

# Fifth Week

## Splitless Injection

For trace analysis<sup>16</sup> of analytes that are less than 0.01% of the sample, **splitless injection** is appropriate. The same port shown for split injection in Figure 23-14 is used. However, the glass liner is a straight, empty tube with no mixing chamber, as shown in Figure 23-15. A large volume ( $\sim 2 \mu\text{L}$ ) of dilute solution in a low-boiling solvent is injected slowly ( $\sim 2 \text{ s}$ ) into the liner, with the split vent closed. Slow flow through the septum purge is maintained during injection and chromatography to remove any vapors that escape from the injection liner. Injector temperature for splitless injection is lower ( $\sim 220^\circ\text{C}$ ) than that for split injection, because the sample spends more time in the port and we do not want it to decompose. The residence time of the sample in the glass liner is  $\sim 1 \text{ min}$ , because carrier gas flows through the liner at the column flow rate, which is  $\sim 1 \text{ mL/min}$ . In splitless injection,  $\sim 80\%$  of the sample is applied to the column, and little fractionation occurs during injection.

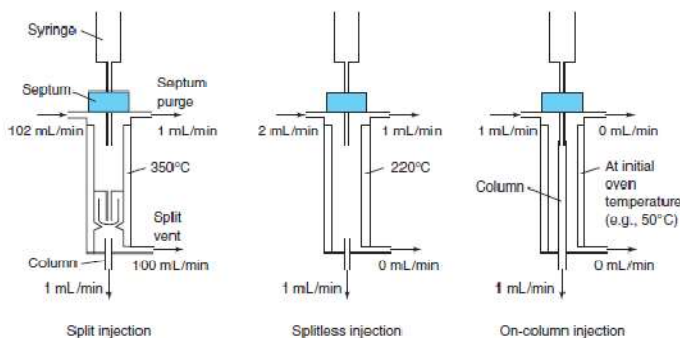
The initial column temperature is set  $40^\circ\text{C}$  below the boiling point of the solvent, which therefore condenses at the beginning of the column. As solutes catch up with the condensed plug of solvent, they are trapped in the solvent in a narrow band at the beginning of the column. This **solvent trapping** leads to sharp chromatographic peaks. Without solvent trapping, the bands could not be sharper than the 1-min injection time. Chromatography is initiated by raising the column temperature to vaporize the solvent trapped at the head of the column.

An alternative means of condensing solutes in a narrow band at the beginning of the column is called **cold trapping**. The initial column temperature is  $150^\circ\text{C}$  lower than the boiling points of the solutes of interest. Solvent and low-boiling components are eluted rapidly, but high-boiling solutes remain in a narrow band at the beginning of the column. The column is then rapidly warmed to initiate chromatography of the high-boiling solutes. For low-boiling solutes, *cryogenic focusing* is required, with an initial column temperature below room temperature.

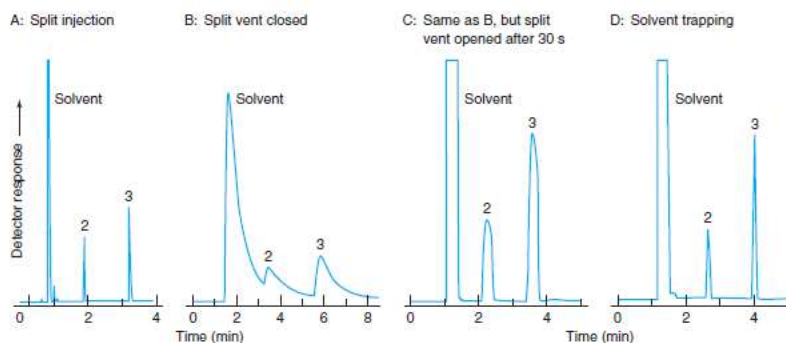
Figure 23-16 shows effects of operating parameters in split and splitless injections. Experiment A is a standard split injection with brisk flow through the split vent in Figure 23-15. The column was kept at  $75^\circ\text{C}$ . The injection liner was purged rapidly by carrier gas, and peaks are quite sharp. Experiment B shows the same sample injected in the same way, except the split vent was closed. Then the injection liner was purged slowly, and sample was applied to the column over a long time. Peaks are broad, and they tail badly because fresh carrier gas continuously mixes with vapor in the injector, making it more and more dilute but never completely flushing the

For solvent trapping, sample should contain  $10^4$  times as much solvent as analyte and column temperature should be  $40^\circ\text{C}$  below the solvent's boiling point.

