



LC Troubleshooting

**Craig S. Young and
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Guest author Craig Young and column editor John Dolan provide some tips and techniques to get the most out of evaporative light-scattering detection.

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LC Troubleshooting column

Success with Evaporative Light-Scattering Detection

During the past 20 years, evaporative light-scattering detection (ELSD) has moved into the mainstream of detection choices for liquid chromatography (LC) separation. The evaporative light-scattering detector is a preferred detector for some applications — for example, carbohydrate, lipid, or polymer analysis — but it also is an attractive complement to spectroscopic detectors for a host of other applications. ELSD offers freedom from some of the limitations of spectroscopic detection because it is not limited to compounds that contain UV-absorbing chromophores, and it is immune to mobile-phase variations and gradient baseline shifting. This month's "LC Troubleshooting" column presents some practical aspects of ELSD for those considering its use in their laboratories.

Operating Principle

Evaporative light-scattering detectors for LC measure, in an absolute sense, the amount of light scattered by particles of mobile phase that have been dried through evaporation (1,2). In general, evaporative light-scattering detectors deliver a signal for all compounds that do not evaporate or decompose during the mobile-phase evaporation stage, as we will discuss below. Although design characteristics differ from manufacturer to manufacturer, a general detection mechanism is common to all evaporative light-scattering detectors, and it comprises three stages: nebulization, mobile-phase evaporation, and detection (Figure 1).

Nebulization: A nebulizer combines a gas flow of air or nitrogen with the column effluent to produce an aerosol of minute droplets.

Mobile-phase evaporation: The aerosol is introduced into a heated drift tube in which the mobile phase evaporates and leaves behind a particulate form of the target compound. Evaporation is in a heated zone, with a temperature set by users, and the useful temperature range is a matter of

distinction between the various instruments on the market.

Detection: Light striking the dried particles that exit the drift tube is scattered, and the photons are detected by a photodiode or photomultiplier tube at a fixed angle from the incident light (3–5).

Theoretically, except for highly volatile analytes (for example, ethanol in wine), most compounds can be detected. Furthermore, unlike optical absorption detectors, the detection sensitivity in ELSD is independent of the compound's spectral properties and based roughly upon the absolute quantity of compound.

Advantages of ELSD

Evaporative light-scattering detectors are considered to be universal, as is the more traditional refractive index detector. However, ELSD offers advantages over refractive index detection in that it is compatible with a much wider range of solvents and modifiers, and it produces stable baselines during gradient elution chromatography because its response is independent of the spectral properties of the analyte and sol-

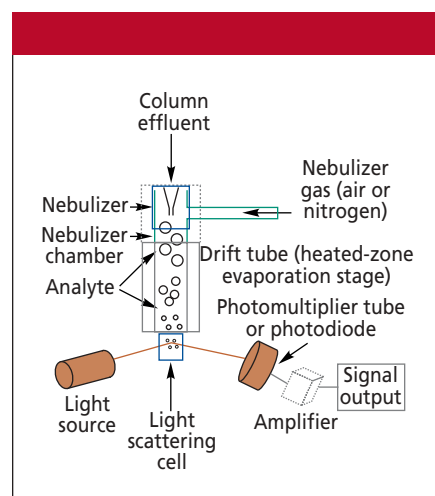


Figure 1: Evaporative light-scattering detector schematic. Note that the nebulizer and gas inlet are part of the nebulization chamber and may or may not be part of the heated zone.

vent (6–8). Compared with spectroscopic detectors, evaporative light-scattering detectors produce more-uniform detection sensitivity for most analytes, regardless of their physical and chemical properties (9).

Changes in either the drift-tube temperature or the inlet gas flow can cause the ELSD signal and noise levels to change (10). These parameters are changed neither frequently nor haphazardly. Assuming that gas flow is constant and that the evaporation temperature is sufficient to evaporate all solvent proportions and is held constant, an ELSD signal will not vary because of changes in the mobile phase's solvent proportion, temperature, or viscosity. In everyday use, this statement means that mobile-phase proportions can be changed to extremes from run to run without causing any extra equilibration time for the detector. Furthermore, the evaporative light-scattering detector will not respond adversely to the pulsations of a problematic pump, as would a refractive index or UV detector, because the baseline is not a response to a solvent bulk property.

