LC Troubleshooting

Solvent Selection, Part I — UV Absorption Characteristics

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This column about solvents used in liquid chromatography is the first of a series that will appear every few months. In this series, we will examine some of the characteristics that make solvents suitable for certain applications. Solvents are often blamed for spurious chromatographic peaks, so we will learn some techniques for troubleshooting solvent problems. The authors draw upon their customer-service experiences at a major solvent manufacturer.

One of the most important steps in liquid chromatography (LC) is selecting mobile-phase and sample-preparation solvents. Many chromatographers overlook the importance of selecting the best solvent for their current application. Some users stick with a particular solvent because of cost or previous success using it. Unfortunately, this approach may lead to unanticipated difficulties.

To obtain the best results, you should consider the inherent chemical and physical characteristics and the required purity of the solvents. Whether you are developing a completely new method or modifying an existing one, you should always take these properties into account.

UV CUTOFF

Our first topics in this series of columns are the UV cutoff, the UV–vis absorption curves of the solvent, and using these characteristics to begin the solvent-selection process. The UV cutoff is the most universally used parameter for solvent selection. Most manufacturers label every solvent bottle with the UV cutoff obtained from an actual solvent-lot analysis. A solvent's UV cutoff is determined by its molecular structure, and it is an important criterion when selecting solvents for use with a UV detector either for HPLC or sample preparation.

The solvent UV cutoff is defined as the wavelength at which the absorbance of the solvent in a 1-cm cell is equal to 1 AU, using water in the reference cell. The UV radiation at the cutoff wavelength is not completely blocked by the solvent in the cell but is equal to 10% of the incident radiation, so the solvent has absorbed 90% of the incident radiation. At or below the UV-cutoff wavelength, the solvent should not be used with a UV detector. In special circumstances, the solvent can be used near the cutoff value as a modifier at ≤10% levels in the mobile phase. Impurities in the solvent can alter the solvent cutoff, but these usually are noticeable in the UV spectrum of a contaminated solvent.

Many chromatographers overlook the UV-absorbance properties of the solvents they have chosen for their method. Each solvent has a unique UV spectrum that must be considered when using UV absorbance detection. Most solvent manufacturers offer solvent guides or compilations of data about their solvents that include typical UV spectra. The word "typical" is key here — solvents can have lot-to-lot UV-spectra variations that may or may not be noticeable in your application. Usually very small, these variations depend on the solvent manufacturer, the available raw manufacturing material, the solvent-purification process, and the manufacturer's quality-control parameters. Selecting a reputable solvent manufacturer will ensure minimum lot-to-lot quality variations.

Before we look at the UV characteristics of individual solvents, let's examine how these characteristics can affect chromatographic analysis. The primary side effect of using high-absorbance solvents as part of the mobile phase is the appearance of *system peaks*.

System peaks most often are recognized as a pair of peaks — one positive and the other negative — that represent enrichment and depletion zones eluted from the column, as shown in Figure 1. System peaks vary in retention time and size, depending on the sample matrix, injection volume, mobile-phase composition, and the stationary phase. You can prevent system peaks by carefully selecting all the solvents (including the sample diluent)

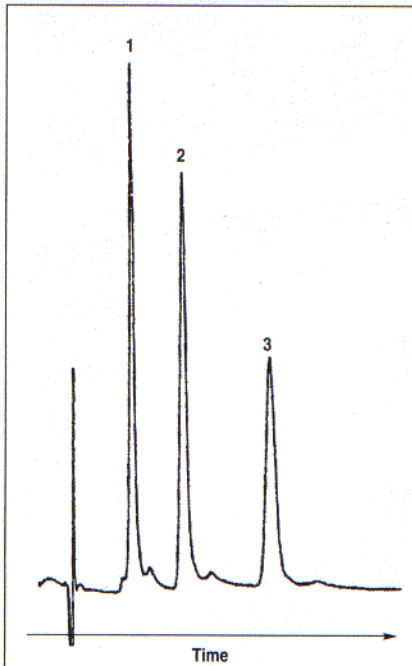

FIGURE 1: Chromatogram showing positive and negative system peaks near the void volume. Column: 15 cm × 4.6 mm B&J 9575 OD5 octadecyl; mobile phase: 85:15:0.1 (v/v/v) water–acetonitrile–trifluoroacetic acid; flow rate: 2.0 mL/min; detection: UV absorbance at 234 nm; sample matrix: 75:25 (v/v) acetonitrile–water. Peaks: 1 = salicylglycine, 2 = acetylsalicylic acid, 3 = gentisic acid.

