Chapter 31

Gas Chromatography Mass Spectrometry

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Summary

General Uses

- Identification and quantitation of volatile and semivolatile organic compounds in complex mixtures
- Determination of molecular weights and (sometimes) elemental compositions of unknown organic compounds in complex mixtures
- Structural determination of unknown organic compounds in complex mixtures both by matching their spectra with reference spectra and by a priori spectral interpretation

Common Applications

- Quantitation of pollutants in drinking and wastewater using official U.S. Environmental Protection Agency (EPA) methods
- Quantitation of drugs and their metabolites in blood and urine for both pharmacological and forensic applications

- Identification of unknown organic compounds in hazardous waste dumps
- Identification of reaction products by synthetic organic chemists
- Analysis of industrial products for quality control

Samples

State

Organic compounds must be in solution for injection into the gas chromatograph. The solvent must be volatile and organic (for example, hexane or dichloromethane).

Amount

Depending on the ionization method, analytical sensitivities of 1 to 100 pg per component are routine.

Preparation

Sample preparation can range from simply dissolving some of the sample in a suitable solvent to extensive cleanup procedures using various forms of liquid chromatography.

Analysis Time

In addition to sample preparation time, the instrumental analysis time usually is fixed by the duration of the gas chromatographic run, typically between 20 and 100 min. Data analysis can take another 1 to 20 hr (or more) depending on the level of detail necessary.

Limitations

General

Only compounds with vapor pressures exceeding about 10^{-10} torr can be analyzed by gas chromatography mass spectrometry (GC-MS). Many compounds with lower pressures can be analyzed if they are chemically derivatized (for example, as trimethylsilyl ethers). Determining positional substitution on aromatic rings is often difficult. Certain isomeric compounds cannot be distinguished by mass spectrometry (for example, naphthalene versus azulene), but they can often be separated chromatographically.

Accuracy

Qualitative accuracy is restricted by the general limitations cited above. Quantitative accuracy is controlled by the overall analytical method calibration. Using isotopic internal standards, accuracy of ±20% relative standard deviation is typical.

Sensitivity and Detection Limits

Depending on the dilution factor and ionization method, an extract with 0.1 to 100 ng of each component may be needed in order to inject a sufficient amount.

Complementary or Related Techniques

- Infrared (IR) spectrometry can provide information on aromatic positional isomers that is not available with GC-MS; however, IR is usually 2 to 4 orders of magnitude less sensitive.
- Nuclear magnetic resonance (NMR) spectrometry can provide detailed information on the exact molecular conformation; however, NMR is usually 2 to 4 orders of magnitude less sensitive.

Introduction

Like a good marriage, both gas chromatography (GC; see Chapter 8) and mass spectrometry (MS; see Chapter 30) bring something to their union. GC can separate volatile and semivolatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them. Therefore, it was not surprising that the combination of the two techniques was suggested shortly after the development of GC in the mid-1950s.

Gas chromatography and mass spectrometry are, in many ways, highly compatible techniques. In both techniques, the sample is in the vapor phase, and both techniques deal with about the same amount of sample (typically less than 1 ng). Unfortunately, there is a major incompatibility between the two techniques: The compound exiting the gas chromatograph is a trace component in the GC's carrier gas at a pressure of about 760 torr, but the mass spectrometer operates at a vacuum of about 10^{-6} to $l0^{-5}$ torr. This is a difference in pressure of 8 to 9 orders of magnitude, a considerable problem.

How It Works

The Interface

The pressure incompatibility problem between GC and MS was solved in several ways. The earliest approach, dating from the late 1950s, simply split a small fraction of the gas chromatographic effluent into the mass spectrometer (1). Depending on the pumping speed of the mass spectrometer, about 1 to 5% of the GC effluent was split off into the mass spectrometer, venting the remaining 95 to 99% of the analytes into the atmosphere. It was soon recognized that this was not the best way to maintain the high sensitivity of the two techniques, and improved GC-MS interfaces were designed (2). These interfaces reduced the pressure of the GC effluent from about 760 torr to $l0^{-6}$ to 10^{-5} torr, but at the same time, they

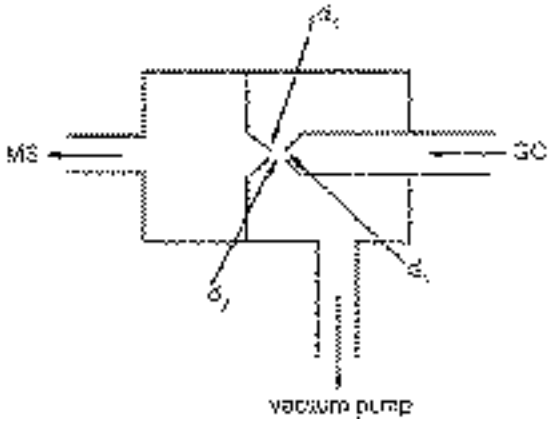
passed all (or most) of the analyte molecules from the GC into the mass spectrometer. These interfaces were no longer just GC carrier gas splitters, but carrier gas separators; that is, they separated the carrier gas from the organic analytes and actually increased the concentration of the organic compounds in the carrier gas stream.

The most important commercial GC carrier gas separator is called the jet separator; see Fig. 31.1 (3). This device takes advantage of the differences in diffusibility between the carrier gas and the organic compound. The carrier gas is almost always a small molecule such as helium or hydrogen with a high diffusion coefficient, whereas the organic molecules have much lower diffusion coefficients. In operation, the GC effluent (the carrier gas with the organic analytes) is sprayed through a small nozzle, indicated as d_1 in Fig. 31.1, into a partially evacuated chamber (about 10^{-2} torr). Because of its high diffusion coefficient, the helium is sprayed over a wide solid angle, whereas the heavier organic molecules are sprayed over a much narrower angle and tend to go straight across the vacuum region. By collecting the middle section of this solid angle with a skimmer (marked d_3 in Fig. 31.1) and passing it to the mass spectrometer, the higher-molecular-weight organic compounds are separated from the carrier gas, which is removed by the vacuum pump. Most jet separators are made from glass by drawing down a glass capillary, sealing it into a vacuum envelope, and cutting out the middle spacing (marked d_2 in Fig. 31.1). It is important that the spray orifice and the skimmer be perfectly aligned.