Limitations of ELSD

Sensitivity: Small-molecule sensitivity with ELSD is limited to 1–50 ng on-column in the best cases. More commonly, 50–100 ng on-column is a generally observed limit of detection. This amount is suitable for many analyses but could fall short for some needs. By comparison, some UV and fluorescence analyses can yield detection limits in the femtogram range on-column (11,12).

Linearity: Because the evaporative light-scattering detector is not a spectroscopic detector, its response does not obey Beer's Law (11). Instead, the light-scattering phenomenon is described by three mathematical terms, all of which are influenced by particle size. The observed peak area (A) is related to the quantity of analyte on-column (m) through the relationship

$$A = am^x \quad [1]$$

where x is the slope of the response line and a is the response factor. Thus, logarithmic values for A and m will produce a linear trend ($\log A = a + x \log m$). This data treatment is well established in the literature (3,11,12). This function limits the detector's ability to be used for high-accuracy quantitative work.

Volatility: ELSD has a limitation with regard to small-molecule analyte volatility. Given the mechanism of mobile-phase evaporation, analysts should strive to find

the lowest evaporation or drift-tube temperature that will efficiently evaporate the solvent, so that semivolatile analytes of interest are not lost to evaporation as well. This limitation underscores the importance of minimizing the evaporation temperature for a given analysis. Drift-tube temperatures of 40 °C and 80 °C might not provide appreciably different analyte response for sucrose and propylparaben, but semivolatile substances such as glycerol and urea will yield much higher signals at the lower evaporation temperature (13).

Practical Considerations

Inlet gas: Aerosol formation demands an inlet gas of either air or an inert gas such as nitrogen, helium, or argon, the last gas being very costly. Most commonly, workers use instrument-grade air or nitrogen. The gas need not be highly pure, but it must be free of particulate matter. If house air is used, it must be free of oil and particulate matter. Therefore, the inlet gas must be filtered properly to remove these impurities. By their nature, evaporative light-scattering detectors consume a high volume — 2–4 L/min — of nebulizer gas (3–5).

Exhaust requirement: Because the evaporative light-scattering detector produces an aerosol, the unit must be vented to a suitable fume hood. This requirement is for health considerations; venting the unit is not important to the detector's operation, but the exhaust could present a significant health problem to individuals in the laboratory.

Need for a volatile mobile phase:

Mineral acids and bases and nonvolatile buffers such as potassium phosphate cannot be used with ELSD. These modifiers, although commonly used to adjust the pH of mobile phase when using other detectors, will in the worst cases foul the drift tube and optical cell of the evaporative light-scattering detector and in the best cases produce an unacceptably noisy baseline. Some examples of acceptable volatile modifiers are trifluoroacetic acid, ammonium formate, ammonium acetate, acetic acid, ammonium carbonate, and ammonium hydroxide.

Optimizing Settings

Evaporation or drift-tube temperature:

For analyses that involve semivolatile substances, analysts should strive to find the lowest evaporation or drift-tube temperature that will evaporate the mobile phase efficiently. This temperature is the lowest temperature that produces an acceptably low noise signal. Low temperatures mini-

mize the chance of missing semivolatile analytes that could be lost to evaporation at higher drift-tube temperatures. When analyzing natural products, for example, it is desirable to achieve near-ambient evaporation temperatures so that all but the most volatile substances can be observed. In practice, it is common to find one or two evaporation temperatures that suit most needs.

