CHAPTER 6

Chromatography

Oh, no, you forgot to put the cap on your ballpoint pen, so you now have a big inkspot on the pocket of your best shirt as a "reward" for your negligence. You dab it with a wet cloth, only to see the spot grow even larger. New colors seem to be appearing as the spot widens. Congratulations! You've just performed a form of chromatography in which you have crudely separated some of the dyes that comprise the ink. In this chapter, we'll be learning some of the basic chromatographic techniques that are used to separate mixtures by taking advantage of the differential distribution of the individual compounds between two immiscible phases—in the case of your shirt, these were its fibers and the water you used in an effort to remove the stain. These techniques are now so powerful and sophisticated that even enantiomers (Sec. 7.1) may be separated efficiently by such means.

6.1 INTRODUCTION

We described the common laboratory techniques of recrystallization, distillation, and extraction for purifying organic compounds in Chapters 3, 4, and 5. In many cases, however, the mixtures of products obtained from chemical reactions do not lend themselves to ready separation by any of these techniques because the physical properties of the individual components are too similar. Fortunately, there are a number of chromatographic procedures available that we can use to effect the desired purification, and some of them are described in this chapter.

The word **chromatography** was first used to describe the colored bands observed when a solution containing plant pigments is passed through a glass column containing an adsorbent packing material. From that origin, the term now encompasses a variety of separation techniques that are widely used for analytical and preparative purposes.

All methods of chromatography operate on the principle that the components of a mixture will distribute unequally between two immiscible phases, which is also the basis for separations by extraction (Chap. 5). The **mobile phase** is generally a liquid or a gas that flows continuously over the fixed **stationary phase,** which may be a solid or a liquid. The individual components of the mixture have different affinities for the mobile and stationary phases, so a dynamic equilibrium is established in which each component is selectively, but temporarily, removed from the mobile phase by binding to the stationary phase. When the equilibrium concentration of that substance in the mobile phase decreases, it is released from the stationary phase and the process continues. Since each component partitions between the two

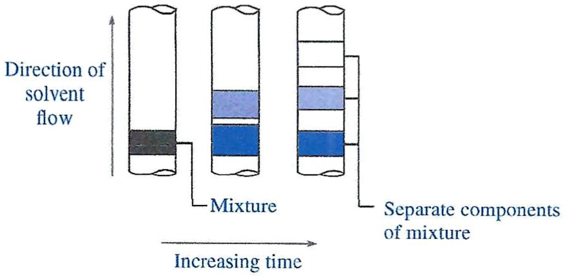


Figure 6.1
Separation of mixture by chromatography.

phases with a different equilibrium constant or **partition coefficient,** the components divide into separate regions termed **migratory bands** (Fig. 6.1). *The component that interacts with or binds more strongly to the stationary phase moves more slowly in the direction of the flow of the mobile phase.* The attractive forces that are involved in this selective adsorption are the same forces that cause attractive interactions between any two molecules: electrostatic and dipole-dipole interactions, hydrogen bonding, complexation, and van der Waals forces.

The chromatographic methods used by modern chemists to identify and/or purify components of a mixture are characterized by the nature of the mobile and stationary phases. For example, the techniques of **thin-layer** (TLC), **column,** and **high-performance liquid chromatography** (HPLC) each involve *liquid-solid* phase interactions. **Gas-liquid partition chromatography** (GLC), also known as **gas chromatography** (GC), involves distributions between a mobile *gas* phase and a stationary *liquid* phase coated on a solid support. These important techniques can be used as tools to analyze and identify the components in a mixture as well as to separate the mixture into its pure components for preparative purposes. Although there are other chromatographic techniques, such as ion exchange and paper chromatography, a review of those methods is beyond the scope of this discussion.

6.2 THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) is a form of **solid-liquid adsorption** chromatography and is an important technique in organic chemistry for rapid analysis of small quantities of samples, sometimes as little as 10^{-9} g. Thus, TLC is frequently used to monitor the progress of reactions and of preparative column chromatographic separations as well as to determine the optimal combinations of solvent and adsorbent for such separations (Sec. 6.3). An important limitation to TLC, and to column chromatography as well, is that it cannot be used on volatile compounds having boiling points below about 150 °C (760 torr).

To execute a TLC analysis, a *small* amount of the sample to be analyzed, or a solution of it, is first applied to a solid **adsorbent** bound to a rectangular glass or plastic plate (Fig. 6.2a). The adsorbent serves as the stationary phase. Next, the plate, with its spotted end down, is placed in a closed jar, called a **developing chamber** (Fig. 6.3). The chamber contains a *saturated atmosphere* of a suitable **eluant** or **eluting solvent,** which is the mobile phase and may be comprised of either a single solvent or mixture of two or more. A folded filter paper is often used to help maintain solvent equilibration in the chamber. It is important that the level of solvent in the chamber be *below* the location of the spot on the TLC plate. Otherwise, the sample

6.4 GAS-LIQUID CHROMATOGRAPHY

Gas-liquid chromatography (GLC), which is also called **gas chromatography** (GC), is a technique that may be used to separate mixtures of volatile compounds whose boiling points may differ by as little as 0.5 °C. It can also be applied as an analytical tool to identify the components of a mixture or in preparative applications when quantities of the pure components are desired.

