Waters™

アプリケーションノート

Waters 2420 Evaporative Light Scattering Detector

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief describes Waters 2420 Waters 2420 Evaporative Light Scattering Detector.

Benefits

- High signal-to-noise performance from unique design features, low noise electronics and sophisticated control
- Application flexibility by fully controlling all parameters, especially temperature
- Full control and time programming whether standalone or by a data system means that methods development can be automated and streamlined
- PMT gain control by time programming allows the user to optimize S/N for each peak
- Low temperature solvation is available when required for labile compounds
- Increasing the temperatures at the nebulizer and the drift tube allows the unit to support high flow rates efficiently without losing sensitivity, avoiding blockages and minimizing dispersion, in conjunction with multiple nebulizer availability
- Multiple high-power heating supports fast warm up times
- Full qualification methodology: IQ/MQ/OQ/PQ for the detector, system, and software including test solutions are available
- Supports multiple flow rates and dispersion requirements by providing multiple nebulizers

Introduction

What is Evaporative Light Scattering Detection?

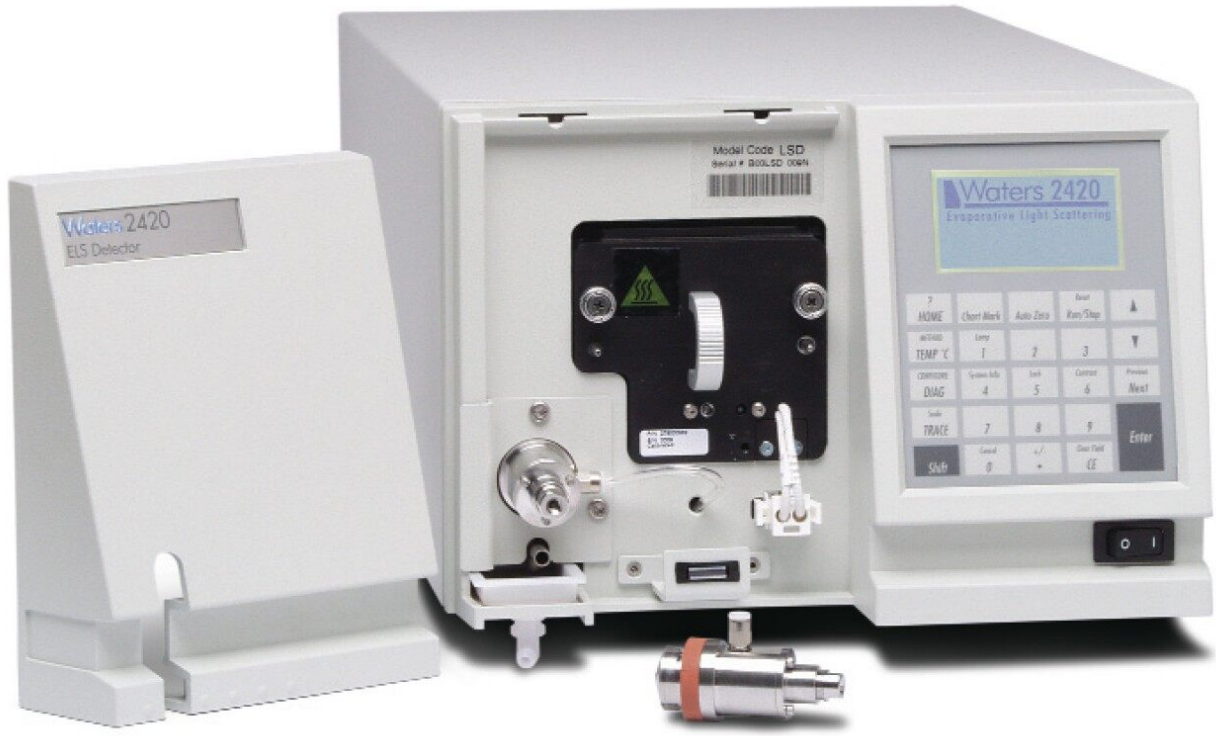
Evaporative Light Scattering detection (ELSD) is a HPLC detection technique based on the ability of particles to scatter light when they pass through a beam of light. The detector will respond to compounds that are less volatile than the mobile phase. Evaporative light scattering therefore offers an alternative detection strategy for compounds that do not have a UV chromophore, and, unlike refractive index detectors, ELSD is compatible with gradient analyses. Evaporative light scattering detection is compatible with a wide range of flow rates and mobile phase compositions and can be part of a multi-detection scheme (for example: PDA/MS/ELSD).

