Agilent 1260 Infinity Method Development Solution

Automatic Scaling of Gradient Times and Flow Rates for Different Column Lengths and Diameters Using the Agilent ChemStation Method Scouting Wizard

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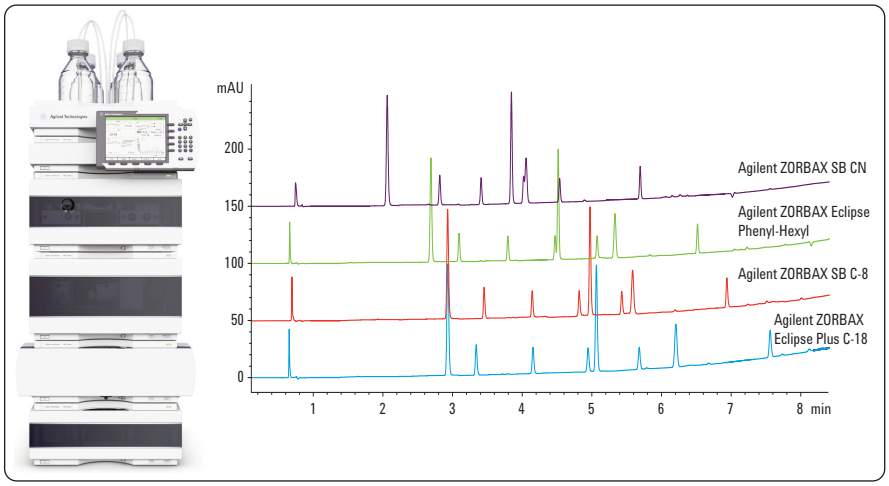
Application Note

Drug Discovery



Abstract

The Agilent 1260 Infinity Method Development Solution offers:

- Automated setup of method development sequences with all needed methods for column scouting, variation of mobile phases, gradients, and temperatures using the Agilent ChemStation Method Scouting Wizard
- Possible installation of eight columns of different selectivity, length, and internal diameter
- Installation of up to eight columns of different scaling of flow rate and gradient times according to the column internal diameter and length

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Introduction

Analytical HPLC method development is important in the chemical and pharmaceutical industries. Typically, a set of columns is tested in combination with a set of mobile phases. The scope of the method scouting process is to find the best column and mobile phase combination for the separation of a specific set of compounds. After the column scouting runs are finished fine-tuning of the gradient, column temperature, pH, and speed of the complete analysis is performed with the Method Scouting Wizard. The entire, time-consuming procedure can be shortened if columns and solvents can be changed automatically during a sequence.

The Agilent 1260 Infinity Method Development Solution offers a highly flexible system for one through eight columns up to 100 mm in length, or one through six columns up to 300 mm in length. Several independent heated zones are available to optimize the temperature for different columns. In addition, solvent selection valves can be clustered with the pump to enhance the selection of available mobile phases.

In combination with the Agilent ChemStation Method Scouting Wizard, sequences with different methods can be created automatically, where columns and solvents become method parameters. This allows automated, unattended change of columns and solvents within a sequence.

This Application Note demonstrates:

- The use of the Agilent 1260 Infinity Method Development Solution for column scouting to find optimum separation conditions for a specific application problem.
- The use of the Method Scouting Wizard to scale flow rate, run times and gradient times, if columns of different internal diameter and length are used in the same sequence.

Equipment

An Agilent 1260 Infinity Method Development Solution was used including:

- Agilent 1260 Infinity Quaternary Pump (G1311B)
- Agilent 1260 Infinity Autosampler (G1367E)
- Two Agilent 1260 Infinity Thermostatted Column Compartments (G1316C)
- Agilent 1260 Infinity Diode Array Detector (G4212B)
- Agilent Method Development Valve Kit, high pressure (G4230B) with Agilent Method Development Capillary Kit low dispersion, for short columns (p/n 5067-1595)
- Several Agilent ZORBAX columns of different lengths, internal diameters and chemistries
- Agilent ChemStation B04.02 with Agilent ChemStation Method Scouting Wizard add-on

Results and discussion

Method development using columns of different selectivity

In this study, we used the compounds shown in Figure 1, which contain acidic, basic and neutral compounds.

