Fluorescence-based isolation of formononetin from red clover extract with the Agilent 1100 Series purification system

Application

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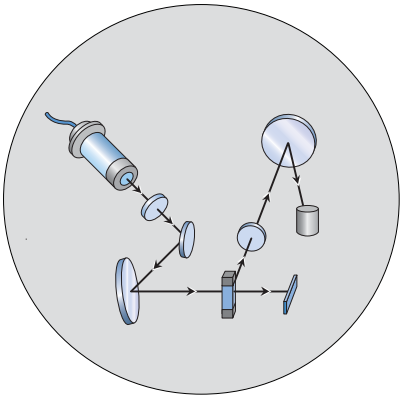

Abstract

With the Agilent 1100 Series purification system it is possible to trigger fractions according to retention time, peaks and on mass. All Agilent 1100 Series UV detectors can be used directly for peak-based fraction triggering without any additional hard- or software. It is also possible to connect other Agilent or non-Agilent detectors using additional hardware, such as the Universal Interface Box (UIB) and the dual channel A/D interface. This Application Note describes how the Agilent 1100 Series fluorescence detector is integrated into the purification system and how it is used to isolate formononetin from red clover extract – a complex natural product.

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Introduction

UV-based purification of compounds from complex mixtures can be difficult due to the relatively low selectivity of UV detection. This problem can be overcome if the mass of the target compound is known. It is then possible to isolate the compound by mass-based fraction collection. In cases where the target mass is not known but the compound shows fluorescence, it is possible to integrate an Agilent 1100 Series fluorescence detector[1,2] into the purification system AS (analytical scale) or PS (preparative scale)[3]. In this Application Note we show how to set up such a purification system. As an example we isolated fromonoetin, a fluorescent compound, from red clover extract — a complex natural product.

Equipment and system setup

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

- Agilent 1100 Series vacuum degasser
- Agilent 1100 Series quaternary pump
- Agilent 1100 Series well-plate autosampler
- Agilent 1100 Series thermostatted column compartment
- Agilent 1100 Series diode array detector
- Agilent 1100 Series fluorecence detector
- Agilent 1100 Series fraction collector AS
- Agilent 1100 Series universal interface box

System setup and configuration

The Agilent 1100 Series fluorescence detector (FLD) is set up and configured as described in the manual delivered with the module. It is incorporated into the liquid flow path between the Agilent 1100 Series diode array detector (DAD) and the Agilent 1100 Series fraction collector AS. Since the firmware of the FLD is not able to trigger peaks it is necessary to send the analog output signal of the detector to the UIB for peak-based fraction collection. The UIB is connected via CAN cable to the other modules (figure 1). For proper peak triggering it is important to connect the UIB to the FLD analog output labeled as *ANALOG 1*.

When configuring the FLD in the ChemStation it is important to set the *Analog Output Range* of *Output 1* to 1 Volt. The *Analog Output Range* setting can be found in the *FLD Control* window.

Delay volume calibration for the FLD

For proper collection of fractions the delay volume between the FLD and the diverter valve of the fraction collector must be measured[6]. This is done, as for any UV detector, using the Agilent fraction delay sensor which is built into the fraction collector. The delay volume calibration procedure using the standard delay calibrant (G1946-85020) is described in the Agilent 1100 Series Purification System User's Guide[5]. The standard delay volume calibration method of the ChemStation must be enhanced with the FLD settings (FLD default settings can be used) and saved under a different name. It is important to perform the delay calibration with the same peakwidth (response time) setting of the FLD as used in the purification run. Furthermore, it is strongly recommended to set the peak width (response time) to *> 0.005 min (0.12 s)* or lower[6].

