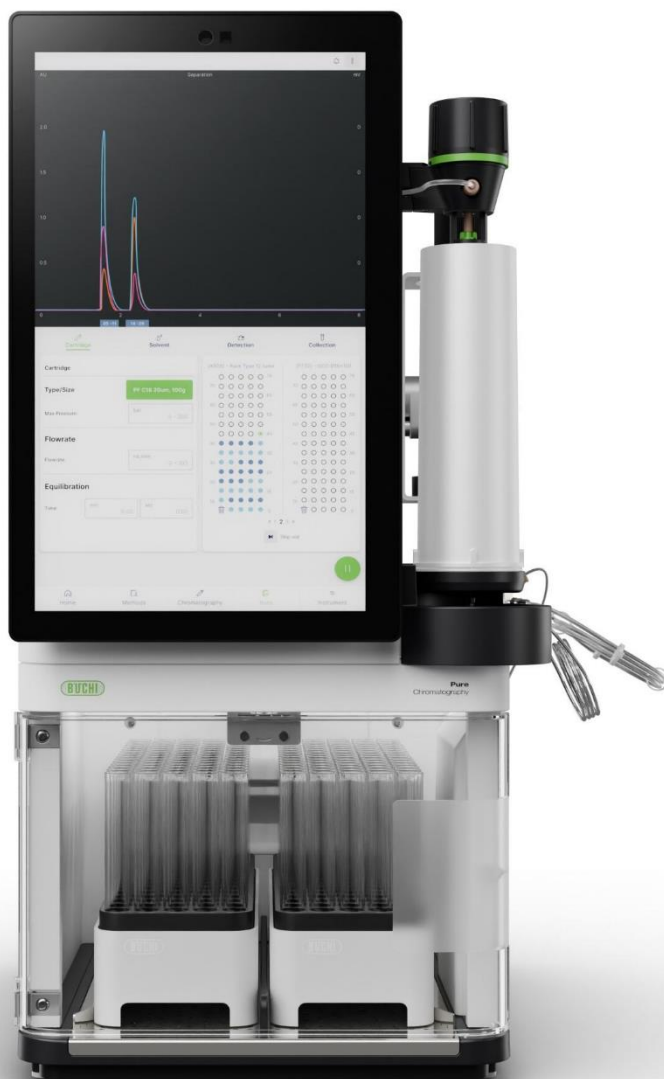




Technical Note – N°. 889/2025

# Pure Excellence ELSD

## Abstract:

This Technical Note assists users in understanding the operation and optimal utilization of the Pure Excellence ELSD (Evaporative Light Scattering Detector) for flash/prep HPLC chromatography. It provides an overview of the underlying principles, key guidelines for use, and a comprehensive performance review to ensure accurate and efficient ELS detection across various applications.



## 1. Introduction

### 1.1 Why using Evaporative Light Scattering Detection (ELSD)?

Molecules lacking certain functional groups or configurations, such as surfactants, sugars, fats, and steroids, cannot be detected by UV detectors, as only compounds with specific functional groups or configurations absorb light in the UV range. Furthermore, UV detection can be complicated when the mobile phase, such as ethanol or ethyl acetate, absorbs at the measured wavelength, interfering with analyte detection.

In contrast, ELSD requires no specific chemical characteristics for detection, making it ideal for non-chromophore applications. It enables precise analyte detection without interference from the solvent signal, ensuring accurate sample collection. Additionally, since the ELSD signal is directly proportional to the analyte's mass, it is highly effective for quantitative analysis, with no limitations related to sample structure.

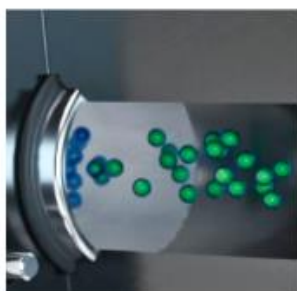
The primary drawback of ELSD detection is that it is a destructive technique, though only a small portion of the sample is consumed during analysis. Since the detection principle relies on solvent evaporation, its key limitation is that the target compound must be less volatile than the solvent.

