Agilent 1200 Series LC Method Development Solution – Automated method development with up to eight columns

Technical Note

Introduction

Analytical HPLC method development is an important task for the creation of final test methods for products in the chemical and pharmaceutical industries. Typically, a set of columns is tested in combination with a set of solvents. This method scouting process helps to find the best column and mobile phase for the separation of a set of compounds. In addition, a fine tuning process is often needed to optimize the gradient, column temperature, pH, or speed of the complete analysis. The entire procedure is often time-consuming if columns and solvents cannot be changed automatically in a sequence.

The Agilent 1200 Series LC Method Development Solution offers a highly flexible system that can be used for up to eight columns up to 100 mm in length and up to six columns with lengths of up to 300 mm. Several independent heated zones are available to optimize the temperature for different columns. The system uses a sequence with different methods to automate column and solvent changes. The system can be combined with sophisticated method development software like:

- ACD/AutoChrom for ChemStation from ACD/Labs
- ChromSword Auto for ChemStation from ChromSword Baltic.

In this Technical Note, we will describe:

- Hardware: How valves, capillaries, columns, and solvent selection valve are integrated into an Agilent 1200 Series Rapid Resolution LC (RRLC) system
- Software: How to set up a method development system in Agilent ChemStation
- Application example using six different columns
- Comparison of performance with a standard Agilent 1200 Series RRLC system, using columns with 3.0 and 2.1 mm internal diameters on both

Agilent Technologies

Hardware description

LC modules

For the experiments described in this Technical Note, the Agilent 1200 Series LC Method Development Solution was built upon an Agilent 1200 Series Rapid Resolution LC system. The system was comprised of the following modules with firmware revisions A.06.01 or higher:

- Agilent 1200 Series binary pump SL with micro degasser
- Agilent 1200 Series high performance autosampler SL plus
- Two Agilent 1200 Series thermostatted column compartments SL plus with installed valve drives
- Agilent method development valve kit, *high* pressure with Agilent method development capillary kit, low dispersion, for short columns (option #001)
- Agilent 1200 Series diode-array detector (DAD) SL
- Agilent ZORBAX Rapid Resolution High Throughput (RRHT) columns with 1.8 µm particle size
- Agilent ChemStation B04.01

Description of the system

The Agilent 1200 Series LC Method Development Solution consists of two or three clustered Agilent thermostatted column compartments SL plus that are integrated into an Agilent 1200 Series LC or RRLC system. One column compartment contains the valve that is connected to the pump and delivers the flow to the columns. The second column compartment contains the valve that is connected to the detector and delivers the flow coming from the active column to the detector. A maximum of eight columns up to 100 mm length can be installed in two clustered column compartments using the low dispersion heat exchangers (see figure 1). Up to six columns of lengths between 100 mm and 300 mm can be installed in three column compartments, if solvent preheating is required (recommended).

To set up a complete Agilent 1200 Series LC Method Development Solution, the parts and modules shown in figure 1 must be integrated. The valve drives are factory-installed. The valve heads are interchangeable and can be easily mounted by the user. The valves are mounted on a pull-out rail that facilitates very easy plumbing of capillaries. The Agilent method development valve kits (G4230A or G4230B) integrate:

- Valve heads in different pressure ranges
- Three different optional capillary kits
- Optional column kits