Basic Principles of Gas-Liquid Chromatography

Gas-liquid chromatography operates on the principle of partitioning the components of a mixture between a mobile gaseous phase and a stationary liquid phase. In practice, a sample is injected into a heated chamber where it is immediately vaporized and carried through a column by a flowing inert gas such as helium or nitrogen, which is called the **carrier gas.** This gaseous mixture is the **mobile phase.** The column is packed with a finely divided solid support that has been coated with a viscous, high-boiling liquid, which serves as the **stationary phase.** As the mobile phase moves through the column, its components are continuously partitioned between the two phases. Those components that show a higher affinity for the mobile phase move through the column more quickly, whereas those with a stronger attraction to the stationary phase migrate more slowly, and separation occurs. As with a fractional distillation column (Sec. 4.4), a GLC column may be characterized by the number of theoretical plates it provides. GLC columns typically have many more theoretical plates, however, so they can effect separations that would be impossible using fractional distillation.

The **retention time** of a component is the elapsed time required for the compound to pass from the point of injection to the detector, and it may be used for purposes of identification. The retention time of a component is *independent* of the presence or absence of other components in the sample mixture. There are four experimental factors that influence retention time of a compound: (1) the *nature* of the stationary phase, (2) the *length* of the column, (3) the *temperature* of the column, and (4) the *flowrate* of the inert carrier gas. Thus, for a particular column, temperature, and flowrate, the retention time will be the same for a specific compound.

Although a large number of **stationary liquid phases** are available, only a few are widely used (Table 6.1). Each liquid phase has a maximum temperature limit above which it cannot be used. This temperature depends upon the stability and

Table 6.1 *Common Stationary Phases for Gas-Liquid Chromatography*

Liquid Phase	*Type*	*Property*	*Maximum Temperature Limit, °C*
Carbowax 20M	Hydrocarbon wax	Polar	250
OV-17	Methylphenyl silicone	Intermediate polarity	300
QF-1	Fluorosilicone	Intermediate polarity	250
SE-30	Silicone gum rubber	Nonpolar	375

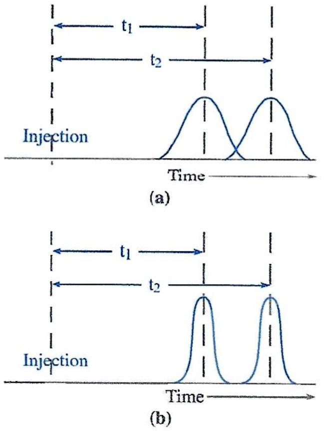


Figure 6.12
Effect of bandwidth on resolution. (a) Wide bands. (b) Narrow bands.

volatility of the liquid phase; at higher temperatures, the liquid phase will vaporize and "bleed" from the column with the mobile phase.

The differences in the *partition coefficients* (Secs. 5.2 and 5.3) of the individual components of a mixture in GLC depend primarily upon the differences in solubility of each of the components in the liquid phase. Two important factors determining the solubility of a gas in a liquid are its vapor pressure at the **ambient temperature,** which is the temperature of the column, and the magnitude of its interactions with the liquid phase. Regarding the first factor, the solubility of a gas in a liquid decreases as its vapor pressure increases. This means the more volatile components of a gaseous mixture tend to pass through the column more rapidly and elute before the less volatile ones. The impact of the second factor is understandable from the principle that *like dissolves like* (Sec. 3.2). Polar samples are most effectively separated by using a polar liquid phase, whereas nonpolar compounds are best separated using a nonpolar liquid phase.

The stationary liquid phase is normally coated as a thin film on an inert solid support. The support is composed of small, uniformly meshed granules, so that a large surface area of the liquid phase is available for contact with the vapor phase to ensure efficient separation. Some common types of solid supports include Chromosorb P and Chromosorb W, which are composed of diatomaceous earth. The surface areas of these supports vary from 1 m^2/g to 6 m^2/g. Columns are now commercially available with a wide variety of liquid phases on different solid supports. An alternative method of supporting the liquid phase is used in capillary columns. In these columns, the liquid is coated directly onto the inner walls of the tubing. These columns are highly efficient but relatively expensive.

In general, the efficiency, or **resolution,** of a column increases with increasing length and decreasing diameter. Increasing the pathlength increases the difference in retention times between bands, whereas decreasing the diameter of the column gives rise to narrower bands. With a small band separation, as measured from the band centers, wide bands are more likely to overlap (Fig. 6.12a) than narrow bands (Fig. 6.12b).

