

Preparative high throughput HPLC – alternating column regeneration with the Agilent 1100 Series valve solutions

Application

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<u>Abstract</u>

Driven by the demand to analyze and purify large numbers of samples in drug discovery, researchers nowadays do not only need high throughput analytical HPLC equipment but also ask for fully automated and reliable preparative HPLC systems. As described previously for analytical HPLC¹ the throughput on a system can not only be enhanced by optimizing the hardware and method but also by alternating two identical columns in the system. In this Application Note we describe how to configure and set up a high throughput purification system², equipped with a mass selective detector (MSD) and an external 2-position/10-port valve³.





Introduction

Alternating column regeneration in analytical scale HPLC can be performed using a 2-position/10port valve built into the Agilent 1100 Series thermostatted column compartment.

For alternating column regeneration at preparative-scale flow rates up to 100 mL/min the external 2-position/10-port valve must be used. The configuration of the valve, the capillary connections and the general method setup for the pumps are identical for both internal and external valves. This Application Note only describes the differences in the software screens. For further details please refer to a previously published Application Note¹.

Equipment

The system used comprised the following modules:

- Two Agilent 1100 Series preparative pumps
- Agilent 1100 Series preparative autosampler
- Agilent 1100 Series column organizer
- Agilent 1100 Series 2-position/ 10-port valve
- Agilent 1100 Series diode array detector
- Agilent 1100 Series fraction collector PS
- Agilent 1100 Series mass selective detector
- Agilent 1100 Series isocratic pump (as make-up pump)
- Agilent active splitter
- Agilent 1100 Series isocratic, quaternary or preparative pump

(as regeneration pump) The system was controlled using the Agilent ChemStation (rev. A.09.03) and the Purification/ HighThroughput software (rev. A.01.02).

<u>Results</u>

Valve set-up

The external 2-position/10-port valve is controlled from the *Setup Valve* window shown in figure 1. For automated column regeneration the box *Next position after run* must be checked. It sets *Position* automatically to *Use current*. This setting switches the valve automatically to the next column after a run is finished and this position is kept when the run starts. Further details, for example, configuration of the capillary connections can be found by selecting *Help*.

Sequential runs

Without column regeneration the

purification run steps "draw & inject", "gradient run", "column wash" and "column equilibrate" are performed sequentially as shown in figure 2. This leads to an overall cycle time of 13 minutes for the method used in this Application Note.

Alternating column regeneration with isocratic regeneration pump

If an isocratic pump is used for alternating column regeneration the step "column equilibrate" can be done while the next purification run is already being performed on the second column (figure 3). To ensure that the volume (V1) between the pump and the valve is filled with mobile phase of the gradient starting composition, an additional rinse time has to be added after the column wash. The duration of this rinse time depends on the system setup, for example, on the delay volume of the autosampler¹. In the example in figure 3 the cycle time could be reduced to 10.5 minutes.

| Positior | 1 | Timetable |
|------------------------|-------------------------|--------------------------------------------------|
| Use | current | 9 Line Time Position Insert 9 Append 8 Cut |
| Valve <u>N</u> Colu | lame mn regeneration | Copy Paste IX Next position after run |
| | | |
| Position | n Descriptions | |
| 1 | CombiHT BC1041 | 2 CombiHT BC1096 |
| | | <u> </u> |



The following isocratic pumps can be used for column regeneration in a purification system:

- Agilent 1100 Series isocratic pump (max. flow rate 10 mL/min at 200 bar), and
- Agilent 1100 Series preparative pump (maximum flow rate 100 mL/min at 400 bar).

Alternating column regeneration with gradient regeneration pump

When using a gradient pump as regeneration pump the steps "column wash" and "column equilibrate" can be done while the next purification run is already being performed on the second column (figure 4). Then the rinse time has to be added directly after the gradient is finished.

The following gradient pumps can be used for column regeneration in a purification system:

- Agilent 1100 Series isocratic pump (max. flow rate 10 mL/min at 200 bar) with Agilent 1100 Series 12-position/13port solvent selection valve, and
- Agilent 1100 Series quaternary pump (max. flow rate 10 mL/min at 200 bar).

Alternating column regeneration with gradient regeneration pump and overlapped injection

With the overlapped injection feature⁴ of the Agilent 1100 Series preparative autosampler it is possible to draw the next sample while the previous purification run is still being performed. Especially in preparative HPLC where high sample volumes, possibly requiring multi-draw, have to be injected this can lead to an immense time saving (figure 5).



Figure 2 Sequential runs



Figure 3





Figure 4

Alternating column regeneration with gradient regeneration pump



Figure 5



Conclusion

References

In this Application Note we showed how to perform high throughput purification runs using alternating column regeneration with an external Agilent 1100 Series 2-position/10-port valve. Depending on the type of regeneration pump either column wash, or column wash and column equilibrate can be done while the next purification run is already being performed on the second column. Furthermore, the possibility of overlapped injection with the autosampler reduces the cycle time, especially for preparative HPCL, where high injection volumes and therefore long injection times are common.

1.

"High throughput HPLC Alternating column regeneration with the Agilent 1100 Series valve solutions", Agilent Technologies Technical Note, **2002**, publication number 5988-7831EN.

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3.

"New dimensions for HPLC applications", Agilent Technologies Brochure, **2002**, publication number 5988-6707EN.

4.

"Optimizing the Agilent 1100 Series System for High Sample Throughput", Agilent Technologies Technical Note, **1998**, publication number 5968-0467E.

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