Automated Scouting of Stationary and Mobile Phases Using the Agilent 1290 Infinity II Method Development Solution

Technical Overview

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Abstract

This Technical Overview demonstrates the use of the Agilent 1290 Infinity II Method Development Solution for automated scouting of stationary and mobile phases. The solution is equipped with an Agilent 1290 Infinity II Multicolumn Thermostat that enables automated switching between up to eight columns. The Agilent 1290 Infinity II Flexible Pump is clustered with two solvent-selection valves for the connection of up to 26 different solvents. The Agilent Method Scouting Wizard of the Agilent OpenLAB CDS ChemStation Edition Software facilitates easy setup of methods for different combinations of columns, mobile phases, and temperatures in a single sequence.

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Introduction

In today's pharmaceutical, food, and environmental analysis laboratories, many chemically different compounds are analyzed by UHPLC methods. For these analyses, many different stationary phases, solvents, and modifiers are used. As a consequence, the development of corresponding methods to solve these analytical problems can be time-consuming. To overcome this challenge, the Agilent 1290 Infinity II Method Development Solution can be used for automated scouting of stationary and mobile phases. The Agilent 1290 Infinity II Multicolumn Thermostat can handle up to eight different columns, and enables precise column-temperature control over a broad temperature range. Heat transfer is done by heat exchangers with lowest internal volume and highest efficiency to achieve excellent retention time stability for highly reproducible retention times[1]. The Agilent 1290 Infinity II Flexible Pump is equipped with two solvent-selection valves, providing up to 26 channels for different solvents and buffers. This capability enables more than 1,000 possible analytical conditions. To set up the corresponding methods, the Agilent Method Scouting Wizard is available as a plug-in tool for Agilent OpenLAB CDS ChemStation Edition Software. This wizard enables the scouting of columns, solvents, gradients, and temperatures in an easy setup scheme. At the end of the setup process, a sequence comprising methods for all possible combinations is created. This sequence also contains the necessary flushing methods, column equilibration methods, and column storage conditions.

This Technical Overview demonstrates the typical setup of the 1290 Infinity II Method Development Solution. In particular, the setup of the Agilent Infinity II Multicolumn Thermostat and the 1290 Infinity II Flexible Pump is demonstrated from a hardware perspective. The setup of the experiments in the Method Scouting Wizard for column, solvent, and temperature scouting with the final creation of the scouting sequence is described from a software perspective.

Experimental

Instrumentation

The Agilent 1290 Infinity II Method Development Solution comprised the following modules:

- Agilent 1290 Infinity II Flexible Pump (G7104A)
- Agilent 1290 Infinity II Multisampler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) including valve drive (option #058) equipped with Agilent Quick-Change column selection valve (G4239C, 1,300 bar) including capillary kit (option #005) for installation of up to eight columns
- Agilent 1290 Infinity II Diode Array Detector (G7117B)
- Agilent 1290 Infinity valve drives (2x G1170A) equipped with Agilent Quick-Change 12-position/13-port solvent selection valves (2x G4235A)

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, revision C.01.07 (minimum driver requirement A.02.11 SP1) with Method Scouting Wizard, revision A.02.05

