Peak-based fraction collection using an evaporative light scattering detector with the Agilent 1100 Series purification system

Application

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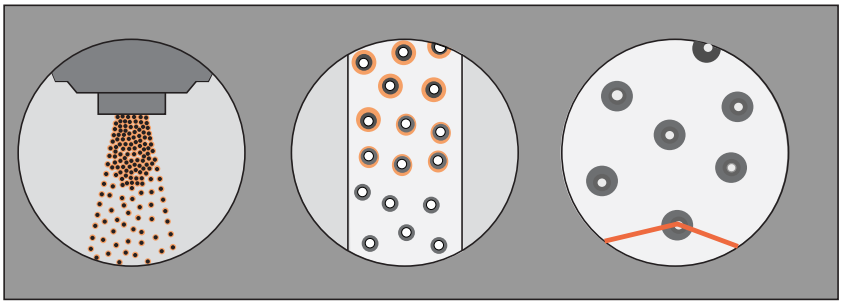

Abstract

The Agilent 1100 Series purification system allows for peak-based fraction collection using the signal of any Agilent 1100 Series UV-visible detector. In another Application Note[1] we showed that by adding the Agilent 1100 Series universal interface box (UIB), it is also possible to perform peak-based fraction collection using the signals of other Agilent detectors, for example, the Agilent 1100 Series fluorescence or refractive index detectors. In this Application Note we explain how a non-Agilent detector can be built into the purification system and how it can be used to trigger peak-based fraction collection.

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Introduction

The evaporative light scattering detector (ELSD) is a detector commonly used in the pharmaceutical industry. It can detect compounds with no chromophoric group and the detector response depends only on the amount of eluted compound. Therefore, it is ideal for purity checks. Typical applications include analysis of amino acids, peptides, carbohydrates, and lipids.

In this Application Note we show how an ELSD can be used with the Agilent 1100 Series purification system[2] for peak-based fraction collection. The system setup described can also be used to add other non-Agilent detectors for peak triggering, such as a nitrogen detector. As an example we show the separation of four glucocorticoid standards. The collected fractions were re-analyzed to demonstrate the purification performance of the system.

Equipment and system setup

Equipment

All experiments were performed on an Agilent 1100 Series purification system AS containing the following modules:

- Agilent 1100 Series quaternary pump with degasser
- Agilent 1100 Series well-plate autosampler
- Agilent 1100 Series thermostatted column compartment
- Agilent 1100 Series diode array detector
- Agilent splitter 1:10/1:20
- Agilent 1100 Series fraction collector AS
- Sedere Sedex Model 75 ELS detector
- Agilent dual-channel A/D interface
- Agilent 1100 Series universal interface box

The system was controlled using the Agilent ChemStation (rev. A.09.01) and the Purification/Highthruput software (rev. A.01.01). The ELSD was controlled using its keypad.

System setup and configuration

Since the ELSD is a destructive detector it was set up in the flow path using a splitter (Agilent 1:10/1:20 splitter). The main flow went to the fraction collector, the split flow to the ELSD (figure 1).

To trigger fractions on the ELSD signal the analog output of the detector was connected to a universal interface box (UIB). The UIB was connected to the 1100 Series modules via a CAN cable. If the ELSD signal is also needed for integration or calibration in the