For cold trapping, stationary phase film thickness must be  $\approx 2 \mu\text{m}$ .



**FIGURE 23-15** Representative injection conditions for split, splitless, and on-column injection into an open tubular column.



**FIGURE 23-16** Split and splitless injections of a solution containing 1 vol% methyl isobutyl ketone (b.p. 118°C) and 1 vol% *p*-xylene (b.p. 138°C) in dichloromethane (b.p. 40°C) on a BP-10 moderately polar cyanopropylphenyl methyl silicone open tubular column (0.22 mm diameter  $\times$  10 m long, film thickness = 0.25  $\mu$ m, column temperature = 75°C). Vertical scale is the same for A–C. In D, signal heights should be multiplied by 2.33 to be on the same scale as A–C. [From P. J. Marriott and P. D. Carpenter, "Capillary Gas Chromatography Injection," *J. Chem. Ed.* 1996, 73, 96.]

sample from the injector. Peak areas in B are much greater than those in A because the entire sample reaches the column in B, whereas only a small fraction of sample reaches the column in A.

Experiment C is the same as B, but the split vent was opened after 30 s to rapidly purge all vapors from the injection liner. The bands in chromatogram C would be similar to those in B, but the bands are truncated after 30 s. Experiment D was the same as C, except that the column was initially cooled to 25°C to trap solvent and solutes at the beginning of the column. This is the correct condition for splitless injection. Solute peaks are sharp because the solutes were applied to the column in a narrow band of trapped solvent. Detector response in D is different from A–C. Actual peak areas in D are greater than those in A because most of the sample is applied to the column in D, but only a small fraction is applied in A. To make experiment D a proper splitless injection, the sample would need to be much more dilute.

### On-Column Injection

**On-column injection** is used for samples that decompose above their boiling points and is preferred for quantitative analysis. Solution is injected directly into the column, without going through a hot injector (Figure 23-15). The initial column temperature is low enough to condense solutes in a narrow zone. Warming the column initiates chromatography. Samples are subjected to the lowest possible temperature in this procedure, and little loss of any solute occurs. The needle of a standard microliter syringe fits inside a 0.53-mm-diameter column, but this column does not give the best resolution. For 0.20- to 0.32-mm-diameter columns, which give better resolution, special syringes with thin silica needles are required.

### 23-3 Detectors

For *qualitative analysis*, a mass spectrometer (Chapter 21) can identify a chromatographic peak by comparing its spectrum with a library of spectra. For mass spectral identification, sometimes two prominent mass spectral peaks are selected in the electron ionization spectrum. The *quantitation ion* is used for quantitative analysis. The *confirmation ion* is used for qualitative identification. For example, the confirmation ion might be expected to be 65% as abundant as the quantitation ion. If the observed abundance is not close to 65%, then we suspect that the compound is misidentified.

Another method to identify a peak is to compare its retention time with that of an authentic sample of the suspected compound. The most reliable way to compare retention times is by **spiking**, also called *co-chromatography*, in which an authentic compound is added to the unknown. If the added compound is identical with a component of the unknown, then the relative area of that one peak will increase. Identification is tentative when carried out with one column but it is firmer when carried out on several columns with different stationary phases.

**TABLE 23-4** Detection limits and linear ranges of gas chromatography detectors

Detector	Approximate detection limit	Linear range
Thermal conductivity	400 pg/mL (propane)	$>10^5$
Flame ionization	2 pg/s	$>10^7$
Electron capture	As low as 5 fg/s	$10^4$
Flame photometric	$<1$ pg/s (phosphorus)	$>10^4$
	$<10$ pg/s (sulfur)	$>10^3$
Nitrogen-phosphorus	100 fg/s	$10^5$
Sulfur chemiluminescence	100 fg/s (sulfur)	$10^5$
Photoionization	25 pg to 50 pg (aromatics)	$>10^5$
Fourier transform infrared	200 pg to 40 ng	$10^4$
Mass spectrometric	25 fg to 100 pg	$10^5$

SOURCE: Most data are from D. G. Westmorland and G. R. Rhodes, "Detectors for Gas Chromatography," *Pure Appl. Chem.* 1989, 61, 1147.