The two other experimental factors that may be varied to alter the degree of separation of the bands are the *temperature* at which the column is maintained and the *flowrate* of the carrier gas. Increasing the temperature results in shorter retention times, because the solubility of gases in liquids decreases with increasing temperature. The partition coefficients are affected, and the bands move through the column at a faster rate. Higher flowrates also cause retention times to decrease. In spite of the decreased resolution and band separation obtained at higher temperatures and flowrates, these conditions are sometimes necessary for substances that would otherwise have very long retention times.

Instrumentation

All commercially available gas-liquid chromatographs (GLCs) have a number of basic features in common. These are illustrated schematically in Figure 6.13. Parts **1–5** are associated with supplying the dry **carrier gas,** usually helium or nitrogen, and allowing an operator to control its flow. The mixture to be separated is injected using a gas-tight syringe through a septum into the **injection block (6)**, an individually heated chamber in which the sample is immediately vaporized. The sample then enters the flowing stream of carrier gas and is swept into and through the **column (7)**, which is located in an **oven (8)** and consists of coiled aluminum, stainless steel, or glass tubing containing an appropriate stationary phase. In the column, the individual components separate into bands that ultimately pass through

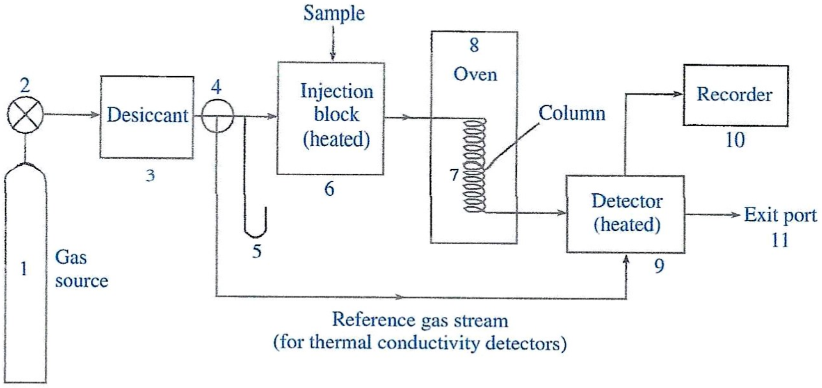


Figure 6.13
Schematic diagram of apparatus for GLC.

a **detector (9)**, producing a signal whose voltage is proportional to the amount of material other than carrier gas present in the mobile phase. One type of detector that is commonly used is the **thermal conductivity detector** (TCD), which operates on the basis of differences in the thermal conductivity of the mobile phase as a function of its composition. A **flame ionization detector** (FID) is much more sensitive and functions by detecting the number of ions produced by passing the mobile phase through a hydrogen flame. The **recorder (10)** plots the changes in voltage measured by the detector as a function of time to give the **gas chromatogram.** The vapors then pass from the detector into either the atmosphere or a collection device at the **exit port (11)**.

The GLCs available in the introductory organic laboratory often can only be operated at a constant oven temperature, the so-called **isothermal** mode of operation. Some GLCs, however, have a temperature-programming option, which allows the temperature of the oven, and consequently that of the column, to be varied over a range of temperatures. This is particularly useful when the sample being analyzed contains components having widely varying boiling points. Thus, the oven temperature may be held at a constant temperature for a specified period of time at the beginning of an analysis but then may be increased to higher temperatures as a function of time. For example, the temperature program illustrated in Figure 6.14 involved an initial temperature of 100 °C. After 5 minutes, the temperature was ramped up to 125 °C at a rate of 5 °C/min, held at that temperature for 5 additional minutes, and then further ramped up at a rate of 10 °C/min to a final temperature of 175 °C for completing the analysis. The programming option allows the higher-boiling components of the mixture to elute in a reasonable period of time because rates of elution increase with increasing temperatures. If the analysis had been performed at the higher temperature, the lower-boiling components might have eluted too quickly to be separated from each other.

Another modification of a GLC involves its direct connection to a mass spectrometer (MS) to produce a hybrid instrument commonly called a **GC-MS**. This combination provides a powerful analytical technique because the GC-MS combines the separating power of GLC with the ability of mass spectrometry (Sec. 8.5) to determine molar masses using very small amounts of material (see the Historical Highlight *Who's Taking What? Analysis of Biological Fluids for Illegal Substances*, which is available online). Thus, a GC-MS allows the mass spectrum of each component of a mixture of volatile compounds to be obtained as each individual

