Transferring HPLC methods from the HP 1090 Series to the Agilent 1100 Series HPLC system

Technical Note

Introduction

The HP 1090 Series HPLC system, introduced in 1983, is regarded to be an outstanding instrument in terms of performance and reliability. It was developed with the objective to create an HPLC system with optimized pump performance at 50–5000 µl/min flow rates and with a new type of detector that could acquire spectral data online. The result was a high performance solvent delivery system (DR5) and a new UV detector based on diode array detection technology (DAD).

Research activities in the later 1980s switched their focus to designing a pump that cost less to manufacture and maintain than the HP 1090 Series DR5 pump, but still provide equal or better performance. For more flexibility a modular design was chosen, resulting in the Agilent 1100 Series modules and systems for HPLC.

The Agilent 1100 Series features the following new design elements:

- New high performance pump, with a simple, easy-to-maintain design.
- New diode array detector offering a wavelength range from 190–950 nm and a noise specification of $\pm 1 \times 10^{-5}$ AU.
- Variable wavelength detector (VWD).
- New Peltier controlled column compartment from 10 degrees below ambient up to 80 °C and high temperature stability.
- Degassing with online vacuum degassing.
- New injector and autosampler design, featuring a robotic arm to transport the vials under the injector needle for more flexibility, sample cooling using Peltier elements, injection volume up to 1800 µl, 6-ml and 2-ml vials.
- Low benchspace requirements.

After introducing the Agilent 1100 Series and particularly now that the HP 1090 Series has been fully replaced by the Agilent 1100 Series, a common question is being asked by current and prospective users:

"*How do I transfer my well evaluated and established HP 1090 Series method to an Agilent 1100 Series system?*"

In this note we explain

- how HP 1090 Series methods can be transferred to the Agilent 1100 Series,
- which Agilent 1100 Series instrumentation to use, and
- which parameters to select for the Agilent 1100 Series modules.

For a better understanding of the processes, we also include some theoretical background information.

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What are the hardware differences between Agilent 1100 and HP 1090 Series?

Pumps

The design of the Agilent 1100 Series pumps is completely different. In terms of performance such as flow rate and gradient precision, the Agilent 1100 Series pumps achieve comparable or even better results. The delay volume of the HP 1090 Series DR5 pump is comparable to that of the Agilent 1100 Series binary pump.

Degassing

The HP 1090 Series uses helium for solvent degassing, whereas the Agilent 1100 Series uses vacuum degassing. This difference should not have any great impact on most applications.

Injector and Autosampler

The Agilent 1100 Series uses a completely new design.The vials are moved by a robotic arm and samples can now be cooled without using external devices. This new design has negligible influence on method transfer.

Column Compartment

A completely new design is used in the Agilent 1100 Series, allowing ambient and subambient operation without external devices. The improved temperature stability means the influence of ambient temperature on retention time precision should be minimized—especially for ambient runs.

DAD

The DAD in the Agilent 1100 Series has been improved with regard to wavelength range and limits of detection and quantification.

What Agilent 1100 Series modules are needed for smooth method transfer from HP 1090 Series?

To achieve similar or even better performance table 1 shows which Agilent 1100 Series module is equivalent to which part of the HP 1090 Series system.

HP 1090 Series	Agilent 1100 Series	Comments on Agilent 1100 Series
DR5 solvent delivery system	binary pump (high pressure gradient pump)	flow range 0.05 to 5 ml/min
PV5 solvent delivery system	quaternary pump (low pressure gradient pump)	flow range >0.2 to 10 ml/min
Diode-array detector (DAD)	DAD	wavelength range 190 to 950 nm BL noise ± 1 x 10^{-5} AU
Filter photometric detector (FPD)	VWD	wavelength switching
Column compartment	Peltier controlled thermostatted column compartment	10 °C below ambient up to 80 °C Temperature stability ± 0.15 °C
Helium degassing	online vaccum degasser*	high degassing efficiency
Autosampler/injector (air pressure driven actuators)	electrically driven injector/ autosampler, robotics-arm moves vials*	sample cooling via peltier elements, injection volume up to 1800 µl, 6 ml and 2 ml vials

* For a standard Agilent 1100 Series system, gas supplies are not needed.