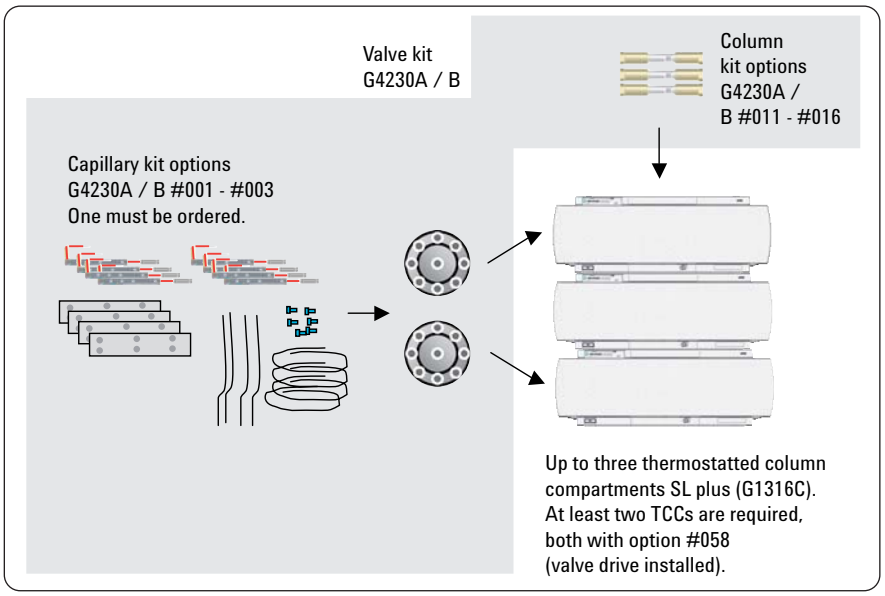

Figure 1
Modules and parts needed for an Agilent 1200 Series LC Method Development Solution.

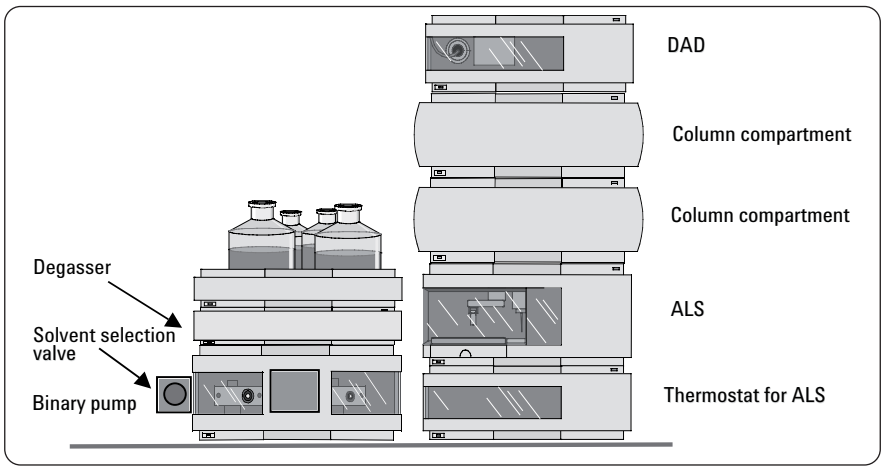

Figure 2
Agilent 1200 Series RRLC system configuration for method development. ALS = autosampler.

Figure 3 shows the setup that was used for the experiments presented here. It had two column compartments, IN and OUT valves, and six columns. Three heated zones were used to keep columns at different temperatures. This enables, for example, raising the temperature for one column to 80 °C while keeping another column at 30 °C. A waste and a bypass capillary were installed at the remaining two valve positions, to enable flushing of the system. (Note: The waste line can be used to quickly flush the solvent delivery system at high flow rates without passing the detector, for example, during solvent changes.) By eliminating the waste and/or the bypass line, this instrument setup could support up to eight columns. If required, a separately available post-column cooler could be installed at the free heater position in the lower column compartment.

