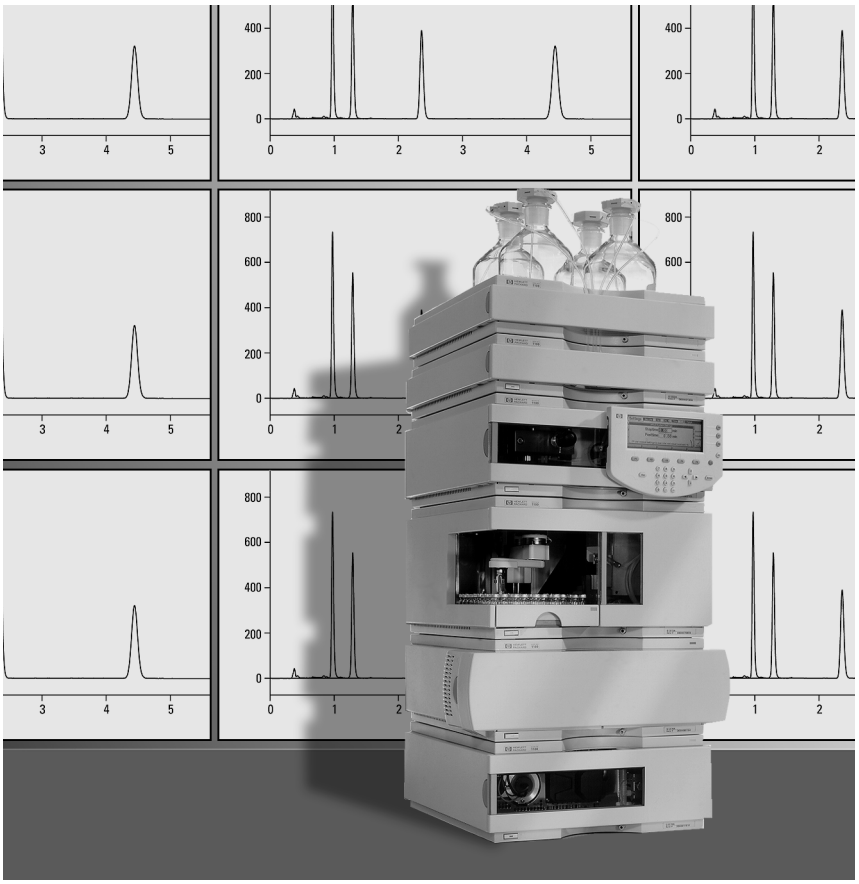
Optimizing the Agilent 1100 Series System for Highest Performance

Technical Note



Introduction

This document will help you to efficiently prepare an Agilent 1100 Series HPLC system for best performance. It describes how to achieve

- highest sensitivity,
- highest precision,
- low flow rates,
- lowest delay volume,
- lowest carry-over, and
- best results in normal phase chromatography.

We first describe a short standard check-out procedure, which is recommended before starting to optimize the instrument. This ensures that the instrument has no defects which may cause ghost peaks, increased noise or unstable retention times.

In the second section, we then explain how to optimize the performance of an Agilent 1100 Series HPLC system.[1]

Agilent Technologies
Innovating the HP Way

1. Standard Check-out Procedure

What is needed for the standard check-out procedure

- Column:
  125 × 4 mm Hypersil ODS, 5 µm (Agilent part number 7982618-564) Use a new column or a column of known history.

- Solvents:
  bi-distilled water, acetonitrile

- Isocratic standard sample (Agilent part number 01080-68704)

Checking the equipment for leaks and contamination

1. Fill one solvent bottle with bi-distilled water (channel A) and the other one with acetonitrile (channel B).

2. Open the purge valve and purge both channels with 5 ml/min for at least 10 min. Check outlet tubing of the purge valve. If no air bubbles are observed purging can be stopped.

3. Adjust compressibility and stroke under `INSTRUMENT, MORE PUMP`. Use the Help key to find appropriate compressibility values for the different solvents. With the quaternary pump you only need one compressibility value, which should be set to 100. For the binary pump set A to 46 and B to 115. The stroke should be set to auto for both A and B.

