Method scale-up from analytical to preparative scale with the Agilent 1100 Series purification system PS

Application

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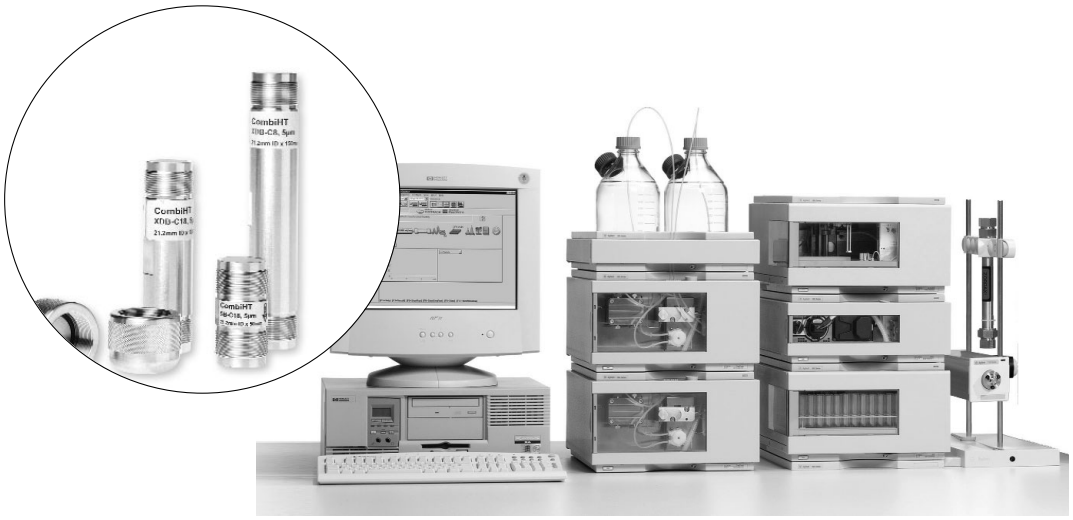

Abstract

Preparative HPLC is an essential tool to purify compounds for activity testing during the discovery and development process of new drugs. Depending on the compound amount, preparative HPLC can be carried out at analytical scale on columns with 5 mm id or less, or at preparative scale on much larger columns. In this Application Note we show purification of three model compounds from analytical to preparative scale on the Agilent 1100 Series purification system PS to demonstrate the excellent performance of the system even at low flow rates.

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Introduction

During drug discovery and development compounds have to be purified from natural extracts or combinatorial libraries for activity testing. Due to the rise of high throughput synthesis and high throughput screening, traditional low throughput purification techniques, such as preparative TLC or crystallization, can create a bottleneck in a synthesis laboratory. A modern and completely automated technique for compound purification is preparative HPLC. In the early stages of drug discovery a large number of compounds are usually synthesized in minute amounts. Therefore, purification can be carried out on columns of 5 mm id or less, which is called analytical scale preparative HPLC. In the later stages of drug discovery and development the compound amount to purify and, therefore, the column id increases.

Agilent Technologies offers two basic purification systems[1] for both analytical and preparative scale purification applications. The Agilent 1100 Series purification system AS (analytical scale) is particularly designed for flow rates below 5 mL/min, the 1100 Series purification system PS (preparative scale) for flow rates up to 100 mL/min. For the PS system Agilent offers the new 1100 Series preparative pumps[2], which deliver a flow of up to 100 mL/min at a maximum backpressure of 400 bar. The pumps and the system were developed for high flow rates, however, it is also possible to use the system at flow rates below 5 mL/min due to the excellent performance of the Agilent 1100 Series preparative pumps.

In this Application Note we show a gradient analysis of a model sample on a 3 mm id column at a flow rate of 0.35 mL/min as well as on a 50 mm id column at a flow rate of 100 mL/min without changing the configuration of the Agilent 1100 Series purification system PS.