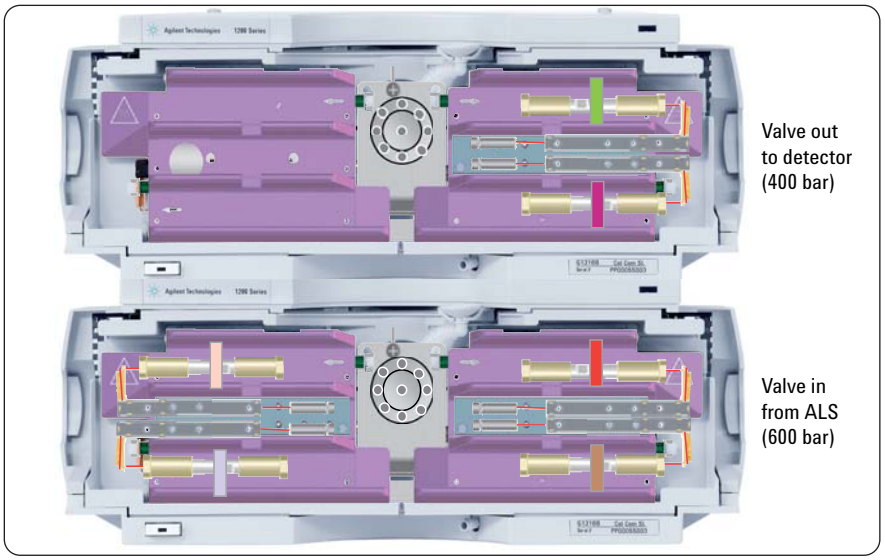

Figure 3
Clustered Agilent 1200 Series thermostatted column compartments SL plus with six columns installed.

Configuring the Agilent 1200 Series LC Method Development Solution in the Agilent ChemStation

Clustering and configuring the column compartments must be done only once during system setup. It is done by marking the appropriate check boxes, as shown in figure 4. This screen defines which thermostat contains the IN valve that is connected to the autosampler and which thermostat contains the OUT valve that is connected to the detector inlet.

Next, the positions of the columns and the waste and the bypass positions are defined (figure 5). The positions are color-coded and should reflect the color of the column clips used during installation of the columns.

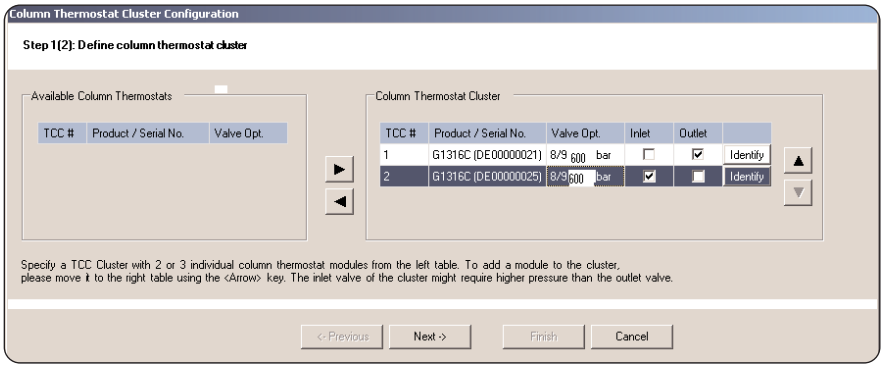

Figure 4
Configuring the two column compartments.

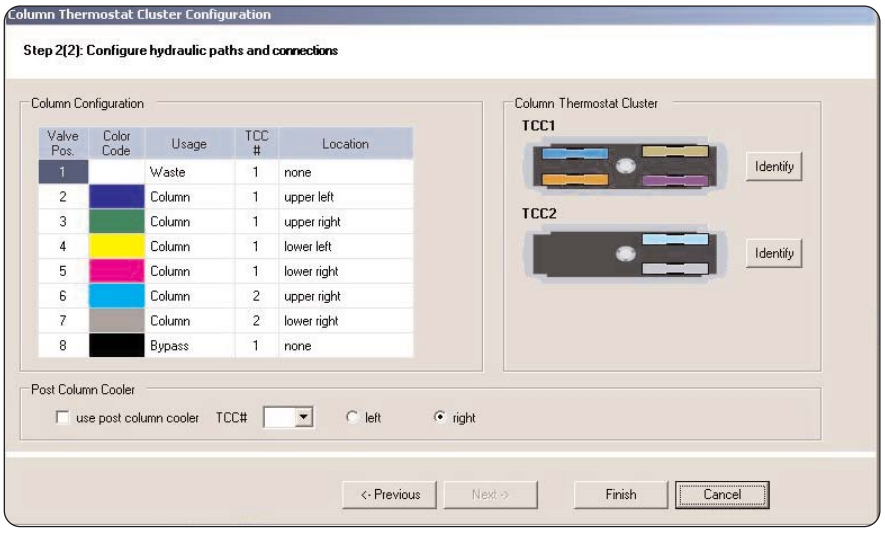

Figure 5
Definition of the column, waste, and bypass positions, as well as an optional post-column cooler.