These jet separators work well at the higher carrier gas flow rates used for packed GC columns (10 to 40 mL/min); however, there are certain disadvantages. Packed GC columns are an almost infinite source of small particles upstream of the jet separator. If one of those particles escapes from the column, it can become lodged in the spray orifice and stop (or at least severely reduce) the gas flow out of the GC column and into the mass spectrometer. Part of this problem can be eliminated with a filter between the GC column and the jet separator, but eventually a particle will plug up the orifice. In fact, sometimes it is not a particle at all, but rather tar (mostly pyrolyzed GC stationary phase) that has accumulated in the spray orifice over time. Clearly, these devices require maintenance.

Currently, the most common strategy, which is ideally suited for capillary GC columns, is to pass all of the carrier gas flow into the ion source of the mass spectrometer (4). This works only if the GC gas flow is sufficiently small and the pumping speed of the mass spectrometer's vacuum system is suf-

Figure 31.1 The jet separator, a device for interfacing a packed column GC with an MS. The three distances are typically d_1, 100 μm; d_2, 300 μm; and d_3, 240 μm.

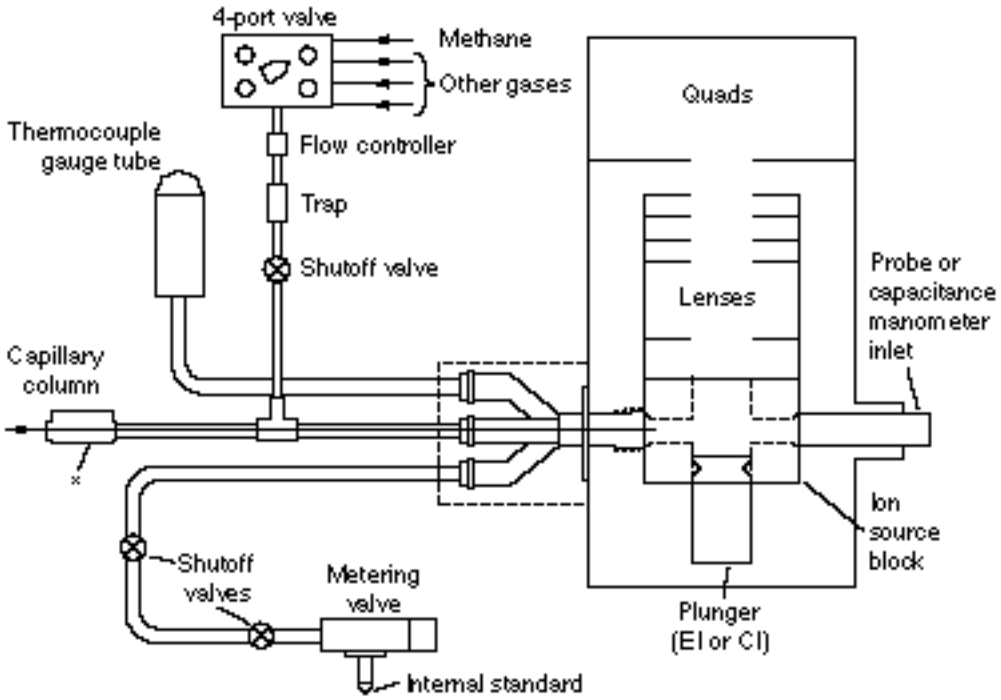


ficiently high to handle the gas flow. For most capillary GC columns, the gas flow is 1 to 2 mL/min, and for most modern mass spectrometers, the pumping speed is at least 300 L/sec. The development of flexible, fused silica capillary columns has made this approach routine. In fact, the only time a jet separator is now used is for a few applications that require packed or thick stationary phase GC columns (for example, for permanent gas analysis).

In practice, most GC-MS interfacing is now done by simply inserting the capillary column directly into the ion source. Fig. 31.2 is a diagram of one such system. The fused silica column runs through a 1/16-in.-diameter tube directly into the ion source. Other gases, such as methane for chemical ionization, are brought into the ion source by a T joint around the capillary column. One of the other two lines into the ion source is used for a thermocouple vacuum gauge tube so that the pressure in the ion source can be roughly measured. The remaining line into the ion source is for the delivery of the mass spectrometer calibration standard, perfluorotributylamine. Most joints are welded together to avoid leaks when this inlet system is thermally cycled or vented. The only removable (Swagelok) fitting is at the junction of the GC column and the far end of the inlet tube (marked with an asterisk in Fig. 31.2). This fitting uses Vespel ferrules. Once the ferrules are on the GC column and it is in the ion source, it is desirable to cut off a few centimeters of the column, if possible. This eliminates the possibility of fine particles partially occluding the end of the column.

If the end of the column cannot be placed directly in the ion source, the material in the GC-MS interface becomes important. The interface is held at 250 to 280 °C; thus, it should not include a reactive metal (such as copper). In some interfaces, glass-lined stainless steel tubing has been used, even though this tubing is difficult to bend properly.

Figure 31.2 A typical GC-MS interface for fused silica capillary GC columns. The end of the GC column enters the ion source of the mass spectrometer.



In summary, for capillary GC-MS, the best interface is no interface at all; run the flexible, fused silica GC column directly into the ion source. Using a column that is 25 to 30 m long by 220 to 250 μm inner diameter gives an ion source pressure of 10^{-6} to 10^{-5} torr, a more than acceptable pressure at which to obtain electron impact spectra. This gives a helium or hydrogen GC carrier gas velocity of 25 to 35 cm/sec or a flow of about 1 to 2 mL/min. The GC columns most widely used for GC-MS are those in which the stationary phase has been chemically bonded to the fused silica; DB-5 is a common trade name. Occasionally, there have been problems with the plastic cladding on the outside of the GC column. This cladding is usually hot (typically 250 °C) and under vacuum. Thus, it may decompose, giving background ions in the mass spectrum or weakening the fused silica itself.

The Data System

The amount of data that can be produced during one GC-MS experiment is overwhelming. In a typical GC-MS experiment, the mass spectrometer might be scanned every 2 sec during a 90-min GC run, whether GC peaks are entering the mass spectrometer or not. Assuming that each mass spectrum has an average of 100 mass/intensity measurements, one such GC-MS experiment will give 270,000 mass/intensity pairs. Because these data have several significant figures and because other ancillary data are also obtained, the data output from a typical GC-MS experiment is about 1 megabyte. To manage this high data flow, computers are required; thus, it is virtually impossible to purchase a GC-MS system without a powerful (but small) computer acting as a data system.

