Troubleshooting

Success with Evaporative Light-Scattering Detection, Part II: Tips and Techniques

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Evaporative light-scattering detection offers a complementary detection mode that can detect nearly all eluted compounds in either isocratic or gradient conditions.

John W. Dolan
LC Troubleshooting column

Evaporative light-scattering detection (ELSD) for liquid chromatography (LC) applications was the subject of a previous installment of "LC Troubleshooting" (1). That column described the working principles and basic attributes of ELSD. There are three steps in the operation of evaporative light-scattering detectors: nebulization, evaporation, and detection. First, the mobile phase is nebulized into small droplets with the aid of a nebulizing gas, typically air or nitrogen. Next, the droplets of column effluent pass through a heated drift tube, where the volatile components evaporate and leave a particulate residue containing the sample molecules and other nonvolatile materials. These particles pass through the detection section, where they scatter light from a lamp, resulting in varying amounts of the light reaching a photodetector. Evaporative light-scattering detectors are more or less universal detectors, as are refractive index (RI) detectors, in that they will respond to any compound that survives the evaporation stage. Of course, one must be careful to use volatile mobile-phase components, because nonvolatile components such as phosphate buffers also will remain as particulates after the evaporation stage and give a high background signal. A more detailed discussion of the operating principles of ELSD can be found in reference 1.

The previous article raised several common questions from readers regarding the practical application of ELSD to laboratory samples. This month's "LC Troubleshooting" addresses some of those questions and builds upon the previous discussion in terms of detector applications.

Balancing Detector Attributes — Always a Compromise

To determine the suitability of a detector for a given application, issues of detector selectivity, sensitivity, and gradient compatibility must be considered. This is no less true when considering ELSD for an application. As a rule, the detector of choice for a given application represents a compromise of many factors.

The *selectivity* of a detector describes its ability to detect only certain compounds of interest from among many other compounds, including the mobile phase. For a given application, a highly selective detector might be a limitation or an advantage. For example, fluorescence detectors are highly selective because relatively few compounds fluoresce. For this reason, derivatization procedures are developed to create a target fluorophore. Fluorescence methods usually are quite sensitive, offering femtogram-level detection. Ultraviolet (UV) detectors are moderately selective in that the analysis wavelength determines their response to analytes based upon the analytes' molar absorptivity at that wavelength. A sample's UV chromatographic profile at 220 nm could be very different from that at 254 nm or 280 nm due to the sometimes large differences in UV response by the different sample constituents. Although not as sensitive as fluorescence methods, UV detectors can determine picograms on-column. Both techniques generally are compatible with gradient operation.

On the other end of the selectivity scale are differential RI detectors. RI detectors are not selective and are nearly universal, responding to compounds whose RI differs from that of the mobile phase. The RI is a bulk property, influenced greatly by temperature and pressure. RI detectors can be used to determine methanol in a mobile phase of acetonitrile and water. However, RI detectors lack sensitivity (lower limits of detection range from hundreds of nanograms to micrograms on-column), a matter made worse by the presence of organic components in the mobile phase, which diminish the RI differential. RI detectors provide their greatest sensitivity when the mobile phase is pure water. Because they respond to bulk property changes in the mobile phase, RI detectors

are incompatible with gradient elution and often require long equilibration times (2).

Evaporative light-scattering detectors also are nearly universal detectors, with the caveat that they will miss truly volatile sample constituents such as low molecular weight alcohols. As described in the previous column (1), ELSD will respond to all light-scattering particle aggregates that remain after the mobile phase has evaporated. In this regard, ELSD offers a more accurate record of the relative abundance of compounds in a sample than RI detection. Because it is not influenced by the bulk properties of the solvent, ELSD is fully gradient compatible (3).

ELSD serves as a complement to other detection methods. Although not the most sensitive, it is sensitive enough for most applications, commonly offering limits of detection in the hundreds of picograms on-column. Used in this way, evaporative light-scattering detectors frequently reveal more components in a sample than can be seen with light-absorbing detectors. Also, ELSD offers gradient compatibility and relatively turnkey operation. At other times, ELSD is the detection method of choice in terms of sensitivity to weakly chromophoric or nonchromophoric compounds such as carbohydrates and phospholipids. A very powerful detector combination is an in-series connection of a photodiode-array UV–vis detector for multiwavelength determination of chromophoric constituents and an evaporative light-scattering detector for determining both chromophoric and nonchromophoric compounds with relative abundance profiling (4).