If an external solvent selection valve (G1160A) is installed, it is possible to cluster it with a pump, as shown in figure 6. This means that this control area network (CAN) valve no longer appears as an independent module in the Agilent ChemStation software. Instead, the solvent selection is done by choosing the predefined solvent in the pumps user interface.

After this clustering procedure, one restart of the software is required. From then on, the clustered modules do not appear individually in the Agilent ChemStation software. The new user interfaces make column and solvent selection very simple.

In the "More column thermostat cluster" menu, the system can be told which columns are currently installed and which standby temperatures should be used if a column is not in the flow path (figure 7). The columns can be selected from the columns database in the Agilent ChemStation, which can store all the columns you are using regularly in your lab. In our example, valve position 1 is the waste position; this means the flow enters the IN valve and is then guided to waste. Valve position 8 is the bypass position, which allows flushing the system up to the detector with no column installed. In positions 2 through 7, we have six different columns installed.

Once all columns are configured, setup of a chromatographic method can begin. With the user interface afforded by the column compartment cluster, the column to be used can be selected by one mouse-click. The predefined position in the column compartment and the predefined color code will be shown. Finally, the temperature of the solvent for this method is set (figure 8).

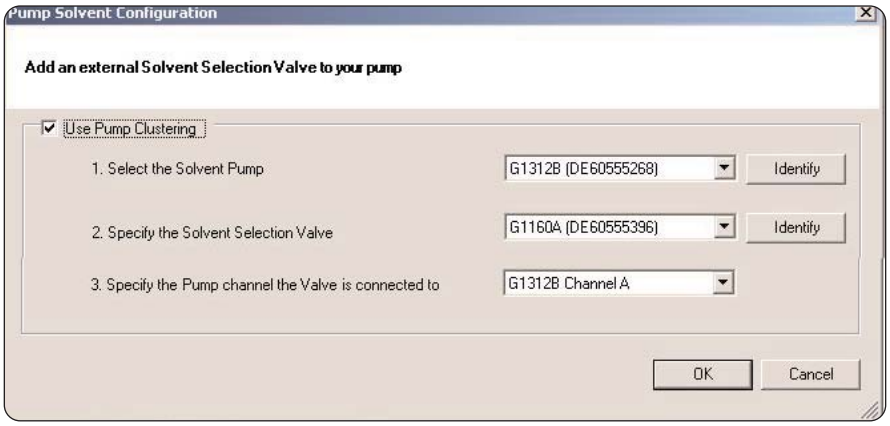

Figure 6
Clustering of solvent selection valve.

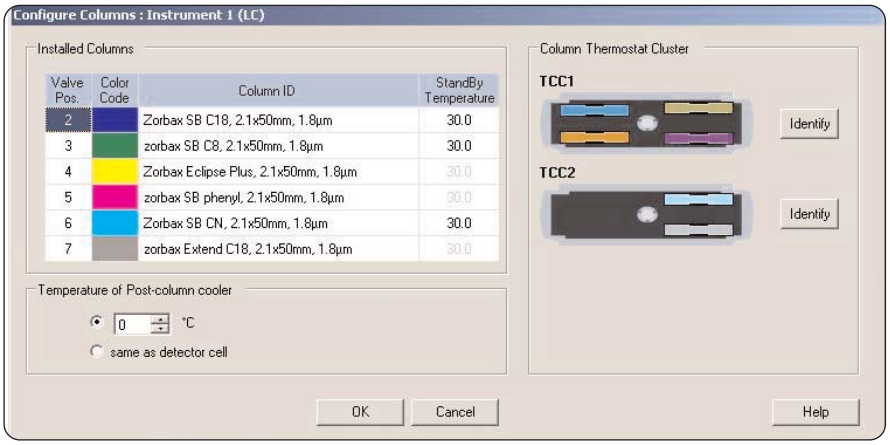

Figure 7
Defining column type and valve position.