How do data systems work? Two things are going on at the same time (5). There are two different rates within the system. There is a slow rate that times the start and stop of the mass spectrometer scan. This is usually set such that 10 to 15 mass spectra are obtained across a typical GC peak. Because these peaks are usually on the order of 20 to 30 sec wide, the mass spectrometer scan speed is usually set at 2 to 3 sec per spectrum. While this scan is going on, the computer must read the output of the electron multiplier at a rate fast enough to define the mass peak profile. In most commercial GC-MS data systems, the voltage output from the preamplifier on the electron multiplier is converted from an analog signal to a digital value (using an analog-to-digital converter) at a rate of 10,000 to 100,000 times per sec. This process generates large amounts of data: If the analog-to-digital converter worked at 10,000 conversions/second, each minute of the GC-MS experiment would generate 600,000 numbers. This would quickly fill most bulk storage devices; thus, to avoid saving all of these data, most data systems find the mass peaks in real time and convert them into mass intensity pairs, which are then stored on the computer's hard disk. Once the most recent mass spectral scan is stored, this cycle is repeated until the end of the gas chromatogram is reached. Each of the spectra stored on the hard disk has a retention time associated with it, which can be related directly to the gas chromatogram itself. The latter is usually reconstructed by the GC-MS data system by integrating the mass spectrometer output. All modern GC-MS data systems are capable of displaying the mass spectrum on the computer screen as a bar plot of normalized ion abundance versus mass-to-change (m/z) ratio (often called mass). Like the other parts of the GC-MS instrument, the data system must be calibrated. Typically this is done by running a standard compound, such as perfluoro-tributylamine.

What It Does

Gas chromatographic mass spectrometry is the single most important tool for the identification and

quantitation of volatile and semivolatile organic compounds in complex mixtures. As such it is very useful for the determination of molecular weights and [sometimes] the chemical compositions of unknown organic compounds in complex mixtures. Among other applications, GC-MS is widely used for the quantitation of pollutants in drinking and wastewater. It is the basis of official EPA methods. It is also used for the quantitation of drugs and their metabolites in blood and urine. Both pharmacological and forensic applications are significant. GC-MS can be used for the identification of unknown organic compounds both by matching spectra with reference spectra and by a priori spectral interpretation. The identification of reaction products by synthetic organic chemists is another routine application, as is the analysis of industrial products for control of their quality.

To use GC-MS, the organic compounds must be in solution for injection into the gas chromatograph. The solvent must be volatile and organic (for example, hexane or dichloromethane). Depending on the ionization method, analytical sensitivities of 1 to 100 pg per component are routine. Sample preparation can range from simply dissolving some of the sample in a suitable solvent to extensive cleanup procedures using various forms of liquid chromatography. In addition to the sample preparation time, the instrumental analysis time is usually fixed by the duration of the gas chromatographic run, typically between 20 and 100 min. Data analysis can take another 1 to 20 hr (or more) depending on the level of detail necessary.

GC-MS has a few limitations. Only compounds with vapor pressures exceeding about 10^{-10} torr can be analyzed by GC-MS. Many compounds that have lower pressures can be analyzed if they are chemically derivatized (for example, as trimethylsilyl ethers). Determining positional substitution on aromatic rings is often difficult. Certain isomeric compounds cannot be distinguished by mass spectrometry (for example, naphthalene versus azulene), but they can often be separated chromatographically. Quantitative accuracy is controlled by the overall analytical method calibration. Using isotopic internal standards, accuracy of ±20% relative standard deviation is typical.

Mass Spectrometer Components

Electron ionization (Chapter 30) is most commonly used to produce ions from the compounds separated by the GC. Chemical ionization may also be used. Quadrupole (p. 656), ion trap (p. 656), and time-of-flight analyzers may be used to separate ions in the MS. These analyzers have rapid response times and relatively low costs.

Analytical Information

GC-MS is used both for the qualitative identification and for the quantitative measurement of individual components in complex mixtures. There are different data analysis strategies for these two applications.

Qualitative

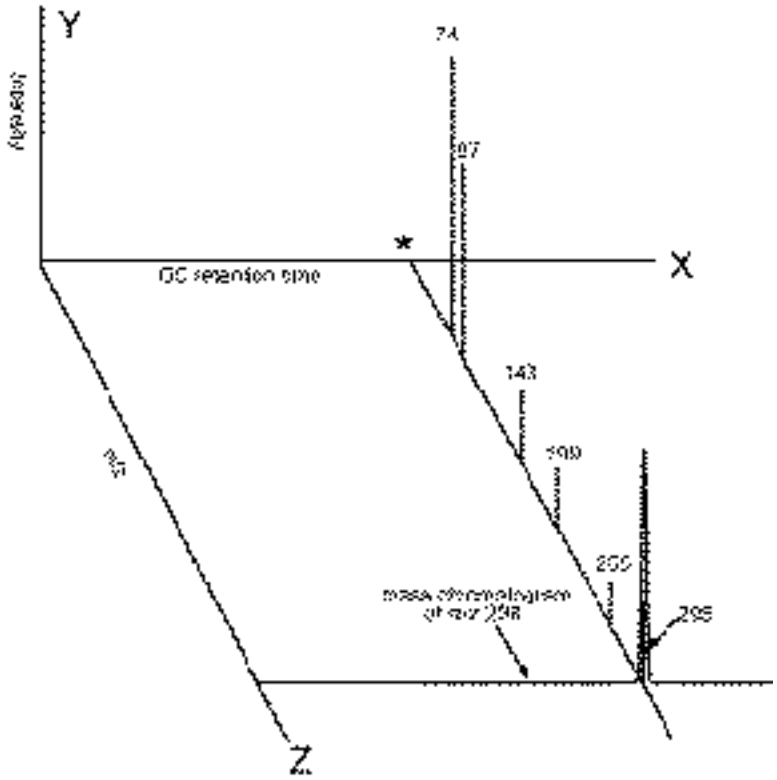
There are three ways of examining GC-MS data. First, the analyst can go through the gas chromatogram (as reproduced by the mass spectrometer) and look at the mass spectra scanned at each GC peak maximum. This has the advantage of being relatively quick but the disadvantage of missing components of the mixture that are not fully resolved by the GC column. The second approach is to look at each mass

spectrum in turn, in essence stacking up the mass spectra one behind the other and examining them individually. This has the advantage of completeness but the disadvantage of tedium. The third approach is to look at the intensity of one particular mass as a function of time.