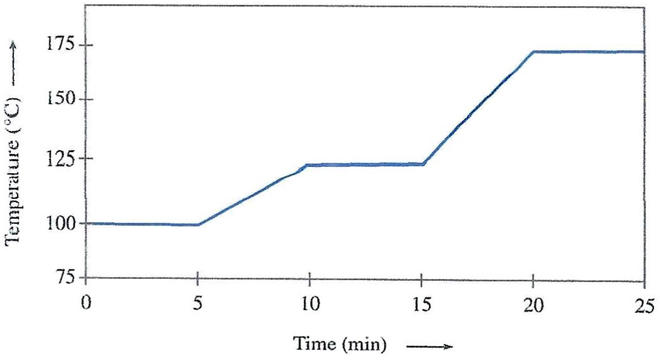


Figure 6.14
Example of temperature versus time program for a GLC analysis.

component emerges from the GLC. If the components of a mixture are known, as they would be if you were analyzing a fraction obtained by normal-phase column chromatography of a mixture of benzyl alcohol (7) and methyl benzoate (8) (Optional Discovery Experiment, Sec. 6.3), you could use the information provided by the mass spectrum, as discussed below, to confirm the identity and order of elution from the chromatographic column of each of these colorless compounds. If the components are unknown, on the other hand, knowledge of the molar mass of each compound, coupled with information about the origin of the mixture being analyzed, may allow assignment of structures to the unknown substances. Other spectral data (Chap. 8) are generally required before structural assignments can be made, however.

A partial schematic of a GC-MS is shown in Figure 6.15; the elements of the GLC that precede the column have been omitted but are the same as those shown in Figure 6.13. By comparing Figures 6.13 and 6.15, you see that a GC-MS has the outlet of the column (1) leading to a **stream splitter** (2), which sends part of the eluant to a **detector** (3), usually an FID, and part of it to a **mass spectrometer** (4); **recorders** (5, 6) provide the necessary records of when components are eluting from the GLC and what their mass spectra are. Both the GLC and the MS units are vented to the atmosphere (7).

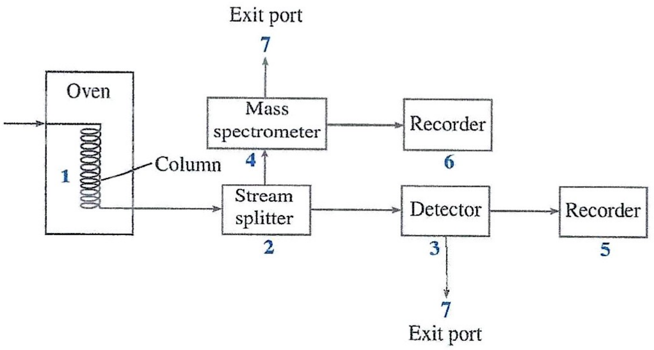


Figure 6.15
Partial schematic diagram of a GC-MS.

Experimental Techniques

Qualitative Analysis. The *retention time* of a pure compound is constant under a specified set of experimental conditions, including the column, temperature, and flowrate. Consequently, this property may be used as a *first* step to identify an unknown compound or the individual components in a mixture. In a typical experiment, an unknown compound or mixture is injected into the injection port of a GLC, and the retention time(s) of the component(s) is (are) measured. A series of known samples are then injected under the same conditions. Comparison of the retention times of the standard samples with those of the unknown allows a preliminary identification of the component(s) of the unknown. A convenient way of confirming that the retention times of a standard and the unknown are the same involves injecting a sample prepared by combining equal amounts of the two. If a *single* peak is observed in the chromatogram, the *retention times* of the standard and the unknown are identical. However, observation of the same retention time for a known and an unknown substance is a *necessary but not sufficient* condition to establish identity, because it *is* possible for two different compounds to have the same retention time. Independent confirmation of the identity of the unknown by spectral (Chap. 8) or other means is imperative.

An example of the use of GLC as a qualitative, analytical tool is illustrated in Figure 6.16. These sets of peaks represent a gas chromatographic separation of the distillation fractions of a mixture of cyclohexane and toluene similar to those obtained in the distillation experiment described in Section 4.3. The notations A,

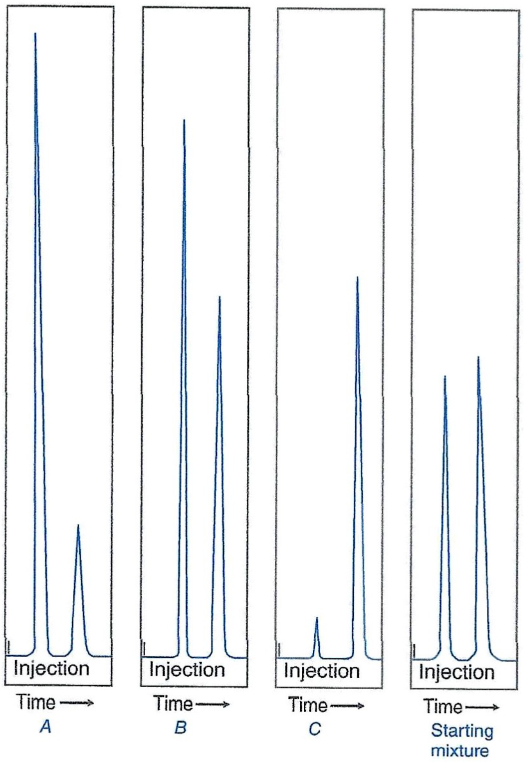


Figure 6.16
GLC analysis of the distillation fractions from the distillation experiment in Section 4.3.