A water/acetonitrile or water/methanol gradient was used and 5% of a TFA solution (2 % TFA in water) was added over the complete run time to keep the ionic strength constant. The A channel contained the aqueous mobile phase, the B channel contained acetonitrile, the C channel methanol and the D channel the 2% TFA solution.

Four columns of different selectivity, but the same length, internal diameter and particle size were used for method scouting to find appropriate chromatographic conditions. The Method Scouting Wizard was used to create a method scouting sequence.

A basic method must be set up to use as a starting point for the Method Scouting Wizard. This method is then used to create other methods needed for the different columns. In this example, the same flow rate and the same

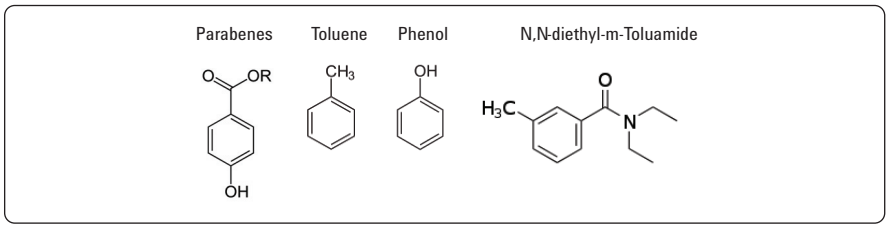
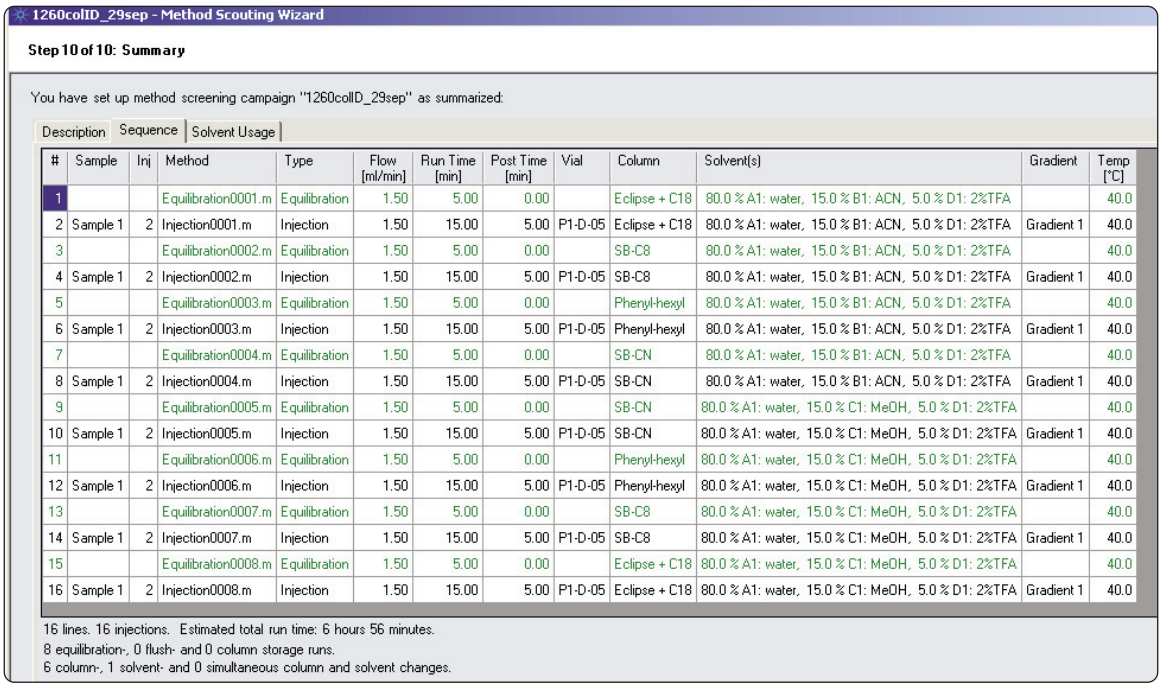


Figure 1
Chemical structure of first application example.

gradient were applied for all columns. The resulting method development sequence is shown in Figure 2.