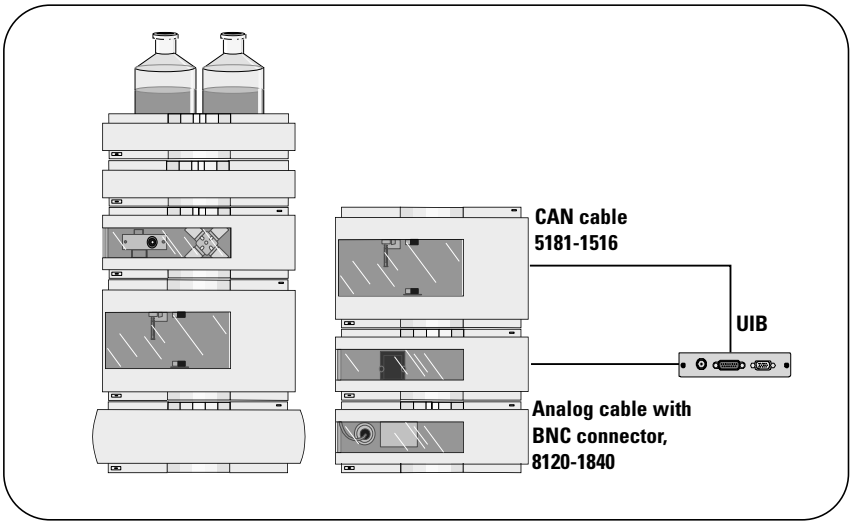

Figure 1
Incorporating the FLD for peak-based fraction collection

Results and Discussion

Fraction collection triggered on the FLD signal

To trigger fraction collection on the FLD signal the UIB must be selected as *Peak Detector* in the *Setup Fraction Collector* window of the ChemStation. The parameters *Up Slope*, *Down Slope* and *Threshold* can be selected as for any UV detector. To isolate formononetin the values for up slope and down slope were re-moved from the table to trigger on threshold only. The threshold value was set to 50 mV and the peak based fraction collection was performed in a timetable between 12 and 17 minutes. Figure 2 shows the DAD, FLD and UIB signal of the red clover plant extract – vertical lines mark the collected fraction. Reanalysis of the collected fraction was monitored using both FLD and DAD to make sure any impurities were detected, even if they show no fluorescence. Figure 3 shows the chromatogram of the reanalyzed fraction. The FLD as well as the DAD signal confirm the excellent purity of the collected fraction.

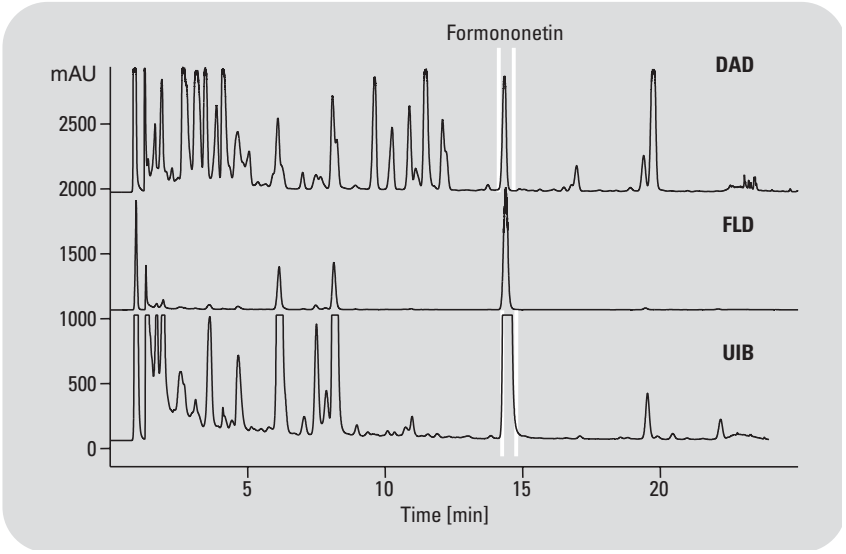

Figure 1
Fractionation triggered on the UIB signal

Columns	Zorbax SB-C18 3 x 150 mm, 5 µm
Mobile phases:	A= water + 0.1 % AcOH B= acetonitrile + 0.1 % AcOH%
Gradient:	at 0 min 20 % B at 20 min 45 % B at 21 min 100 % B
Column wash	at 24.5 min 100 % B at 25 min 20 % B
Stop time:	25 min
Post time:	5 min
Flow:	0.7 mL/min
Injection:	20 µl
Column temp.	35 °C
UV detector:	DAD: 260 nm/16 (ref. 800 nm/100), standard flow cell (10 nm)
FLD:	excitation 250 nm, emission 450 nm, standard flow cell

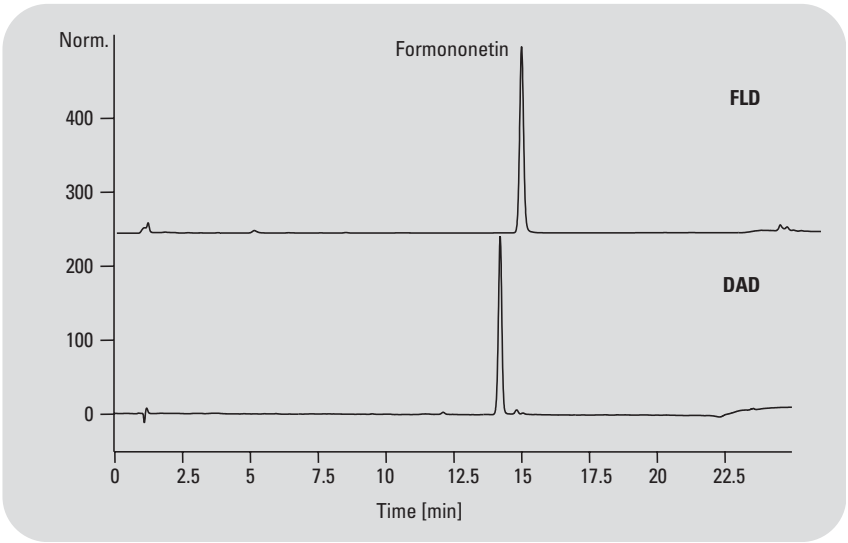

Figure 2
Reanalysis of collected fractions

Conclusion

In this Application Note we showed how to trigger the Agilent 1100 Series fraction collector with the Agilent 1100 Series fluorescence detector. We described how the detector is built into the purification system AS, how it is set up, configured and used for fraction triggering via the UIB. We also explained how to perform the delay volume calibration for such a system. As an application example we isolated the fluorescent compound formononetin from red clover extract.

References

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