Table 1
Performance overview of HP 1090 Series and Agilent 1100 Series systems

Minimizing the influence of design differences on chromatographic performance

Nowadays HPLC methods in routine use must pass a validation process, demonstrating that the method is adequate for the desired analytical purpose. The parameters usually examined in the validation process include:

- limits of detection and quantification,
- accuracy,
- precision,
- selectivity and specificity,
- linearity,
- range, and
- ruggedness.

Limits and ranges of these parameters are influenced by the HPLC equipment being used, for example, the detection limit is strongly influenced by the noise behavior of the detector.

Therefore, if a method is transferred from an existing instrument to another instrument type such as the Agilent 1100 Series, the difference in instrument design has to be taken into consideration. For example, the Agilent 1100 Series diode-array detector will in many cases provide lower LODs/LOQs because of far better signal-to-noise ratios for the evaluated peaks.

Instrument characteristics influence the validation parameters mentioned earlier to different degrees. Table 2 gives a rough overview of the influence of HPLC instrument components on chromatographic performance.

Further, design differences often result in differences in chromatographic performance. The following sections take a more detailed look at Agilent 1100 Series modules to find out what must be done to obtain similar performance as with HP 1090 Series systems.

	Pump	Degasser	Injector	Column Compartment	Detector
Limit of detection and quantitation	+	+	+	+	+++++
Accuracy and and precision	++++	+	+++	+	++++
Selectivity/specifity	+++++	-	-	+	+++
Linearity/rang	-	-	+++	-	++++
Ruggedness	+++++	+	+++	++	++++

+++++ = strong influence, + = low influence, - = no influence

Table 2
Influence of HPLC instrument components on chromatographic performance

1. HP 1090 Series DR5 pump and Agilent 1100 Series binary pump

The DR5 pump of the HP 1090 Series has been considered as the best overall commercially available pump. To achieve the same performance with an Agilent 1100 Series system, the binary pump should be chosen. This pump is based on high-pressure gradient design and offers:

- flow range from 50 µl/min to 5 ml/min
- low delay volume, 180–480 µl without mixer, 600–900 µl with mixer, depending on backpressure
- high flow and composition precision.

Each solvent is pumped by its own pump assembly and solvent mixing takes place on the high pressure side. The binary pump should be chosen when flow rates below 0.5 ml/min are used. It is also recommended for rapid gradient analysis and in cases where highest performance in terms of retention time precision for binary gradient analysis is needed over the complete range from 0 to 100% B.

The Agilent 1100 Series binary pump should also be chosen if lowest delay volumes are mandatory for the analysis. The delay volume of the pump—besides the colum—influences the separation of compounds especially if low flow rates with gradient analysis are used. An Agilent 1100 Series system with high-pressure gradient pump achieves comparable system delay volumes and similar gradients will yield almost identical chromatograms.

Method transfer from HP 1090 Series DR5 pump to Agilent 1100 Series binary pump

Hints:

- Compressibility has to be set correctly for both solvents on the Agilent 1100 Series.
- Stroke volume for both channels has to be set—for optimum baseline stability the stroke should be set to 20 µl for both channels, because then the lowest possible stroke volume is automatically used.
- The mixer in the Agilent 1100 has to be removed, if the HP 1090 mixer was not installed. The mixer can be replaced by a capillary, part number 01090-87610.
- Ternary gradients are not possible with Agilent 1100 Series binary pump.
- Flushing of the column with a third or fourth solvent is possible with the optional solvent selection valve.

2. HP 1090 Series PV5 pump and Agilent 1100 Series quaternary pump

Both pump types, the HP 1090 Series PV5 pump and the Agilent 1100 Series quaternary pump are low-pressure gradient pumps, in which solvent composition is achieved using a multi-channel gradient valve. Solvent mixing is done on the low pressure side.