The Method Scounting Wizard leads through 10 steps to set up the complete sequence. The ternary gradient is defined in Step 4 (Figure 3).



#	Sample	Inj	Method	Type	Flow [ml/min]	Run Time [min]	Post Time [min]	Vial	Column	Solvent(s)	Gradient	Temp [°C]
1			Equilibration0001.m	Equilibration	1.50	5.00	0.00		Eclipse + C18	80.0 % A1: water, 15.0 % B1: ACN, 5.0 % D1: 2%TFA		40.0
2	Sample 1	2	Injection0001.m	Injection	1.50	15.00	5.00	P1-D-05	Eclipse + C18	80.0 % A1: water, 15.0 % B1: ACN, 5.0 % D1: 2%TFA	Gradient 1	40.0
3			Equilibration0002.m	Equilibration	1.50	5.00	0.00		SB-C8	80.0 % A1: water, 15.0 % B1: ACN, 5.0 % D1: 2%TFA		40.0
4	Sample 1	2	Injection0002.m	Injection	1.50	15.00	5.00	P1-D-05	SB-C8	80.0 % A1: water, 15.0 % B1: ACN, 5.0 % D1: 2%TFA	Gradient 1	40.0
5			Equilibration0003.m	Equilibration	1.50	5.00	0.00		Phenyl-hexyl	80.0 % A1: water, 15.0 % B1: ACN, 5.0 % D1: 2%TFA		40.0
6	Sample 1	2	Injection0003.m	Injection	1.50	15.00	5.00	P1-D-05	Phenyl-hexyl	80.0 % A1: water, 15.0 % B1: ACN, 5.0 % D1: 2%TFA	Gradient 1	40.0
7			Equilibration0004.m	Equilibration	1.50	5.00	0.00		SB-CN	80.0 % A1: water, 15.0 % B1: ACN, 5.0 % D1: 2%TFA		40.0
8	Sample 1	2	Injection0004.m	Injection	1.50	15.00	5.00	P1-D-05	SB-CN	80.0 % A1: water, 15.0 % B1: ACN, 5.0 % D1: 2%TFA	Gradient 1	40.0
9			Equilibration0005.m	Equilibration	1.50	5.00	0.00		SB-CN	80.0 % A1: water, 15.0 % C1: MeOH, 5.0 % D1: 2%TFA		40.0
10	Sample 1	2	Injection0005.m	Injection	1.50	15.00	5.00	P1-D-05	SB-CN	80.0 % A1: water, 15.0 % C1: MeOH, 5.0 % D1: 2%TFA	Gradient 1	40.0
11			Equilibration0006.m	Equilibration	1.50	5.00	0.00		Phenyl-hexyl	80.0 % A1: water, 15.0 % C1: MeOH, 5.0 % D1: 2%TFA		40.0
12	Sample 1	2	Injection0006.m	Injection	1.50	15.00	5.00	P1-D-05	Phenyl-hexyl	80.0 % A1: water, 15.0 % C1: MeOH, 5.0 % D1: 2%TFA	Gradient 1	40.0
13			Equilibration0007.m	Equilibration	1.50	5.00	0.00		SB-C8	80.0 % A1: water, 15.0 % C1: MeOH, 5.0 % D1: 2%TFA		40.0
14	Sample 1	2	Injection0007.m	Injection	1.50	15.00	5.00	P1-D-05	SB-C8	80.0 % A1: water, 15.0 % C1: MeOH, 5.0 % D1: 2%TFA	Gradient 1	40.0
15			Equilibration0008.m	Equilibration	1.50	5.00	0.00		Eclipse + C18	80.0 % A1: water, 15.0 % C1: MeOH, 5.0 % D1: 2%TFA		40.0
16	Sample 1	2	Injection0008.m	Injection	1.50	15.00	5.00	P1-D-05	Eclipse + C18	80.0 % A1: water, 15.0 % C1: MeOH, 5.0 % D1: 2%TFA	Gradient 1	40.0

