

Evaporative Light Scattering Detectors

as an Alternative Detection Method for Flash Chromatography

Abstract

The CombiFlash® Rf system has an input that allows external detectors to send an analog peak signal for display and peak cutting. Examples of detectors that can be used with the CombiFlash Rf system include Refractive Index (RI), fluorescence, visible light¹, Evaporative Light Scattering Detectors (ELSD), or other devices generating a voltage range of 0–1 volt². This feature is available on CombiFlash Rf systems with an external detector connector (serial number 209C20075 and higher) and running software version 1.5 or higher. The connection and use of an ELSD is discussed with examples.

Background

ELSD works by measuring the light scattered from solute particles remaining after nebulization and evaporation of the mobile phase. An ELSD consists of three main parts:

- The *nebulizer* where the compound and mobile phase are sprayed into the detector as droplets.
- The *drift tube* evaporates the solvent leaving the compounds as a fine powder. The drift tube is generally heated to facilitate evaporation of the solvent.
- A *detector* containing a light source and sensor.

ELSD is used when a compound has no chromophore or a weak chromophore, or the chromophore is masked by the absorbance of the mobile phase. ELSD is also used when the absorbing wavelength of the desired compound is unknown.

An ELSD should be configured properly for best results. The nebulizer and drift tube temperatures need to be set properly. The gas flow needs to be set properly on some units. High quality solvents should be used with an ELSD to reduce noise. Refer to your ELSD documentation for more information.

Note

Buffers containing metal cations are not compatible with ELSD because they also produce particles after solvent removal that masks eluted compounds.

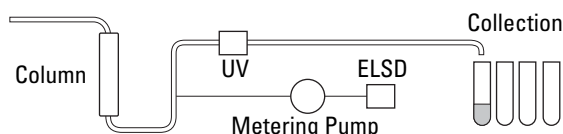


Figure 1: Simple diagram of fluid path

1. The CombiFlash Rf-200 with UV-vis detection is available as part number 68-5230-008
2. Contact the factory for special cable if the voltage range exceeds 1 volt.

Discussion

Connection to CombiFlash Rf

Evaporative light scattering detectors are considered “destructive detectors” where the sample is lost as part of the detection technique. The actual amount of sample lost is small (less than 5%).

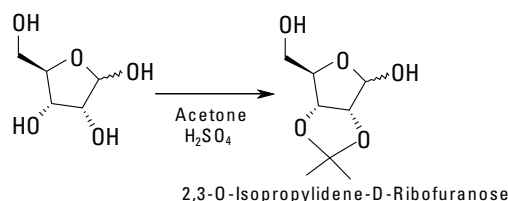
CombiFlash Rf systems deliver flow rates that range from 5 through 200 mL/minute. Teledyne Isco provides the CombiFlash Rf External Detector Interface Kit (P/N 60-5237-063) which meters off the optimal sample rate for evaporative light scattering detectors regardless of the flow rate of the CombiFlash Rf system (Figure 1).

The CombiFlash Rf system can accept an external signal with a range of 0 to 1 volt which the software can display and collect fractions. A voltage range greater than 1 volt can be accepted with a special cable.

An external ELSD can be shared among different CombiFlash Rf or other chromatography systems offering a cost effective and space-saving solution to changing projects. Almost any ELSD available on the market is compatible with the CombiFlash Rf system.

Example 1:

2,3-O-Isopropylidene-D-Ribofuranose



This compound contains no chromophores and is impossible to fractionate with UV detection. The compound is prepared from the acid catalyzed reaction of ribose suspended in acetone.

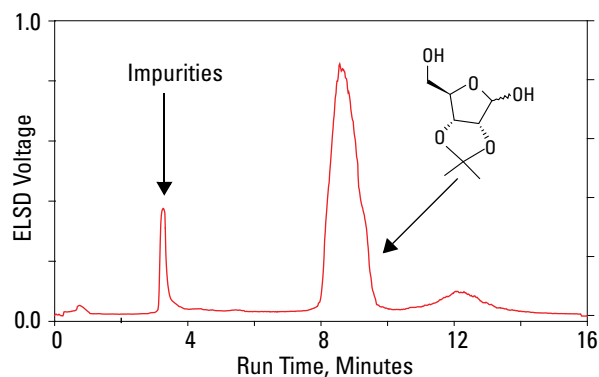


Figure 2: Purification of 2,3-O-isopropylidene-D-ribofuranose

Example 2: Test Mix A (acetophenone, methyl paraben, and p-aminobenzoic acid)

Acetophenone, methyl paraben, and p-aminobenzoic acid were dissolved in ethyl acetate and injected onto a RediSep® Rf column.