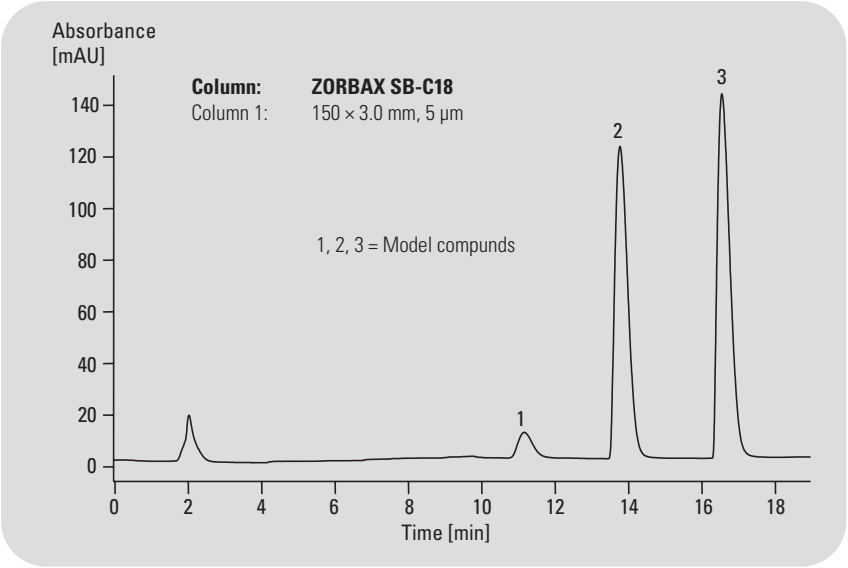

Figure 1
Chromatogram at 0.35 mL/min flow rate

Equipment

The system used included:

- Two Agilent 1100 Series preparative pumps
- Agilent 1100 Series preparative autosampler
- Agilent 1100 Series column organizer
- Agilent 1100 Series diode array detector
- Agilent 1100 Series fraction collector PS

The system was controlled using the Agilent ChemStation (rev. A.09.01).

Results and Discussion

Analytical scale method development

The method to analyze the three model compounds was developed on an Agilent 1100 Series system including two Agilent 1100 Series preparative pumps to form the required gradient. A 3.0-mm id ZORBAX SB-C18 column was used at a flow rate of 0.35 mL/min. The peaks in figure 1 show a good resolution and peak shape, which confirms the good performance of the Agilent 1100 Series preparative pump even at low flow rates as described in another technical note[3].

Columns	ZORBAX SB-C18 150 × 3 mm, 5 µm
Mobile phases:	A= water B= acetonitrile
Gradient:	5 % B to 25 % B in 10 min 25 % B for 9.9 min 25 % B to 5 % B in 0.1 min
Stop time:	20 min
Post time:	5 min
Flow rate:	0.35 mL/min
Injection:	0.8 µL
Column temp.:	ambient
UV detector:	DAD: 220/16nm (ref. 360/60 nm), Preparative flow cell (3 mm path length

Scale-up calculations

The complete scale-up procedure was calculated according to the equations in figure 2.

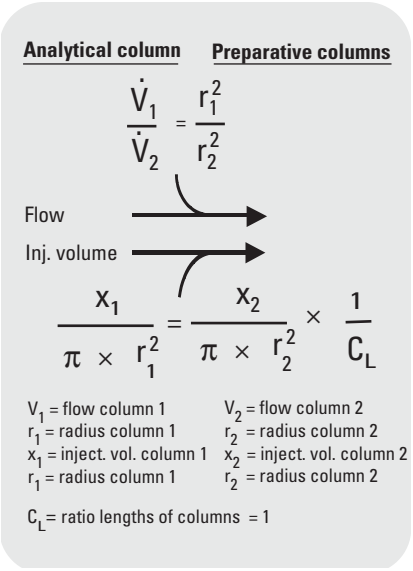

Figure 2
Equations used for scale-up calculations

To calculate the flow rates using the upper equation the 3.0 mm id column with a flow rate of 0.35 mL/min was chosen as the starting point. The lower equation was used to calculate the injected sample volume.

Due to higher flow rates and applied sample amounts it was necessary to change from the 3-mm pathlength SST preparative flow cell to a 0.3-mm pathlength quartz cell for the larger columns. This causes the tenfold increase in the calculation of the injection volume. The calculated flow rates and injection volumes for all columns and flow cells are summarized in table 1.

Analytical and preparative scale purification

Analytical and preparative scale runs were performed on four different columns (4.6 mm id, 9.4 mm id, 21.2 mm id and 50 mm id) using the flow rates and injection volumes as outlined in table 1. The resulting chromatograms in figure 3 show two things – first, that the Agilent 1100 Series preparative pump performs very well over a wide flow rate range, even below the specified minimum flow rate of 5 mL/min. And, second, that it is possible to scale-up on the ZORBAX columns[4,5] without losing resolution, which is very important for a fast scale-up process without any method redevelopment.