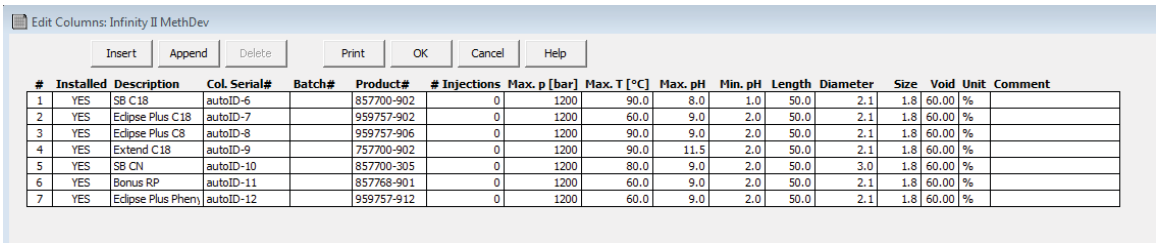
Columns

- Agilent ZORBAX RRHD SB-C18, 2.1 × 50 mm, 1.8 µm (p/n 857700-902)
- Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)
- Agilent ZORBAX RRHD Eclipse Plus C8, 2.1 × 50 mm, 1.8 µm (p/n 959757-906)
- Agilent ZORBAX RRHD Extend C18, 2.1 × 50 mm, 1.8 µm (p/n 757700-902)
- Agilent ZORBAX RRHD SB-CN, 2.1 × 50 mm, 1.8 µm (p/n 857700-305)
- Agilent ZORBAX RRHD Bonus RP, 2.1 × 50 mm, 1.8 µm (p/n 857768-901)
- Agilent ZORBAX RRHD Eclipse Plus Phenyl-Hexyl, 2.1 × 50 mm, 1.8 µm (p/n 959757-912)

Instrument setup

The columns used for all applications were defined in the ChemStation columns list (Figure 1). For each column, a description, geometric data, particle size, and limitations such as pH, pressure, and temperature can also be given. Installed columns should be marked with YES in the first column of the table (Figure 1) to ensure that the column description is assigned to the respective results.

The column list is connected directly to the column assignment in the 1290 Infinity II Multicolumn Thermostat (Figure 2). In this assignment, the position of the column at the right or left side of the 1290 Infinity II Multicolumn Thermostat can be chosen, and associated with a color code.



#	Installed	Description	Col. Serial#	Batch#	Product#	# Injections	Max. p [bar]	Max. T [°C]	Max. pH	Min. pH	Length	Diameter	Size	Void	Unit	Comment
1	YES	SB C18	autoID-6		857700-902	0	1200	90.0	8.0	1.0	50.0	2.1	1.8	60.00	%	
2	YES	Eclipse Plus C18	autoID-7		959757-902	0	1200	60.0	9.0	2.0	50.0	2.1	1.8	60.00	%	
3	YES	Eclipse Plus C8	autoID-8		959757-906	0	1200	90.0	9.0	2.0	50.0	2.1	1.8	60.00	%	
4	YES	Extend C18	autoID-9		757700-902	0	1200	90.0	11.5	2.0	50.0	2.1	1.8	60.00	%	
5	YES	SB CN	autoID-10		857700-305	0	1200	80.0	9.0	2.0	50.0	3.0	1.8	60.00	%	
6	YES	Bonus RP	autoID-11		857768-901	0	1200	60.0	9.0	2.0	50.0	2.1	1.8	60.00	%	
7	YES	Eclipse Plus Phen	autoID-12		959757-912	0	1200	60.0	9.0	2.0	50.0	2.1	1.8	60.00	%	

Figure 1. An Agilent ChemStation column list. It provides an overview on all columns available.

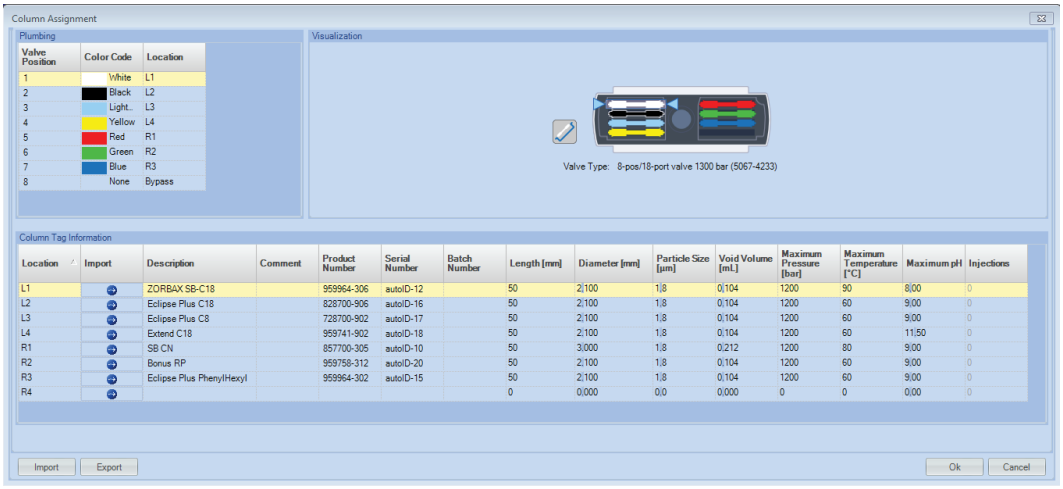


Figure 2. Column assignment in the Agilent 1290 Infinity II Multicolumn Thermostat. Each column is assigned to an unambiguous location.

