Chapter 12: End-of-Chapter Solutions

1.

(a)

Chromatographic detector	Use
ECD (electron capture detector)	GC only
FID (flame ionization detector)	GC only
fluorescence	HPLC only
mass spectrometer	GC and HPLC
RI (refractive index) detector	HPLC only
TCD (thermal conductivity detector)	GC only
UV/Vis absorption detector	HPLC only

(b) Of the choices, only the mass spectrometer provides molecular structure information. UV-Vis absorption can provide some identification, but many organic analytes have similar spectra.

(c) The refractive index and UV-vis absorption detectors (for HPLC) and the thermal conductivity detector (for GC) are non-destructive.

2.

GC detector	advantage	disadvantage
TCD (thermal conductivity detector)	universal non-destructive	less sensitive than other detectors
FID (flame ionization detector)	sensitive high dynamic range	destroys analyte
ECD (electron capture detector)	selective for electronegative functional groups very sensitive for select analytes non-destructive	limited dynamic range
mass spectrometer	provides structural information for solute identification	more complex maintenance and operation destroys analyte

There are a number of other more specialized GC detectors that are not discussed in the text. Some examples include:

- photoionization (PID): selective for aromatic compounds, e.g., benzene, touluene, ethylbenzene, and xylenes (BTEX)
- flame photometric detector (FPD): flame chemiluminescence for compounds containing sulfur and phosphorous
- nitrogen phosphorous detector (NPD): plasma ionizer for compounds containing nitrogen and phosphorous

- dry electrolytic conductivity detector (DELCD): an ECD replacement for chlorine and bromine containing compounds

3.

(a) reverse-phase partition liquid chromatography:

n-hexanol, benzene, and n-hexane (more polar to less polar, the nonpolar analytes will interact more strongly and be retained longer)

(b) normal-phase partition liquid chromatography:

n-hexane, benzene, and n-hexanol (less polar to more polar, the more polar analytes will interact more strongly and be retained longer)

(c) capillary GC with nonpolar stationary phase

In this case we expect the analytes to elute in order of boiling point (b.p.) from lowest to highest b.p. The expected order is:

n-hexane (b.p. = 68 C)
benzene (b.p. = 80 C)
n-hexanol (b.p. = 157 C)

The deciding factor between reversed-phase and normal-phase partition chromatography is usually based on solubility of the solutes. Give the reasonable solubility of the listed solutes in a water-based mobile phase, reversed-phase will be the preferred method for these analytes.

The main advantage of GC is the superior resolution compared to HPLC. If these solutes must be separated from other components, then GC will be advantageous. Given that the alcohol is more polar than the other solutes, the disadvantage of GC is that there may not be an optimum stationary phase to achieve narrow symmetric peaks for all analytes.

4.

Ions in solution have an electrostatic attraction to immobilized ions on the stationary phase. The strength of the interaction depends on the charge and size of the analyte ions. Ions of higher charge and smaller size, which can approach the stationary phase closer, are attracted more strongly and are retained longer on the column.

5.

(a) The analytes are anions so use the anion-exchange HPLC column. Attempting to inject inorganic ions into a GC results in decomposition or depositing non-volatile salts in the injector.

(b) The analytes are inorganic and organic cations so use the cation-exchange HPLC column. The rationale is the same as for anions.

(c) The analytes are volatile organic compounds so use capillary GC column. Since the analytes are nonpolar, choose the nonpolar polydimethylsiloxane stationary phase.

(d) Answering this question requires some research into the structure of barbiturates. Based simply on the structures, either GC or HPLC should be suitable techniques. Earlier analytical

methods used GC analysis. A blood matrix will require an extraction step and the barbiturates are often derivatized. The stationary phase will depend on the product of the derivatization reaction. Since these molecules are water soluble, the C18 HPLC column is being developed as a standard analytical method. Again SPE cleanup will be needed due to the complex blood matrix.

6.