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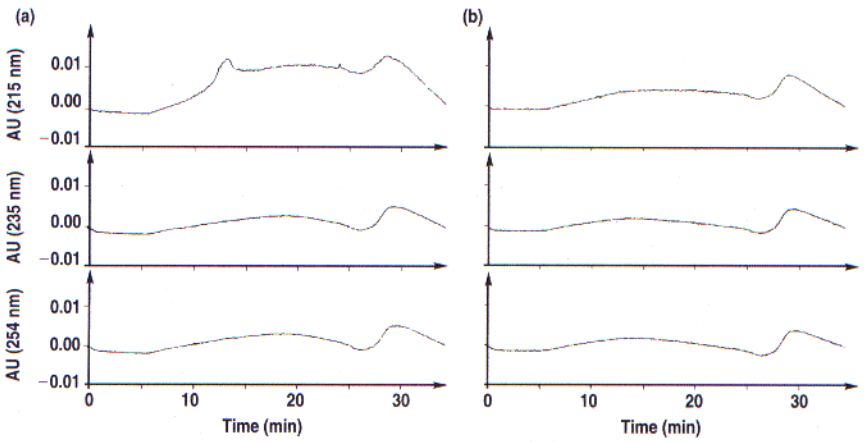

FIGURE 2: Gradient chromatograms generated using (a) unacceptable and (b) finished-product acetonitrile. Column: 15 cm × 4.6 mm B&J 9511, HLD OC5 octyl; mobile phases and gradient: 70:30 (v/v) water–acetonitrile to 100% acetonitrile over 20 min, hold for 5 min, and ramp back to original conditions over 5 min; flow rate: 1.5 mL/min; detection: UV photodiode-array, 190–400 nm. No sample was injected.

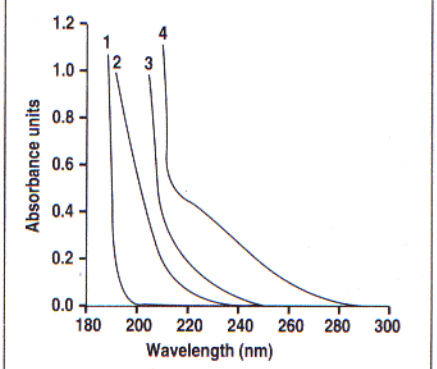

FIGURE 3: UV spectra of various solvents. Spectra: 1 = acetonitrile, 2 = hexane, 3 = methanol, 4 = tetrahydrofuran.

used during a separation. Dissolving samples in the mobile phase and working at wavelengths at which the sample matrix and mobile phase have near-zero absorbance are the best ways to minimize the system-peak effects. Solvents used as modifiers at ≤10% can be used at or near their UV cutoff, but their contribution to the total absorbance should only be 0.1 AU, which allows an acceptable working absorbance range.

Next, let's look at some common solvents and their unique characteristics when used as mobile-phase components.

COMMON REVERSED-PHASE SOLVENTS

Acetonitrile: Acetonitrile is one of the most popular solvents for reversed-phase LC. It has a very low UV cutoff of <190 nm, which contributes to its popularity. It also provides low UV absorbance (≥200 nm), so it often is the first choice for detection near 200 nm.

The challenge for a solvent manufacturer is to remove from the raw materials any trace levels of low-wavelength, UV-absorbing compounds that may interfere with the chromatography. Chromatographers using gradient applications will see these trace impurities at very low UV wavelengths as ghost peaks, such as those illustrated in Figure 2. Chromatographers using isocratic applications or higher UV wavelengths will not notice the trace impurities, even if the solvent manufacturer fails to remove them.

If you are using a gradient, low UV wavelength (<210 nm), and high detection sensitivity, it is well worth the effort to compare acetonitrile from different suppliers or to select a quality manufacturer to ensure consistently good solvent lots.

Methanol: The next solvent of choice is methanol. Many chromatographers start with methanol instead of acetonitrile because it typically is less expensive than acetonitrile but provides similar UV spectral characteristics when comparing cutoffs. Methanol's UV cutoff is <205 nm — only slightly higher than acetonitrile (Figure 3).

The difference between methanol and acetonitrile is in the region near their cutoffs — the methanol curve is not as flat as acetonitrile's, especially as it approaches the UV cutoff. Methanol starts absorbing significantly at 250 nm and continues with a gradual rise until the absorbance increases sharply at 220 nm, leading to a UV cutoff at 205 nm.