The Agilent 1100 Series quaternary pump can be used from < 0.2 ml/min up to 10 ml/min. Up to four solvents can be used to form a gradient.

Method transfer from HP 1090 Series PV5 pump to Agilent 1100 Series quaternary pump

Hints:

- The delay volume of the Agilent 1100 Series quaternary pump is twice as low as the HP 1090 Series PV5 pump. For a smooth transfer, additional internal volume has to be added to the Agilent 1100 Series quaternary pump. This can be done using sample loops with well-known internal volumes. The loop can be installed between the pump and the injector.
- Compressibility and stroke volume must be set on the Agilent 1100 Series quaternary pump. The stroke volume should be set to automatic for best baseline performance. One compressibility value has to be set. If the solvents used for gradient operation have very different compressibility values, the compressibility should be set to 100.

3. HP 1090 Series helium degasser and Agilent 1100 Series vacuum degasser

The main difference is that the HP 1090 Series uses helium for degassing whereas the Agilent 1100 Series uses vacuum degassing. The main advantage of helium degassing is that no additional internal volume is added and changing the solvent in one channel only takes a few minutes. The disadvantages are that a gas-supply installation is needed and that helium is expensive.

The main advantage of vacuum degassing is that degassing is convenient and very effective. This can improve chromatographic performance, for example, in the analysis of PNAs with fluorescence detector, where remaining oxygen in the mobile phase is responsible for quenching effects.

Method transfer from HP 1090 Series helium degasser to Agilent 1100 Series vacuum degasser

Hints:

- Degassing is very efficient with the vacuum degasser, even improving the signal-to-noise ratio for some special applications with specific detectors.
- Each degasser channel has to be purged thoroughly if a new solvent needs to be used because of the internal volume of the vacuum degasser. A rule of thumb is 10 minutes purging with 5 ml/min per channel.

4. HP 1090 Series and Agilent 1100 Series injectors and autosamplers

The injector on the HP 1090 Series uses glass syringes as metering device which can be used at low pressures. Glass syringes in general have the disadvantage that they cannot be permanently in the flow path at the high pressure side for continuous flushing. A special wash cycle has to be used to flush the syringe when solvents need to be changed or when an air bubble has to be removed from the syringe.

The Agilent 1100 Series injector's metering device has a stainless steel body and sapphire plunger. This design can be used with pressures up to 400 bar and can be flushed permanently while in the high pressure flow path. Another advantage is that exchange of the metering device is not necessary for injection volumes larger than 25 µl. For injection volumes above 100 µl multiple-draw routine or an optional 900-µl metering device can be used.

Method transfer from HP 1090 Series to Agilent 1100 Series injector and autosampler

Hints:

- Vial numbering is different because of different tray designs. The Agilent 1100 has no vial position 0.
- Significant low carry-over using the injection mode *injection with needle wash*.
- Mixing of drawn-up sample and derivatization reagents can be done *in air* or *in seat*. *In air* is the mixing procedure used in the HP 1090 Series. *In seat* is a mixing procedure where mixing takes place in the seat capillary for better turbulent mixing.
- No exchange of syringes for injection volumes larger than 25 µl.
- Cooling of thermally-labile samples can now be done using the thermostatted Agilent 1100 Series autosampler, which provides peltier temperature control from 4–40 °C.

5. HP 1090 Series and Agilent 1100 Series column compartments

Temperature control in the HP 1090 Series column compartment is done by preheating the eluent as it enteres the compartment and by circulating heated air inside the compartment. The Agilent 1100 Series uses a completely different temperature-control design. Heating and cooling of the eluent and the inner compartment is done using Peltier elements. The compartment temperature range from 10 degrees below ambient up to 80 °C is extended to subambient operation with a temperature stability of ± 0.15 degrees. External cooling devices—if used on HP 1090 Series systems—are not needed with Agilent 1100 Series systems.