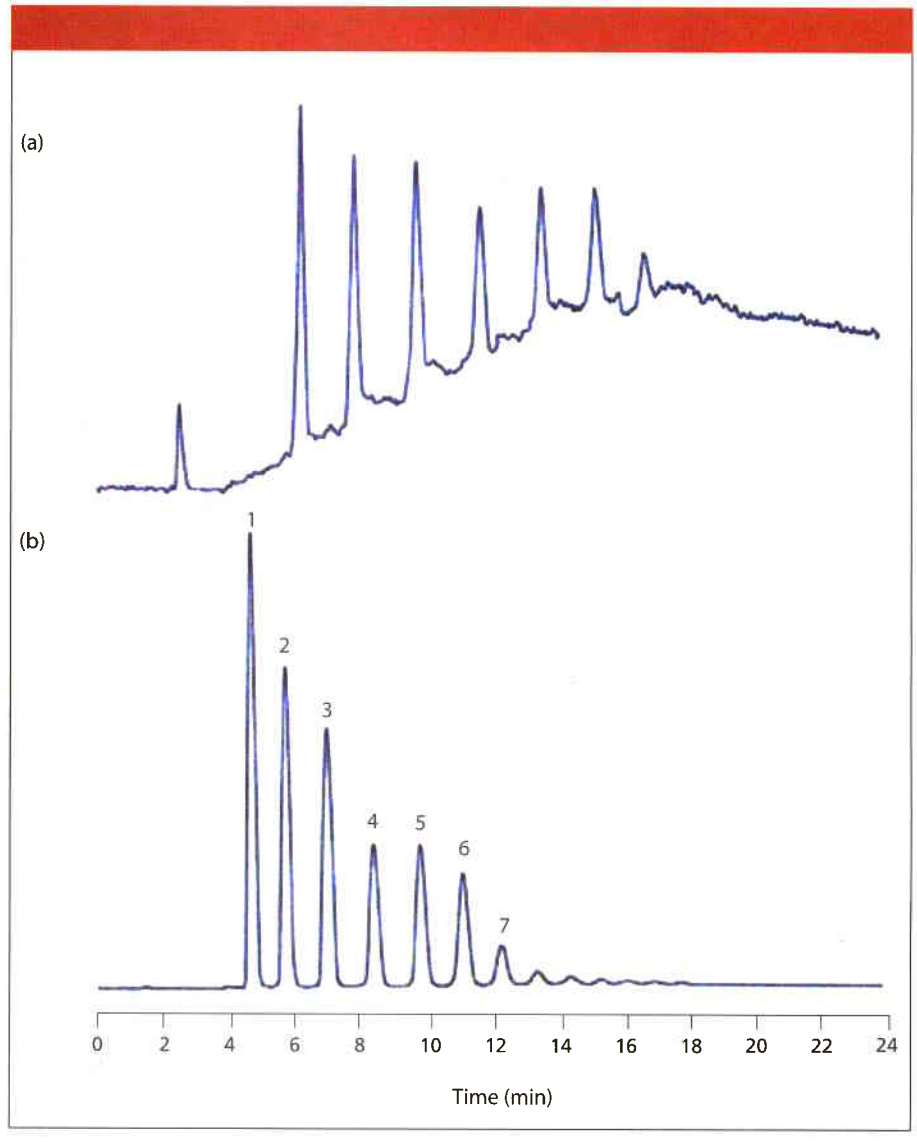

Figure 1: A comparison of ELSD response to carbohydrates using (a) silica-based and (b) polymer-based amino columns. Mobile phase A: acetonitrile; mobile phase B: water; gradient: 35–50% B over 26 min; flow rate: 1.0 mL/min; detector: Shimadzu ELSD-LT. Peaks: 1 = glucose, 2 = maltose, 3 = maltotriose, 4 = maltotetraose, 5 = maltopentaose, 6 = maltohexaose, 7 = maltoheptaose. (Courtesy of Shimadzu Scientific Instruments, Inc.)