Therefore, ELSD is highly effective at detecting a wide range of analytes regardless of their chemical structure, while UV detection specifically identifies compounds with chromophores. When an analyte is more volatile than the solvent, a limitation for ELSD, UV detection complements this by providing reliable identification. Together, these techniques compensate for each other's shortcomings, delivering efficient and comprehensive analysis across diverse sample types.

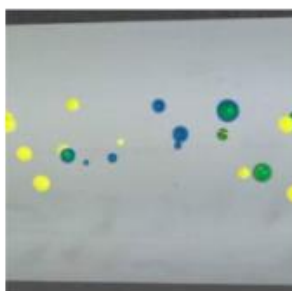
|             | UV                                                                                                                                           | ELSD                                                                                                                   |
|-------------|----------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|
| Advantages  | <ul style="list-style-type: none"><li>· Compound identification</li><li>· Non-destructive</li></ul>                                          | <ul style="list-style-type: none"><li>· Universal detection</li><li>· Quantitative</li></ul>                           |
| Limitations | <ul style="list-style-type: none"><li>· Poor response for non-UV-absorbing compounds</li><li>· Solvent limitation due to UV cutoff</li></ul> | <ul style="list-style-type: none"><li>· Destructive</li><li>· Sample must be less volatile than mobile phase</li></ul> |

### 1.2 How does ELSD work?

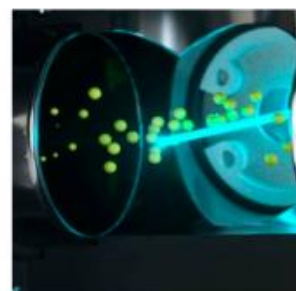
The general mechanism of ELSD involves measuring the amount of light scattered by particles of solvent which have been dried through evaporation. The process consists of three steps: nebulization, solvent evaporation and detection. During nebulization, a nebulizer combines a gas flow of air or nitrogen with the column or cartridge effluent to produce an aerosol of tiny droplets. In the next step, the droplets are introduced into a drift tube where the mobile phase evaporates and leaves behind a particulate form of the target compound. In the last step, light strikes the dried particles which exit the drift tube, the light is scattered, and the resulting photons are detected by a photodiode.



Step 1. Nebulization



Step 2. Mobile Phase Evaporation



Step 3. Detection

### 1.3 Solvents and buffers

ELSD relies on evaporating the mobile phase to detect the analyte. Selecting the right solvents and buffers is therefore crucial to optimize the ELSD performance while ensuring good chromatographic separation.

#### 1.3.1 Solvents

The choice of the solvent is based on its boiling point, which values can be found below:

| Solvent               | Boiling Point (°C) | Solvent         | Boiling Point (°C) |
|-----------------------|--------------------|-----------------|--------------------|
| Dimethyl sulfoxide    | 189                | Benzene         | 80                 |
| N,N-Dimethylformamide | 153                | Ethyl acetate   | 77                 |
| Toluene               | 111                | Ethanol         | 78                 |
| Water                 | 100                | Hexane          | 69                 |
| Propanol              | 97                 | Tetrahydrofuran | 66                 |
| Dipropylether         | 90                 | Methanol        | 64                 |
| Dichloroethane        | 84                 | Chloroform      | 62                 |
| Acetonitrile          | 82                 | Acetone         | 56                 |
| Cyclohexane           | 81                 | Dichloromethane | 40                 |

It is recommended to choose volatile solvents that evaporate easily through the ELSD, the most common being: Hexane, Ethanol, Methanol, Acetonitrile, Ethyl Acetate, Dichloromethane and water.

#### 1.3.2 Buffers

Buffers are sometimes necessary to maintain pH stability for analyte solubility and separation. For ELSD, volatile buffers are essential, such as: Trifluoroacetic acid, Formic acid, Acetic acid, Ammonia, Ammonium bicarbonate.

