Performance of the Agilent 1100 Series preparative pump at low flow rates

Technical Note

Introduction

The Agilent 1100 Series preparative pump[1,2] forms the basis of the Agilent 1100 Series purification system. It is an isocratic high performance pump with dual piston design for a flow rate range of 5–100 ml/min at 400 bar. To form a gradient system two pumps have to be combined. The extremely small delay volume (figure 1) of the mixer and the attached capillaries, about 0.7 ml, allows fast and steep gradients over a wide flow rate range. Since automated re-analysis of collected fractions is possible with this system it is important that the pump also performs well at flow rates below the specified range.

In this Technical Note we compare the Agilent 1100 Series preparative pump with the Agilent 1100 Series quaternary pump when both are connected to the Agilent 1100 Series purification system. We will demonstrate that the Agilent 1100 Series preparative pump shows similar performance. However, since the purification system is built for high flow rates the performance of the complete system is not as good as for an analytical system, especially when using fast gradients at very low flow rates.

Equipment

The system included:

- two Agilent 1100 Series preparative pumps or one Agilent 1100 Series quaternary pump,
- Agilent 1100 Series diode array detector with standard flow cell, and
- Agilent 220 micro plate sampler (MPS) modified with the Agilent 220 MPS preparative fraction collection accessory.

The system was controlled using the Agilent ChemStation (revision A.08.04) and the micro plate sampling software (revision A.03.02).

To compare the performance of the two pumps both were connected in the same way to an Agilent 1100 Series purification system PS (preparative scale).

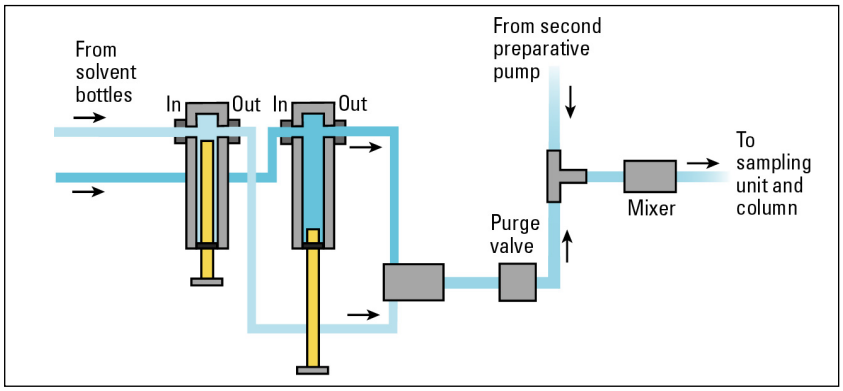

Figure 1
Schematic drawing of preparative pump

Tracer experiments

For the tracer experiments water was used for solvent A and water plus 0.5 % acetone for solvent B. Restriction capillaries were used to achieve a sufficient back pressure on the system of about 100 bar. To determine the gradient performance parameters (composition ripple, composition accuracy, composition precision and composition linearity) the same method as in the ChemStation OQ/PV gradient composition test was used. Details of the method are described in figure 2.

The gradient composition test was performed at flow rates of 10, 5, 3 and 1 ml/min for the preparative pump and at flow rates of 5, 3 and 1 ml/min for the quaternary pump.

Results

Flow rate 10 ml/min

Figure 2 shows the chromatogram and method for the gradient composition test at 10 ml/min. The performance data is shown in table 1. Except for the composition ripple, all performance data are within the limits.