The column used for the individual method can be selected in the method tab of the 1290 Infinity II Multicolumn Thermostat (Figure 3). In contrast to previous versions, columns are assigned to respective positions in the 1290 Infinity II Multicolumn Thermostat, and then selected accordingly. The appropriate column can be selected either by the drop-down menu, which shows all assigned columns, or by just clicking the column with the correct color code in the image of the 1290 Infinity II Multicolumn Thermostat. The current valve position, which connects automatically to the chosen column, is shown. For quick information, the valve position, the color code of the chosen column, and its product number is shown in Agilent ChemStation (Figure 4).

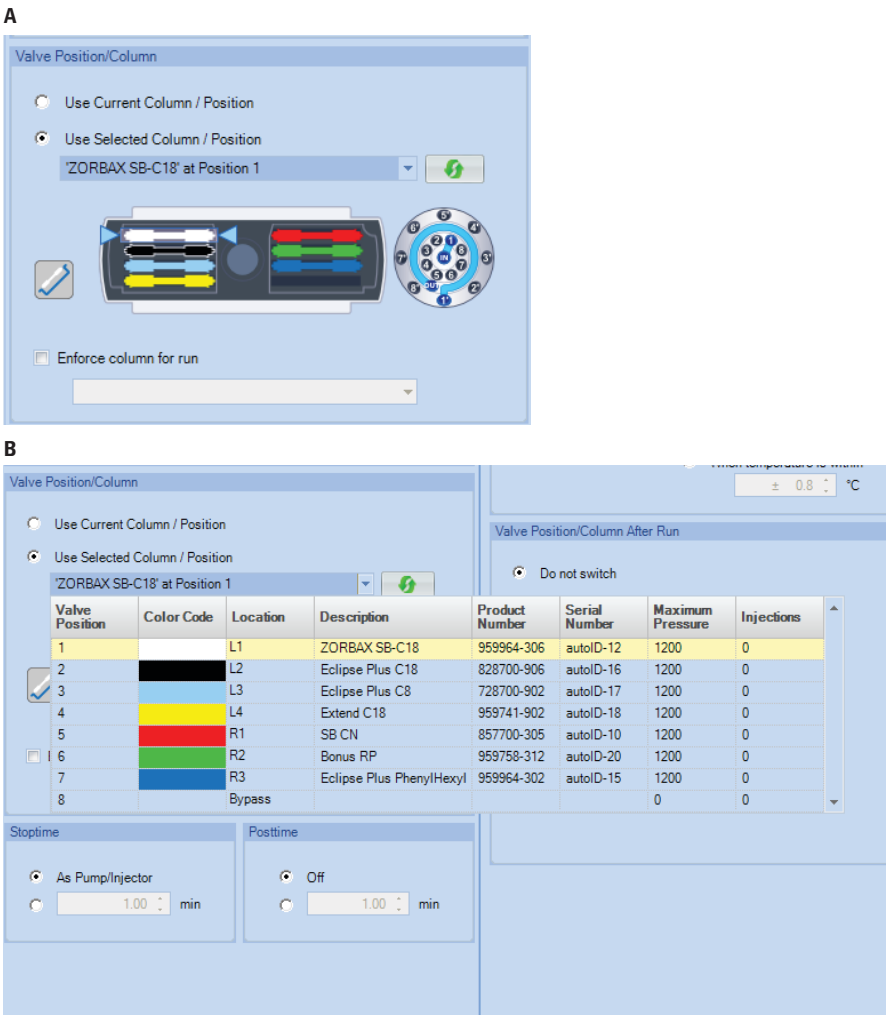


Figure 3. Column selection in the method tab of the 1290 Infinity II Multicolumn Thermostat using the drop-down menu.