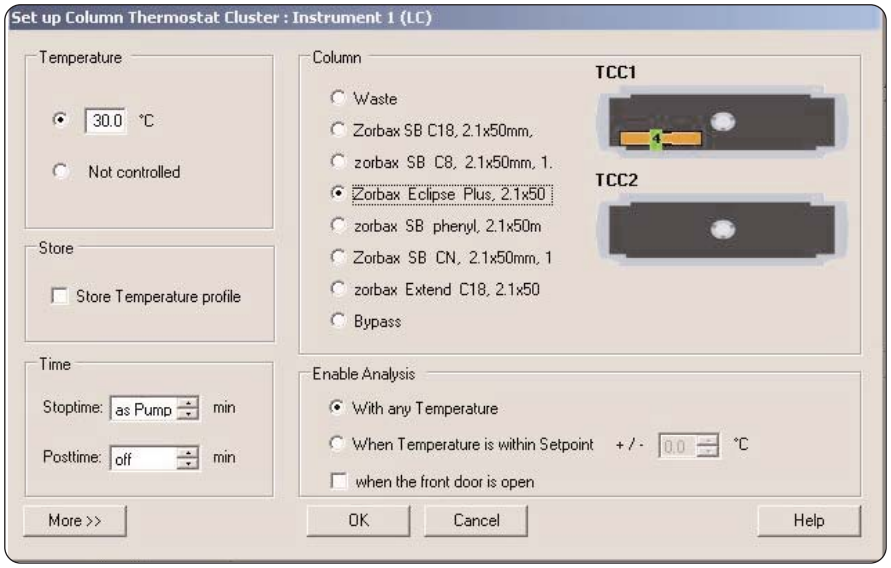

Figure 8
Selecting a column and the appropriate temperature for a chromatographic method.

Having defined column and column temperature, the other chromatographic conditions for the pump, autosampler, and detector can be set as usual and stored under a specific method name. Each column can use the same conditions or unique conditions. All methods are added to the standard sequence table.

Application example using six columns for method scouting

For the following practical example, a sample was chosen that contained a main compound and four impurities. The impurities were added in the low-percentage range.

First experiment

An initial experiment was done on an Agilent ZORBAX Eclipse Plus C18 column; see chromatogram and conditions in figure 9. Acidic conditions were chosen, using trifluoroacetic acid (TFA) as the modifier for the mobile phases. This first experiment was done on a 4.6 x 50 mm column. It indicated that a gradient range from 10 to 50 % organic solvent was a good start. A sequence was set up based on the information obtained from the initial experiment. The remaining experiments were done using columns with 2.1 mm internal diameters and lengths of 50 mm.

Column scouting

For the method scouting experiments, six different columns were chosen (figure 8). The same chromatographic parameters were used for all columns. After completion of the sequence, the resulting chromatograms were evaluated. The obtained chromatograms are shown in figure 10.

Table 1 shows the performance characteristics of the six chromatograms.

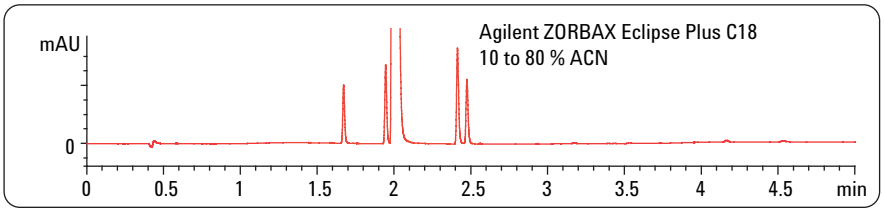

Figure 9
First test chromatogram.

Chromatographic conditions
Sample: Main compound 2 mg/mL, four impurities in the range from 1.3 to 2.2 % (spiked)
Column: 4.6 x 50 mm
Mobile phase: Water + 0.2 % TFA, acetonitrile (ACN) + 0.16 % TFA
Gradient: 10 to 80 % ACN in 5 min
Flow: 1.5 mL/min
Column compartment: 40 °C
Injection volume: 3 µL
Detector: DAD SL, 270/10 nm, Ref 360/100 nm, PW > 0.01 min, slit 4 nm, 10 mm detector cell