16 lines. 16 injections. Estimated total run time: 6 hours 56 minutes.
8 equilibration-, 0 flush- and 0 column storage runs.
6 column-, 1 solvent- and 0 simultaneous column and solvent changes.

Figure 2
Sequence created by the Method Scouting Wizard; column wash and equilibration runs can be included.

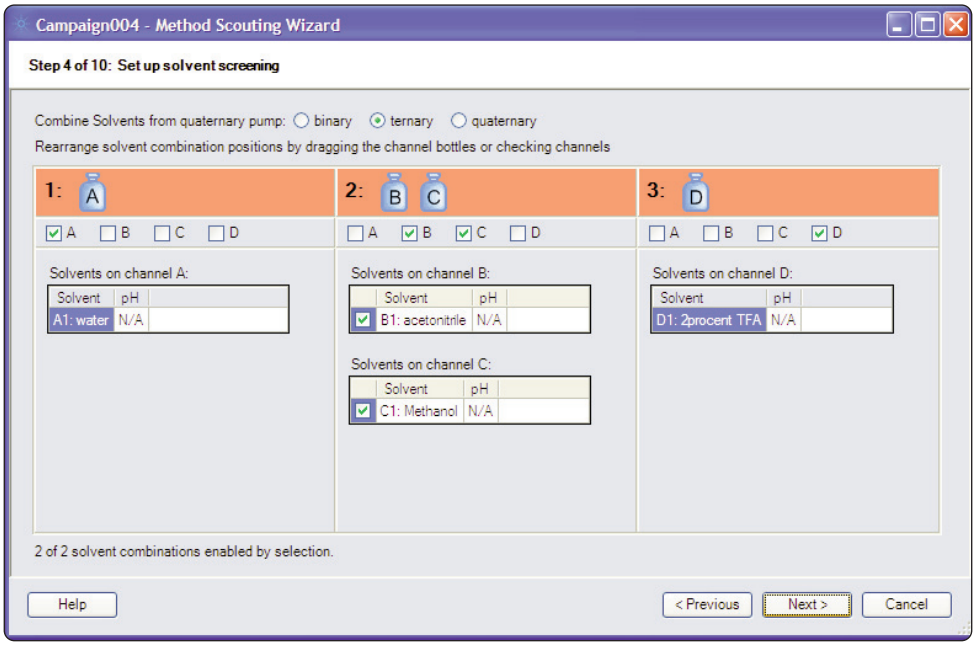
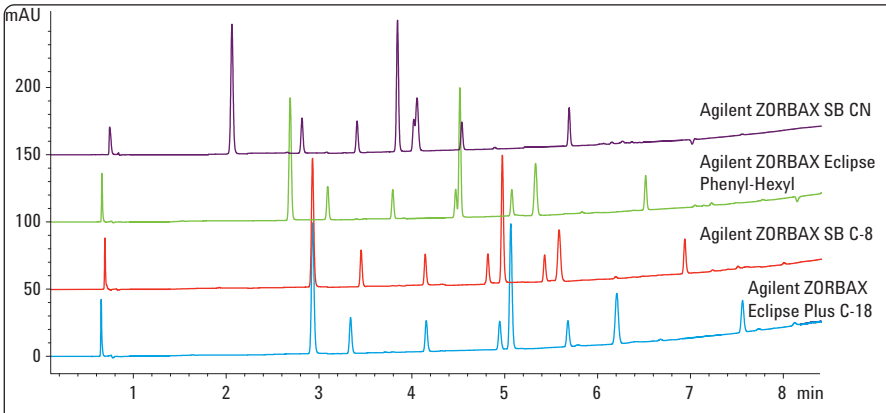


Figure 3
Method Scouting Wizard screen for setting up ternary gradients.