### 1.4 Typical applications

Some substances and compounds do not possess any chromophoric structures, such as conjugated double bonds or aromatic groups. This makes them undetectable by UV detectors and can only be purified with ELSD:

- Essential oils
- Ginkgolides
- Natural products
- Phospholipids
- Polymers
- Fats
- Oils
- Sugars
- Carbohydrates
- Fatty acids
- Amino acids
- Dextran
- Steroids
- Vitamins
- Histidine
- Polystyrene
- Lipids
- Surfactants

## 2. Equipment

Pure Excellence C-915 or C-950.

## 3. Chemicals

**Sample 1:** Mixture of chromophoric and non-chromophoric compounds.

**Sample preparation** (20 g of the solid sample).

1. Weigh the following compounds in a 50 mL bottle.
  - 200 mg of cholesteryl acetate (non-chromophoric).
  - 400 mg of methyl paraben (chromophoric).
  - 400 mg of 4-aminobenzoic acid (chromophoric).
2. Add 20 mL of ethyl acetate and put the solution into an ultra sonification bath for 15 minutes.
3. Transfer the solution into a 2 L evaporation flask and add 19.00 g of silica gel 60 and 30 mL ethyl acetate.
4. Evaporate the solvent on the Rotavapor with following parameters:
  - Bath temperature: 60 °C.
  - Rotation: 200 RPM.
  - Cooling temperature: 20 °C.

A vapor duct with frit is used to prevent the silica going into the condenser.

5. Empty receiving flask and change to drying mode with a vacuum of 5 mbar for 10 minutes.
6. Load 1.00 g of the solid mixture in a 15 g solid loader tube.

**Sample 2:** Non-chromophoric Sodium Chlorid solution.

12 g NaCl diluted in 100 mL, sonicated for 15 min.

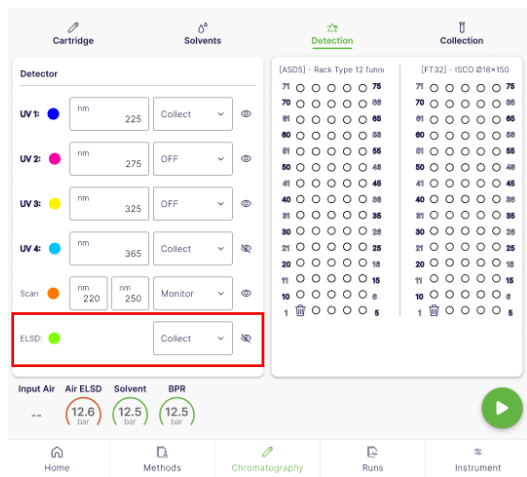
## 4. Procedure

The samples are run on a Pure Excellence instrument and the following describes the setup and handling of its ELSD.

The ELSD is built in the instrument without needing any additional lab space and positioned between the cartridge/ column and the UV detector. A small portion of the sample/solvent flow is diverted to the ELSD, while the main flow continues to the UV detector and fraction collector.