For high-sensitivity gradient analyses, the evaporation temperature should be set to minimize the baseline noise, which is a measure of nebulization efficiency, for the gradient condition that presents the greatest evaporative burden. For example, in reversed-phase chromatography with a binary gradient of water to acetonitrile, the beginning condition of the gradient will be the most prone to baseline noise because the water, which is more difficult to evaporate, is in the highest proportion. Thus, the drift-tube temperature should be set to minimize baseline noise under the initial gradient conditions. Conversely, for normal-phase chromatography with a binary gradient of acetonitrile to water, the final condition of the gradient will be the most prone to baseline noise as water reaches its highest proportion. Accordingly, the drift-tube temperature should be set to minimize baseline noise under the final gradient conditions. For analyses not

requiring high sensitivity, baseline noise could be irrelevant.

Figure 2 shows noise characteristics for some common mobile phases at the same evaporation temperature. Once again, it is common practice to find one or two drift-tube temperatures that suit most needs.

Mobile-phase flow rate also affects the choice of evaporation temperature. Higher flow rates will increase the evaporative burden of the drift tube, particularly when water is present. Increasing the flow rate for fast chromatography could require an increase in drift-tube temperature for the same mobile phase.

Inlet gas pressure: Nebulizer efficiency plays a role in determining the sensitivity of the evaporative light-scattering detector. Accordingly, an optimum inlet gas flow rate (usually 2–4 L/min) will produce a good signal-to-noise ratio. Although aerosol formation can show some dependence upon solvent viscosity, it is recommended to find one inlet pressure (usually 35–60 psi) that will produce acceptable performance for most analyses (3–5). A few experiments to bracket the inlet pressure changes around the manufacturer's recommended setting could be necessary.

Correcting Common Misconceptions

Although most chromatographers have seen manufacturers' advertisements for evaporative light-scattering detectors, few of them are well versed in the details of evaporative light-scattering detector operation that make it unique among detector alternatives. Misconceptions about ELSD abound; some of these misconceptions are addressed below.

- *The evaporative light-scattering detector is not a spectroscopic detector.* This statement was made clear previously in the description of operation. However, the difference must be kept in mind during routine operation of the detector because its behavior is unlike that of a spectroscopic detector. As an example, it is common to believe that an analytical column is clean of analytes after monitoring by low-wavelength UV detection, only to find that the ELSD response is at maximum, an indication that non-UV-absorbing materials are being eluted from the column. Similarly, the evaporative light-scattering detector will respond to bonded-phase loss in a column under harsh conditions. This response is easy to see when using silica-based amino columns with aqueous mobile phases — hydrolysis of the bonded phase results in

contamination of the column effluent with hydrolyzed bonded phase that does not absorb in the UV region but which produces a noisy, rising baseline in the ELSD response. The phenomenon can go unnoticed when using a UV or refractive index detector, although analysts might observe a slow reduction in column performance (reduced peak symmetries and resolution) (9).

- *The evaporative light-scattering detector makes an absolute measurement, not a difference measurement.* The detector response arises from an absolute photon count from light-scattering particles rather than a difference measurement between analyte and mobile phase. Mobile-phase solvents and modifiers are lost to evaporation before a light-scattering measurement is made. As a result, the evaporative light-scattering detector signal does not vary with solvent proportion, temperature, or viscosity. The only observed equilibration time is caused by changing the temperature at the evaporation drift tube stage. As soon as the detector's actual drift-tube temperature reaches its set temperature, it produces a stable signal.
 - *The evaporative light-scattering detector is not a mass spectrometer, and it cannot give molecular mass information.* This unfortunate misconception arises from a mistaken interpretation of a phrase used in marketing — *mass sensitive*. Casual readers frequently misinterpret this phrase and think that the detector delivers mass information. It does not. The evaporative light-scattering detector is mass sensitive in the sense that the light-scattering phenomenon is dependent upon the size of the dried particle aggregates that remain after the evaporation stage. Hence, the detector output truly reflects the quantity or mass of total analyte responsible for the light scattering. In other words, the evaporative light-scattering detector is sensitive to the total mass of analyte that reaches its optical cell (1).
- ### Other Considerations
- *The evaporative light-scattering detector is a destructive detector.* The evaporative light-scattering detector generates an aerosol of sample and matrix that is lost to the exhaust. Thus, the detector must be last in line if it is used in series with other detectors.
 - *ELSD presents negligible back pressure.* Because the inlet is a nebulizer, the evap-

