

## Agilent 1100 Series purification system: Fractionation and automated re-analysis of fractions

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Purification of a compound is a typical task in preparative liquid chromatography. The first step in the purification process is fractionation of the crude product. In the second step the fractions obtained are re-analyzed to identify those with sufficient purity of the desired product. With the Agilent 1100 Series purification system both steps, fractionation and re-analysis, can be done with the same instrument without any hardware modification. Furthermore, the software allows automated re-analysis of the fractions, which means the whole fractionation/re-analysis process can be performed automated and unattended.

The fractionation was performed on a 21.2-mm id column with a flow rate of 25 ml/min. For fraction analysis it was necessary to switch to a column with a smaller column id – here a 3-mm id column was used. The column switching valve of the Agilent 1100 Series thermostatted column



### Figure 1

Column switching valve of the Agilent 1100 Series thermostatted column compartment



# Figure 2 Fractionation based on retention time windows on column 1

compartment was used for column switching (figure 1). The fractionation result is shown in figure 2.

# Conditions

Column 1 21.2 x 150 mm Zorbax SB-C18, 5 µm Column 2 3 x 150 mm Zorbax SB-C18, 5 µm **Mobile phase** A: water. B: acetonitrile Flow rate 1: 25 ml/min Flow rate 2: 0.6 ml/min Isocratic: 16 % B **UV detector**: diode array detector, 270 nm/16 (reference off) preparative flow cell, 3 mm path length **Column temperature:** ambient **Injection volume 1:** 500 µl Injection volume 2: 20 µl Fractionation

- based on retention time windows
- from 0.8 to 5 min
- 21 fractions collected



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To analyze the collected fractions the column switching valve was switched to column 2 and the pump flow was lowered to 0.6 ml/min. The Agilent 1100 Series preparative pumps can be used even at this low flow rate, however, it is recommended to use a back pressure regulator to avoid air bubbles in the preparative cell of the detector. *Enable automatic analysis* was selected and the method for fraction analysis was chosen. Since setting the column switching valve is part of this method, switching is done automatically which means that the complete fraction analysis process is performed automatically. The results of the fraction analyses are shown in figure 3.

#### Conclusion

In this note we demonstrated the fractionation/fraction re-analysis capabilities of the Agilent 1100 Series purification system. With the column switching valve of the Agilent 1100 Series thermostatted column compartment it was possible to analyze the collected fractions automatically on an analytical column. No hardware modification was necessary. We showed that the Agilent 1100 Series purification system is a helpful tool for unattended, automated and therefore time-saving preparative purification of synthesis products.



Figure 3 Fraction analysis on column 2

## Equipment

#### **Agilent 1100 Series**

- Two preparative pumps
- Thermostatted column compartment
- Diode array detector, preparative flow cell path length 3 mm
- 220 micro plate sampler
- Micro plate sampling software
- Agilent ChemStation
- Recommended: back pressure regulator

#### Columns

- 21.2 x 150 mm Zorbax SB-C18, 5 μm (part number 870150-902)
- 3 x 150 mm Zorbax SB-C18, 5 μm (part number 883975-302)

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