**Linear response** means that peak area is proportional to analyte concentration. For very narrow peaks, peak height is often substituted for peak area.

Quantitative analysis with internal standard:

$$\frac{A_x}{|X|} = F \left( \frac{A_S}{|S|} \right)$$

$A_x$  = area of analyte signal  
 $A_S$  = area of internal standard  
 $|X|$  = concentration of analyte  
 $|S|$  = concentration of standard  
 $F$  = response factor

**TABLE 23-5** Thermal conductivity at 273 K and 1 atm

Gas	Thermal conductivity J/(K · m · s)
H <sub>2</sub>	0.170
He	0.141
NH <sub>3</sub>	0.021 5
N <sub>2</sub>	0.024 3
C <sub>2</sub> H <sub>4</sub>	0.017 0
O <sub>2</sub>	0.024 6
Ar	0.016 2
C <sub>2</sub> H <sub>6</sub>	0.015 1
CO <sub>2</sub>	0.014 4
Cl <sub>2</sub>	0.007 6

The energy per unit area per unit time flowing from a hot region to a cold region is given by

$$\text{Energy flux (J/m}^2 \cdot \text{s)} = -\kappa(dT/dx)$$

where  $\kappa$  is the thermal conductivity [units = J/K · m · s] and  $dT/dx$  is the temperature gradient (K/m). Thermal conductivity is to energy flux as the diffusion coefficient is to mass flux.

**Quantitative analysis** is based on the area of a chromatographic peak. In the **linear response** concentration range, the area of a peak is proportional to the quantity of that component. In most instruments, peak area is reported by a computer. Judgment is required to draw baselines beneath peaks and to decide on the boundaries of the area to be measured.<sup>17</sup> If area must be measured by hand, and if the peak is Gaussian, then the area is

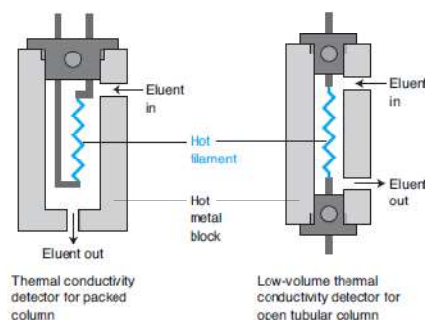
$$\text{Area of Gaussian peak} = 1.064 \times \text{peak height} \times w_{1/2} \quad (23-2)$$

where  $w_{1/2}$  is the width at half-height (Figure 22-9). Quantitative analysis is almost always performed by adding a known quantity of **internal standard** to the unknown (Section 5-4). After measurement of the **response factor** with standard mixtures, the equation in the margin is used to measure the quantity of unknown.

### Thermal Conductivity Detector

In the past, **thermal conductivity detectors** were common in gas chromatography because they are simple and **universal**—responding to all analytes. Thermal conductivity is useful for packed columns. It is less sensitive than other detectors used with open tubular columns (Table 23-4).

**Thermal conductivity** measures the ability of a substance to transport heat from a hot region to a cold region (Table 23-5). Helium is the carrier gas commonly used with a thermal conductivity detector. Helium has the second highest thermal conductivity (after H<sub>2</sub>), so any analyte mixed with helium lowers the conductivity of the gas stream. In Figure 23-17, eluate from the chromatography column flows over a hot tungsten-rhenium filament. When analyte emerges from the column, the conductivity of the gas stream decreases, the filament gets hotter, its electrical resistance increases, and the voltage across the filament changes. The detector measures the change in voltage.



**FIGURE 23-17** Thermal conductivity detectors. [Adapted from J. V. Hinshaw, "The Thermal Conductivity Detector," *JGC* 2006, 24, 38.]