Reasons for Considering ELSD

Analytes that lack strong chromophores: The most common reason to choose ELSD is for the analysis of nonchromophoric compounds. Even photodiode-array detectors capable of simultaneous monitoring from 190 nm to 800 nm in 1-nm steps are limited by the light-absorbing properties of the analytes — the detector might be insensitive to some compounds and might miss other compounds altogether. Historically, low-wavelength analyses at 195 nm or 205 nm attempt to take advantage of the end absorbance of most organic compounds. However, the analysis wavelength resides near the UV cutoff of most HPLC solvents and can produce poor baseline response. ELSD can reveal all sample constituents without regard to chromophores and with much better sensitivity, the only caveat being volatility (5–9). Classic examples of analytes in this category are carbohydrates, phospholipids, and surfactants.

Avoidance of derivatization: The practice of derivatizing analytes before or after the column to produce light-absorbing chromophores adds to the analysis time, yet it can result in highly reproducible and sensitive UV or fluorescence detection. However, some derivatization techniques lack reproducibility, and the added time and expense could be objectionable. In some cases, an evaporative light-scattering detector might be the detector of choice for the underivatized analyte, improving sample throughput and increasing precision of quantification (10–13). However, the resulting sensitivity to the underivatized analyte might be lower. The decision to forego derivatization would represent a compromise of sensitivity in favor of higher sample throughput and precision and lower cost per analysis.

Uniform response factor — universal calibration: Because they respond to the quantity of light-scattering particles, evaporative light-scattering detectors provide a more uniform response to structurally similar analytes than light-absorbing detectors. For many analyte classes, such as lipids, users can create a universal calibration set from a single analyte to quantify all analytes of the same class (14–18).

General Method Development Issues

Setting the drift-tube temperature: The useful temperature range for the evaporation stage (drift tube) of an evaporative light-scattering detector is a matter of distinction between the various instruments on the market. One way to accomplish efficient evaporation at lower temperatures is to lengthen the drift tube. When available, there are advantages to near-ambient (27–30 °C) drift-tube temperatures. The first advantage is the ability to detect semivolatile compounds, and the second is the ability to detect compounds that might decompose at higher temperatures. Examples of semivolatile compounds are urea and glycerol (19,20).

In general, the recommendation for setting the drift-tube temperature is to use the lowest temperature that yields an acceptably low-noise baseline response *and* reveals all analytes of interest. This could involve a compromise. For example, one commercial evaporative light-scattering detector is designed such that operation at 40 °C is relatively turnkey — the setting is suitable for most samples and mobile-phase combinations. However, in method development, analysts should perform two screening runs at different temperatures (for example, at 40 °C and 30 °C) and compare the results. Both chromatograms might give good baseline response, but the lower temperature might reveal new constituent peaks, indicating that semivolatile or temperature-sensitive compounds are present. When using a 0.1% formic acid modifier in the mobile phase, a lower temperature sometimes introduces unacceptable noise in the baseline because the evaporative burden is increased, limiting method flexibility. The first example benefits from a lower drift-tube temperature, whereas the second works better with a higher temperature (or a different mobile phase).

Column stability: ELSD sometimes can yield unexpected results, such as revealing constituent peaks that you've never seen before, suggestive of a problem in methodology. A classic example of problematic methodology is the use of a silica-based amino column in the presence of an aqueous mobile phase. Amino columns are used widely with water–acetonitrile mobile phases for carbohydrate separations. However, this column lacks ruggedness due to chemical degradation of the bonded phase, resulting in a short life span (water from the mobile phase can facilitate a self-hydrolysis mechanism with the amino bonded phase). Furthermore, reducing sugars present in samples can induce Schiff base formation with the column's bonded phase (21–25). A progressive loss of bonded phase results in diminished analyte retention and degradation of peak shapes. Ultimately, column voiding can occur. For users of RI detectors, the shift in chromatographic performance might take place gradually over a few weeks, and then the column is replaced — only the symptoms of column degradation are seen. On the other hand, an evaporative light-scattering detector will respond to the actual bonded phase material (a nonvolatile particulate) leaking from the column with each chromatographic run, producing a noisy and drifting chromatographic baseline (Figure 1). In fact, the noise can be so great that reliable peak identification and quantification might become difficult. Fortunately, several alternative columns are available for such analyses: polymeric amino; diol; ion-exclusion; and resin-based size exclusion (oligomer separations). Each of these has its limitations and advantages, but all offer high-quality, reproducible chromatographic performance with common LC mobile phases and are compatible with ELSD (22,23).