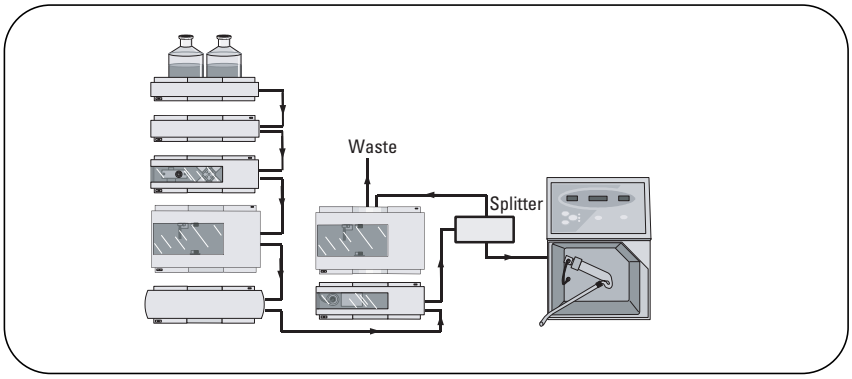


Figure 1
Setup of the ELSD in the flow path using a splitter

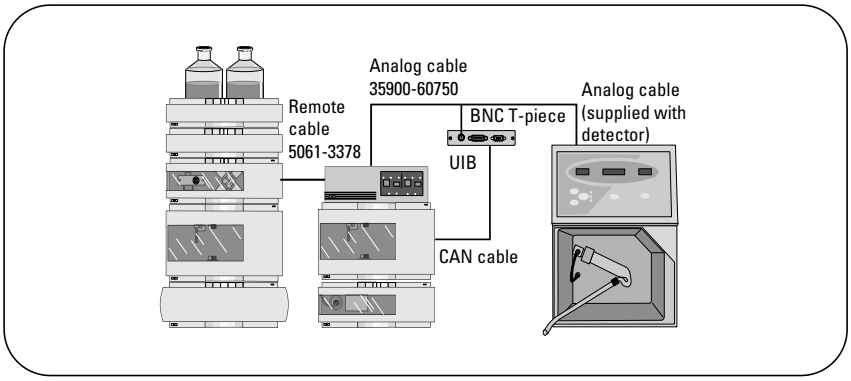


Figure 2
Electronic connections using a UIB and dual-channel A/D interface box

ChemStation, a dual-channel interface is recommended. This is the case especially for signals with low abundance. We therefore connected the analog output of the ELSD to the UIB and the dual-channel A/D interface using a T-piece on the analog output cable (figure 2). To start the dual-channel A/D interface simultaneously with the 1100 Series modules, it was connected to the pump using a remote cable. Any other non-Agilent detector with an analog output can be set up in this way for peak-based fraction collection with the Agilent 1100 Series purification system.

Delay volume calibration for the ELSD

For proper collection of fractions the delay time between the ELSD and the diverter valve of the fraction collector must be measured. This can be done using the Agilent fraction delay sensor built in the fraction collector. The delay calibration procedure is described in the Agilent 1100 Series Purification System User's Guide[3]. The standard delay calibrant (G1946-85020) and the standard delay calibration method of the ChemStation can be used. Since it is absolutely necessary that the desired compound in the split flow arrives at the ELSD before reaching the fraction collector in the main flow, the delay calibration must give delay volume with a positive value. If a negative delay volume is measured the compound would reach the fraction collector before it arrives in the ELSD and would therefore go to waste before it can be triggered. To avoid negative delay volumes the flow connection between the splitter and the ELSD must be kept as short as possible and a capillary with a low internal diameter should be used. If it is not possible to shorten further the capillary leading to the ELSD, the capillary going from the splitter to the fraction collector can be enlarged. However, this would increase dispersion which would result in decreasing fraction collection performance, and should therefore be avoided.

Results and discussion

Fraction collection triggered on the ELSD signal

To trigger fraction collection on the ELSD signal the UIB must be selected as *Peak Detector* in the *Setup Fraction Collector* window in the ChemStation. The parameters *Up Slope*, *Down Slope* and *Threshold* can be selected as for any UV detector. To isolate glucocorticoid standards the values for up and down slope were removed from the table to trigger on threshold only at 70 mV. Figure 3 shows the DAD, dual-channel A/D interface and UIB signal of the glucocorticoid standards — vertical lines mark the collected fractions.