The goal for the Agilent 1100 Series design was to ensure that the set temperature is equal to the temperature at the measuring point in the compartment, in compliance with GLP rules. In the HP 1090 Series the goal was to have the set temperature equal to the temperature in the column. This was implemented by increasing the set temperature by a previously evaluated factor which was dependent on the flow rate and the set temperature.

Table 3 shows the influence of the different design types on the actual temperature in the column.

Consequently during method transfer the compartment temperature on the Agilent 1100 Series has to be elevated in cases where low flow rates are combined with higher temperature.

Set flow flow rate	Set oven temperature	Agilent 1100 temperature at measurement point	Agilent 1100 temperature inside column	HP 1090 temperature at measurement	HP 1090 temperature inside column
0.2 ml/min	65 °C	65 °C	60 °C	70 °C	65 °C
0.2 ml/min	40 °C	40 °C	39 °C	41 °C	40 °C
1 ml/min	65 °C	65 °C	64 °C	66 °C	65 °C
1 ml/min	40 °C	40 °C	40 °C	40 °C	40 °C

Table 3
Comparison of column temperatures

Method transfer from HP 1090 Series to Agilent 1100 Series column compartment

Hints:

- Temperature range is different on the Agilent 1100 Series– 10 degrees above ambient up to 80 °C.
- At higher compartment temperatures, for example, 60 °C the actual temperature in the column is somewhat lower on the Agilent 1100 Series than on the HP 1090 Series because of different heating and measuring routines.

6. HP 1090 Series and Agilent 1100 Series diode array detectors

Hewlett-Packard introduced the first diode array detector (DAD) for liquid chromatography in 1982. Since then significant improvements in the design of both the hardware and software have been made, resulting in the introduction of the Agilent 1100 Series diode array detector. Here, limit of detection and quantification were improved due to the very low noise behavior of the Agilent Series 1100 DAD as compared to the HP 1090 Series DAD. In order to obtain optimum lamp energy in the UV and visible ranges, a tungsten lamp is positioned in line with a deuterium lamp. This allows sensitive measurements in the UV range and in the visible ranges up to 950 nm. Full spectral information can now be obtained from 190 up to 950nm.

The main parameters that differ are shown in table 4.

For smooth method transfer, a detector cell with the same path length should be selected. The Agilent 1100 Series DAD has programmable optical slits which can be selected from the screen—no mechanical exchange is needed. The same slit width should be selected and the tungsten lamp should be switched off. All other detector settings such as detection wavelength, data rate, and so on, should be the same.

Parameter	HP 1090 Series	Agilent 1100 Series
Light source	deuterium lamp	deuterium and tungsten lamps
Short term noise	< 7 x 10^{-5} AU at 254 nm	< 2 x 10^{-5} AU at 254 nm < 2 x 10^{-5} AU at 750 nm
Wavelength range	190 - 600 nm, in steps of 1 nm	190 - 950 nm, in steps of 1 nm
Optical slit width	2, 4, 8 nm	1, 2 4, 8, 16 nm progammable
Diode width	lowest 2 nm	< 1 nm
Flow cells	standard: 6 mm path length 8 µl	standard: 10 mm path length 13 µl
	standard high sensitivity 10 mm path length, 13 µl	semi-micro: 6 mm path length 5 µl
	high pressure/micro: 6 mm path length, 1.7 µl	high pressure/micro: 6 mm path length, 1.7 µl

Table 4
Comparison of diode array parameters

Method transfer from HP 1090 Series to Agilent 1100 Series DAD

Hints:

- A detector cell with the same path length and cell volume should be used.
- The tungsten lamp should be switched off for applications in the UV range.
- The optical slit width should be the same.
- Data rate setting should be the same.
- Detection wavelength and bandwidth should be the same.
- Reference wavelength and bandwidth should be the same.
- The signal-to-noise ratio will be better for most applications, even in the low UV range
- The signal-to-noise ratio will be significantly better for the high UV and the visible range.
- Spectral information up to 950 nm can be achieved with the Agilent 1100 Series DAD.