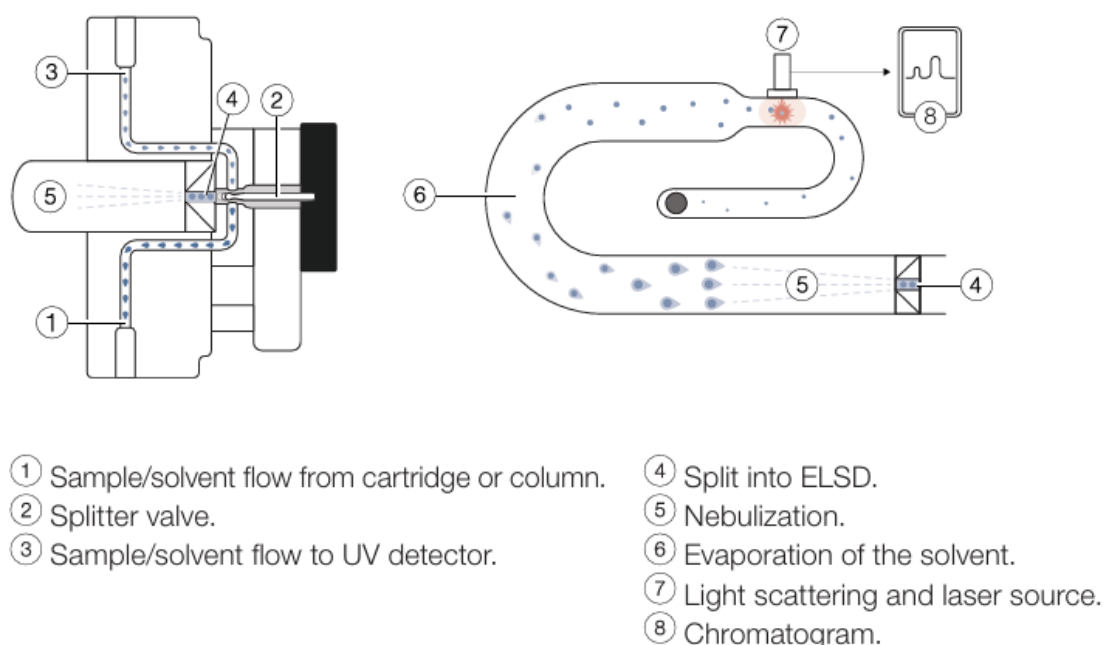
Operation:

The ELSD gets switched on/ off via one button in the software.



#### Process steps:

- **Sample injection:** 30 µL/min get automatically splitted from the main sample/solvent flow into the ELSD. Small droplets are formed and pushed towards the nebulizer. Thanks to a patented nano-pulse mechanism the amount of liquid being split remains constant, even under varying experimental conditions (flow rate, solvent), thereby maintaining consistent signal quality. The sample loss is the lowest on the market.
- **Nebulization:** The droplets are directed into the nebulizer where compressed gas transforms those into an ultra-fine mist entering the evaporation tube.
- **Evaporation:** The mist is carried by the gas through a U-shaped tube towards the detector. Due to the small droplet size, the solvent evaporates efficiently without the need for external heating (optimal performance within a temperature range of 15–40°C). This approach minimizes thermal impact on the sample and optimizes performance.
- **Detection:** A laser serves as the light source, making it highly effective for detecting the ultra-fine mist particles generated by the nebulizer. The analyte particles, now in solid form, pass through a beam of light, scattering it in proportion to their concentration. The resulting light is captured by a trap and focused by a lens onto a photodiode, enabling precise and highly sensitive detection.



#### Gas source:

Air or nitrogen can serve as the carrier gas, provided it is clean, dry, inert, and compressed to at least 4 bars. Back pressure below this level will significantly reduce ELSD performance. Gas purity is critical, as impurities can affect signal quality, and optimizing this aspect is essential for achieving the best results. The Pure drying unit and compressors can optionally be connected to the Pure Excellence instrument to meet all requirements.

#### Cleaning & maintenance:

Thanks to its ingenious functionality, no valve, transfer line, or make-up pump is required for sample transport. This means that almost no spare parts are required, and it is easy to maintain.

For optimal performance of the ELSD regular cleaning is essential. Cleaning the nebulizer is recommended as part of this routine. A clear indication that the nebulizer requires cleaning includes: noisy signal output and elevated baseline readings. No tools are needed to access the nebulizer. Cleaning should be done as following:

- Turn the knob to unlock it.
- Pull out the knob.
- Leave the released knob pulled out.
- Remove the nebulizer.