4. After the purge process, and after installing the column, set the flow rate to 1 ml/min and close the purge valve.

5. Watch for leaks. Leave the thermostatted column compartment open and check each connection for leaks.

6. Watch pressure in the `ONLINE SIGNAL` screen. The ripple should be very regular, no spiking or pressure drops should occur. If they do occur and you can detect no leaks, we recommended contacting the Agilent service organization for possible repair.

7. To check for contamination in your system, select the following settings on your instrument. This is especially important if sensitivity in gradient runs is an issue.

Parameter	Setting
Flow rate	1 ml/min
Detection wavelength	210 nm
Stop time	30
Column compartment temperature	36 °C
Gradient	start with 0 % B and go to 100 % B in 25 min.
Blank run, no injection	no vial number in `SAMPLE INFO` screen

No peaks should be observed if these parameters are set. If, however, the first run does show peaks, run the gradient a second and a third time. Should peaks continue to occur, clean the instrument using different solvents or/and passivate it with 65 % nitric acid.

Checking for baseline noise

Before running the system the detector lamp should have been switched on for at least half an hour. Select the following settings on your instrument:

Parameter	Setting
Flow rate	1 ml/min
Detection wavelength	254 nm
Stop time	30
Column compartment temperature	36 °C
Isocratic:	100 % water
Blank run, no injection	no vial number in SAMPLE INFO screen

Using the Agilent ChemStation choose the PERFORMANCE AND NOISE report and set six 1-minute ranges in the SYSTEM SUITABILITY screen which is located under the REPORT menu. The instrument then calculates the noise automatically. The noise measured over one minute at six different parts in the chromatogram should be close to the following specifications:

- variable wavelength detector (VWD):
  ± 0.75 x 10^{-5} AU at 254 nm
- diode array detector (DAD):
  ± 1 x 10^{-5} AU at 254 and 750 nm

These specifications are based on a cell with a pathlength of 10 mm, a response time of 2 s, a flow of 1 ml/min methanol and a slit width of 4 nm for the diode array detector.

Figure 1 shows how to evalutate the signal noise based on the ASTM method. The Agilent ChemStation software evaluates ASTM noise automatically.

If the noise drastically differs from the specifications, we recommend contacting the Agilent service organization for possible repair.

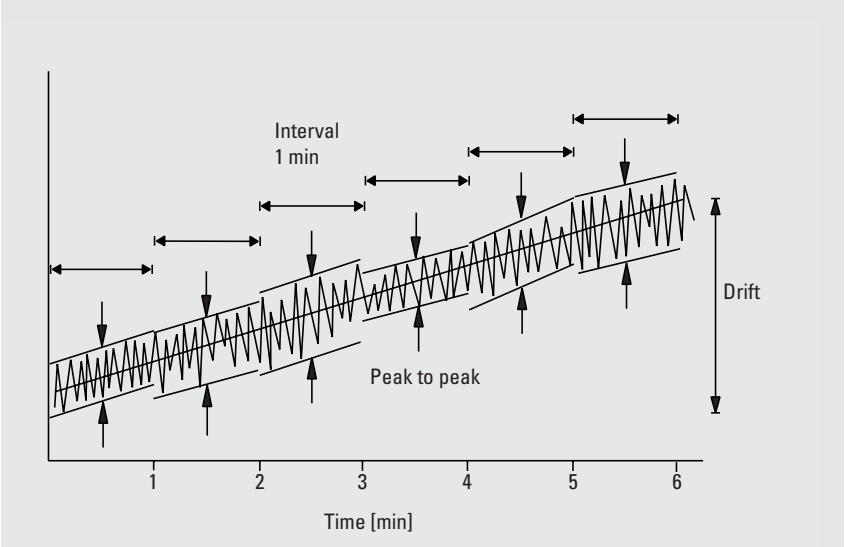


Figure 1
Signal noise based on the ASTM method

Checking retention time and area precision

Retention time and area precision are checked using the isocratic standard sample and gradient analysis with the settings below. Start a sequence with 10 consecutive runs and evaluate the last six runs for retention time and area precision. The RSD for retention times should be <0.5 % and for areas < 2 %.

Figure 2 shows a typical chromatogram for isocratic standard with gradient.