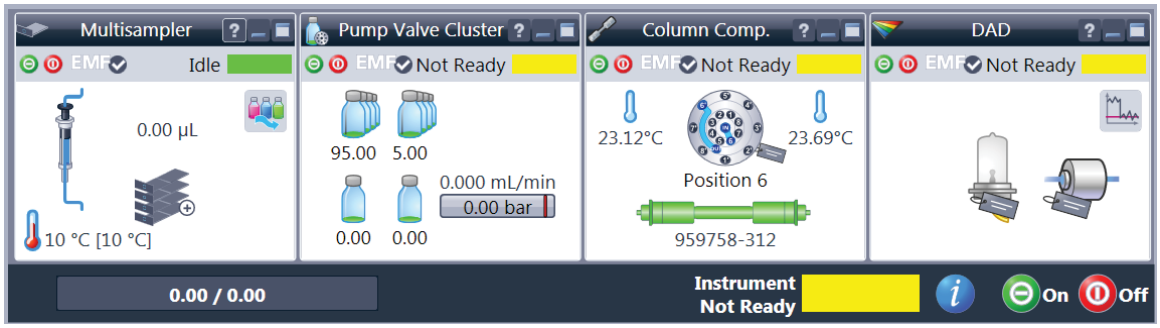


Figure 4. Instrument panel in Agilent ChemStation, highlighting the currently active column.

The assignment of the solvents can be done in a similar way. The solvent selection valves are assigned to the pump channels in the instrument configuration (not shown). In the Pump Valve Cluster Configuration tab, the positions of the solvent selection valve can be named by the connected solvent (Figure 5). The compressibility calibration can be chosen by taking the correct solvent or type of solvent from the drop-down menu, and pH and molarity values can be added. In the Pump Valve Cluster Method tab, the appropriate solvents for channels A and B can be chosen, and the valve will automatically be switched to the correct positions (Figure 6). The accessible multiple solvents are sketched out in the pump section of the ChemStation instrument panel (Figure 4).

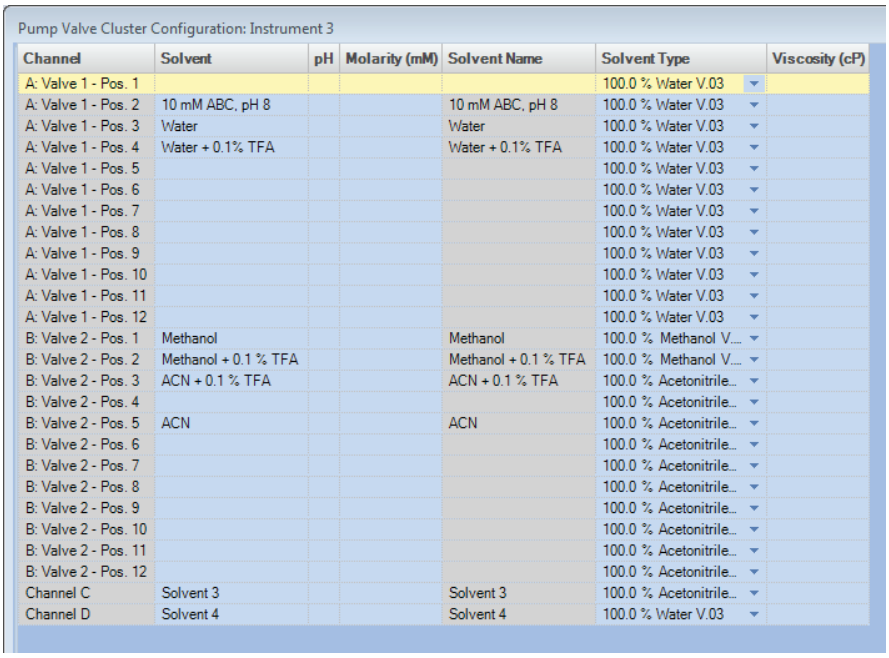


Figure 5. Pump Valve Cluster Configuration tab in Agilent ChemStation.