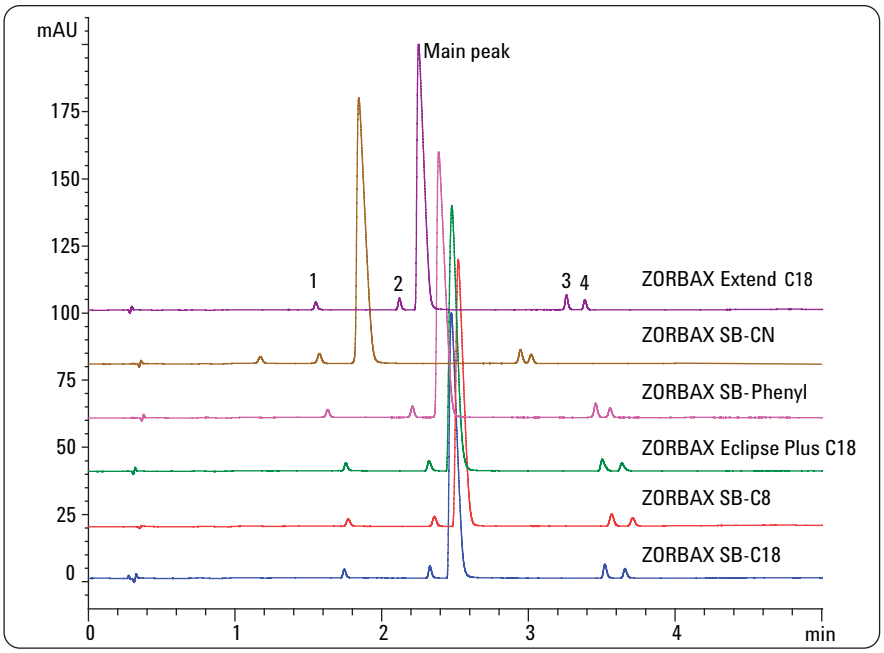

Figure 10
Results obtained from column scouting experiments.

Chromatographic conditions
Sample: Main compound 2 mg/mL, four impurities in the range from 1.3 to 2.2 % (spiked)
Columns: 2.1 x 50 mm columns packed with 1.8 µm particles
Flow rate: 0.5 mL/min
Gradient: 10 to 45 % in 4 min
Stop time: 5 min
Post time: 1 min
Injection volume: 1 µL
Column temperature: all 30 °C
Detector: DAD, 270/10 nm, Ref 360/100 nm, 3 mm detector cell
Configuration: Low-delay-volume configuration for pump, method development capillary kit – low dispersion kit for short columns (G4230B #001)

Parameter	SB-C18	SB-C8	Eclipse Plus	SB-Phenyl	SB-CN	Extend C18
Width of peak 1 (min)	0.0217	0.0275	0.0229	0.0247	0.0289	0.0217
Width of peak 5 (min)	0.0253	0.0304	0.0297	0.0253	0.0281	0.0211
Resolution of main peak	2.20	2.24	2.17	2.52	3.53	2.09
Resolution of peak 5	3.24	2.76	2.62	2.3	1.54	3.50

Table 1
Performance characteristics of the evaluated columns.

The Agilent ZORBAX SB-C18 column was selected as the column with the most appropriate performance. For further evaluation, this column was transferred to a standard Agilent 1200 Series RRLC system. This system has only a single column compartment.

Comparison of performance with standard Agilent 1200 Series RRLC system using 2.1 and 3.0 mm id columns

A very important question is, "How similar are the results obtained from the Agilent 1200 Series LC Method Development Solution compared with results obtained from a standard Agilent 1200 Series RRLC system using the same chromatographic setup?"

For our example, the previously selected Agilent ZORBAX SB-C18 column (2.1 mm id, 1.8 µm particles) was installed in a standard Agilent 1200 Series RRLC system. The same chromatographic conditions were loaded and the obtained chromatogram was compared with the one obtained from the Agilent 1200 Series LC method development solution (figure 11).