Evaporative Light scattering detection is useful for wide range of compounds and compound classes including:

- Carbohydrates (Application Note: 720000727EN)
- Polymers/Copolymers/Blends (Application Note: 720000735EN < https://www.waters.com/nextgen/us/en/library/application-notes/2003/2420-evaporative-light-

scattering-detector-analysis-of-polyethylene-glycol.html>)

- Polymer additives
- Pharmaceuticals (Figures 1 and 2)
- Lipids and fatty acids
- Amino acids (Figure 3)
- Surfactants
- Nutraceuticals (Figure 4)



Waters 2420 Evaporative Light Scattering Detector.

Results and Discussion

Technology and Design

An ELSD has three basic elements: nebulizer, desolvation tube (often referred to as a drift tube) and scattering chamber. In summary, a solvent stream is nebulized and the droplets formed in the nebulizer are entrained in a flow of gas. The droplets are evaporated in the desolvation region and, if there was non-

volatile analyte present in the solvent stream, dry particles remain which are carried along in the flowing gas and solvent vapor stream. A beam of light intersects the path of the flowing stream. If dry particles are present, they scatter light. The scattered light is measured and the intensity of this light is a function of the size and number of such particles.

Nebulization

Column effluent is passed through a narrow needle and mixed with gas (typically nitrogen, but any dry, particulate free gas can be used) to produce an aerosol of droplets. The 2420 nebulizers are tailored to flow rate, a high flow nebulizer for flow rates of 0.30–3.0 mL/minute, and a low flow nebulizer for flow rates of 0.05–0.50 mL/minute. Nebulizer gas flow rate can be adjusted to optimize detector signal and minimize noise. High gas flows give smaller droplets that require less heat to evaporate off the solvent to leave the non-volatile sample. Lower gas flows give large droplets (which generally generate larger particles, giving larger signals), but require more heat to evaporate the solvent, leaving the non-volatile sample and can, if the solvent is not fully evaporated away, cause noise. Droplets that are too large to pass into the drift tube are removed via a siphon tube to waste. The nebulizer region can be heated to improve evaporation and increase signal; this temperature setting should be determined experimentally.