The sequence was completed after approximately seven hours. All four columns were tested with two organic solvents and two replicates for each new chromatographic condition. After the sequence was finished the chromatograms were overlaid and evaluated (Figure 4). Using acetonitrile as the organic phase the Agilent ZORBAX Eclipse Plus C18, 100 × 4.6 mm, 1.8 µm column showed the best resolution for all peaks.

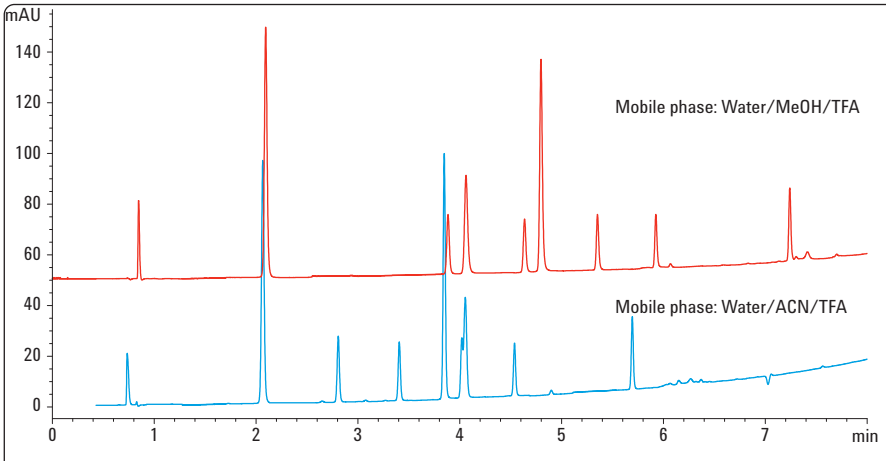
Methanol was used as the second organic phase and chromatograms were compared with those obtained from the previous experiments using acetonitrile as the organic solvent. The best resolution was obtained using the Agilent ZORBAX SB CN column with methanol as the organic solvent (Figure 5). Even the peaks that showed coelution on the CN phase using acetonitrile as the mobile phase could be separated using methanol.



Chromatographic conditions

Columns	4.6 × 100 mm, Agilent ZORBAX Eclipse Plus C-18 or SB C-8 or Phenyl-Hexyl or SB CN, 1.8 µm
Mobil phase:	A = Water B = Acetonitrile C = Methanol D = 2% TFA in water
Gradient ternary:	0 min 80% A, 15% B or C, 5% D
	8 min 5% A, 90% B or C, 5% D
Flow rate:	1.5 mL/min
Stop time:	15 min
Post time:	5 min
Inj vol:	3 µL
Column temp:	40 °C
DAD:	254, 270, 220, 230/10 nm, Ref 360/100 nm,
Flow cell:	10 mm
Peakwidth:	> 0.013 min (20 Hz)

Figure 4
Chromatograms of four columns using acetonitrile as the organic phase.



Chromatographic conditions

Mobil Phase:	A = Water B = Acetonitrile C = Methanol D= 2% TFA in water
Gradient ternary:	0 min 80% A, 15% B or C, 5% D
	8 min 5% A, 90% B or C, 5% D
Flow rate:	1.5 mL/min
Stop time:	15 min
Post time:	5 min
Inj vol:	3 µL
Column temp:	40 °C
DAD:	254, 270, 220, 230/10 nm, Ref 360/100 nm,
Flow cell:	10 mm
Peak width:	> 0.013 min (20 Hz)

Figure 5
Comparison of chromatograms with using either acetonitrile or methanol as the organic solvent on the Agilent ZORBAX Eclipse Plus SB-CN column. The upper chromatogram delivers the best resolution for all peaks.