Parameter	Setting
Sample	Isocratic standard sample (Agilent part number 01080-68704)*
Mobile phase A	water = 35 %
Mobile phase B	acetonitrile = 65 %
Flow rate	1.5 ml/min
Detection wavelength	210 nm
Stop time	6 min
Post time	1 min
Column compartment temperature	36 °C
Gradient	start with 65 % B go to 95 % B in 4 min go to 65 % B in 5 min
Injection volume	5 µl

*Isocratic standard sample contains 0.15 wt.% diethylphthalate, 0.15 wt.% diethylphthalate, 0.01 wt.% biphenyl and 0.03 wt.% o-terphenyl in methanol.

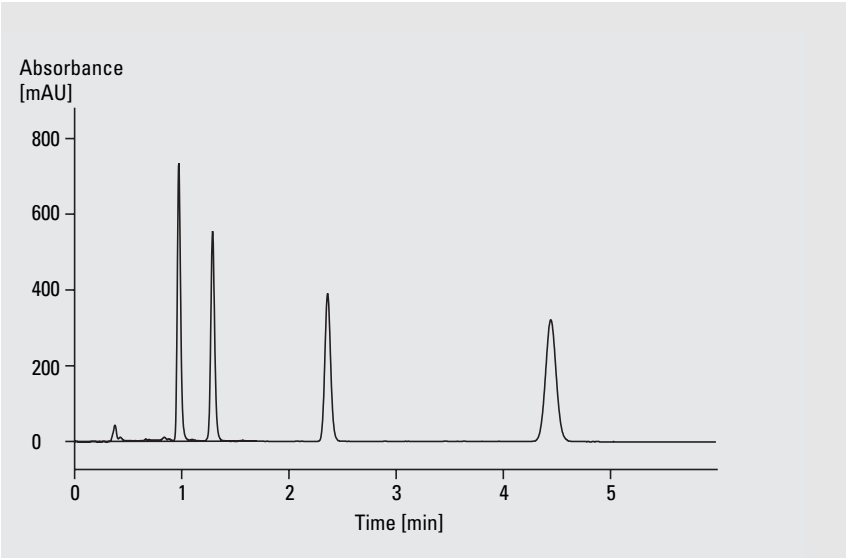


Figure 2
Typical chromatogram for isocratic standard with gradient

2. Optimizing Performance of an Agilent 1100 Series HPLC System

Is sensitivity an issue?

☑ Select a cell with long optical path length, for example, 10 mm.

☑ When using a DAD, set the slit width to high values, for example, 8 nm.

☑ When using a DAD, select a broad bandwidth for the detection wavelength, for example, 30 nm.

☑ Select the reference wavelength such that the reference bandwidth starts close to the end of the sample spectra. For example, if the sample spectra has its maximum at 252 nm and is down to zero mAUs at 300 nm, the optimum reference wavelength here would be 360 nm with a bandwidth of 100 nm.

☑ When using a DAD, select high response times, for example, 0.1 min. For broad peaks, select even higher response times.

Are retention time and area precision an issue?

☑ Select correct compressibility and stroke value for the pump.

☑ Flush instrument thoroughly with selected solvents.

☑ Let column equilibrate for at least 2 hours.

☑ For gradient analysis run the gradient run several times before starting with the test.

☑ Set injection volume to 5 µl.

Is highest performance for gradients in the range from 0 to 5% B and/or from 95 to 100%B an issue?

☑ Select a binary pump for highest performance.

For best performance over the complete gradient range, especially from 0 to 5 % and 95 to 100 %, we recommend using the binary pump.

Are low flow rates an issue?

☑ For flow rates below 200 µl/min use the binary pump with degasser.

☑ Remove the mixer and set the autosampler in bypass mode to reduce the system delay volume below 300 µl depending on the backpressure.

☑ The stroke volume should be 20 µl.

☑ For a diode array detector, you need a semi-micro flow cell (6 mm and 5 µl).