(a) To maintain these acids as anions, the mobile phase pH must be greater than the analyte with the highest pK_a (niacin, $pK_a = 4.85$). To be safe, a pH > 6.85 will keep > 99 % of the niacin deprotonated.

(b) Since these analytes are weak acids, a strong anion exchange stationary phase is preferred. The stationary phase will not change as a function of mobile phase pH. All analytes are anions at the initial pH. As the weakly retained analytes elute from the column, the pH can be lowered to begin protonating the more strongly retained analytes. Since the weak acids become neutral when protonated, these analytes will then elute from the column.

7.

The large size of proteins makes them very non-volatile. If they were injected into a gas chromatograph, they would decompose rather than vaporize intact.

8.

(a) The peak height of each analyte is taken from Table 10.7. The identity of the peaks is made by matching the retention times to the standard data in Table 10.6. The data is summarized here:

analyte	retention time, t_R (min)	peak height (arb. units)
theobromine	1.0	12.2
theophylline	1.7	7.0
caffeine	4.3	2.9

The concentration of each analyte is determined by converting the test portion peak height to concentration using the calibration data in Table 10.6. The calculation for each analyte is a simple proportionality:

$$\frac{c_{\text{theobromine}}}{12.2} = \frac{1.0\ \mu\text{g/mL}}{7.2}$$

$$c_{\text{theobromine}} = 1.7\ \mu\text{g/mL}$$

$$\frac{c_{\text{theophylline}}}{7.0} = \frac{1.0\ \mu\text{g/mL}}{12.4}$$

$$c_{\text{theophylline}} = 0.56\ \mu\text{g/mL}$$

$$\frac{c_{\text{caffeine}}}{2.9} = \frac{1.0\ \mu\text{g/mL}}{3.9}$$

$c_{caffeine} = 0.74\ \mu g/mL$

(b) Given the results in part (a), the weight percent of the analytes in the cocoa sample is determined by correcting for sample preparation. The analyte was contained in 30 mL of extracting solvent, so the amount of each analyte was:

$$wt_{theobromine} = (1.7\ \mu g/mL)(30\ mL) = 50.8\ \mu g$$

$$wt_{theophylline} = (0.56\ \mu g/mL)(30\ mL) = 16.9\ \mu g$$

$$wt_{caffeine} = (0.74\ \mu g/mL)(30\ mL) = 22.3\ \mu g$$

Dividing each weight by the 50-mg sample portion and multiplying by 100% gives:

$$\frac{50.8\ \mu g}{50{,}000\ \mu g} \times 100\% = 0.10\%\ \text{theobromine}$$

$$\frac{16.9\ \mu g}{50{,}000\ \mu g} \times 100\% = 0.034\%\ \text{theophylline}$$

$$\frac{22.3\ \mu g}{50{,}000\ \mu g} \times 100\% = 0.045\%\ \text{caffeine}$$

9.
The total-ion chromatogram (TIC) is the summation of all mass spectral peaks and provides the largest analytical signal. The largest signal provides the greatest sensitivity. Selective-ion monitoring (SIM) displays the ion current signal at only one or a few m/z. This mode provides greater selectivity by picking out the base peak or an intense fragment ion of the analyte of interest. Solutes that do not have a mass fragment at the selected m/z will not appear in the chromatogram. This mode makes it simpler to interpret the chromatogram and reduces the chance of overlapping peaks affecting a quantitative measurement.

10.
Table of MS designs

mass analyzer design	advantage	disadvantage
magnetic-sector	simultaneous detection of several m/z – best precision for isotope ratio measurements	slow scan rate
quadrupole	compact and inexpensive rapid scan rate	moderate resolution limited scan range (≈ 5000

		Da maximum)
time-of-flight	high resolution (15,000) rapid scan rate	pulsed operation requires interface to some ion sources
ion trap	can accumulate ions useful as an interface between a continuous ion source and a time-of-flight mass analyzer	moderate resolution limited scan range (≈ 5000 Da maximum)
orbitrap	highest resolution	expensive

In general, the quadrupole and time-of-flight designs are the most common to be coupled with chromatography. Ion trap and orbitrap mass spectrometers can also be used. The magnetic-sector design is rarely used due to the slow scan rate.