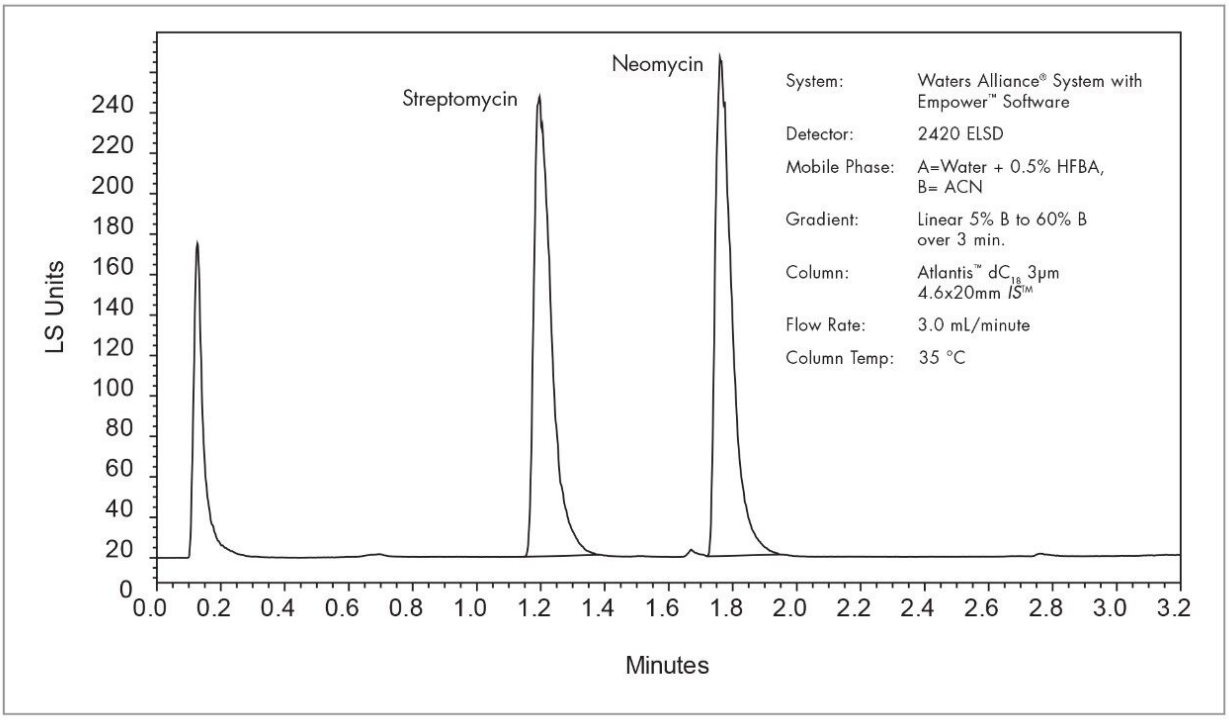


Figure 1. Separation of streptomycin and neomycin.

Desolvation (Drift Tube)

Nebulized droplets are driven into the drift tube by the carrier gas and diffuse down the drift tube. Once in the drift tube, particles are generated by the evaporation of the mobile phase components leaving the non-volatile portion of eluent as particles. Ideally, the drift tube is heated to a temperature that facilitates solvent removal without sample depletion. Higher drift tube temperatures are more efficient at removing solvent, but may evaporate semi-volatile sample components leading to a reduction in signal. Optimal drift tube temperatures are best determined experimentally for each individual compound of interest. When dealing with totally unknown sample components, users should use the lowest drift tube temperature that adequately evaporates the mobile phase at its given flow rate.

Detection

The scattering chamber is the equivalent of the flow cell in UV/Visible detectors. Incident light is generated from a quartz-halogen lamp and passes through a series of lenses and mirrors into the scattering chamber where it interacts with the sample particles. A photomultiplier tube detects light scattered by particles and the resulting signal is output to a data collection device (directly to Empower or MassLynx Software, or other software through a SAT/IN module). The optics bench is also heated to avoid condensation of evaporated mobile phase components. After detection, the particle stream and evaporated solvent are exhausted from the detector.

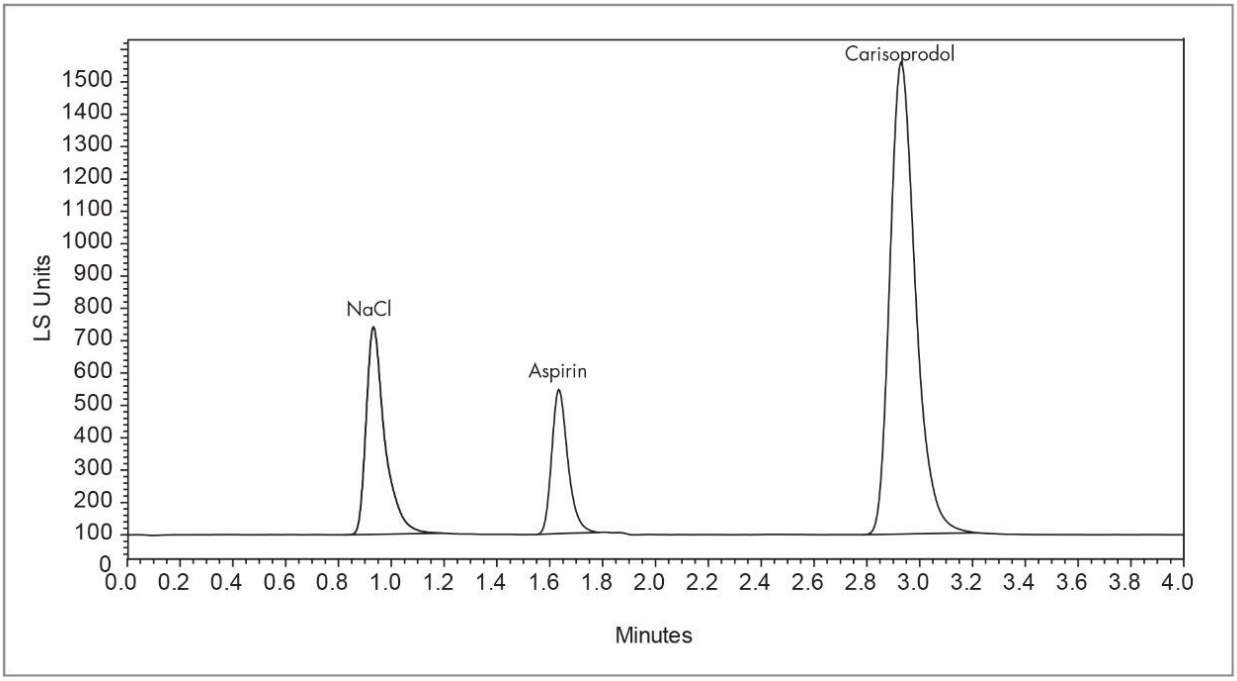


Figure 2. Separation of sodium chloride (void volume marker), aspirin and carisprodol.