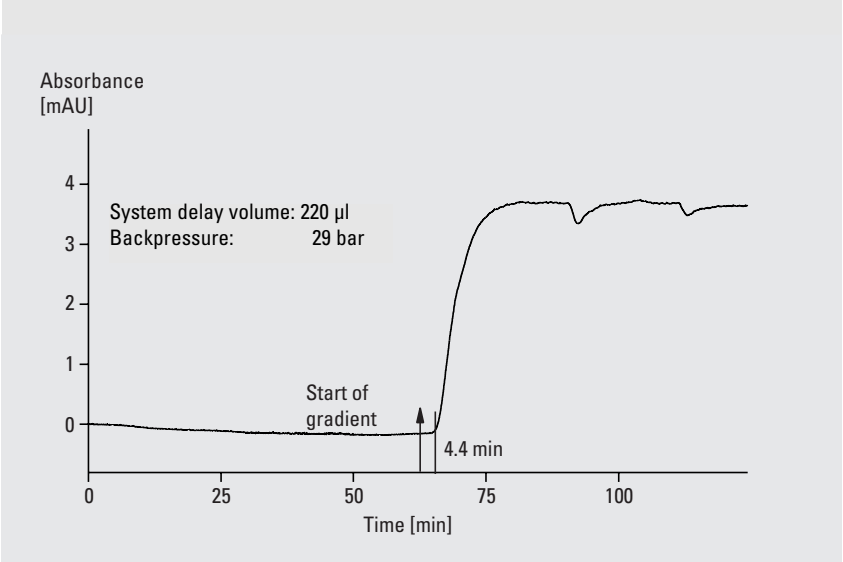
Figure 3 shows the delay volume when no mixer is used and the autosampler valve is set to bypass mode.

For lowest system delay volume, the autosampler valve can be switched into the bypass mode using the injector program. If a sample has been injected the valve should be switched after having flushed the sample loop. The following equation applies:

Wait time = 6 (injection volume + 5 µl) / flow rate

The appropriate injector program should read as follows:

1. DRAW (sample injection volume)
2. INJECT
3. WAIT (calculated wait time)
4. VALVE bypass
5. WAIT (run time minus 1 min)
6. VALVE mainpass



Capillary	4.8 m × 75 µm id Peek restriction capillary with dead volume of 20 µl
Solvent A	methanol
Solvent B	methanol and propylparaben
Flow rate	50 µl/min
Backpressue	29 bar
Autosampler	bypass using injector program
Stroke	20 µl
Step gradient	start with 0 % B up to 10 % B in 1 % steps, each step 60 min.
Detector	diode array, 254/30 nm, ref. 400/100 nm, degasser needed

Figure 3
Optimization of delay volume on a binary pump

Is carry-over an issue?

☑ Use automated needle wash for initializing a needle wash after the sample has been drawn up. For viscous samples which can show high carry-over effects, additional wash steps can be included in the injector program, for example:

1. DRAW (injection volume)
2. INJECT
3. WAIT (calculated wait time)
4. VALVE bypass
5. NEEDLE wash in vial 98, 5 times
6. NEEDLE wash in vial 99, 5 times
7. NEEDLE wash in vial 100, 5 times
8. NEEDLE up
9. DRAW 0.0 µl from seat
10. WAIT (run time minus 1 min)
11. VALVE mainpass
12. VALVE bypass
13. VALVE mainpass
14. VALVE bypass
15. VALVE mainpass

Compared to a standard wash procedure, this procedure substanially reduces sample carry-over.

Optimizing performance for normal phase chromatography

Important for all Agilent1100 Series pumps!

There are several applications, which include normal phase chromatography such as hexane, heptane or toluene as mobile phase. In these cases the pump needs polyethylene seals (Agilent part number 0905-1420). These seals are designed for mobile phases which produce leaks when using standard seals. The disadvantage of the normal phase seals is a shorter lifetime compared to that of the standard seals.

Recommendations for an instrument that will not be running for some time

If an instrument will not be used for several days or weeks, one of the most important things to do before shutting it down, is to flush the system with water.

This ensures that any kind of salts from solvents used in previous runs are flushed away. Salts can crystalize anywhere in the system and may destroy sealings and block frits. Flushing for at least half an hour is highly recommended. The column may be replaced by a low dead volume union.

References

1
"Performance Characteristics of the HP 1100 Series Modules and Systems for HPLC", *Technical Note* **1996**, , Hewlett-Packard publication number 12-5965-1352E

For the latest information and services visit
our world wide web site:
http://www.agilent.com/chem

Publication Number 5968-0468E

Agilent Technologies
Innovating the HP Way