7. HP 1090 Series filter-photometric detector and Agilent 1100 Series variable wavelength detector

The Agilent 1100 Series does not include a single wavelength detector, only a variable wavelength detector.

Method transfer can be done by simply selecting a comparable detector cell, the same detection wavelength and comparable data rate. The performance regarding noise, drift and wander are then similar.

The main parameters that differ are shown in table 5.

Method transfer from HP 1090 Series FPD to Agilent 1100 Series VWD

Hints:

- Select the same detection wavelength on the Agilent 1100 Series VWD.
- Select standard flow cell on the Agilent 1100 Series. The signal-to- noise ratio will be improved due to a longer path length

Parameter	HP 1090 Series	Agilent 1100 Series
Short term noise	5 x 10^{-5} AU	1.5 x 10^{-5} AU at 254 nm
Bandwidth	typically 10 nm	typically 6.5 nm
Wavelength range	190 - 600 nm with interference filter	190 - 600 nm in steps of 1 nm
Flow cells	standard: 6 mm path length 4.5 µl	standard: 10 mm path length 13 µl micro: 5 mm path length 1 µl high pressure 10 mm path length, 14 µl

Table 6
Comparison of detector parameters

8. Application Examples

Comparison of a peptide map application on HP 1090 Series and Agilent 1100 Series systems

One of the most critical applications in terms of pump performance is peptide mapping with 1-mm columns and a flow rate of 50 µl/min. Here the ability of the pump to deliver flow rates as low as 1 µl precisely and a delay volume as low as possible are of significant importance for getting comparable results. As shown in figure 1, the Agilent 1100 Series binary pump performance can be compared to that of the HP 1090 Series.

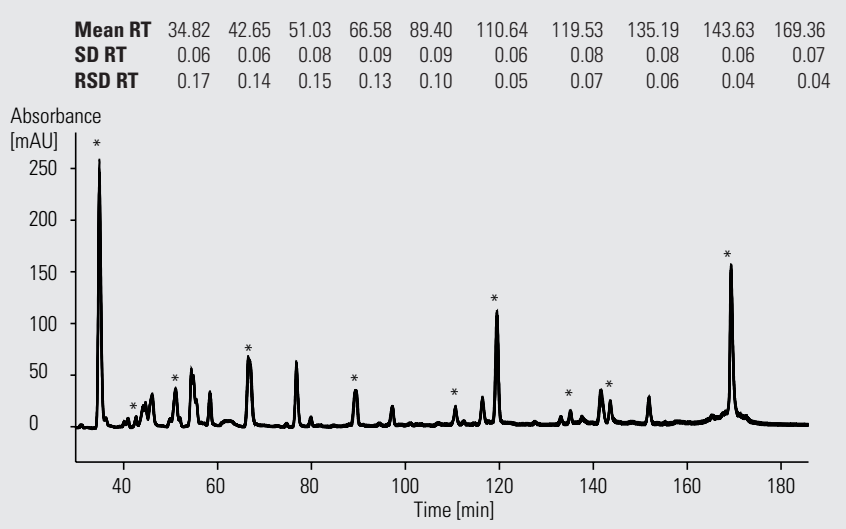


Figure 1
Peptide map application with the HP 1090 Series and the Agilent 1100 Series systems