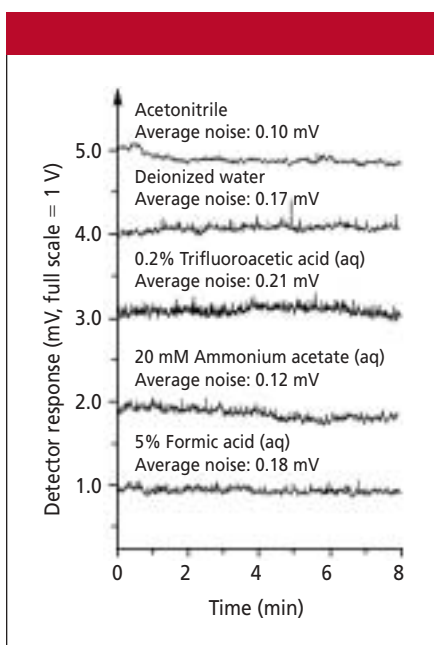


Figure 2: Baseline noise for various mobile phases. Shimadzu ELSD LT detector (gain: 6; drift-tube temperature: 32 °C; inlet gas pressure: 250 kPa), no column installed. Mobile-phase flow: 1 mL/min.

Table I: Troubleshooting the evaporative light-scattering detector

Symptom or Malfunction	Possible Cause	Corrective Action
Excessive noise or continuous spiking	Nonvolatile salts present in the mobile phase	Use a fresh mobile phase that contains no incompatible modifiers
	Unfiltered mobile phases that contain modifiers	Prefilter the mobile phase
	Insufficient drift tube temperature to efficiently evaporate the mobile phase	Raise the evaporation temperature in 5 °C increments as necessary; a high proportion of water in the mobile phase will require a higher evaporation temperature than one with a high proportion of organic solvent; a formic acid modifier in the mobile phase will require a higher evaporation temperature than the same mobile phase without a modifier
	Inlet gas pressure that is either much too low or much too high	Adjust the inlet gas pressure; usual values are 35–60 psi; typically, this adjustment is not finicky and does not need to be done often
	Nebulizer could be dirty and creating a sputtering effect Nebulizer might be damaged and creating a sputtering effect	Clean the nebulizer according to manufacturer's recommendation Harsh conditions could have etched the stainless steel surface of the nebulizer; replace according to the manufacturer's recommendation
Transient spiking	Chemical degradation of the column from incompatible solvents or harsh pH conditions	Monitor the ELSD signal without the column installed to distinguish a column problem from a detector problem; either change the mobile phase or replace the column
	Nonvolatile particles in the drift tube or optical cell Particulates in the inlet gas supply	Stop mobile-phase flow; increase the inlet gas flow to the high end of the range for a period of time Take steps to filter the gas supply
Wandering baseline	Column not equilibrated	Disconnect the column and verify that the signal is stable without a column in-line; equilibrate the column
	Chemical degradation of the column from incompatible solvents or harsh pH conditions	Monitor the ELSD signal without the column installed to distinguish a column problem from a detector problem; either change the mobile phase or replace the column
	Changing drift-tube temperature	Allow sufficient time for the drift tube to achieve the set temperature
UV signal at normal levels but ELSD signal off scale	Non-UV-absorbing compounds emerging from the column	Wait and remember that the evaporative light-scattering detector responds to all semi- and nonvolatile analytes

orative light-scattering detector presents negligible back pressure, and it can be plumbed in serial fashion after any nondestructive detector.

Troubleshooting the Evaporative Light-Scattering Detector

Table I is meant to serve as a guide to common ELSD problems and their solutions. When trying to ascertain detector malfunctions, it is advisable to have established a benchmark of performance under three conditions: gas flow without mobile-phase flow; column off-line with gas flow and mobile-phase flow; and column on-line with gas flow and mobile-phase flow. This practice helps to distinguish between column problems and detector problems.

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