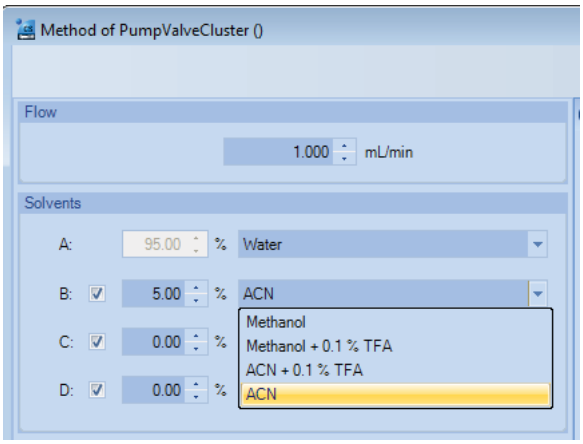
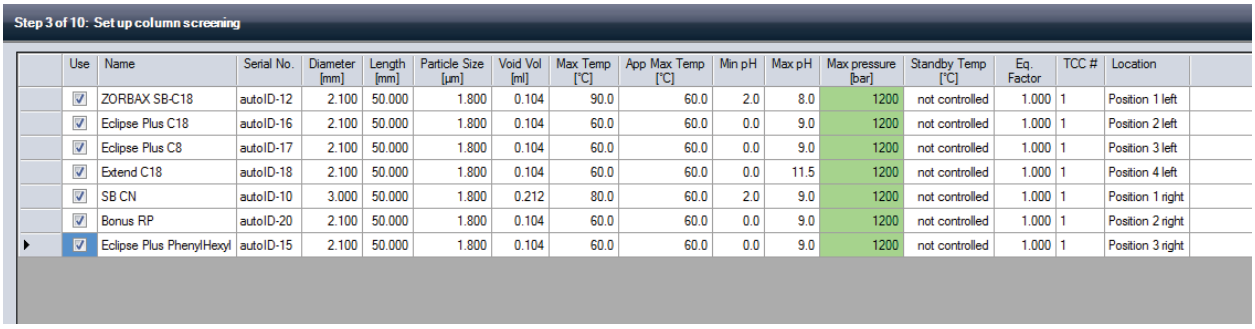


Figure 6. Pump Valve Cluster Method tab in Agilent ChemStation.

Method Scouting Wizard

The Method Scouting Wizard enables the easy setup of many sequences to scout different LC conditions including different columns, solvents, gradients, and temperatures. Finally, the Method Scouting Wizard automatically creates a sequence, which also includes solvent exchanges, re-equilibrations, and column storages when changing columns and solvents. The user is able to choose options of the method scouting campaign by selecting method scouting for columns, gradients, temperature, and solvents. In the dialog box for column scouting, the columns necessary for a study can be checked (Figure 7). If the columns differ by inside diameter and length, the method will be transferred to the current column geometry to make the results comparable.

The different solvents that have to be used can be set in the dialog box for solvent scouting (Figure 8). The Agilent 1290 Infinity II Flexible Pump, which is a quaternary pump, can be used to generate binary, ternary, or quaternary gradients. The current study was set up with the binary pump configuration, in which two solvent channels are connected to deliver the solvent for a binary gradient.



Use	Name	Serial No.	Diameter [mm]	Length [mm]	Particle Size [µm]	Void Vol [ml]	Max Temp [°C]	App Max Temp [°C]	Min pH	Max pH	Max pressure [bar]	Standby Temp [°C]	Eq. Factor	TCC #	Location
☑	ZORBAX SB-C18	autoID-12	2.100	50.000	1.800	0.104	90.0	60.0	2.0	8.0	1200	not controlled	1.000	1	Position 1 left
☑	Eclipse Plus C18	autoID-16	2.100	50.000	1.800	0.104	60.0	60.0	0.0	9.0	1200	not controlled	1.000	1	Position 2 left
☑	Eclipse Plus C8	autoID-17	2.100	50.000	1.800	0.104	60.0	60.0	0.0	9.0	1200	not controlled	1.000	1	Position 3 left
☑	Extend C18	autoID-18	2.100	50.000	1.800	0.104	60.0	60.0	0.0	11.5	1200	not controlled	1.000	1	Position 4 left
☑	SB CN	autoID-10	3.000	50.000	1.800	0.212	80.0	60.0	2.0	9.0	1200	not controlled	1.000	1	Position 1 right
☑	Bonus RP	autoID-20	2.100	50.000	1.800	0.104	60.0	60.0	0.0	9.0	1200	not controlled	1.000	1	Position 2 right
☑	Eclipse Plus PhenylHexyl	autoID-15	2.100	50.000	1.800	0.104	60.0	60.0	0.0	9.0	1200	not controlled	1.000	1	Position 3 right

Figure 7. The Agilent Method Scouting Wizard dialog box for column screening. From the columns installed in the Agilent 1290 Infinity II Multicolumn Thermostat, those necessary for the current study can be checked. Conditions such as flow rate are adjusted automatically to different column geometries.