Method transfer

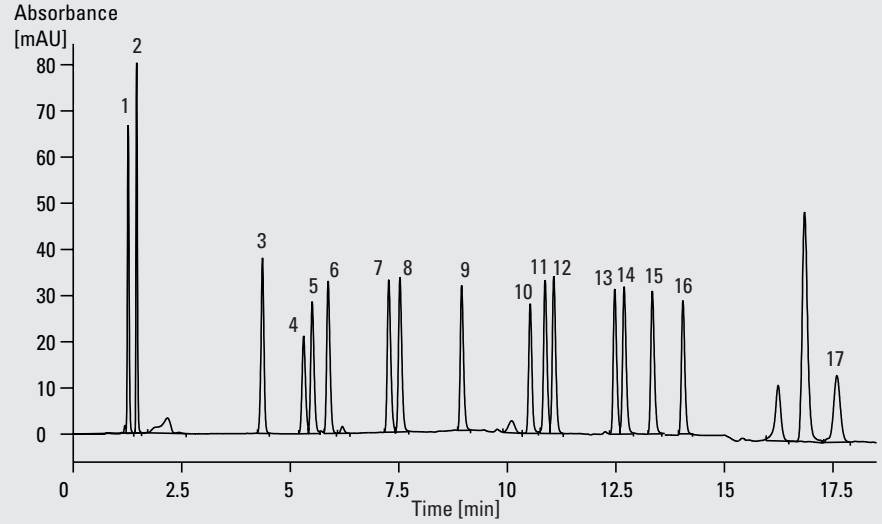
Parameter settings on both systems do not differ much. Flow rate, gradient and detector settings are the same. Differences on the Agilent 1100 Series include compressibility, which is determined by the used eluents, and stroke settings for the binary pump.

Pump parameter	HP 1090 Series DR5 pump	Agilent 1100 Series binary pump
Static mixer	no mixer	no mixer, no other hardware changes needed
Flow rate	50 µl/min	same
Gradient	0 min 1 % B 210 min 51 % B 210 min 70 % B 210.1 min 70 % B, 0.12 ml/min 215 min 98 % B 217 min 98 % B 220 min 1% B 240 min 1 % B 240.1 min 1 %B, 0.05 ml/min 250 min 1 % B	same
Compressibility A	none	46 for water
Compressibility B	none	115 for acetonitrile
Stroke A	none	20 µl
Stroke B	none	20 µl
Column	1 x 250 mm Vydac TP 218	same
Solvent A	0.05 % TFA in water	same
Solvent B	0.043 % TFA in acetonitrile	same
Temperature of column	40 °C	same
Injection	1.1 µl myoglobin digest, 190 pmol	same
UV detection	214/8 nm , reference 450/80 nm	214/8 nm, reference 450/80 nm semi-micro detector cell, 5-µl, 6 mm path length, slit width 4 nm

Table 6
Chromatographic conditions for peptide map

Comparison of amino acid analysis on HP 1090 Series and Agilent 1100 Series systems

Figures 2 and 3 show the analysis of amino acids in a protein hydrolysate. All parameters were set identically except the path length of the detector cell. As a result peak heights were increased for the 10 mm path length cell, therefore, the 10-mm path length is recommended for this type of analysis.



	Compound	Time
1	Asp	1.268
2	Glu	1.463
3	Ser	4.361
4	His	5.309
5	Gly	5.507
6	Thr	5.874
7	Ala	7.270
8	Arg	7.528
9	Tyr	8.949
10	Cys-SS-Cys	10.527
11	Val	10.870
12	Met	11.071
13	Phe	12.474
14	Ile	12.690
15	Leu	13.340
16	Lys	14.045
17	Pro	17.590

Figure 2
Analysis of amino acid hydrolysate on the Agilent 1100 Series with precolumn online derivatization of 250 pmol/µl AA standard using detector cell of 10 mm path length