	Prepara-tive pump	Quater-nary pump	Limit quaternary pump
Composition ripple	0.595	-	0.500
Composition accuracy	0.192	-	1.500
Composition precision	0.018	-	0.500
Composition linearity	0.999999	-	0.99900
Back pressure [bar]	101	-	

Table 1
Performance data of preparative pump at 10 ml/min

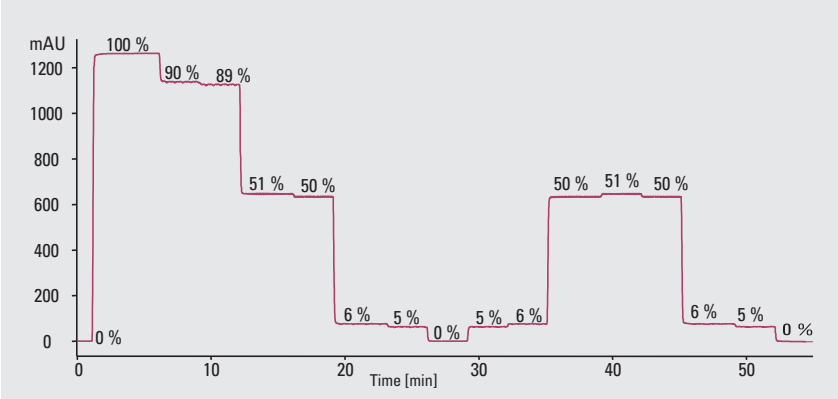

Figure 2
Gradient at 10 ml/min with preparative pump

Mobile Phases:	water = A
	water + 0.5 % acetone = B
Gradient:	see figure 2
Stop time:	55 min
Flow:	10 ml/min
Capillary temp:	40 °C
UV detector:	DAD 265 nm/4 (ref. 450 nm/80)

Flow rate 5 ml/min

Figure 3 shows the chromatogram for the gradient composition test at 5 ml/min. The gradient performance is almost identical. Table 2 shows the performance data.

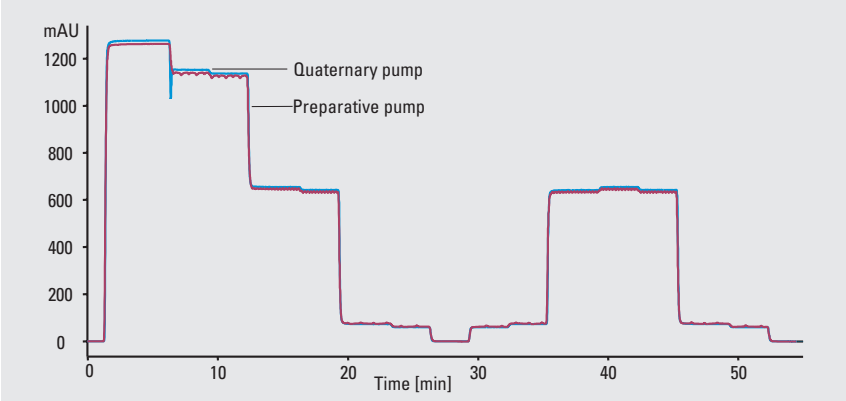

Figure 3
Gradient at 5 ml/min with preparative and quaternary pumps

	Prepara-tive pump	Quater-nary pump	Limit quaternary pump
Composition ripple	0.934	0.148	0.500
Composition accuracy	0.278	0.329	1.500
Composition precision	0.018	0.027	0.500
Composition linearity	0.999998	0.999987	0.99900
Back pressure [bar]	72	92	

Table 2
Performance data of preparative and quaternary pumps at 5 ml/min

Flow rate 3 ml/min

The performance data for the preparative and quaternary pumps are shown in table 3.

	Preparative pump	Quaternary pump	Limit quaternary pump
Composition ripple	0.841	0.051	0.500
Composition accuracy	0.331	0.256	1.500
Composition precision	0.052	0.026	0.500
Composition linearity	0.999997	0.999992	0.99900
Back pressure [bar]	75	93	

Table 3
Performance data of preparative and quaternary pumps at 3 ml/min

Although the specified flow rate range of the preparative pump is from 5–100 ml/min, the performance is good even at a flow rate of 3 ml/min. The relatively high ripple compared to the quaternary pump results from the missing pressure damper. There is no damper built in the preparative pump to keep the delay volume as small as possible. When using the pump at higher flow rates with a large column, this column acts as a damper and the ripple is decreased drastically. To demonstrate this, a small column (4.6 × 30 mm Zorbax SB-C18, 3.5 µm) was built into the system instead of the restriction capillary and the experiment was repeated. Even this small column reduced the ripple from 0.841 to 0.568, which is a reduction of about 30 %.