This third approach makes use of the three-dimensional nature of GC-MS data. Two of these dimensions are the mass versus intensity of the normal mass spectrum; the third dimension is the GC retention time over which the mass spectral data are acquired. This idea is illustrated in Fig. 31.3. The x-axis represents GC retention time, the y-axis represents intensity, and the z-axis represents mass (or more properly, m/z ratios). As shown in Fig. 31.3, a mass spectrum is displayed in the y–z plane. Because a mass spectrum is scanned every 1 to 3 sec, it is also possible to examine the data in the x–y plane. This is a plot of the intensity of one selected mass as a function of time. This plot is called a mass chromatogram (6).

An example may make this concept clear. At the retention time marked with an asterisk in Fig. 31.3, the abbreviated mass spectrum extending into the foreground (the y–z plane) was observed. This happens to be the mass spectrum of methyl stearate; note the relatively abundant ion at m/z 298, which is this compound's molecular weight. The mass chromatogram of m/z 298 is shown in the x–y plane in Fig. 31.3. Note that this mass chromatogram shows one peak, which corresponds to the retention time of methyl stearate. In other words, of all the hundreds of mass spectra taken during this GC-MS experiment, m/z 298 is present in only a very few spectra. Only compounds with m/z 298 in their mass

Figure 31.3 A diagram demonstrating the three-dimensional nature of GC-MS data. The abbreviated mass spectrum extending onto the foreground is that of methyl stearate; the mass chromatogram of m/z 298 (the molecular ion of methyl stearate) is also shown.



spectra will show up in the mass chromatogram of that mass.

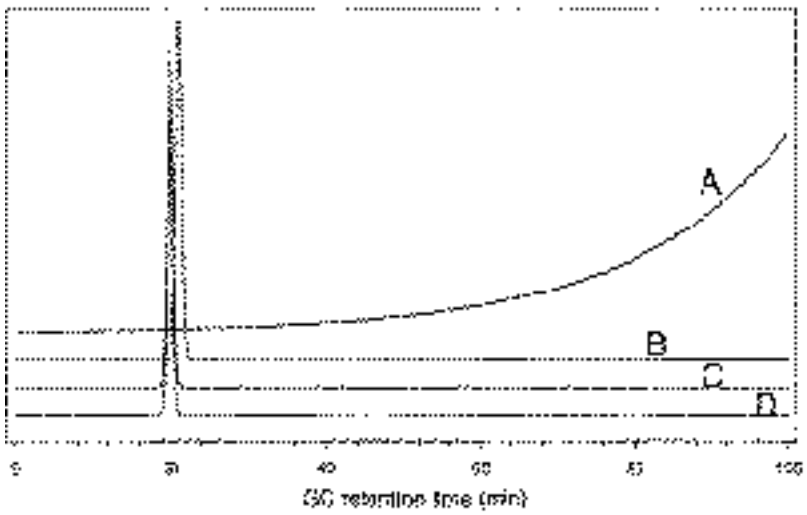
Mass chromatograms can be thought of as a very selective gas chromatographic detector, in this case, one that responds only to methyl stearate. Other compounds can be selectively detected by picking other masses. For example, m/z 320 would be a good mass to use for the selective detection of a tetrachlorodibenzo-p-dioxin because this is its molecular weight. If a mass that is present in the mass spectra of a class of compounds is selected, that compound class can be selectively detected. For example, m/z 149 is present in the mass spectra of alkyl phthalates for alkyl chain lengths greater than two carbon atoms. Thus, the mass chromatogram of m/z 149 would selectively show all the phthalates in a sample.

Mass chromatograms are also useful for determining whether a given mass belongs in a given mass spectrum. For example, if the liquid phase from a GC column is beginning to thermally decompose, all of the mass spectra taken during a GC-MS experiment with that column might show a moderately abundant ion at m/z 207. However, the mass chromatogram of m/z 207 will not show peaks because the source of this ion is bleeding continuously from the column and is not a discrete compound. In fact, the mass chromatogram of 207 will probably track the temperature program of the GC column; see trace (a) in Fig. 31.4. Using this approach, it is easy to distinguish between the ions that really belong in a given mass spectrum and those from background. By looking at sets of mass chromatograms, it is possible to determine whether various ions come from the same compound even if the compounds are not completely resolved by the GC column. If ions belong together (that is, they come from the same GC peak), the mass chromatograms for all these ions will be superimposable in time; see traces (c) and (d) in Fig. 31.4. In fact, these mass chromatograms should all have the same peak shape because they all came from the same GC peak. If the mass chromatograms are not superimposable in time or in shape, the corresponding ions are probably from different compounds, which may have come out of the GC column at slightly different retention times; compare trace (b) to traces (c) or (d) in Fig. 31.4.

Because GC-MS experiments are somewhat complicated, there is always a possibility for something to go wrong. To prevent this, stringent quality assurance procedures are necessary. The following is a nonexhaustive list of some of these problems and what can be done about them.

First, because the analyst is often working at ultra trace levels (a few nanograms, for example),

Figure 31.4 Hypothetical mass chromatograms of four masses. (a) A background ion from the GC column bleed (m/z 207, for example); (b) an ion from a later-eluting compound; (c, d) two ions from an earlier-eluting compound. Note the offset between (b) and (c) or (d).