B, and C refer to the three fractions taken in that experiment. The individual peaks in the mixture may be identified by comparing their retention times with those of pure cyclohexane and pure toluene; the peak with the shorter retention time in the mixture is cyclohexane, whereas the peak with the longer retention time is toluene.

The GC-MS analysis of a mixture of benzyl alcohol (**7**, bp 215.3 °C, 760 torr) and methyl benzoate (**8**, bp 199.6 °C, 760 torr) is provided in Figure 6.17. Figure 6.17a is the GLC trace of the mixture. Because the GLC column being used separates mixtures on the basis of the relative boiling points of its components, Peak A, which emerges first, should be **8**, whereas Peak B should be **7**. This could be confirmed with a GLC if the retention times of **7** and **8** were determined separately. Such additional analyses would not be necessary with GC-MS, however, because the masses of the two compounds are different; that of **7** is 108.14 atomic mass units (amu), and that of **8** is 136.15 amu. Figures 6.17b and 6.17c are the mass spectra corresponding to Peaks A and B, respectively. In Figure 6.17b we see that the molecular ion is at *m/z* 108, whereas it is at *m/z* 136 in Figure 6.17c. This proves that Peaks A and B are benzyl alcohol and methyl benzoate, respectively; our expectation of the reverse order of elution, based on considering boiling points, has been proven wrong. The stationary phase of the column must interact more strongly than expected with **8**. In any case, the GC-MS analysis has provided unambiguous identification of the two peaks through a *single* analysis of the mixture. Moreover, integration of the peaks in the GLC tract would provide a quantitative measure of their relative proportions in the mixture being analyzed.

A feature that may complicate the interpretation of the mass spectrum from a GC-MS analysis may be seen in Figure 6.17c. This feature is a small peak at *m/z* 146, a value that is not associated with either of the components contained in our known mixture of **7** and **8**. Although the substance that is the source of the peak could be an impurity in our sample, it is not. Rather, it is a peak produced from the "stationary" phase of the GLC column being used; the temperature at which the GLC was operating caused a small amount of the high-boiling material comprising this phase to "bleed" from the column into the MS, which results in minor peaks appearing in the spectrum. Such peaks can be confusing if you are not prepared for their possible presence.

A second example illustrating the power of an analysis by GC-MS rather than GLC alone is that of a mixture of methyl benzoate (**8**) and diethyl malonate (**12**), as seen in Figure 6.18. The boiling point of **12** (199.3 °C, 760 torr) is essentially identical to that of **8**, and considering the differing polarities expected for these two esters, predicting their order of elution from a GLC column (Fig. 6.18a) is problematic. However, the mass spectra associated with the two peaks eluting at 150 and 164 seconds in the GLC trace show that Peak A must be diethyl malonate (Fig. 6.18b), whereas Peak B is methyl benzoate (Fig. 6.18c).