In this example, two sets of chromatographic condition resulted in sufficient resolution for the nine peaks. One was based on a C18 phase using acetonitrile and the other solution was based on a CN phase and methanol (Figure 6).

Method development using columns of different length and internal diameter

When using columns with different lengths or internal diameters within the same method development sequence, the gradient times and flow rates must be adjusted to achieve comparable results. The Method Scouting Wizard offers an automated tool to scale both parameters as shown in Figure 7.

Scaling of gradient and run time

Good separation was achieved by the Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm column with acetonitrile as organic phase (Figure 6). The length of the C18 phase used was varied from 50 mm up to 150 mm to discover which column length is the most appropriate for the application discussed. The internal diameter for all columns remained at 4.6 mm.

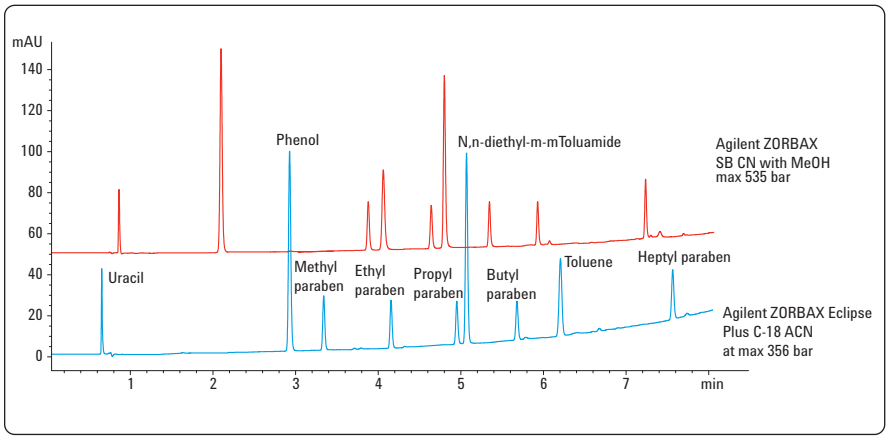


Figure 6
Two solutions for the separation of the nine peaks.

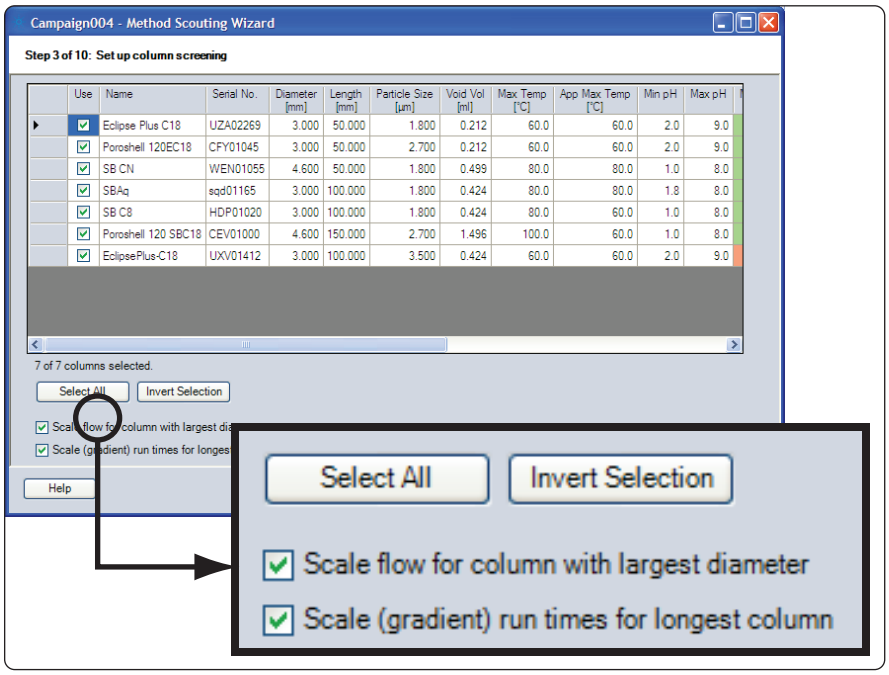


Figure 7
Scaling of flow and gradient (run times) in the Method Scouting Wizard.