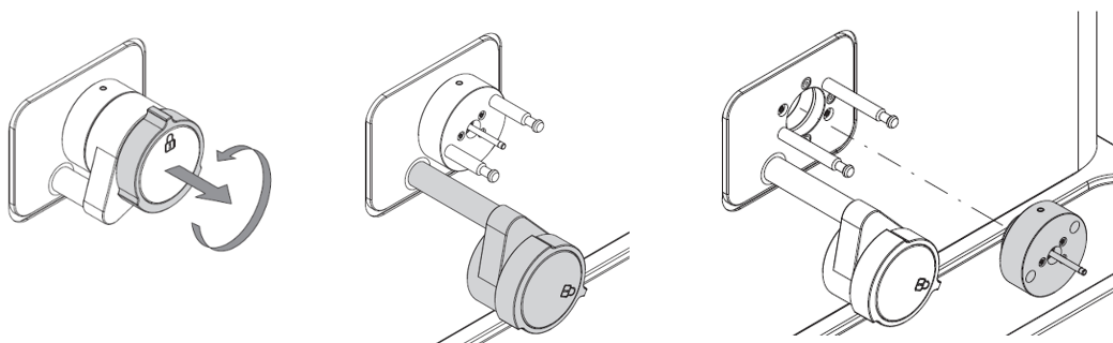


Figure 1: Chromatogram of acarbose and maltose.

- Inspect the Nebulizer, checking for impurities or contamination on its surface.
- Clean the Nebulizer by submerging it in a container filled with isopropanol for ~15 minutes or until complete removal of contaminants.
- Dry the Nebulizer with a clean, lint-free wipe, ensuring no particles or moisture remain.

Once cleaned, insert the nebulizer following those steps:

- Insert the nebulizer.
- Push the knob back in.
- Lock the knob.

## 5. Results

### 5.1 Combination of ELSD and UV detection

A mixture of chromophoric and non-chromophoric compounds have been detected on the Pure Excellence instruments C-915 and C-950. While the chromophoric compounds methyl paraben, 4-aminobenzoic acid can be detected by UV, the non-chromophoric cholesteryl acetate is only visible via ELSD. The results show perfect coordination of the detectors and demonstrate their ideal combination to detect almost any compound.

#### 5.1.1 Experimental conditions

- **Sample:** 1.00 g of the solid mixture (cholesteryl acetate, methyl paraben, 4-aminobenzoic acid) in a solid loader.
- **Flash cartridge:** FlashPure EcoFlex Silica, 12 g.
- **Flow rate:** 30 mL/min.
- **Equilibration:** 4.8 min.
- **Run length:** 7 min.
- **UV wavelengths:** 254, 265, 280, 320 nm.
- **ELSD:** on.
- **Solvent A:** n-Heptane.
- **Solvent B:** Ethyl acetate.

| Step | Time (min) | Ethyl acetate (%) |
|------|------------|-------------------|
| 1    | 0.0        | 0                 |
| 2    | 1          | 0                 |
| 3    | 5          | 100               |
| 4    | 1          | 100               |

