Seventh Week

23-5 Method Development in Gas Chromatography

With the bewildering choice of parameters in gas chromatography, is there a rational way to choose a procedure for a particular problem? In general, there are many satisfactory solutions. As a broad guide to method selection, consider the following topics in this order: (1) the goal of the analysis, (2) sample preparation, (3) detector, (4) column, and (5) injection.[23]

Order of decisions:

1. goal of analysis
2. sample preparation
3. detector
4. column
5. injection

Goal of the Analysis

What is required from the analysis? Is it qualitative identification of components in a mixture? Will you require high-resolution separation of everything or do you just need good resolution in a portion of the chromatogram? Can you sacrifice resolution to shorten the analysis time? Do you need quantitative analysis of one or many components? Do you need high precision? Will analytes be present in adequate concentration or do you need preconcentration or a very sensitive detector for ultratrace analysis? How much can the analysis cost? Each of these factors creates trade-offs in selecting techniques.

Sample Preparation

Garbage in–garbage out!

The key to successful chromatography of a complex sample is to clean it up before it ever sees the column. In Section 23-4, we described solid-phase microextraction, stir bar sorptive extraction, purge and trap, and thermal desorption to isolate volatile components from complex matrices. Other methods include liquid extraction, supercritical fluid extraction, and solid-phase extraction, most of which are described in Chapter 27. These techniques isolate desired analytes from interfering substances, and they can concentrate dilute analytes up to detectable levels. If you do not clean up your samples, chromatograms could contain a broad "forest" of unresolved peaks, and nonvolatile substances will ruin the expensive chromatography column.

Choosing the Detector

The next step is to choose a detector. Do you need information about everything in the sample or do you want to detect a particular element or class of compounds?

The most general purpose detector for open tubular chromatography is a mass spectrometer. Flame ionization is probably the most popular detector, but it mainly responds to hydrocarbons; and Table 23-4 shows that it is not as sensitive as electron capture, nitrogen-phosphorus, or chemiluminescence detectors. The flame ionization detector requires the sample to contain ≥10 ppm of each analyte for split injection. The thermal conductivity detector responds to all classes of compounds, but it is not very sensitive.

Sensitive detectors for ultratrace analysis each respond to a limited class of analytes. The electron capture detector is specific for halogen-containing molecules, nitriles, nitro compounds, and conjugated carbonyls. For split injection, the sample should contain ≥100 ppb of each analyte if an electron capture detector is to be used. A photoionization detector can be specific for aromatic and unsaturated compounds. The nitrogen-phosphorus detector has enhanced response to compounds containing either of these two elements, but it also responds to hydrocarbons. Sulfur and nitrogen chemiluminescence detectors each respond to just one element. Flame photometric detectors are specific for selected elements, such as S, P, Pb, or Sn. A selective detector might be chosen to simplify the chromatogram by not responding to everything that is eluted (as in Figure 23-23). Mass spectrometry with selected reaction monitoring (Figure 23-22) is an excellent way to monitor one analyte of interest in a complex sample.

If qualitative information is required to identify eluates, then mass spectral or infrared detectors are good choices. The infrared detector, like the thermal conductivity detector, is not sensitive enough for high-resolution, narrow-bore, open tubular columns.

Selecting the Column

The basic choices are stationary phase, column diameter and length, and thickness of the stationary phase. A nonpolar stationary phase from Table 23-1 is most useful. An intermediate polarity stationary phase will handle most separations that the nonpolar column cannot. For highly polar compounds, a strongly polar column might be necessary. Optical isomers and closely related geometric isomers require special stationary phases for separation.

Table 23-6 shows that there are only a few sensible combinations of column diameter and film thickness. Highest resolution is afforded by the narrowest columns. Thin-film, narrow-bore columns are especially good for separating mixtures of high-boiling-point compounds that are retained too strongly on thick-film columns. Short retention times provide high-speed analyses. However, thin-film, narrow-bore columns have very low sample capacity, require

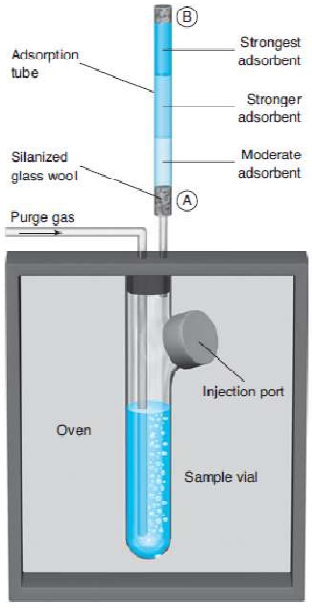

FIGURE 23-27 Purge and trap apparatus for extracting volatile substances from a liquid or solid by flowing gas.