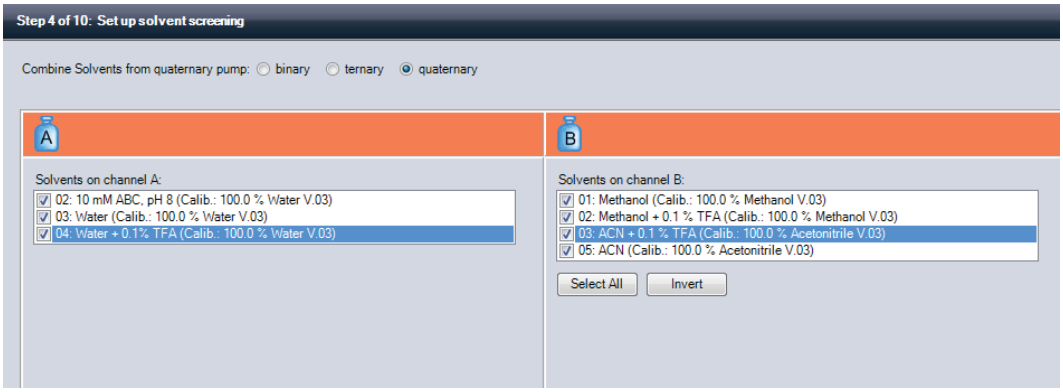
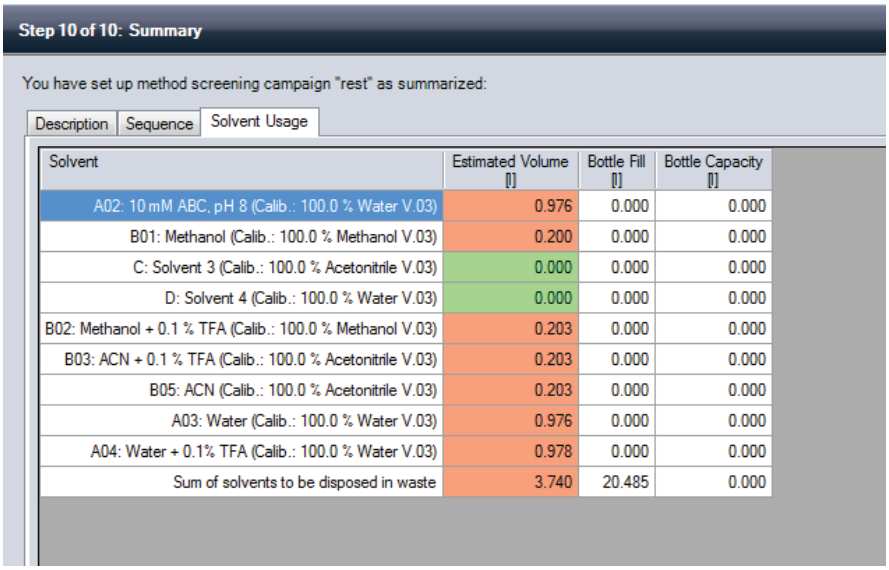


Figure 8. The Agilent Method Scouting Wizard dialog box for solvent screening. The Agilent 1290 Infinity II Flexible Pump is used in a binary mixing setup.

The final sequence was created, and included all necessary solvent exchanges (blue), column equilibration (green), and column storage methods (yellow) as separate runs without injection (Figure 9).

The solvent volumes that are going to be used during the campaign are calculated by the Method Scouting Wizard and shown in the Solvent Usage tab of the Step 10 of 10: Summary (Figure 10).