### 5.1.2 Chromatogram

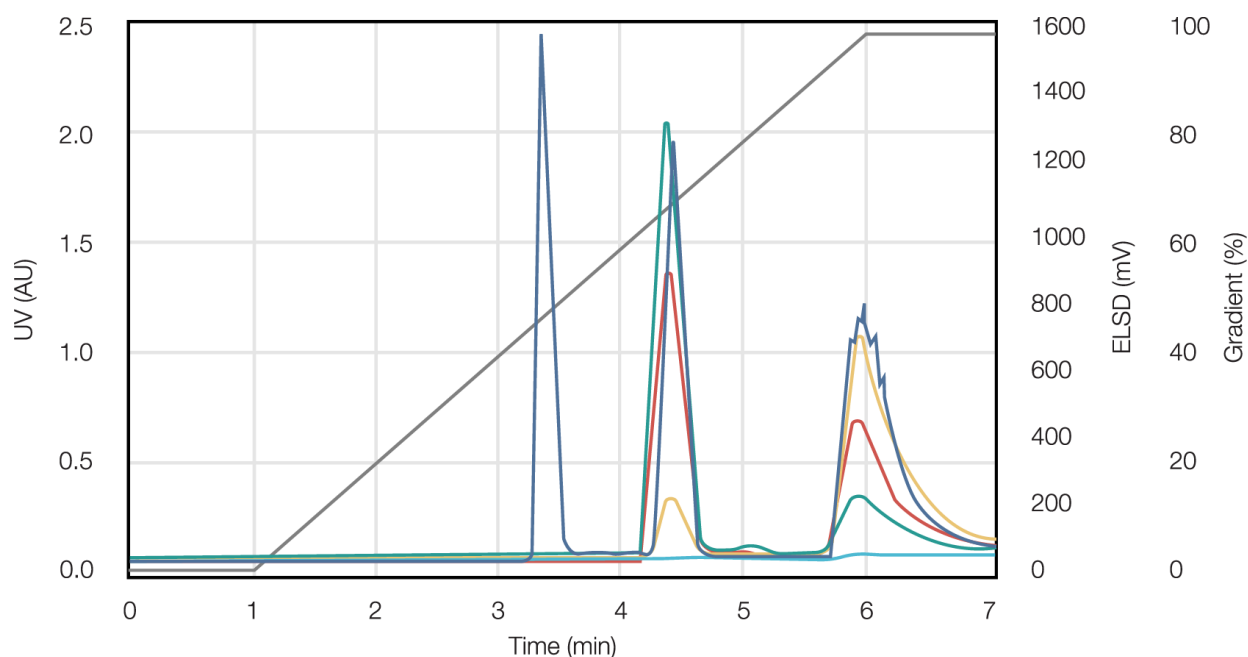


Figure 2: Chromatogram of cholesteryl acetate (non-chromophoric), methyl paraben (chromophoric), 4-aminobenzoic acid (chromophoric), dark blue: ELSD signal; all other colors: UV wavelengths 254, 265, 280, 320 nm.

### 5.2 ELSD signal quality

NaCl was dissolved in water and injected at varying volumes into the Pure Excellence instruments C-915 and C-950 (between 0.2 to 1 mL = 0.024 to 0.12 g) without using a cartridge or column. The results highlight the ELSD's exceptional reliability, sensitivity and precise signal quality thanks to its nano-pulse injection technology. The chromatogram demonstrates consistent signal performance across different concentrations with a steady increase. Despite water's high boiling point, which typically causes noisy signals, the heater-free ELSD maintains smooth signal output, showcasing its superior performance even with challenging solvents.

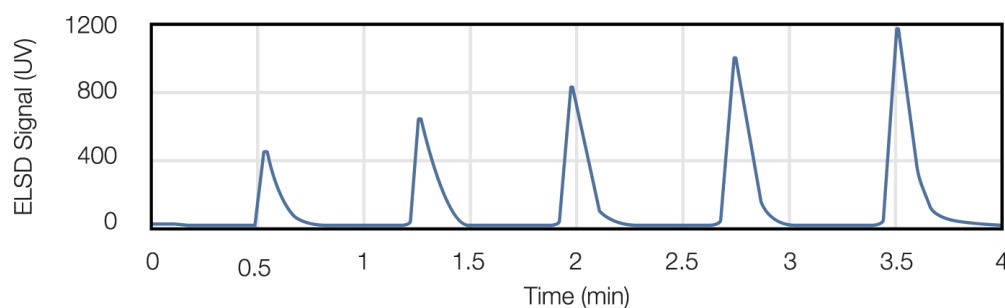


Figure 3: Injection of different volumes of NaCl diluted in water.

## 6. Conclusion

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In summary, the Pure Excellence ELSD combines exceptional performance with practical design features to meet the demands of flash/prep HPLC chromatography. It is perfectly coordinated with the Pure UV detector, highly sensitive and its signal quality ensures accurate and reliable detection. Thanks to its small design (built in the instrument) and its simplified maintenance it is the ideal choice for any chemical synthesis lab.