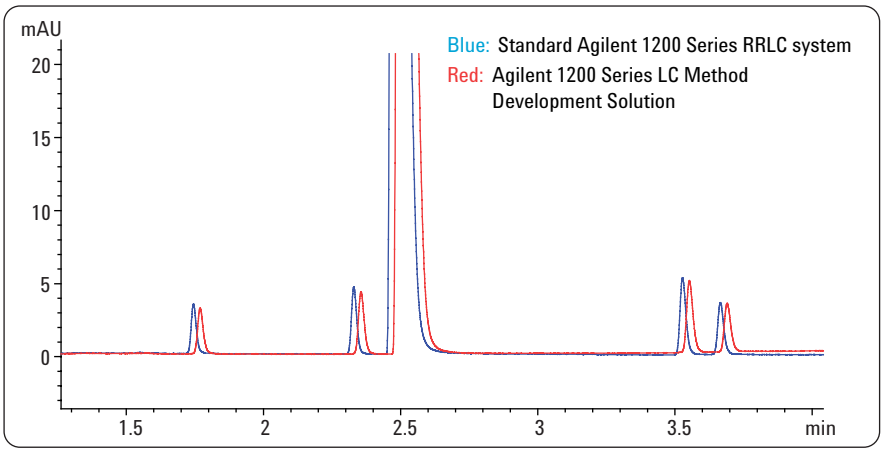

Figure 11
Comparison of standard versus method development chromatogram from analysis of impurities on a 2.1 mm id column packed with 1.8 µm particles.

In table 2, typical chromatographic parameters like retention times, peak widths, peak symmetry, resolution, and USP tailing were compared between the two systems. In all cases, the deviation was below 10 % – even for these demanding conditions. (The lower the column volume and the smaller the particle size, the higher the demands on the system.)

In a further experiment, the two systems were compared using a 100 x 3.0 mm id column packed with 3.5 µm particles. The low-delay-volume configuration was selected for both systems. Both systems used the 1.6 µl low-dispersion heat exchanger, as well as the 2 µL (3 mm path length) flow cell for the diode array detection system. The resulting chromatograms are shown in figure 12.

Because of the significantly larger column and peak volumes of the 3.0 mm id column with 3.5 µm particles, this second test system was less sensitive to extra-column delay volumes. Therefore, the difference between the two systems was even smaller; see table 3.

	Standard Agilent 1200 Series RRLC system					Agilent 1200 Series LC Method Development Solution					Difference				
Peak number	Retention time (min)	Peak width (sec)	Resolution	Symmetry	USP tailing	Retention time (min)	Peak width (sec)	Resolution	Symmetry	USP tailing	Retention time (sec)	Peak width	Resolution	Symmetry	USP tailing
1	1.745	1.164		0.789	1.250	1.769	1.302		0.761	1.257	1.44	111.9%		96.5%	100.6%
2	2.330	1.236	17.09	0.771	1.224	2.356	1.350	15.60	0.767	1.224	1.56	109.2%	91.3%	99.5%	100.0%
3	2.477	3.276	2.30	0.328	2.367	2.502	3.348	2.20	0.356	2.192	1.50	102.2%	95.7%	108.5%	92.6%
4	3.528	1.398	15.79	0.715	1.317	3.553	1.482	15.32	0.734	1.292	1.50	106.0%	97.0%	102.7%	98.1%
5	3.666	1.398	3.47	0.730	1.304	3.690	1.518	3.24	0.730	1.310	1.44	108.6%	93.4%	100.0%	100.5%
										Average:	1.49	107.6%	94.3%	101.4%	98.3%

Table 2
Performance comparison of Agilent 1200 Series LC Method Development Solution versus standard Agilent 1200 Series RRLC system, both with 2.1 mm id columns packed with 1.8 µm particles.