The UV-absorbance specifications provided by manufacturers are not as low as those for acetonitrile at lower wavelengths (at 225 nm, the methanol specification is <0.160 AU, whereas the acetonitrile specification is <0.10 AU), making acetonitrile a better choice for detection at lower UV wavelengths. Methanol can be used successfully at lower wavelengths if analysts pay special attention to the type of chromatography performed (isocratic or gradient) and methanol's UV qualities. Chromatographers also should consider using other alcohols, such as isopropanol or *n*-propanol, that have UV characteristics similar to methanol.

Water: Although high-purity water has few UV characteristics, we must address one major consideration. High-purity water and water with added buffers are notorious for growing bacteria. Bacteria can grow in the water reservoir and cause ghost peaks in the gradients. These ghost peaks correspond to the by-products of bacterial growth such as metabolites and dead bacteria. Adding ≥20% organic solvent or ~0.04% sodium azide to the aqueous part of the mobile phase will help prevent bacterial growth. You should use a bacteriostat or change the aqueous portion of the mobile phase daily to minimize bacterial contamination.

Tetrahydrofuran: Tetrahydrofuran is another popular reversed-phase LC solvent that has a relatively low UV cutoff (212 nm). Like methanol, the UV-absorbance spectrum for tetrahydrofuran is not flat; it actually starts absorbing at 300 nm (the manufacturer's specification is <0.02 AU at 300 nm), as shown in Figure 3. Tetrahydrofuran's spectrum has a broad shoulder from 300 nm to 225 nm and tails gradually to the cutoff.

Many chromatographers recommend using tetrahydrofuran only above 250 nm because of its absorbance properties, but tetrahydrofuran actually has some very useful separating properties and can be used successfully in smaller amounts (<10%) as a mobile-phase modifier at lower wavelengths. Tetrahydrofuran is an unstable solvent that breaks down rapidly, forming peroxides. It can be purchased with or without an oxygen scavenger — butylated hydroxytoluene (BHT). BHT is a very strong UV absorber; therefore, this grade of tetrahydrofuran cannot be used for UV detection but is useful with other types of detection, such as refractive index. When using tetrahydrofuran, be sure to select the appropriate preserved or unpreserved type.

NORMAL-PHASE SOLVENTS

Hexane is the most frequently used solvent in normal-phase applications. Hexane has a UV cutoff of <195 nm and low UV absorbance above 210 nm, making it very useful for normal-phase LC applications using UV detectors. Hexane starts to absorb at ~225 nm (specification is <0.02 AU at 225 nm), so the solvent can be used successfully into the lower UV wavelengths (see Figure 3).

Hexane has a tendency to absorb air while standing. Air absorption affects UV absorbance in the very-low UV range, so sparging hexane with helium sometimes can lower the UV absorbance at lower wavelengths. Other hydrocarbon solvents such as pentane and isooctane have similar UV spectra, so you should follow the same guidelines when selecting these solvents.

Other normal-phase solvents, including methylene chloride, ethyl acetate, acetone, and other polar solvents, can be used as components of normal-phase solvent systems but have unique UV spectra that require attention. These solvents often are used in ≤10% quan-

tities in the mobile phase, so they do not require a detailed study. Remember to review the UV spectrum and cutoff before selecting one of these solvents.

TROUBLESHOOTING

When troubleshooting anomalies and ghost peaks in a gradient run, the solvent should be one of the first problem areas to examine. Run the gradient after a normal equilibration period, then rerun it after a two- to threefold increase in equilibration time. If the ghost peak increases in size as the equilibration time increases, the ghost peak is caused by impurities in the mobile phase. If the mobile phase contains modifiers, rerun the gradient after removing one component. If the ghost peak disappears, it resulted from the missing component. Repeat this test for each mobile-phase component until you identify the problem source. You should use solvent from a freshly opened bottle to verify that the solvent or mobile-phase component — not an inadvertent contaminant — is the problem source.

Impurities associated with solvents can stem from changes in the raw-material composition or the solvent purification process. The compounds that appear as ghost peaks are present at such low concentration levels (<1 ppm) that they are invisible in the UV spectra but become concentrated during a gradient run. Some solvent manufacturers run a gradient that is sensitive to these ghost peaks, monitoring the solvent and ensuring a consistent product, as shown in Figure 2. In isocratic analyses, these abnormalities would cause a baseline shift but generally would be unnoticeable.

SUMMARY

The UV-absorbance characteristics are just one important variable in selecting solvents for LC mobile phases. Review the UV cutoff and spectra carefully when you select solvents. If you must work near the UV cutoff or under other demanding conditions, contact your solvent manufacturer for specific advice about using the solvent.

In the next column in this series, we will look at other physical properties — such as viscosity and miscibility — and how they affect solvent selection during method development. We will show you how to use solvent strength tables and other tools to choose the best solvents for your application.

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