Solvent	Estimated Volume [l]	Bottle Fill [l]	Bottle Capacity [l]
A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03)	0.976	0.000	0.000
B01: Methanol (Calib.: 100.0 % Methanol V.03)	0.200	0.000	0.000
C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03)	0.000	0.000	0.000
D: Solvent 4 (Calib.: 100.0 % Water V.03)	0.000	0.000	0.000
B02: Methanol + 0.1 % TFA (Calib.: 100.0 % Methanol V.03)	0.203	0.000	0.000
B03: ACN + 0.1 % TFA (Calib.: 100.0 % Acetonitrile V.03)	0.203	0.000	0.000
B05: ACN (Calib.: 100.0 % Acetonitrile V.03)	0.203	0.000	0.000
A03: Water (Calib.: 100.0 % Water V.03)	0.976	0.000	0.000
A04: Water + 0.1% TFA (Calib.: 100.0 % Water V.03)	0.978	0.000	0.000
Sum of solvents to be disposed in waste	3.740	20.485	0.000

Figure 10. The Agilent Method Scouting Wizard calculates solvent usage for the complete campaign setup.

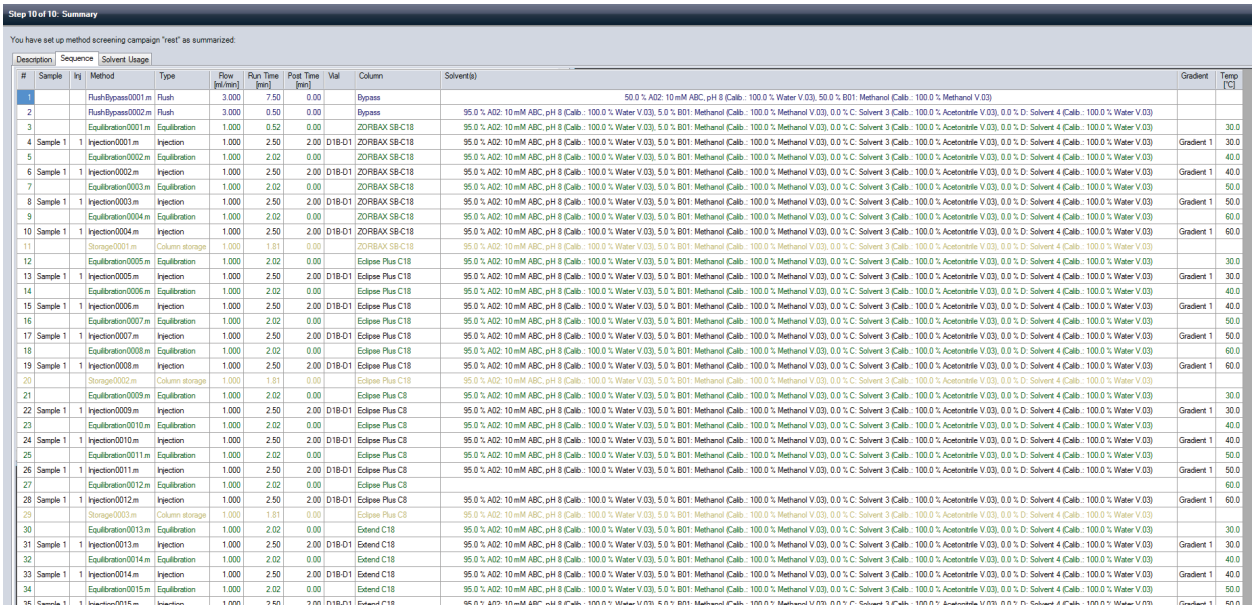


Figure 9. The Agilent Method Scouting Wizard dialog box for sequence setup includes the solvent exchange method, equilibration methods, and column storage methods.

Initial method for the Agilent Method Scouting Wizard

Parameter	Value
Flow	1 mL/min
Gradient	5 %B at 0 minutes, 75 %B at 1.5 minutes, 95 %B at 2 minutes
Stop time	2.5 minutes
Post time	2 minutes
Injection volume	1 µL
Detection	254/10 nm, reference 360/100 nm, data rate 80 Hz
Temperature scouting using 30, 40, 50, and 60 °C	

Solvent scouting using solvents

Mobile phase A

Water, ammonium bicarbonate buffer (ABC), pH 8, water + 0.1 % TFA

Mobile phase B

Methanol (MeOH), MeOH + 0.09 % TFA, acetonitrile (ACN), ACN + 0.09 % TFA

Samples

HPLC standard mixture

Chemicals

All chemicals were purchased from Sigma-Aldrich, Germany. All solvents were purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak).