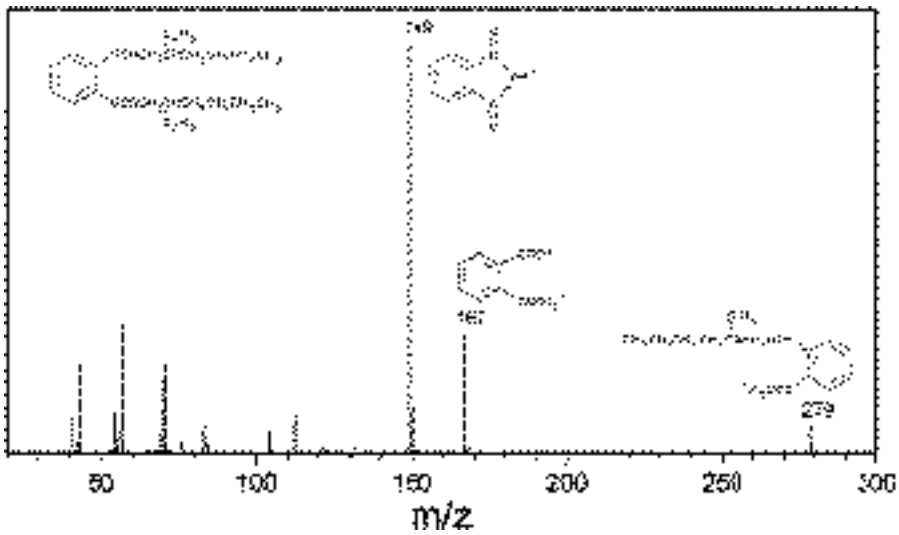
it is possible for a compound that was not originally in the sample to sneak in during the analytical procedure. In particular, sample contamination can come from solvents and glassware. The former problem can be prevented by using high-quality (and expensive) solvents, the latter by heating the glassware to 450 °C after solvent and acid washing. The most common contaminant is probably di(2-ethylhexyl)phthalate. Its mass spectrum is shown in Fig. 31.5. Note the important ions at m/z l49, l67, and 279. It pays to remember this spectrum; it was once published as that of a natural product (7). Phthalates are extremely common as plasticizers. They are particularly abundant (5 to 20%) in polyvinylchloride-based plastic products such as Tygon tubing.

Second, if components in the sample decompose before or after workup, the analyst will not obtain accurate results. Under these conditions, it is possible to identify (and quantitate) a compound that was not originally in the sample, or the analyte of interest could have vanished from the sample. Thus, both false positives and false negatives can result from sample decomposition. This can happen while the sample is waiting to be analyzed or during the analysis itself. For example, a GC injection port held at 250 to 300 °C can cause thermal decomposition of some compounds. One useful procedure is to add (or spike) the analyte into a sample at a known concentration. If there is a substantial loss of this compound or the suspicious formation of another compound, sample decomposition may be a problem.

Third, if the GC column or GC-MS interface is not working properly, the whole GC-MS experiment is in jeopardy. Cold spots are a common problem, as are catalytic surfaces that selectively remove some compounds from the GC gas stream. These problems can be identified using a mixture of standard compounds of varying polarities and acidities.

Fourth, either the mass spectrometer itself or the data system may not be working properly. In this case, incorrect isotope ratios, mass discrimination (ions at higher masses appear less abundant than they should), or mass assignment errors could occur. The key to identifying these problems is to run an overall mass spectrometer performance standard. The one recommended (mandated in many cases) by the EPA is decafluorotriphenylphosphine, the mass spectrum of which is shown in Fig. 31.6. This compound is a good standard. It is easy to run by GC-MS, and it has ions up to about m/z 450. The abun-

Figure 31.5 Mass spectrum of di(2-ethylhexyl)phthalate, a very common experimental contaminant. The structures of some ions are shown.



dances for the various ions, as required by the EPA, are published in the Federal Register (8) and in various other official EPA methods (9). These requirements change from time to time, but they are available from the EPA.

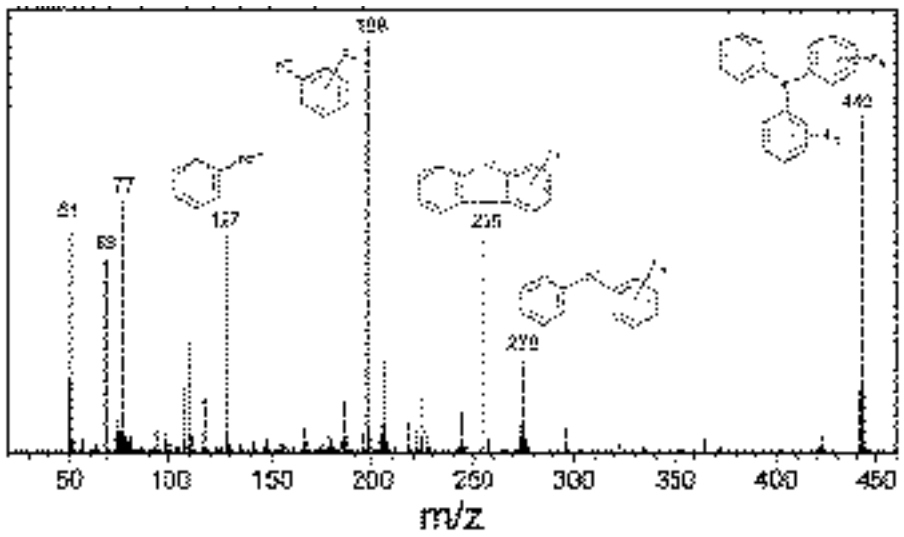
To ensure qualitative identification of an organic compound using GC-MS, several criteria should be met: First, the mass spectra of the unknown compound and of the authentic compound must agree over the entire mass range of the spectra. It is particularly important to compare the patterns within narrow mass ranges (for example, from m/z 50 to 60 in Fig. 31.5); these patterns should agree almost exactly. In this case, the spectrum of the authentic compound might come from a library of reference spectra or from the actual compound itself. In the latter case, the compound could be purchased or synthesized. Second, the GC retention times of the unknown compound and of the authentic compound must agree within about ±1 to 2 sec. It is often convenient to do this experiment by coinjecting the unknown mixture and the authentic compound. The GC peak in question should increase in size by the correct factor. Third, a compound cannot be considered fully identified in a mixture unless two other questions are addressed: Is the identification plausible? Why is it present in a given sample? If an identification is implausible or if there is no reason for a compound to be present in a sample, the identification could be wrong or the compound could be a contaminant.

Quantitative

GC-MS can also be used to measure the concentration of one or more analytes in a complex mixture. Quantitation can be based on peak areas from mass chromatograms or from selected ion monitoring. The latter requires more explanation (10).

With the selected ion monitoring technique, the mass spectrometer is not scanned over all masses; instead, the instrument jumps from one selected mass to another. The advantage of this approach is that the mass spectrometer spends much more time at a given mass, the signal-to-noise ratio at that mass

Figure 31.6 Mass spectrum of decafluorotriphenylphosphine, an EPA-mandated standard. The structures of some ions are shown.



improves, and the overall sensitivity of the experiment increases by a factor of 100 to 1000. For example, the mass spectrometer might integrate for 500 msec at mass m_1, jump to mass m_2 in 10 msec, integrate for 500 msec at that mass, and jump back to m_1 in 10 msec, repeating this cycle for the duration of the GC run. In essence, the intensities of the two masses are recorded as a function of GC retention time, with measurements made every 1.02 sec. In practice, rather than only two masses, 5 to 10 masses are usually monitored simultaneously for 100 to 200 msec each. In this manner, the GC-MS response of 5 to 10 compounds, depending on the specificity of the selected masses, can be measured.