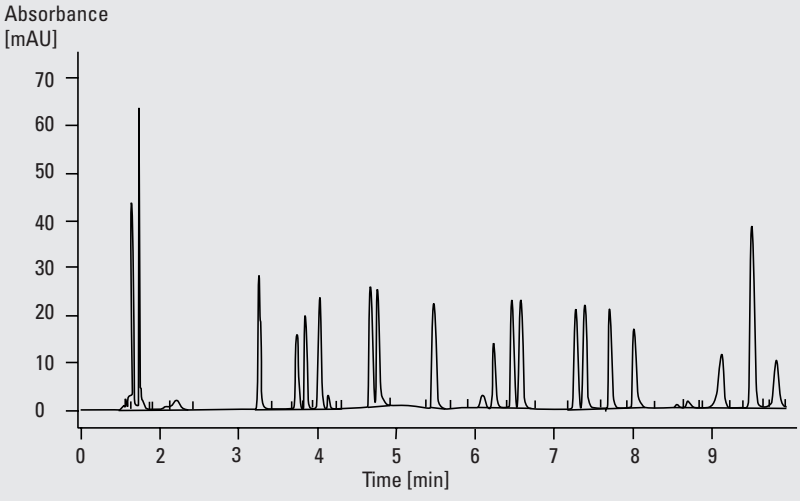


Figure 3
Analysis of amino acid hydrolysate on the HP 1090 Series with precolumn online derivatization of 250 pmol/µl AA standard and 6 mm path length detector cell

Parameter	HP 1090 Series (DR5) without mixer	Agilent 1100 Series binary pump without mixer
Column	200 x 2.1 mm AA column and guard column	same
Mobile phases	A= 20 mMol Na Ac + 0.018 % TEA adjusted to pH 7.2 with 1-2 % acetic acid + 1.5 ml THF B = 20 % of 100 mMol NaAc adjusted to pH 7.2 with 1-2 % acetic acid and 40 % ACN and 40 % MeOH	
Flow rate	0.45 ml/min	same
Compressibility A	-	46
Compressibility B	-	115
Stroke	-	A and B = 20 µl
Gradient	start with 100 % A, at 17 min 60 % B at 18 min 100 % B, at 18. 1 min flow 0.45, at 18.5 min flow 0.8, at 23.9 min flow 0.8, at 24 min 100 % B and flow 0.45, at 25 min 0 % B	same
DAD UV detector	signal A = 338/10 nm, ref = 390/20 nm signal B = 262/16 nm, ref = 324/8 nm at 15 min: signal A = 262/16 nm, ref. = 324/8 nm detector cell: 6 mm pathlength, 8-µl cell volume	same
Oven temperature	40 °C	same
Injector program	1 Draw 5.0 µl from vial 2 (borate buffer) 2 Draw 1.0 µl from vial 0 (OPA reagent) 3 Draw 0.0 µl from vial 100 (water) 4 Draw 1.0 µl from sample 5 Draw 0.0 µl from vial 100 (water) 6 Mix 7 µl cycles 6 7 Draw 1.0 µl from vial 1 (FMOC) 8 Draw 0.0 µl from vial 100 (water) 9 Mix 8 µl, cycles 3 10 Inject	1 Draw 5.0 µl from vial 2 (borate buffer) 2 Draw 1.0 µl from vial 3 (OPA reagent) 3 Draw 0.0 µl from vial 10 (water) 4 Draw 1.0 µl from sample 5 Draw 0.0 µl from vial 10 (water) 6 Mix 8 µl in seat, max. speed six times Draw 1.0 µl from vial 1 (FMOC) 8 Draw 0.0 µl from vial 10 (water) 9. Mix 9 µl in seat, max. speed, 3 times 10. Inject
FLD setting	Excitation: 340 nm, emission: 450 nm PTM gain 12 at 14.5 min excitation: 266 nm, emission: 305 nm PTM gain 11	same

Table 7
Chromatographic conditions for amino acid analysis

Conclusion

For most applications, method transfer from HP 1090 Series to Agilent 1100 Series systems can be performed without losing performance. Differences in selectivity and specificity are minimized if the HP 1090 Series DR5 pump is replaced by the Agilent 1100 Series binary pump and the HP 1090 Series PV5 pump is replaced by the Agilent 1100 Series quaternary pump.

Typically, the performance in terms of precision of retention times and areas is comparable if selection of modules and settings is appropriate on the Agilent 1100 Series.

Improvements in design mean linearity and range are extended on the Agilent 1100 Series and the same performance or better can be expected here.

The noise level of the Agilent 1100 Series DAD and VWD detectors is significantly improved. This means limit of detection and quantification will typically be improved.

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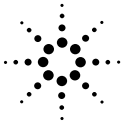

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