The basic method was set up for the longest column. The flow rate was set to 1.5 mL/min and the gradient time to 8 min. The gradient times for the 100, 75 and 50 mm column were automatically scaled to 5.34, 4.02 and 2.69 min by the Method Scouting Wizard (Figure 8).

Chromatographic conditions

Basic method
Mobile phase: A = Water
B = Acetonitrile
D = 2% TFA in water

Basic gradient for 150 mm length:
0 min 80 % A, 15 % B; 5 % D
8 min 5 % A, 90 % B, 5 % D

Gradient for 100 mm length:
0 min 80 % A, 15 % B; 5 % D
5.34 min 5 % A, 90 % B, 5 % D

Gradient for 75 mm length:
0 min 80 % A, 15 % B; 5 % D
4.02 min 5 % A, 90 % B, 5 % D

Gradient for 50 mm length:
0 min 80 % A, 15 % B; 5 % D
2.69 min 5 % A, 90 % B, 5 % D

Flow rate:	1.5 mL/min
Stop time:	15 min
Post time:	5 min
Inj vol:	3 µL
Column temp:	40 °C
DAD	254, 270, 220, 230/10 nm, Ref 360/100 nm,
Flow cell:	10 mm
Peak width:	> 0.013 min (20 Hz)

The results for the different columns regarding resolution and solvent consumption are compared in Table 1.

Column length (mm)	Run time (min)	Gradient time (min)	Resolution marked Peak	Solvent consumption /run (mL)
50	5	2.69	2.15	7.5
75	7.5	4.02	3.07	11.25
100	9	5.34	2.65	13.5
150 (basic method)	15	8	4.22	22.5

Table 1
Results of column scouting with different column length. Flow rate was 1.5 mL/min for all columns.

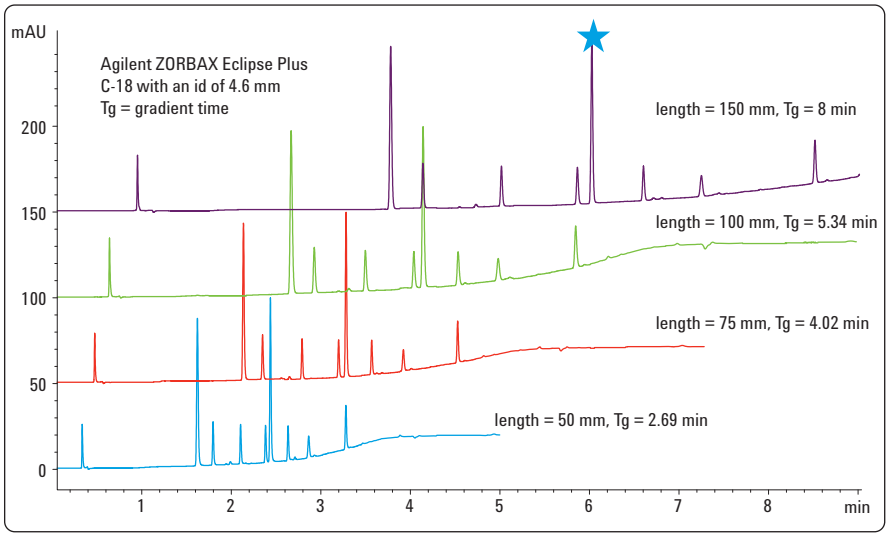


Figure 8
Scaling of run time and gradient time according to column length by the Method Scouting Wizard (Tg= gradient time).