TABLE 23-6 Gas chromatography column comparisons[23]

			
Description	Thin-film narrow-bore	Thick-film narrow-bore	Thick-film wide-bore
Inner diameter	0.10–0.32 mm	0.25–0.32 mm	0.53 mm
Film thickness	~0.2 μm	~1–2 μm	~2–5 μm
Advantages	High resolution Trace analysis Fast separations Low temperatures Elute high-b.p. compounds	Good capacity Good resolution (4 000 plates/m) Easy to use Retains volatile compounds Good for mass spectrometry	High capacity (100 ng/solute) Good for thermal conductivity and infrared detectors Simple injection techniques
Disadvantages	Low capacity (≤1 ng/solute) Requires high-sensitivity detector (not mass spectrometry) Surface activity of exposed silica	Moderate resolution Long retention time for high-b.p. compounds	Low resolution (500–2 000 plates/m) Long retention time for high-b.p. compounds

high-sensitivity detectors (flame ionization might not be adequate), do not retain low-boiling-point compounds well, and could suffer from exposure of active sites on the silica.

Thick-film, narrow-bore columns in Table 23-6 provide a good compromise between resolution and sample capacity. They can be used with most detectors (but usually not thermal conductivity detectors) and with compounds of high volatility. Retention times are longer than those of thin-film columns. Thick-film, wide-bore columns are required for use with thermal conductivity and infrared detectors. They have high sample capacity and can handle highly volatile compounds, but they give low resolution and have long retention times.

To improve resolution, use a
- longer column
- narrower column
- different stationary phase

If a particular column meets most of your requirements but does not provide sufficient resolution, then a narrower column of the same type could be used (Equation 22-35b). To obtain similar retention times for the same length of column, the thickness of the stationary phase should be decreased in proportion to the diameter. A column diameter of 0.15 mm is sensible for maximizing resolution without requiring a different gas chromatograph designed for narrower columns.[24]

Doubling the length of a column doubles the number of plates and, according to Equation 22-30, increases resolution by $\sqrt{2}$. Doubling the column length is not the best way to increase resolution because it doubles retention time. A narrower column increases resolution with no penalty in retention time. Selecting another stationary phase changes the unadjusted relative retention (γ in Equation 22-30) and might resolve components of interest.

To increase the speed of analysis without decreasing resolution, a shorter, narrower column can be chosen. Another way to decrease retention time without sacrificing resolution is to switch carrier gas from He to H_2 and increase flow rate by a factor of 1.5 to 2 (Figure 23-11).

If you have measured resolution of a few key components of a mixture under a small number of conditions, commercial software is available to optimize conditions (such as temperature and pressure programming) for the best separation.[25]

Figure 23-28 suggests approximate upper and lower analyte mass limits for different columns and detectors. The abscissa shows the mass of a given analyte that reaches the column or detector. A typical volume of injected liquid is 1 μL, with a mass of 1 mg. If analyte concentration is 1 ppm, the mass of analyte in 1 μL is 10^{-9} g = 1 ng. A vertical line at 10^{-9} g falls in the operating range of all columns and four of the detectors. The sample mass is too small for thermal conductivity, too great for electron capture, and near the upper limit for selected ion monitoring. For a 100:1 split injection, the mass of analyte introduced onto the column would be 100 times smaller, or 10^{-11} g. This mass is in range for all but the thermal conductivity detector. For columns, the region to the left of 10^{-11} g is shaded because chromatography becomes progressively more problematic as the

alumina, and molecular sieves. The retention index measures elution times in relation to those of linear alkanes. Temperature or pressure programming reduce elution times of strongly retained components. Without compromising separation efficiency, the linear flow rate may be increased when H_2 or He, instead of N_2, is used as carrier gas. Split injection provides high-resolution separations of relatively concentrated samples. Splitless injection of very dilute samples requires solvent trapping or cold trapping to concentrate solutes at the start of the column (to give sharp bands). On-column injection is best for quantitative analysis and for thermally unstable solutes.

Quantitative analysis is usually done with internal standards in gas chromatography. Co-elution of an unknown peak with a spike of a known compound on several different columns is useful for qualitative identification of the unknown peak. Mass spectral and infrared detectors provide qualitative information to help identify unknowns. The mass spectrometer is more sensitive and less subject to interference when selected ion monitoring or selected reaction monitoring are employed. Thermal conductivity detection has universal response but is not very sensitive. Flame ionization detection is sensitive enough for most columns and responds to most organic compounds. Electron capture, nitrogen-phosphorus, flame photometry, photoionization, chemiluminescence, and atomic emission detectors are specific for certain classes of compounds or individual elements.

You need to define the goal of an analysis before developing a chromatographic method. The key to successful chromatography is a clean sample. Solid-phase microextraction, stir-bar sorptive extraction, purge and trap, and thermal desorption can isolate volatile components from complex matrices. After sample preparation, the remaining decisions for method development are to select a detector, a column, and the injection method, in that order.