The difference between mass chromatograms and selected ion monitoring is significant. With the latter technique, the responses from only a few preselected masses are recorded. With mass chromatograms, all of the masses are scanned; thus, no preselection is required. This is the necessary tradeoff for higher sensitivity. Clearly, the mass spectrum of the analyte must be known so that the masses that uniquely characterize it can be selected. This information can be obtained from the literature (or from a library of reference spectra) or from the laboratory. Each selected set of masses can be monitored for the duration of the complete GC run or for only selected GC retention times (often called time windows). Selected ion monitoring is almost fully software driven; thus, it is very flexible. Different sets of masses, different time windows, and different integration times can be easily set up.

To convert the peak areas to mass of analyte, whether from mass chromatograms or from selected ion monitoring, the peak areas must be calibrated. The two main strategies are based on external and internal standards. With external standards, the area of one or more mass chromatogram is calibrated with a known amount of the analyte injected into the GC-MS in a different experiment. Detection limits of a few nanograms can be achieved with this technique. However, the strategy that gives the most accurate quantitative results is the use of internal standards, which are known amounts of compounds added to the sample before isolation of the analytes begins. After sample extraction and cleanup, only the ratio of response between the analyte and the internal standard must be measured. This ratio multiplied by the amount of the internal standard gives the amount of the analyte injected into the GC-MS system. This can be converted to concentration using the correct dilution factors.

The best internal standards are chemically very similar to the analyte; thus, any losses of the analyte during the analytical procedure are duplicated by losses of the internal standard, so it is a self-correcting system. Homologues of the analyte can be used as internal standards, but the very best are isotopically labeled versions of the analyte. Using isotopically labeled standards and selected ion monitoring, it is possible to get sensitivities of less than 1 pg. Depending on the relationship of the internal standard to the analyte, the precision and accuracy of most analyses are improved by at least a factor of 2 to 3 over external calibration. The tradeoff is complexity and cost. Ideally, an internal standard for each analyte in a mixture should be used, and isotopic standards can sometimes cost several hundred dollars for a few milligrams.

Applications

The analysis of octachlorodibenzo-p-dioxin (OCDD) in sediment from Lake Ontario is a useful example of a quantitative measurement made with GC-MS. This example also demonstrates the use of isotopically labeled internal standards. These experiments were done in the author's laboratory using electron capture negative ionization, but the principle is the same regardless of the ionization technique selected.

OCDD has a molecular weight of 456 (usually called M). The mass spectrum of the unlabeled (native) compound is dominated by an ion cluster corresponding to M-Cl, the most intense peak of which

is m/z 423, which is the first isotope peak (the one containing one ^{37}Cl) in this cluster. The isotopic standard used for this experiment was per-^{37}Cl-labeled OCDD, which has a molecular weight of 472. Its M-Cl ion is at m/z 435. Because there is no ^{35}Cl in this molecule, there are no isotope peaks. Selected ion monitoring of m/z 423 and 435 was used for the measurements.

The first step in this procedure is to calibrate the internal standard against a known amount of native OCDD. This calibration results in a response factor relating the response of the native compound to the labeled compound. Standard solutions of each were prepared such that 1 pL had 85 pg of native and 40 pg of labeled OCDD. Injection of 1 μL of this standard gave the selected ion monitoring results shown in Fig. 31.7(a); the areas of the two peaks are given as values of *A* in the figure. The response factor is

$$\text{Response factor} = \frac{\text{Area}_{423}/\text{pg Native OCDD}}{\text{Area}_{435}/\text{pg Labeled OCDD}} \tag{31.1}$$

In this case, the response factor is (16,581/85 pg) divided by (24,073/40 pg), which is 0.32. This is what it should be, given equimolar responses of the two compounds and given their difference in isotopic composition.

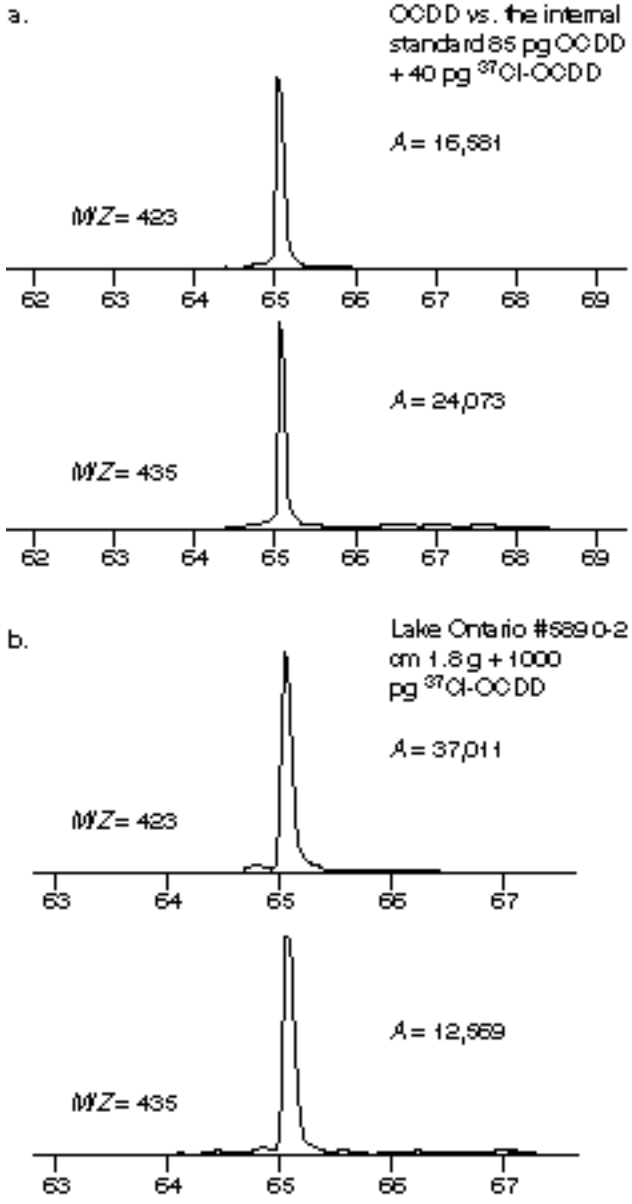
The selected ion monitoring data for an unknown sample are shown in Fig. 31.7(b). In this case, 1000 pg of the labeled OCDD was added to 1.8 g (dry weight) of Lake Ontario sediment before extraction. After extensive cleanup on silica and alumina, two clean GC-MS peaks were obtained with the areas shown in the figure. The calculation of the final concentration of OCDD in the sediment proceeds as follows: First, the amount of the internal standard in the sediment is divided by the weight of dry sediment; in this case, 1000 pg/1.8 g = 560 pg/g. Second, a corrected area of the native OCDD is calculated by dividing the area of this peak by the response factor; in this case, this corrected area is 37,011/0.32 = 115,660. (Note that these areas have consistent but arbitrary units.) Third, the ratio of the native and labeled areas is 115,660/12,569 = 9.2. This is the factor by which the concentration of native OCDD exceeds the labeled compound. Fourth, the concentration of the labeled material is multiplied by this factor to obtain a final concentration of native OCDD in the sediment. This value is 9.2 × 560 pg/g = 5100 pg/g. This measurement was part of a larger study that indicated that the major source of polychlorinated dibenzo-p-dioxins and dibenzofurans to the atmosphere was the combustion of municipal or chemical waste rather than coal (11).