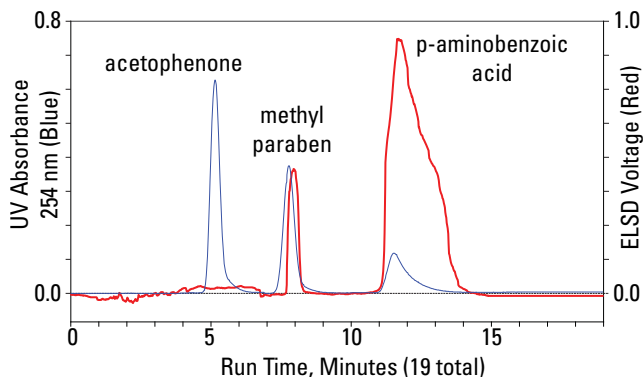


Figure 3: Purification of acetophenone, methyl paraben, and p-aminobenzoic acid on a RediSep Rf column with UV detection overlaid on an ELSD run.

Test Mix A is used because it contains a mixture of compounds with a wide range of volatility and melting points. The melting point of a compound was found to play a role in ELSD detection¹. The acetophenone does not appear on the ELSD despite having a boiling point of 200 °C (Figure 3).

Troubleshooting Air on ELSD

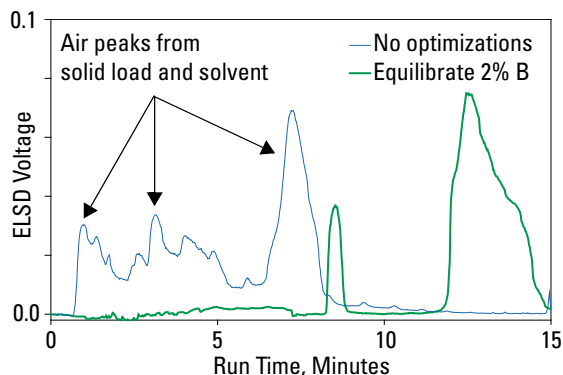


Figure 4: ELSD showing the symptoms of air in the system.

1. Webster, G.K.; Jensen, J.S.; Diaz, A.R. "Investigation into Detector Limitations Using Evaporative Light-Scattering Detectors For Pharmaceutical Applications." *J. Chromatographic Science*, Oct 2004, 42, 484

Figure 4 shows how air may generate false peaks on some ELSD units. These peaks are likely caused by sputtering at the nebulizer as a mixture of gas and solvent enters the ELSD and generates false peaks. Equilibrating the column with 1 or 2 percent of B solvent often removes these solvent effect peaks. Likewise, remove air in the solid sample cartridge by filling it with the weak "A" solvent at the start of the run.

A liquid injection instead of solid loading is another technique that reduces air in the system. However, a liquid injection may cause peak broadening and increase the possibility of the sample precipitating ("crashing") as it comes in contact with the weak solvent in the column. (Refer to Application Note 15, available at www.isco.com/lcappnotes).

Advantages & Disadvantages of ELSD

Advantages	Disadvantages
Detects UV insensitive compounds	Not a universal detector <ul style="list-style-type: none"> • Volatile compounds can evaporate with the solvent • Compounds with low melting points can be difficult to detect
Collect compounds that would be masked by the mobile phase spectrum	Non-volatile buffers can't be used
Purify compounds with unknown UV-visible spectrum	Air in the system can create noise and false peaks
Ability to use a shared detector for different projects	Increased system complexity due to the second detector Signal easily "maxed out"
	Destructive detector with sample loss of up to 5%
	Warm-up time (up to ½ hour) needed on some systems
	Solvent quality important since poor quality solvent may contain interfering compounds that hinder ELSD detection
	Gas source may be required

Conclusion

The CombiFlash Rf system has the ability to display the input from external detectors and collect fractions from this input. In this case, an ELSD was used as the external detector. The advantages of an ELSD are that it enables collection of peaks not visible to the UV detector built into the CombiFlash Rf system. However, the ELSD fails to detect low-melting point or volatile compounds so it is not a universal detector. ELSD requires a degree of optimization for best results. The ELSD is easily connected to the CombiFlash Rf with the external adapter package 60-5237-063.

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