The chromatograms show all the same patterns. The best resolution was obtained on the 150 mm column, but with a 15 min run time leading to higher solvent consumption. A resolution > 2 was obtained on the 50 mm column within 5 min, which is typically sufficient for a separation. The 75 mm length column provided the best compromise between run time (7.5 min) and resolution (3.07).

Scaling of flow rate

In a third experiment the internal diameter of the columns was varied because columns from different vendors are not necessarily available in the same dimensions. For example, the inner diameter of the 4 mm columns can be 4 mm or 4.6 mm depending on the manufacturer. It is necessary to adjust the flow rate according to the diameter to get comparable results for these columns. We selected two columns with the same length and same particle size but with internal diameters of 4.6 and 3 mm to demonstrate flow rate scaling. The chromatograms are shown in Figure 9.

The flow rates were set automatically according to the internal diameter of the column used. For example, the flow rate of the 3 mm column was reduced to 0.638 mL/min. Typically slightly longer retention times for columns with smaller internal diameters are observed (Figure 9). This is because the flow rate scaling calculation is based on column volume and does not take the system delay volume into account. Another advantage of the flow scaling option is minimal risk of errors due to over-pressure, because a column of smaller internal diameter is switched into the flow path. Otherwise the complete sequence would be stopped and aborted.

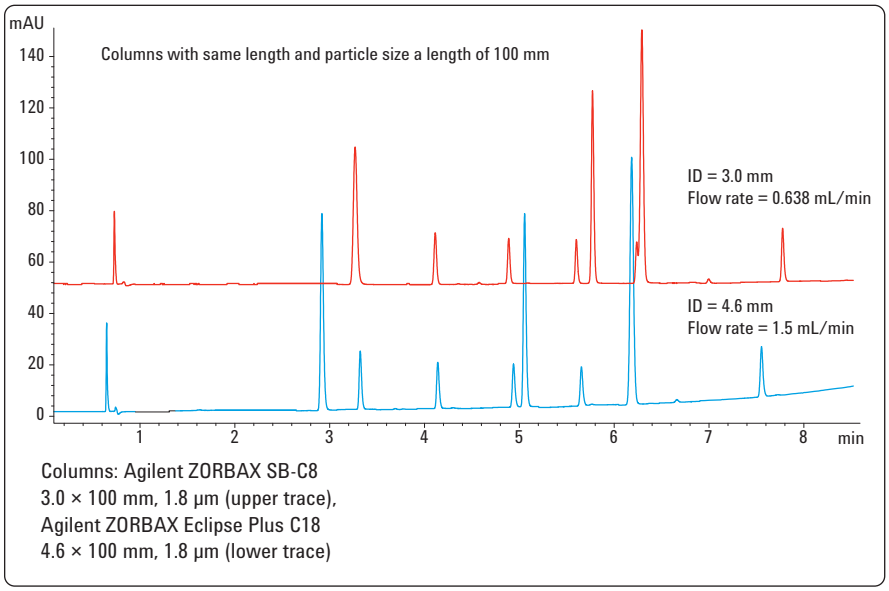


Figure 9
Automatic scaling of flow rate for different column IDs by the Method Scouting Wizard.

Conclusion

The Agilent 1260 Infinity Method Development Solution in combination with the Method Scouting Wizard allows automated column, gradient, mobile phase and temperature scouting with up to eight columns with different selectivity, different length and internal diameter. The user is guided by the Method Scouting Wizard through all setup screens. The sequence, methods and required rinse and re-equilibration steps are created automatically once all the necessary information is completed. In addition, the software adapts flow rate, gradient time and run time according to the used column dimensions specified. This optimizes the method for resolution or shortest run time in one sequence. Finally, this software minimizes the risk that the maximum allowed pressure for a column is exceeded or the sequence will be stopped due to an error.

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