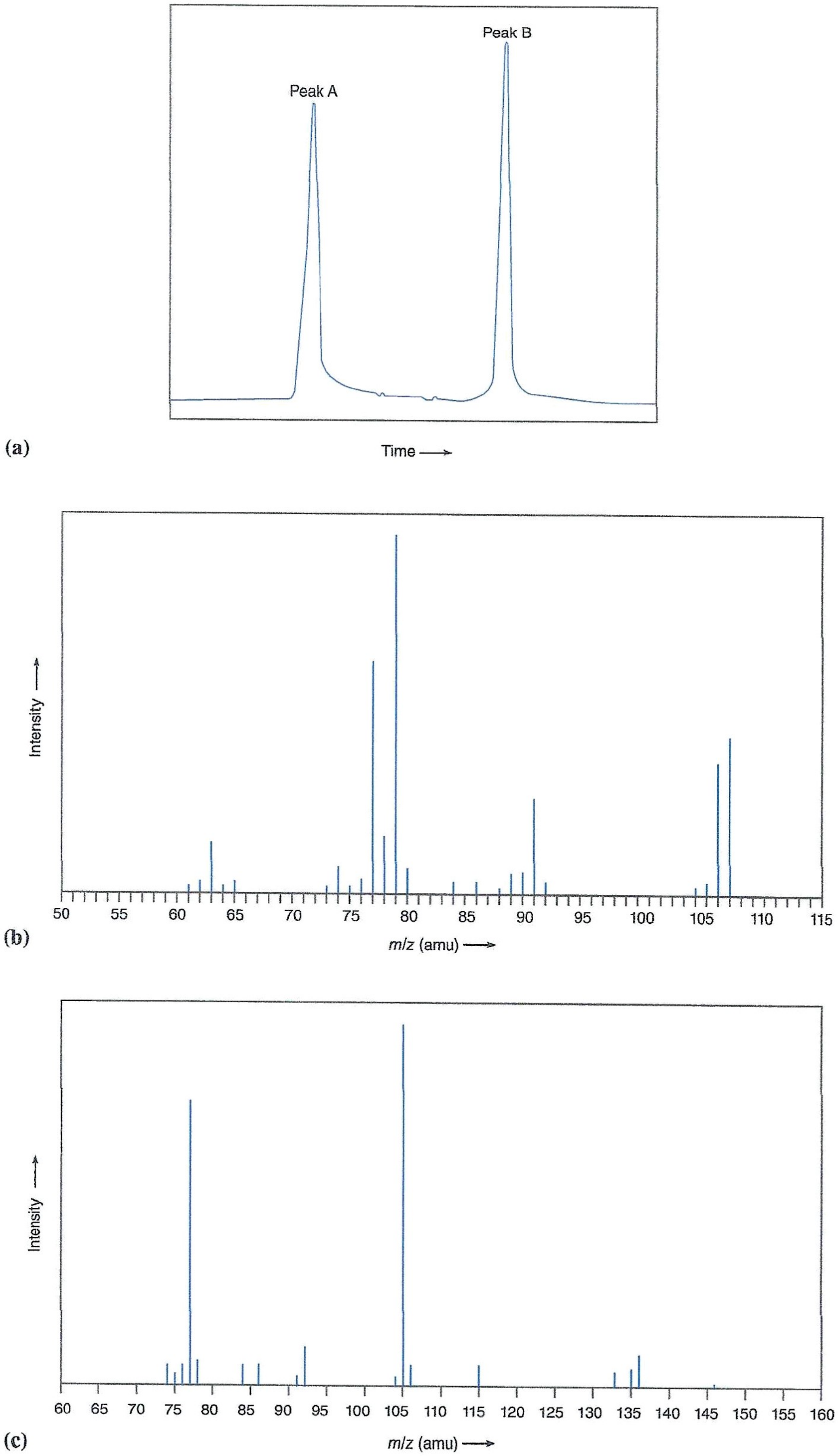


Figure 6.17
GC-MS analysis of a mixture of benzyl alcohol and methyl benzoate. (a) GLC trace. Column and conditions: 0.5-mm × 15-m, 0.25-μ film of BPX5 (DB-5); initial column temperature: 50 °C (2.0 min); ramp rate: 15 °C/min; final column temperature: 260 °C (30 min); flowrate: 1.5 mL/min. (b) MS spectrum of Peak A. (c) MS spectrum of Peak B.