Flow rate 1 ml/min

The performance data for the preparative and quaternary pumps are shown in table 4. At a flow rate of 1 ml/min the performance of both pumps decreases drastically. This is not only caused by low pump performance. Here the physical limitations of the purification system, such as large id capillaries, determine the performance.

	Preparative pump	Quaternary pump	Limit quaternary pump
Composition ripple	1.033	2.793	0.500
Composition accuracy	0.593	1.193	1.500
Composition precision	0.207	0.4	0.500
Composition linearity	0.999982	0.999954	0.99900
Back pressure [bar]	114	133	

Table 4
Performance data of preparative and quaternary pumps at 1 ml/min

Chromatographic experiments

To demonstrate the performance of the preparative pump at low flow rates a chromatographic experiment was also performed. A gradient from 5 to 95 % B within 10 minutes was set up at a flow rate of 1 ml/min. The experiment was repeated ten times and the precision of retention time was determined to demonstrate the reproducibility of the gradient. The table in figure 4 shows that the precision of retention time is between 0.2 and 0.43 %, which is not as good as for an analytical system but still in an acceptable range.

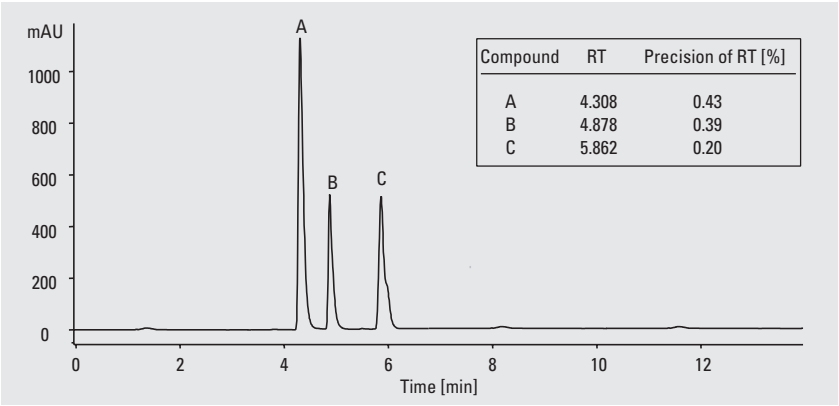

Figure 4
Gradient performance

Column:	4.6×75 mm Zorbax SB-C18, 5 µm
Mobile Phases:	A = water, B = acetonitrile
Gradient:	5 % B to 95 % B in 10 min
	95 % B for 3 min
	95 % B to 5 % B in 1 min
Stop time:	14 min
Post time:	10 min
Flow:	1 ml/min
Injection:	20 µl
Column temp:	ambient
UV detector:	DAD 210 nm/4 (ref. 360 nm/100)
	SST flow cell
	(path length 0.3 cm)

Conclusion

In this Technical Note we demonstrated the performance of the Agilent 1100 Series preparative pump at flow rates below the specified range of 5 to 100 ml/min. The pump connected to the Agilent 1100 Series purification system showed good performance even at 3 or 1 ml/min. However, since the system is built for high flow rates it naturally shows limitations at low flow rates. One reason is, for example, dispersion due to the capillaries used in the system, which leads to broadened peaks. This means, whether or not an analytical application is possible, for example the re-analysis of collected fractions, depends on the application itself. If some prerequisites are met, for example high compound concentration and not-too-fast gradient, analytical runs are possible using the Agilent 1100 Series preparative pump.

References

1.
"Optimized solutions for sample purification from µg to gram quantities" Agilent Technologies Brochure, **2000**, publication number 5980-2808EN

2.
"Agilent 1100 Series Pumping Systems" Agilent Technologies Quick Reference, **2000**, publication number 5988-0023EN

www.agilent.com/chem

Published March 1, 2001
Publication Number 5988-2435EN

Agilent Technologies
Innovating the HP Way