# A Single Evaporative Light Scattering Detector for Universal Detection in LC and Rapid Resolution LC (RRLC)

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## Introduction

There is a clear trend in pharmaceutical, food and hydrocarbon processing industries to detect every compound of a sample. This is the case for R&D- but also for QA/QC laboratories. "Show me all compounds and impurities" is a typical requirement in these laboratories.
An ideal detector for this task is the evaporative light scattering detector (ELSD) because its response is independent of a compound's absorbance, fluorescence or electro-activity. It also sees compounds not detected by LC/MS because they are non-ionizable. An important feature is furthermore that it can be used in isocratic and gradient analyses. The requirement to show all compounds is easier to fulfill when columns filled with particles smaller than 2 µm are used. They were introduced in 2003 and result in a significant increase in chromatographic performance. Besides the improved resolution an up to 30 times reduction of analysis times and an increase in signal heights and areas is observed. In the present paper we show that the new ELSD can be used successfully with "sub-two-micron columns" (STM) in the Rapid Resolution (RRLC) mode but also in the standard LC modes with columns filled with standard 5 µm-particles. Depending on the analytical task a fast change of the nebulizer is all what needs to be done.

## Equipment

Agilent 1200 Rapid Resolution System with with the new 1200 Series ELSD

## Conclusions

The 1200 Series ELSD is enabled for all modes of HPLC including the fast chromatograms in RRLC. With the standard flow nebulizer peak width of one seconds and larger are typical, with the RRLC nebulizer peaks as narrow as 0.65 seconds and larger are possible. Thus theoretical peak capacities of close to 950 in 10 minutes are calculated. Peak shapes and widths are comparable to those of the diode array detectors (DAD), which have got only the cell contributing to band broadening.

## Results and Discussion

**Comparison of nebulizers**
To cover the whole flow rate range of HPLC with one ELSD 5 nebulizers are available, which can be easily exchanged by the user.

| Nebulizer | Useable Flow Rate [ml/min] | Optimum FlowRate [ml/min] | Backpressure [bar at 1 ml/min] |
|---|---|---|---|
| Large Flow | 0.8 - 5 | 1.5 – 4.0 | 4 |
| Standard Flow | 0.2 – 2.5 | 0.5 – 2.0 | 4 |
| RRLC (April 01) | 0.2 – 1.4 | 0.4 - 1.0 | 24 |
| Semi Micro Flow | 0.02 – 1.2 | 0.04 – 1.0 | 44 |
| Micro flow | 0002 – 0.08 | 0.003 – 0.04 | 24 at100 µl/min |

**Table 1: Characteristics of nebulizers**

The standard flow nebulizer is recommended for HPLC andRRLC with 4.6 mm i.d. columns while the new RRLC nebulizer is recommended for 2.1- and 3.0- mm i.d. columns when the focus is on peak capacity. The below figure shows with a separation of 4 parabens in 0.5 minutes that the peak volume is decreased from 16.5 to 10.7 µl when the standard flow- is exchanged for the RRLC nebulizer. This very small peak volume is achieved by a minimized flow path and the overall innovative cell design which minimizes band broadening by gas supported focussing.

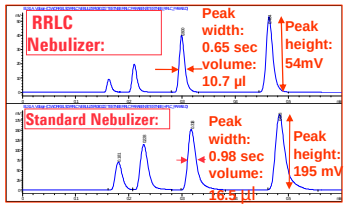


**Figure 1: Comparison of RRLC separation of 4 parabens with RRLC- and standard flow nebulizer showing very narrow peak width**

Almost the same situation for peak width is observed when we compare the RRLC nebulizer with the semi micro flow nebulizer, which can evaporate the flow rates of 2.1- and 3.0 mm i.d. STM columns. The reduction of the peak width by 35% and thus an improved peak capacity has to be paid by a reduction of the sensitivity by a factor of 2.6 (refer to table 2). This can be explained with a less favorable solvent-nitrogen ratio.

## Results and Discussion

| Nebulizer | Peak width [µl] | Theoretical peak capacity (10 minutes)* | Sensitivity (signal/noise*) |
|---|---|---|---|
| Standard Flow | 16.5 | 606 | 1625 |
| Semi Micro Flow | 16.5 | 606 | 1167 |
| RRLC | 10.7 | 935 | 450 |

* Sample Paraben mix, 500 ppm each, Flow 1 ml/min
**Table 2: Performance data for nebulizers**

**Comparison of Peakwidth in DAD- and ELSD detection**
The most important detectors in HPLC and RRLC are UV-based. Therefore any other detectors are compared with them. Figure 3 shows with the anlysis of 6 antiepileptic drugs with DAD- and ELSD detection that the two detctors are equivalent in terms of peak shape and width. The peak volumes of, for example the 3rd peak are with 15.5 µl(DAD) and 15.2 µl (ELSD) almost the same although the ELSD is the last detector in series and more complex. In UV based detection only the flow cell and the capillaries contribute to band broadening while in ELSD detection the nebulizer, the evaporation tube, the cell and the capillaries contribute. The data show that the 1200 Series ELSD with it´s innovatively designed nebulizers and gas supported focussing is fully enabled for LC and RRLC.

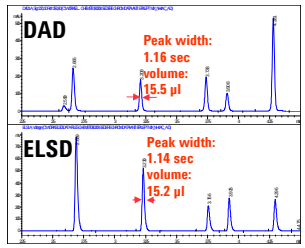


**Figure 2: RRLC Analysis of 6 antiepileptic drugs showing equivalent peak shape and width with DAD and ELSD**