A qualitative application has been selected from the work of a colleague in the author's department who has been carrying out studies of the electrochemical reduction of phthalide (12); see Fig. 31.8 for all structures and data. Dimethylformamide was the solvent and tetra-n-butylammonium perchlorate was the supporting electrolyte. Products derived from the electrolysis were analyzed by GC-MS, and the gas chromatogram and mass spectra shown in Fig. 31.8 were obtained. The peak at 18.16 min was due to phthalide, and the peak at 18.34 min was due to n-tetradecane, which had been added in a known amount as an internal standard for purposes of quantitating the products. The identities of the other two peaks were not immediately clear.

The mass spectrum of the peak at 15.21 min (see Fig. 31.8, middle) indicates that this compound has a molecular weight of 185 Da; because this is an odd number, this compound probably has at least one nitrogen atom. There is a substantial loss of 43 Da to give the ion at m/z 142, and there is also a major loss of 42 Da to give the ion at m/z 100. These two losses suggest the presence of at least two C_3H_7 moieties. Remembering that the supporting electrolyte was tetra-n-butylammonium perchlorate, it was reasonable to suggest that this peak was tri-n-butylamine. This assignment was verified by comparison of this mass spectrum and gas chromatographic retention time with those of authentic material. The structures of the ions at m/z 100, 142, and 185 are suggested on the mass spectrum; however, it is important to remember that these ion structures are a result of the interpretation of the spectrum and are not produced by the data system.

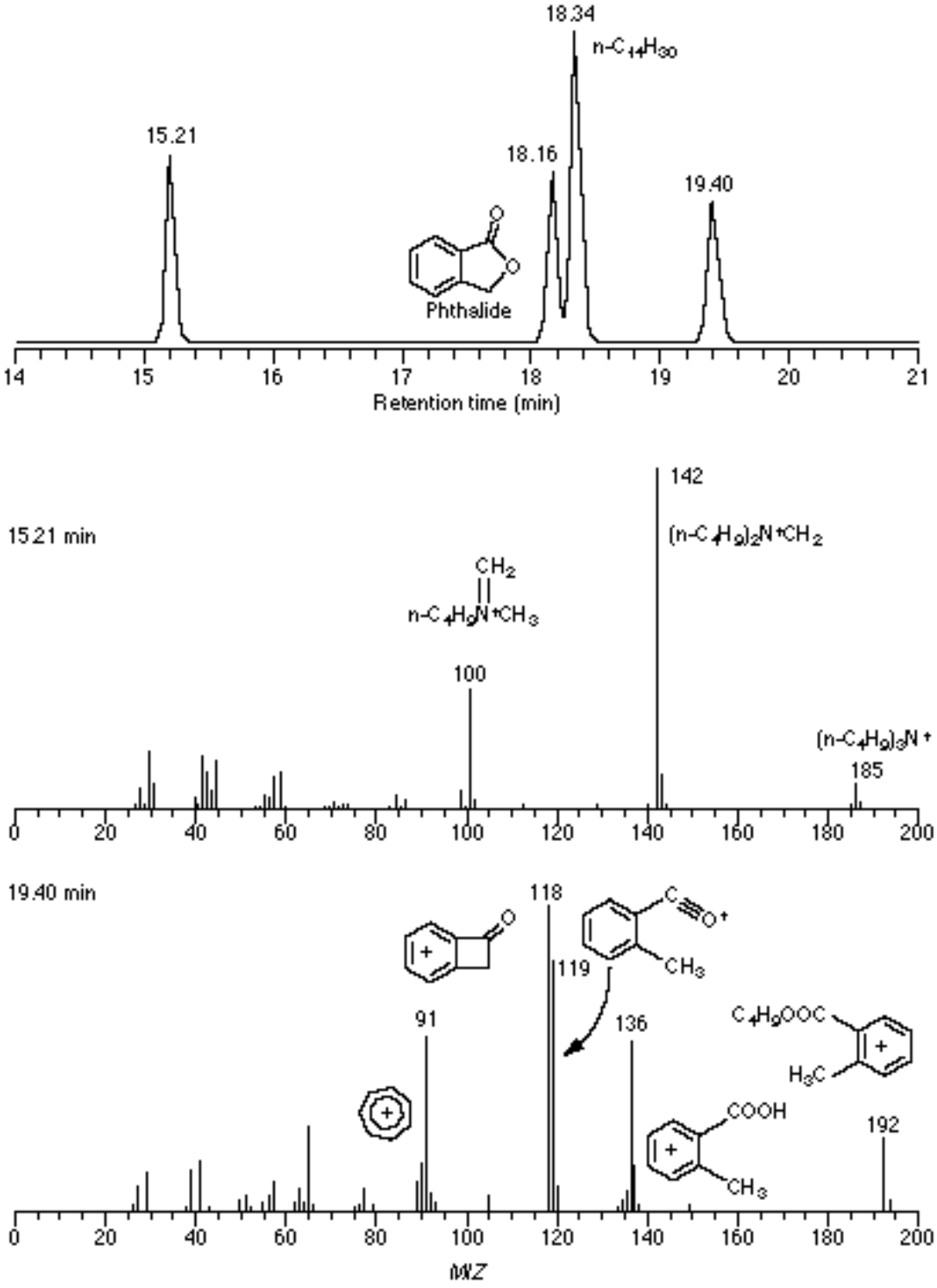
The mass spectrum of the peak at 19.40 min (see Fig. 31.8, bottom) indicates that this compound