	Standard Agilent 1200 Series RRLC system					Agilent 1200 Series LC Method Development Solution					Difference				
Peak number	Retention time (min)	Peak width (sec)	Resolution	Symmetry	USP tailing	Retention time (min)	Peak width (sec)	Resolution	Symmetry	USP tailing	Retention time (sec)	Peak width	Resolution	Symmetry	USP tailing
1	2.356	1.386		0.840	1.119	2.377	1.416		0.870	1.098	1.26	102.2%		103.6%	98.1%
2	2.969	1.464	15.16	0.850	1.104	2.990	1.476	14.82	0.850	1.096	1.26	100.8%	97.8%	100.0%	99.3%
3	3.113	3.402	2.10	0.200	3.228	3.136	3.402	2.11	0.220	2.302	1.38	100.0%	100.5%	110.0%	93.7%
4	4.224	1.572	15.57	0.770	1.174	4.252	1.626	15.53	0.790	1.152	1.68	103.4%	99.7%	102.6%	98.1%
5	4.375	1.626	3.32	0.800	1.131	4.403	1.650	3.24	0.810	1.119	1.68	101.5%	97.6%	101.3%	98.9%
										Average:	1.45	101.6%	98.9%	103.5%	97.6%

Table 3
Performance comparison of Agilent 1200 Series LC Method Development Solution versus standard Agilent 1200 Series RRLC system, both with 3.0 mm id columns packed with 3.5 µm particles.

Conclusion

The Agilent 1200 Series LC Method Development Solution offers highest flexibility together with highest performance. Up to eight columns with lengths up to 100 mm can be installed. Up to six columns with lengths of up to 300 mm can be installed. Multiple heated zones are available for holding different columns at different temperatures. Installation of connecting capillaries is easy to perform using the pull-out valves. Agilent ChemStation software offers easy configuration, setup, and – most important – easy use of the system.

The experiments discussed in this Technical Note show that a method can be transferred from the Agilent 1200 Series LC method development solution to a standard Agilent 1200 Series RRLC system without significant differences in retention times, resolution, and peak tailing. Typically, the differences will be less than 10 %, even under demanding chromatographic conditions with narrow-diameter columns that are packed with sub-2-micron particles.

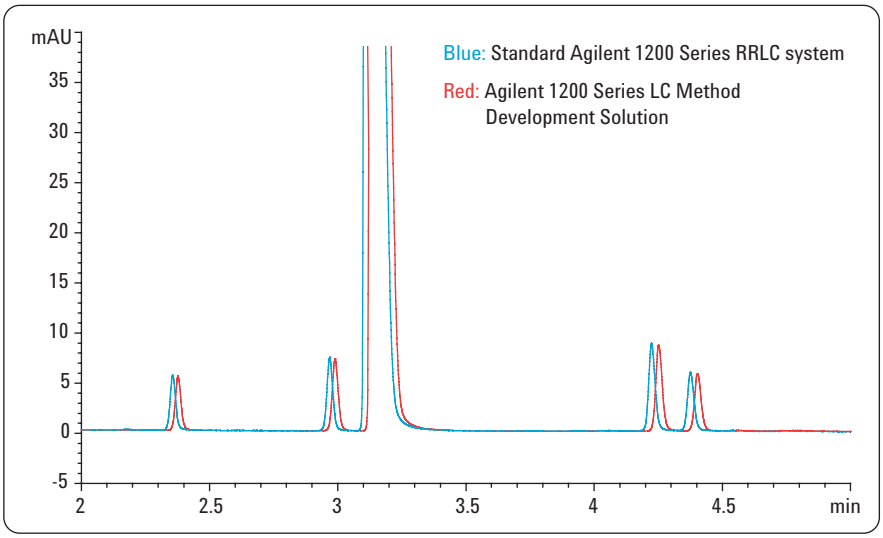

Figure 12
Comparison of chromatograms on an Agilent 1200 Series LC Method Development Solution versus a standard Agilent 1200 Series RRLC system, both using a 3.0 mm id column packed with 3.5 µm particles.

Chromatographic conditions
Sample: Main compound 2 mg/mL, four impurities in the range from 1.3 to 2.2 % (spiked)
Column: 3.0 x 100 mm packed with 3.5 µm particles
Flow rate: 0.8 mL/min
Gradient: 10 to 45 % in 4 min
Injection volume: 3 µL
Column temperature: 30 °C
Detector: DAD, 270/10 nm, Ref 360/100 nm, 3 mm path length detector cell
Configuration: Low-delay-volume configuration with 0.12 mm capillaries and low-dispersion heat exchanger

www.agilent.com/chem/1200mds

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