Solvent purity issues: Occasionally, when using ELSD, an abnormally elevated or noisy (> 0.5 mV peak-to-peak) baseline signal is seen for a normally well-behaved mobile phase such as acetonitrile–water. This mobile-phase combination serves as a benchmark for ease of evaporation at low temperatures and low noise (< 0.2 mV peak-to-peak). Further confounding the matter, an in-line UV detector might show no such behavior. In such cases, light-scattering particles have contaminated the mobile phase. The origin of the particles might be from impurities in the mobile phase, a lack of proper filtration, or the analytical column or plumbing.

Solvent contamination problems can be isolated by removing the column and noting whether the problem persists. Solvent problems can be particularly vexing. A high performance liquid chromatography (HPLC)–grade solvent from two vendors might give different results with evaporative light scattering if different levels of light-scattering impurities are present. HPLC-grade solvent purity specifications are relative to the level of UV-absorbing impurities — these impurities might be unrelated to the solvent's light-scattering impurities. Solvent modifiers prepared from crystalline salts are another source of elevated and noisy ELSD baselines; proper filtering is mandatory.

Column bleed: If the column is implicated, late-eluted sample components might cause high background signals. Flushing the column with a strong solvent can solve this problem. Sometimes the column might bleed light-scattering material from its own degradation. For example, traditional silica-based columns are stable only within the pH range of 2–7.5. A silica-based column under acid pH conditions (pH < 2) potentially can lose bonded phase due to hydrolysis, whereas basic conditions (pH > 7.5) can result in silica dissolution. Either situation can cause poor peak shapes for basic analytes (26–31). Modern Type-B silica-based materials are less prone to such instability. Column degradation could result in mobile-phase contaminants that show up with ELSD, but that would not be apparent with UV or RI detection. However, all detectors would show a progressive erosion of the chromatography: analyte retention, resolution, and peak shape.

ELSD and LC–MS: ELSD is increasingly recognized for its value in liquid chromatography–mass spectrometry (LC–MS) detection applications (36). Because evaporative light-scattering and MS detectors both use an evaporative stage, a mobile phase that is suitable for MS also is compatible with ELSD. This commonality allows evaporative light-scattering detectors to act as surrogate MS detectors in some development activities, freeing up the more expensive MS detectors for other work. ELSD commonly will detect everything that might be discerned by MS, albeit with sensitivity lower by one or more orders of magnitude. For most analyses, the mobile phase — including volatile modifiers for purposes of column selectivity or MS ionization — and gradient proportioning can be chosen using ELSD. The resulting methodology then can be transferred directly to MS detection (32,33).

It might be advantageous to simultaneously use ELSD with photodiode-array and MS detection (34–39). (Because both evaporative light-scattering and MS detectors are destructive to samples, the mobile-phase stream must be split to provide flow for both.) By using complementary detectors, more-complete peak information can be gained in one chromatography run. Photodiode-array and evaporative light-scattering detectors respond to all eluted compounds, both chromophoric and

nonchromophoric. ELSD provides further record of the relative abundance of compounds. A mass spectrometer can be focused on one specific mass for highly sensitive and selective detection or a range of masses to yield mass information about sample components.

Preparative applications: There is an increasing trend toward using ELSD for preparative applications, either alone or in conjunction with UV detection. A fraction of the column effluent is split to feed the evaporative light-scattering detector, which can be used to trigger fraction collection of non-UV-absorbing compounds. For example, one might have a preparative system operating at 50 mL/min with a postcolumn flow splitter that feeds a UV detector and a fraction collector and sends a 5000:1 or 10,000:1 split to an evaporative light-scattering detector. A delay volume between the flow splitter and UV detector can be used to achieve simultaneous detection of peaks at both detectors. Either detector can be used to trigger fraction collection.

Conclusions

ELSD can be a very useful addition to the liquid chromatographer's set of analytical tools. The ability to detect nearly all eluted compounds in either isocratic or gradient conditions gives ELSD a broad range of applications. ELSD's universal detection nature and dependence upon volatile mobile phases make it a useful adjunct to LC–MS applications, particularly during method development. As with any LC detector, evaporative light-scattering detectors have their own unique set of potential caveats, mostly centered on their sensitivity to unwanted sample components, mobile-phase contaminants, and column bleed. Systematic problem isolation is the key to determining the source of ELSD problems.

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