System:	Waters Alliance System with Empower Software
Detector:	2420 ELSD
Column:	Symmetry C_{18} Column 5 µm 3.9 x 150 mm
Mobil phase:	A=Water + 1% Formic Acid, B= Methanol
Composition:	Isocratic 36% A, 64% B
Flow Rate:	1.0 mL/minute
Column temp.:	30 °C

Linearity

ELSD’ s are not spectrometric detectors; therefore, they do not obey Beer’ s Law and are fundamentally non-linear. Scattering is independent of the particle’ s chemical properties. It is a function of multiple processes including Rayleigh scattering, Mei scattering, refraction, and reflection. The size and shape of the particle, number of particles, and the wavelength of the incident light all impact light scattering. The absolute detector response is a mixture of all scattering types, although any one type may predominate in a given sample. Calibration curves produced by an ELSD best fit to quadratic equations, but may also be fit to log/log curves.

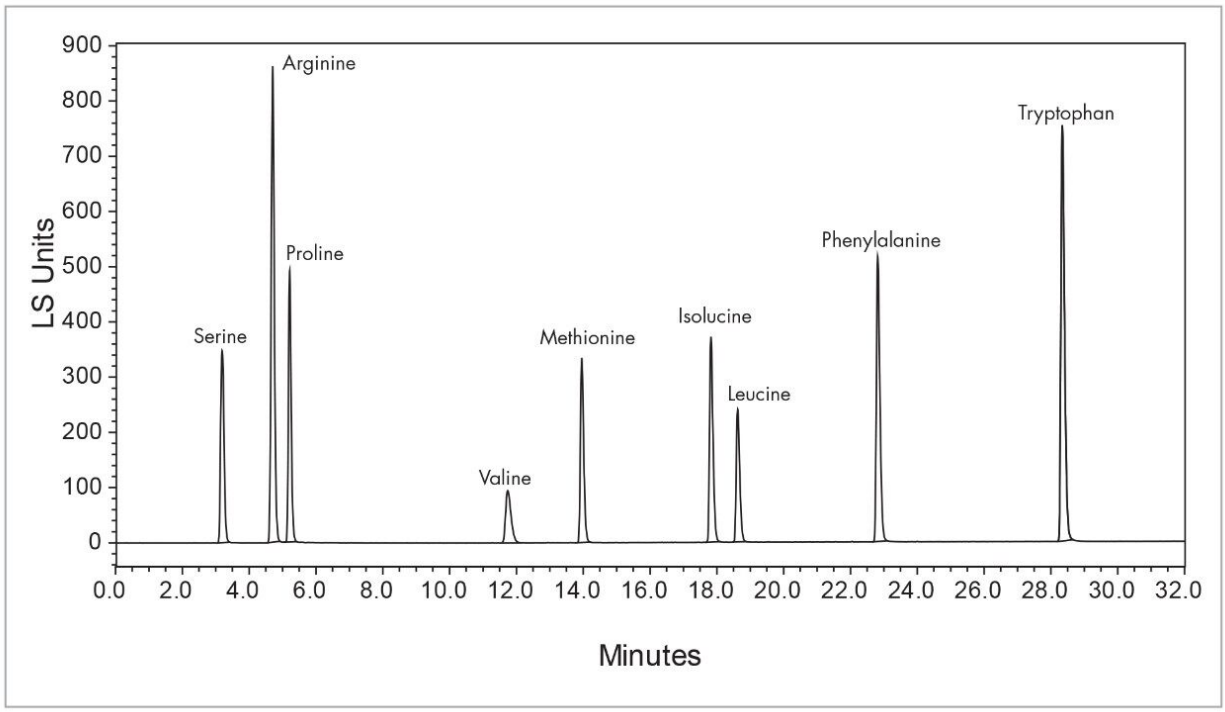


Figure 3. Separation of a 9 amino acid mixture.