Delay volume reduction

The compounds in figure 1 have rather long retention times caused by the delay volumes of the pumps (approx. 0.7 mL) and autosampler (approx. 0.9 mL). The delay volume of the autosampler can be removed from the flow path of the system by using an injector program in the ChemStation. This program switches

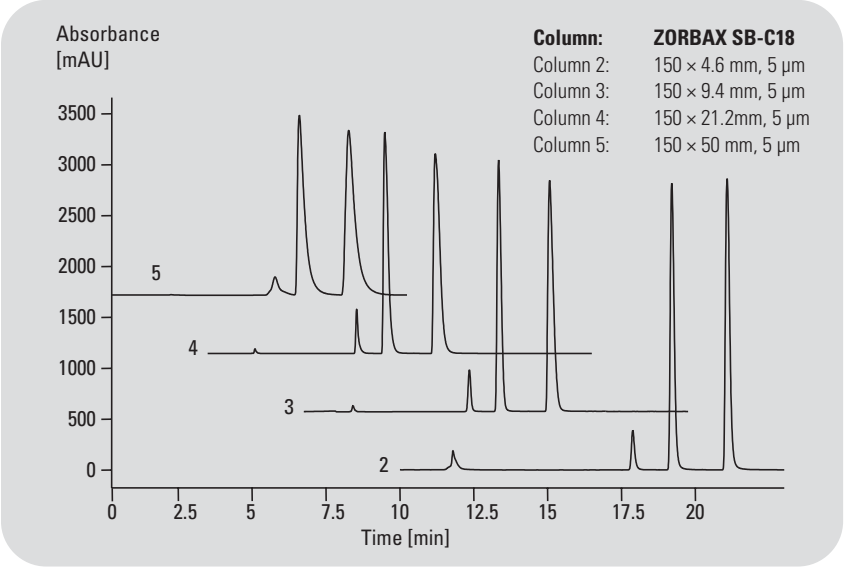

Figure 3
Results of scale-up calculations

Column	Dimension	Flow	Injection volume	Detector cell
1 (figure 1)	150 x 3.0 mm	0.35 mL/min	0.8 µl	3 mm SST
2 (figure 3)	150 x 4.6 mm	0.85 mL/min	2.0 µl	3 mm SST
3 (figure 3)	150 x 9.4 mm	3.5 mL/min	80 µl (8 µl x 10)	0.3 mm quartz
4 (figure 3)	150 x 21.2 mm	18 mL/min	400 µl (40 µl x 10)	0.3 mm quartz
5 (figure 3)	150 x 50 mm	100 mL/min	2200 µl (220 µl x 10)	0.3 mm quartz

Table 1
Flow rates,injection volumes and detector cells for different column sizes

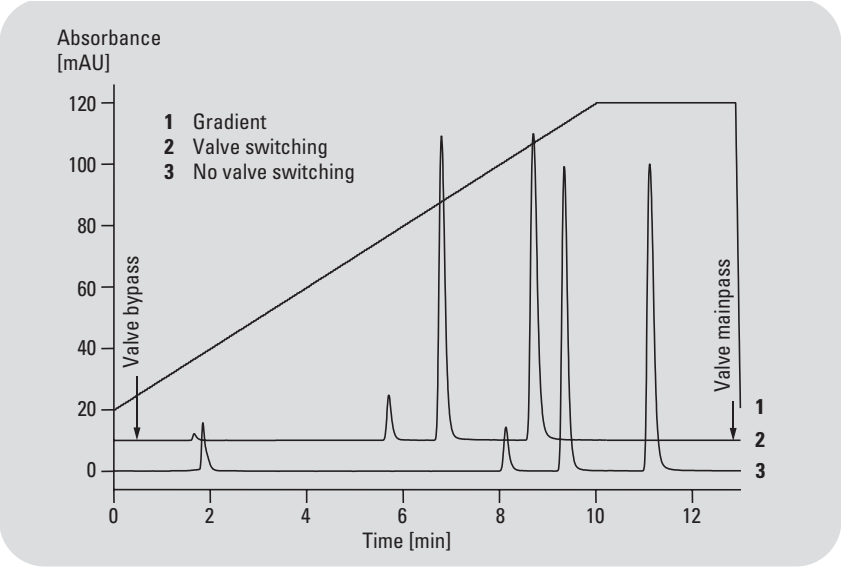

Figure 4
Chromatogram with and without delay volume reduction

the autosampler valve in bypass after the injection and back in mainpass at the end of the run[6,7]. Curve 2 in figure 4 shows the resulting chromatogram performed at a flow rate of 0.85 mL/min on a 4.6 mm id column.

Conclusion

In this Application Note we showed the excellent performance of the Agilent 1100 Series preparative pump over a wide flow rate range from 0.35—100 mL/min. The complete flow rate range can be covered at a maximum backpressure of up to 400 bar without changing pump heads. Of course, the Agilent 1100 Series preparative pump is fully compatible with the ChemStation's OQ/PV and EMF (Early Maintenance Feedback) features. We also showed that it is possible to scale-up an application from a ZORBAX column of 3 mm id to one with 50 mm id without any loss of resolution. This increases throughput by reducing the time required for re-developing or adjusting the method.

References

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