Figure 31.7 Selected ion monitoring records of m/z 423 (from native octachlorodibenzo-p-dioxin) and m/z 435 (from $^{37}Cl_8$-octachlorodibenzo-p-dioxin) showing quantitation by the isotopic internal standard method. Only the gas chromatographic retention time range between 62 and 69 min is shown. The values of *A* are the areas of the peaks, in arbitrary units.



has a molecular weight of 192 Da. There was a substantial loss of 56 Da to give the ion at m/z 136. The ion at m/z 91 was probably a tropylium ion ($C_7H_7^+$), and this suggested that this molecule was derived from phthalide. The meaning of the ions at m/z 118 and 119 was not obvious, but the difference between 91 and 119 suggested the presence of a carbonyl group (28 Da) in this molecule. Putting these ideas together and noting the components of the reaction mixture, it was hypothesized that this compound was butyl 2-methylbenzoate. Again, the structures of the major ions are suggested on the mass

Figure 31.8 Gas chromatogram of a reaction mixture from the electrochemical reduction of phthalide. The solvent was dimethylformamide and the supporting electrolyte was tetra-n-butylammonium perchlorate. The two compounds at 15.21 and 19.40 min were unknown; their mass spectra are shown.



spectrum. The validity of the identification of this GC peak was verified by comparison of the mass spectrum and gas chromatographic retention time with those of the authentic compound. Apparently, this compound results from a reaction between an electrogenerated intermediate (radical anion of phthalide) and the tetra-n-butylammonium cation (of the supporting electrolyte). Moreover, there is reason to believe that the process leading to butyl 2-methylbenzoate actually occurs in the heated injection port of the gas chromatograph.

Nuts and Bolts

Relative Costs

Although in principle GC-MS experiments can be performed on magnetic sector instruments, in practice almost all GC-MS today is done on quadrupole or ion trap instruments. These instruments are relatively inexpensive and are simple to control by a computer. The major factor influencing the cost of a quadrupole- or ion-trap–based GC-MS system is the ionization methods available on the instrument and the mass range of the mass spectrometer. Simple quadrupole or ion trap instruments that use only electron impact ionization and have a mass range of 20 to 700 cost about $50,000. Those capable of both positive and negative chemical ionization and with mass ranges of 20 to 2000 cost about $200,000. Operating costs include instrument maintenance, GC carrier gases and columns, and spare parts. In most laboratories, these costs are about 5% of the instrument cost per year.

Vendors for Instruments and Accessories

The following list is not exhaustive; several smaller companies enter the field each year and several leave. The following are some of the larger companies that deal with complete GC-MS systems. See Chapter 28 (p. 564) for a detailed list of vendors, including addresses for the following vendors.

The Finnigan Corp. (San Jose, CA) sells several instruments, some based on traditional quadrupole technology, some based on ion trap technology, some based on triple quadrupole technology, and a few based on magnetic sector technology. Various ionization methods are available.

Micromass UK (Manchester, U.K.) also has a wide range of instruments available with a wide range of ionization methods. Some are quadrupole based; some are magnetic sector based.

The Hewlett-Packard Corp. (Palo Alto, CA) markets several quadrupole-based instruments ranging from small benchtop instruments designed for the chromatographer to versatile, stand-alone instruments that can accommodate both gas and liquid chromatographic inlet systems.

Varian Associates, Inc. (Walnut Creek, CA) sells ion-trap–based GC-MS systems. These are typically small instruments with a good price-to-performance ratio.

With all of these companies, the recent trend has been to produce smaller and smaller instruments. Thirty years ago a GC-MS system with its data system occupied a whole room (or even two). Now these systems fit on the top of a small bench or table. Not only does this trend save space, but it saves manufacturing costs, some of which are passed on to the instrument purchaser. These smaller instruments

also have fewer parts, making them less costly to maintain. There is also a trend toward increasing automation. Modern instruments are often equipped with an automatic injection system, and once filled with samples, the data system can control all functions of the instrument including sample introduction. Thus, once a trained person has developed the methodology and set up the data system, the instrument will almost run itself.

Required Level of Training

The required level of training and expertise varies as a function of the level of data interpretation. At the simplest level, because of the computer interface, most GC-MS instruments can be operated by people with no formal training in mass spectrometry or chemistry. A high school education is often sufficient. Maintenance of the instrument requires some mechanical and electronic skills, but again no formal training in mass spectrometry or chemistry is needed. For interpretation of the data, some chemistry training is needed, particularly organic chemistry. Many graduates of high-quality undergraduate programs in chemistry and most graduates of graduate programs in analytical or organic chemistry acquire these skills through their course work. Given a normal undergraduate course sequence in organic chemistry, most technically trained people can acquire specific training in mass spectrometry through 1- to 2-week courses offered through professional societies (such as the American Chemical Society or the American Society for Mass Spectrometry).

Service and Maintenance

Unlike most other spectrometers, in which radiation is passed through the sample, with mass spectrometry, the sample is inserted directly into the instrument. Thus, these instruments require more care than most others. The analyst should expect to clean the ion source every 2 to 4 mo and change the GC column every 3 to 6 mo. The instrument is electronically complex but highly modularized; thus, most electronic failures are corrected by replacement of a printed circuit board. The data systems are relatively hardy and are furnished with diagnostic software for both the computer and the mass spectrometer. Downtime for most modern instruments should be less than 5 to 10%, and maintenance costs should be less than 5% of the instrument cost per year.

Suggested Readings

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MCLAFFERTY, FRED W., AND FRANTISEK TURECEK, *Interpretation of Mass Spectra*, 4th ed. Mill Valley, CA: University Science Books, 1993.

NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY, *NIST/EPA/NIH Mass Spectral Library for Windows™* (61.4 megabytes). Gaithersburg, MD: NIST Standard Reference Data, 1995.

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