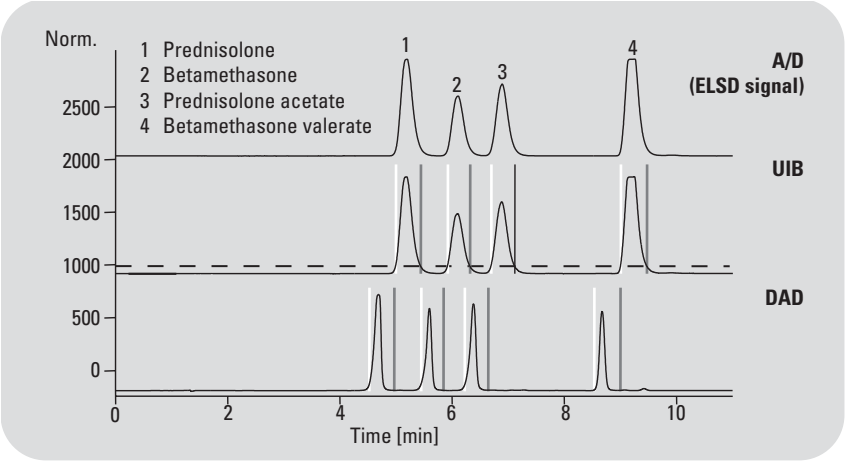
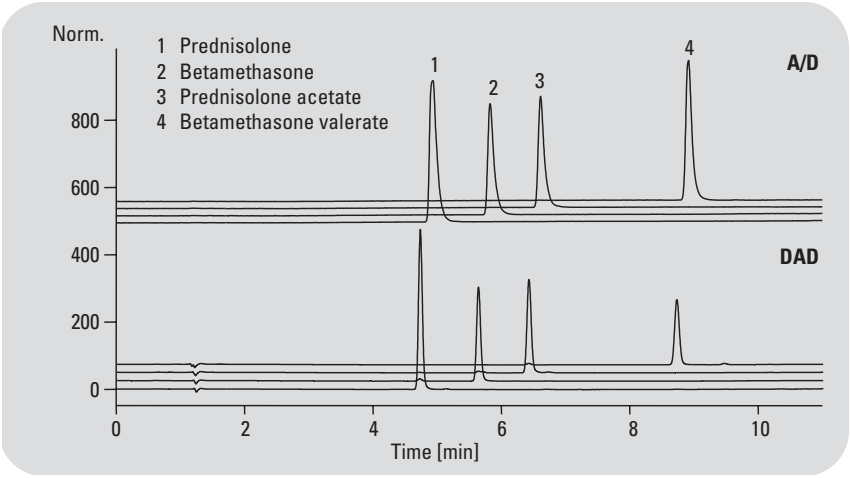


Figure 3
Peak-based fraction collection on the ELSD signal

Columns	Hypersil ODS 4 × 125 mm, 5.0 µm
Mobile phases:	A= water B= acetonitrile
Gradient:	at 0 min 20 % B at 10 min 80 % B
Column wash:	at 11 min 20 % B
Stop time:	11 min
Post time:	5 min
Flow rate:	1.0 mL/min
Injection:	20 µL
Column temp.:	25 °C
UV detector:	DAD: 254/16nm (ref. 360/100 nm), standard flow cell (10 mm pathlength)
ELSD:	45 °C, nitrogen pressure at 2 bar, gain setting at 8



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Injection:	20 µL
Column temp.:	25 °C
UV detector:	DAD: 254/16 nm (ref. 360/100 nm), standard flow cell (10 mm pathlength)
ELSD:	45 °C, nitrogen pressure at 2 bar, gain setting at 11

Figure 4
Re-analysis of fractions

To confirm the purification results the fractions were re-analyzed on the same system without using the splitter. Figure 4 shows the resulting chromatograms. They clearly show that the four compounds could be separated without any impurities. This confirms the excellent performance of the Agilent 1100 Series purification system with the non-Agilent ELS detector.

Conclusion

In this Application Note we showed how to trigger peak-based fraction collection on the Agilent 1100 Series purification system with a non-Agilent detector. As an example we used the Sedere Sedex Model 75 ELS detector, however, it is possible to set up any other non-Agilent detector in the same way as long as it has an analog signal output. As an example we showed the separation of four glucocorticoid standards in analytical scale preparative HPLC.

References

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