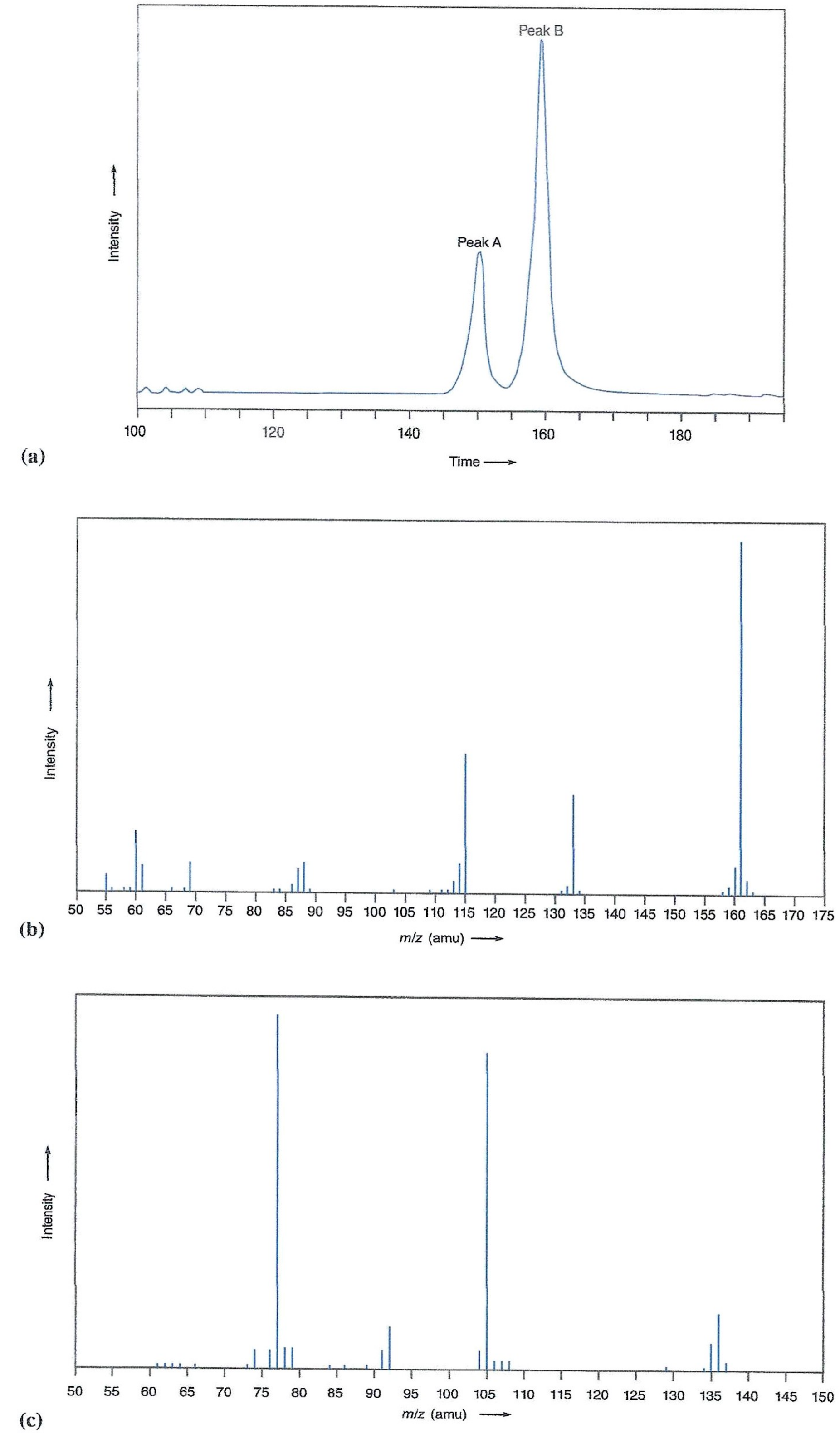
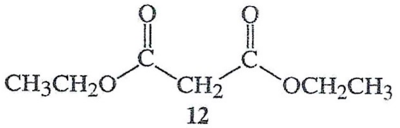


Figure 6.18
GC-MS analysis of a mixture of methyl benzoate and diethyl malonate. (a) GLC trace. Column and conditions: 0.5-mm × 15-m, 0.25-μ film of BPX5 (DB-5); initial column temperature: 50 °C (2.0 min); ramp rate: 15° C/min; final column temperature: 260 °C (30 min); flowrate: 1.5 mL/min. (b) MS spectrum of Peak A. (c) MS spectrum of Peak B.

You may be perplexed by the fact that the molecular ion from **12** appears to have *m*/*z* 161 rather than the expected value of 160.17. This could be the result of poor calibration of the instrument, so that the *m*/*z* values are inaccurate; proper calibration of the instrument is critical for proper interpretation of GC-MS data. The actual explanation in this case, though, is that diethyl malonate apparently has a propensity to be protonated under the conditions of the mass spectrometric analysis, so it is this ion rather than the molecular ion that is being detected. This is yet another phenomenon that can make interpretation of GC-MS data difficult for a beginner. In any event, a single GC-MS analysis of the mixture of **8** and **12** allows unambiguous determination of the order of elution from the GLC column of these esters, just as we saw in the analysis of the mixture of **7** and **8.**


$CH_3CH_2OC(=O)CH_2C(=O)OCH_2CH_3$

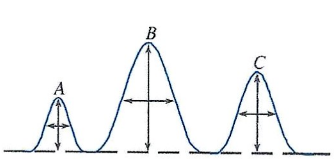
12

Diethyl malonate

Quantitative Analysis. The voltage output of the detector is related to the mole fraction of the material being detected in the vapor, so there is a correlation between the *relative areas* under the peaks in the chromatogram and the *relative amounts* of each of the components in the mixture. The quantitative evaluation of the chromatogram thus requires reliable methods for determining these peak areas.

An **electronic integrator,** which measures the intensity of detector output as a function of time, is the most accurate method for determining peak areas. However, these devices are expensive, so they are usually found only in research laboratories. Since the thickness and density of chart paper are reasonably uniform, another means of determining the relative areas involves carefully cutting the peaks out with scissors; the peak areas are then assumed to be proportional to their weight, as measured on an analytical balance. The original chromatogram should be saved as a permanent record, so the peaks should be cut from a photocopy of the chromatogram.

If the peaks are symmetrical, as are those shown in Figure 6.16, the areas may be approximated by assuming them to be equilateral triangles. The area of a symmetrical peak is then determined by multiplying the width of the peak at its half-height times its height. The percentage of each component in the mixture may be computed as the area of the peak corresponding to that component, expressed as a percentage of the sum of the areas of all peaks in the chromatogram. A sample calculation of this type is shown in Figure 6.19.