11.
The electrodes provide the electrostatic driving force to move the charged proteins (or other type of macromolecules). Using SDS surfactant gives all of the proteins a negative charge, so they migrate from the negative electrode towards the positive electrode. The buffer maintains the system at a pH where the proteins are stable. In discontinuous gel electrophoresis, the change in buffer pH "stacks" the proteins before entering the running gel. Large proteins are hindered to a greater extent by the pore size of the gel. Smaller proteins migrate faster and travel farther in gel electrophoresis. Since preparation of the gel can vary day-to-day, measurements are made relative to molecular weight standards in one or more separate lanes of the gel.

12.
The biomolecules in a gel are usually visualized by adding a stain. The most common stains are Coomassie brilliant blue for proteins and ethidium bromide for nucleic acids. Silver stain works for either type of biomolecule and provides greater sensitivity. In some applications it is possible to irradiate the gel with ultraviolet light to detect the biomolecules using fluorescence.

13.
The acrylamide concentration and the relative amount of cross linker (usually bis-acrylamide) added during preparation of the gel determines the pore size. Since proteins have a large range of sizes, the pore size must be appropriate to match the proteins being separated. Other additives besides the buffer and SDS surfactant include urea to denature proteins, a reducing agent to break disulfide bonds, and reagents to fine tune the polymerization. Most labs that use SDS-PAGE will have detailed recipes and protocols that are optimized for their applications.

14.

When multiple analytical methods are suitable for a given class of analytes, in this case amino acids, it can often be difficult to know what method to choose. I provide a general discussion here to help you understand the methods that you might have discovered. In many cases the method selection is determined by practical reasons, e.g., what instrument is available, or by constraints such as time or cost. A chief scientific reason is the complexity of the sample matrix. The high resolution of GC allows rapid chromatography, but requires derivatization of the amino acids. HPLC is readily amenable to amino acids in aqueous solution, but will usually require longer run times than GC. Capillary electrophoresis provides the highest resolution. Despite the greater complexity to achieve repeatable results, CE might be the best choice is the amino acids must be separated from similar interferences.

15.

Common forms of arsenic are arsenite, As(III), arsenate, As(V), methylarsonic acid, As(V), and dimethylarsinic acid, As(V). Since all of these compounds can form anions at high pH (≈11 or higher), they can be separated by anion-exchange chromatography. The effluent of the chromatography column can enter an ICP-MS to measure each arsenic species separately.

16.

I did a Google search on “capillary electrophoresis application note” to find manufacturer literature. There is certainly many, many more papers in the primary literature. A few examples that I found are applications in fragment analysis (DNA and RNA sequencing), food testing, pesticides, pharmaceuticals, and forensics analysis. A common aspect that I found in my limited search was a focus on biological analytes.

17.

Total-ion chromatograms (TIC) are the GC or HPLC chromatograms produced when counting peaks of all m/z created by the analytes. (In practice the mass range when using a quadrupole mass analyzer will be something like $m/z = 40 – 1000$. The low masses are avoided to reduce baseline noise due to atmospheric gases and the high masses have low sensitivity.

Selected-ion chromatograms (SIC, also called selected-ion monitoring) records a chromatogram measuring only one or a few mass spectral peaks at selected m/z. The advantage is more time measuring the peaks of interest rather than scanning a full spectrum. The selected-ion chromatogram is therefore more selective and more sensitive for the targeted analyte(s) than a TIC.

Extracted-ion chromatograms (EIC) actually use data from total-ion chromatogram but only displays chromatographic peaks that have mass spectral peaks at certain m/z, as selected by person performing data analysis.