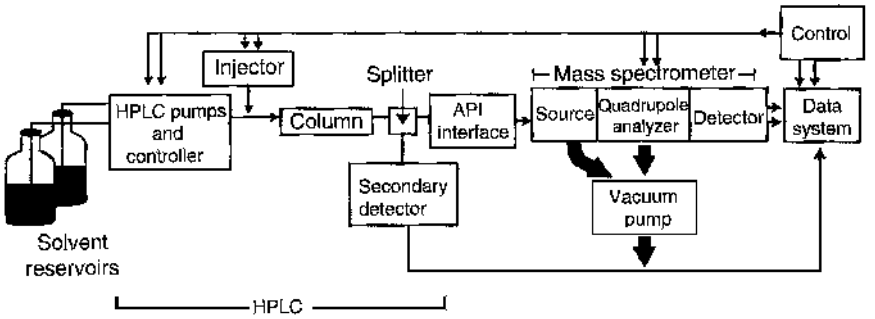

FIGURE 1.9 LC/MS system diagram.

instrument for the laboratory. These LC/MS systems aimed at the production as well as the general research laboratory began to appear on the market about 10 years ago. They now claim major improvements in ease of maintenance and operator training, in calibration stability, in interface flexibility, and in system pricing (Fig. 1.9).

Chromatographically, the HPLC offers flexibility in media and in isocratic and solvent gradient separation technology. Almost anything that can be dissolved can be separated, generally without much sample preparation or derivatization. Large molecules such as proteins and restriction fragments can be separated and analyzed using electrospray techniques.

Limitations to using the technique are due to the current failure of LC/MS systems to provide molecular ion fragmentation without going to a LC/MS/MS system and greatly increasing the cost and experience need to use these systems. The chemical ionization used in atmospheric pressure chemical ionization (APCI) interfaces is a soft ionization and does not usually fragment the molecular ion. LC/MS currently can be used to provide retention times and molecular weights of the separated materials but not definitive compound identification. Existing analytical techniques and calibration standards are just appearing; few have been accepted by and approved by regulatory agencies. Price and reliability are still considerations for general laboratory application. Existing spectral libraries may require modification to be used with LC/MS/MS analysis and definitely will need additional compounds added to them.

1.5.2 Capillary Zone Electrophoresis/Mass Spectrometry (CZE/MS)

Another research tool of growing popularity, capillary electrophoresis interfaced with a mass spectrometer offers a powerful but limited tool for analytical separations (Fig. 1.10).

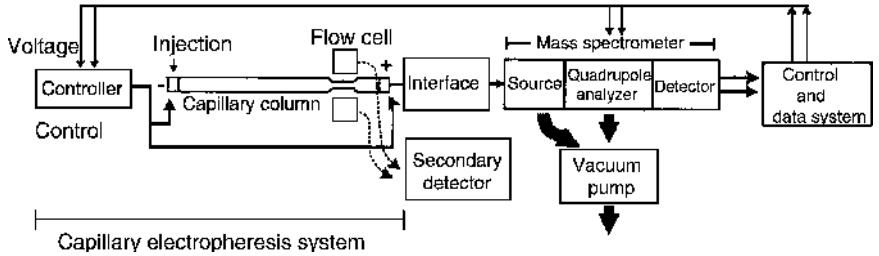

FIGURE 1.10 CZE/MS system diagram.

Capillary electrophoresis uses electromotive force to separate charged molecules in a capillary column filled with buffer or buffer containing gel. A very strong electrical voltage potential is applied to either end of the column. Ionized compounds moved toward the electrode with the opposite charge at a rate dependent on their size and charge strength. It is designed to work with very small amounts of material and delivers a very concentrated compound disc to the mass spectrometer interface. Very high efficiency separation can be achieved. It has proved very useful for analyzing multiple charged molecules such as proteins and DNA restriction fragments when combined with an electrospray MS interface. Limitations for general application have been injector design problems, necessity to work with very high voltages and high concentrations of volatile buffer, and problems eluting samples into the MS interface. Current systems cost, high levels of maintenance, and calibration stability problems have prevented this technique from wider application, but these appear to be coming under better control. Like LC/MS, there are few approved methods for production applications.

1.5.3 Supercritical Fluid Chromatography/Mass Spectrometry (SFC/MS)

Widely considered to be only a laboratory curiosity, this technique has been adopted by manufacturers of flavors, essential oils, and may be developed into a useful environmental analysis tool. One of its most attractive features is its use in combination with supercritical fluid sample extraction in automated sample preparation and analysis.

In SFC/MS, gases such as carbon dioxide can be used in their supercritical fluid state as a mobile phase for separation of injected material on a normal phase HPLC column. Equipment from the injector to the detector interface must be operated under the pressures needed to maintain the gas in its supercritical state (Fig. 1.11).

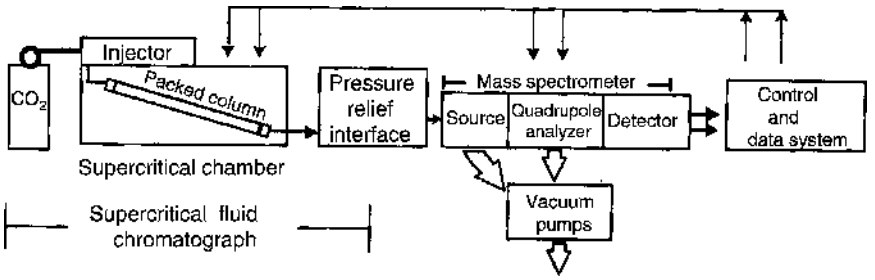

FIGURE 1.11 SFC/MS system diagram.

The major advantage to the techniques is that mobile phase is dispersed simply by reducing pressure in the ionizing interface. Except for minor carbon dioxide contamination, there is almost no solvent background in the mass spectrometer operation. Limitations are the requirements for high pressure chromatographic operation, limited mobile phase selection, and lack of availability of commercial equipment and methodology. The latter two problems may quickly disappear if a specific application develops that only SFC/MS can answer.