System:	Waters Alliance System with Empower Software
Detector:	2420 ELSD
Column:	Atlantis dC_{18} 5 µm 4.6 x 250 mm
Mobil phase:	A=Water + 0.1% TFA, B= ACN + 0.1% TFA
Gradient:	5 min Isocratic hold, Linear 0% B to 30% B over next 30 min.
Flow Rate:	1.0 mL/minute
Column temp.:	20 °C

Waters 2420 ELSD features:

- High sensitivity and low noise performance
- Time-controlled temperature, photomultiplier tube (gain) and gas pressure

- Controlled by Waters Empower Software and MassLynx version 4.0 SP2 Software
- Front panel control and up to 10 method storage
- Controls Waters column heater module (CHM)
- Ultimate temperature control utilizing heat at both the nebulizer and drift tube as well as a heated optics bench
- Smallest commercially available detector allowing it to be stacked with other Waters detectors
- Full qualification methodology: IQ/MQ/OQ/PQ for the detector, system and software including test solutions are available
- Supports multiple flow rates and dispersion requirements by providing multiple nebulizers

Serviceability and Usability:

- Front-mounted user replaceable long-life lamp
- Snap in nebulizer mounting
- Calibrated high sensitivity long life PMT
- User diagnostics, including lamp history
- Fail-safe devices such as automatic solvent shut-off switch and leak management

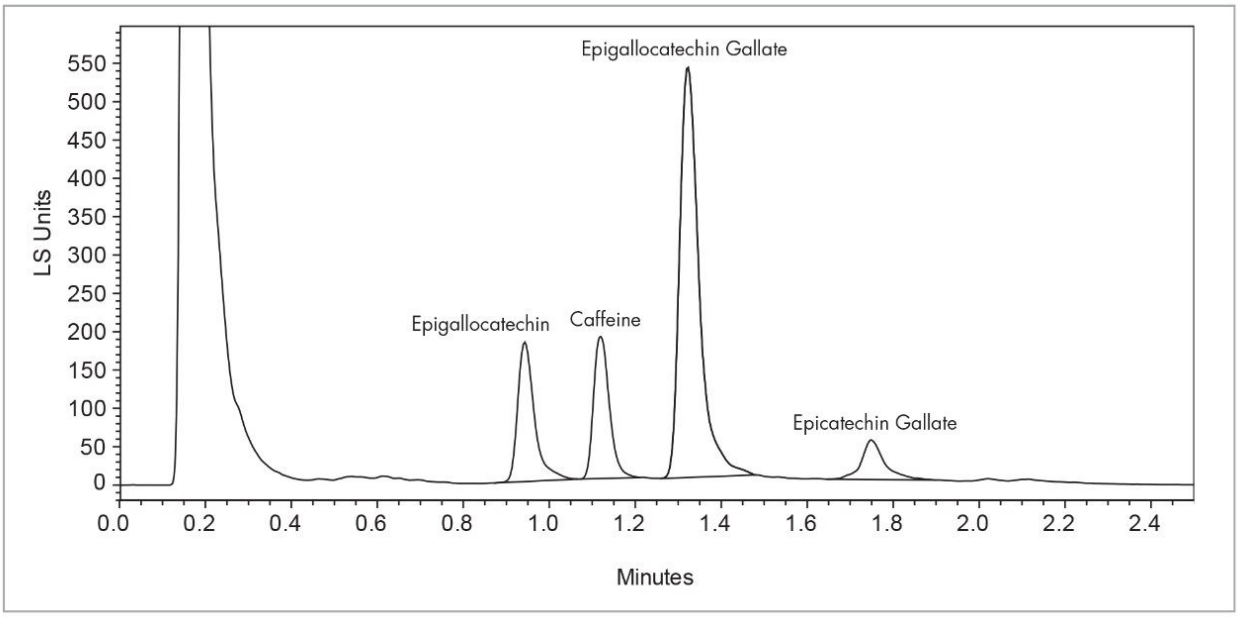


Figure 4. Separation of a green tea extract.

System:	Waters Alliance System with Empower Software
Detector:	2420 ELSD
Column:	Atlantis dC_{18} 3 µm 4.6 x 20 mm IS
Mobil phase:	A=Water B= Methanol
Gradient:	Linear 5% B to 30% B over 2 min.
Flow Rate:	3.0 mL/minute
Column temp.:	40 °C

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