Areas: $A = 17 \times 8 = 136 \text{ mm}^2$

$B = 34 \times 17 = 578$

$C = 25 \times 12 = 300$

Total area $= 1014 \text{ mm}^2$

$\%A = \frac{136}{1014} \times 100 = 13.4\%$

$\%B = \frac{578}{1014} \times 100 = 57.0\%$

$\%C = \frac{300}{1014} \times 100 = 29.6\%$

100%

Figure 6.19
Determination of percentage composition of a mixture by GLC.

Although the peak areas are related to the mole fraction of the component in the mobile phase, they are not *quantitatively* related because the response of the detector varies with the class of the compound. Not all compounds have the same thermal conductivity (TCD), nor do they ionize in a hydrogen flame to form the same types or number of ions (FID). Thus, it is necessary to *correct* the measured areas in the chromatogram using the appropriate **response factor** to obtain an accurate quantitative analysis of the mixture. Although response factors for different compounds may be determined experimentally, approximate values are published in monographs on gas chromatography. The response factors for thermal conductivity and flame ionization detectors for compounds that you may encounter in this experiment are given in Table 6.2. Notice that the correction factors vary more

Table 6.2 *Weight (W_f) and Mol (M_f) Correction Factors for Some Representative Substances**

Substance	*Thermal Conductivity*		*Flame Ionization*	
	Wf	*Mf*	*Wf*	*Mf*
Benzene	1.00	1.00	1.00	1.00
Toluene	1.02	0.86	1.01	0.86
Ethylbenzene	1.05	0.77	1.02	0.75
Isopropylbenzene	1.09	0.71	1.03	0.67
Ethyl acetate	1.01	0.89	1.69	1.50
n-Butyl acetate	1.10	0.74	1.48	0.99
Heptane	0.90	0.70	1.10	0.86
o-Xylene	1.08	0.79	1.02	0.75
m-Xylene	1.04	0.76	1.02	0.75
p-Xylene	1.04	0.76	1.02	0.75
Ethanol	0.82	1.39	1.77	3.00
Water	0.71	3.08	—	—

*McNair, H.M.; Bonelli, E.J. *Basic Gas Chromatography*, 5th ed., Varian Aerograph, Walnut Creek, CA, 1969.

widely for flame ionization detectors than for thermal conductivity detectors. In the experimental section, you have the opportunity to calculate the response factor for an unknown compound.

To analyze a mixture of substances quantitatively when **weight factors** are known, the peak area for each component is simply *multiplied* by the weight factor for that particular compound. The resulting *corrected* areas are used to calculate the percentage composition of the mixture according to the procedure outlined in Figure 6.19. Note that using these factors provides the composition on a *weight percentage* basis.

Calculation of the composition of a mixture on a mole percentage basis requires the use of **mole factors,** M_f. These are obtained by dividing the weight factors by the molar masses of each component of the standard solution and normalizing the resulting numbers. A sample calculation utilizing mole correction factors is provided below in the analysis of a mixture of ethanol, heptane, benzene, and ethyl acetate with a GLC equipped with a thermal conductivity detector. The last column shows the percentage composition calculated directly from the measured peak areas, without correction for detector response. The dramatic differences in the calculated composition with and without this correction, as noted in the last two columns, underscore the importance of this correction for quantitative analysis.

Compound	*Area (A) (mm^2)*	*Uncorrected % (A/207.1 × 100)*	M_f	$A \times M_f$	*Mol % ($A \times M_f/194 \times 100$)*
Ethanol	44.0	21.2	1.39	61.2	31.5
Heptane	78.0	37.7	0.70	54.6	28.1
Benzene	23.2	11.2	1.00	23.2	11.9
Ethyl acetate	61.9	29.9	0.89	55.4	28.5
Total	207.1	100		194.4	100

Use of Syringes. Liquid samples are injected into the heated injection port of a gas chromatograph using a gas-tight syringe with a capacity of 1−10 μL. The sample is either injected neat or dissolved in a volatile liquid such as diethyl ether or pentane. The sample should not contain nonvolatile substances that may eventually clog the injection port or contaminate the stationary phase of the column.

Gas-tight syringes are precision-made and expensive. You should handle them carefully and adhere to the following procedure when using them. To fill the syringe, draw slightly more than the desired volume of the sample into the barrel by withdrawing the plunger as needed, point the syringe needle up, push out the excess liquid, and then wipe the tip of the needle with a tissue. To inject the sample, insert the needle *straight* into the septum as far as it will go, *push the plunger all the way in with one quick motion*, and remove the syringe from the septum while holding the plunger in place. If the sample is injected slowly, it will produce a wider band on the column and the peaks will be broadened. Be careful to *avoid bending the needle or the plunger* during the injection. It is important to *clean the syringe immediately after use.* Rinse it with a volatile solvent such as acetone and dry it by drawing a stream of air through it.