Results and Discussion

For the method scouting campaign, seven columns were used in combination with three aqueous and four organic solvents. The eighth position in the multicolumn thermostat was used as a bypass to flush the system quickly when a solvent exchange was necessary. In addition, temperature scouting was performed at 30, 40, 50, and 60 °C. A generic gradient was used during the complete campaign. The necessary sequence was created by the Method Scouting Wizard, resulting in a total of 166 sample injections as well as corresponding flush, equilibration, and storage runs.

Figure 11 shows the column scouting of the separation of the standard sample mix at 40 °C using water and acetonitrile as solvents. Seven chromatograms representing the seven used columns are compared. Using this temperature and solvent combination, the Agilent ZORBAX SB-C18 and Agilent ZORBAX Eclipse Plus C8 columns showed the best separation, followed by the Agilent ZORBAX Eclipse Plus C18, Agilent ZORBAX Extend C18, and Agilent ZORBAX Bonus RP columns. The separations obtained on the Agilent ZORBAX SB-CN and the Agilent ZORBAX Eclipse Plus Phenyl-Hexyl columns were not promising for further optimization.

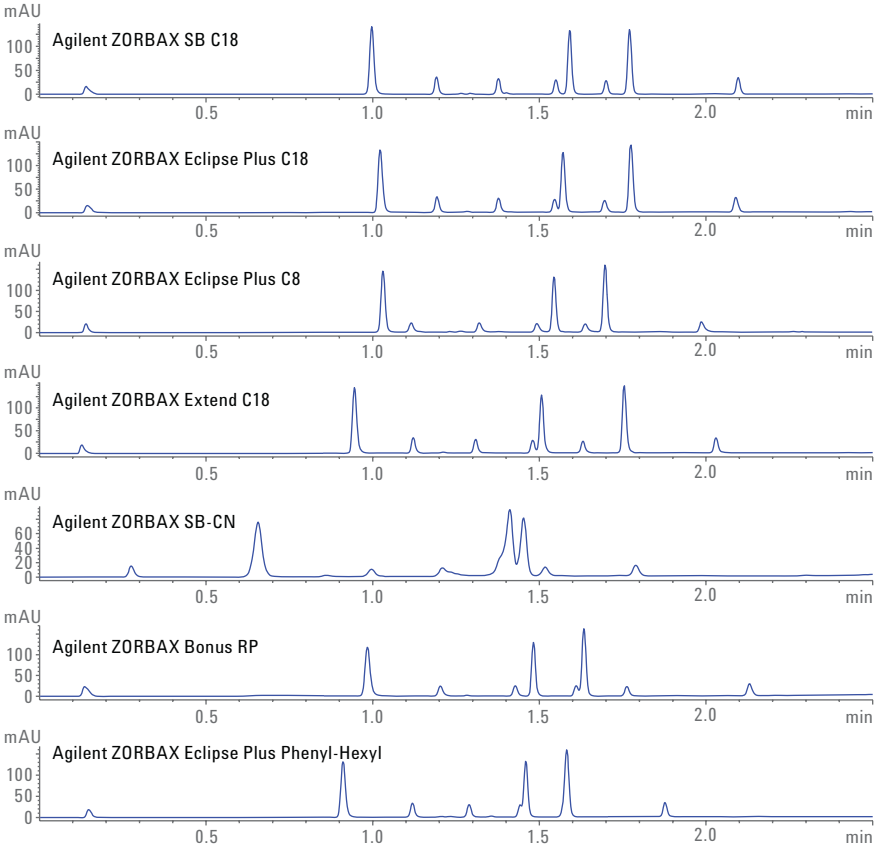


Figure 11. Column scouting using seven different columns, using water (A) and acetonitrile (B) at 40 °C.

Figure 12 shows the solvent scouting on a ZORBAX Eclipse Plus C18 column at 40 °C. For this column and temperature combination, a 10 mM ammonium bicarbonate buffer and acetonitrile delivered the best resolution.

Figure 13 shows the temperature scouting on a ZORBAX Eclipse Plus C18 column with water and acetonitrile. For this column and solvent combination, 60 °C delivered the best resolution.

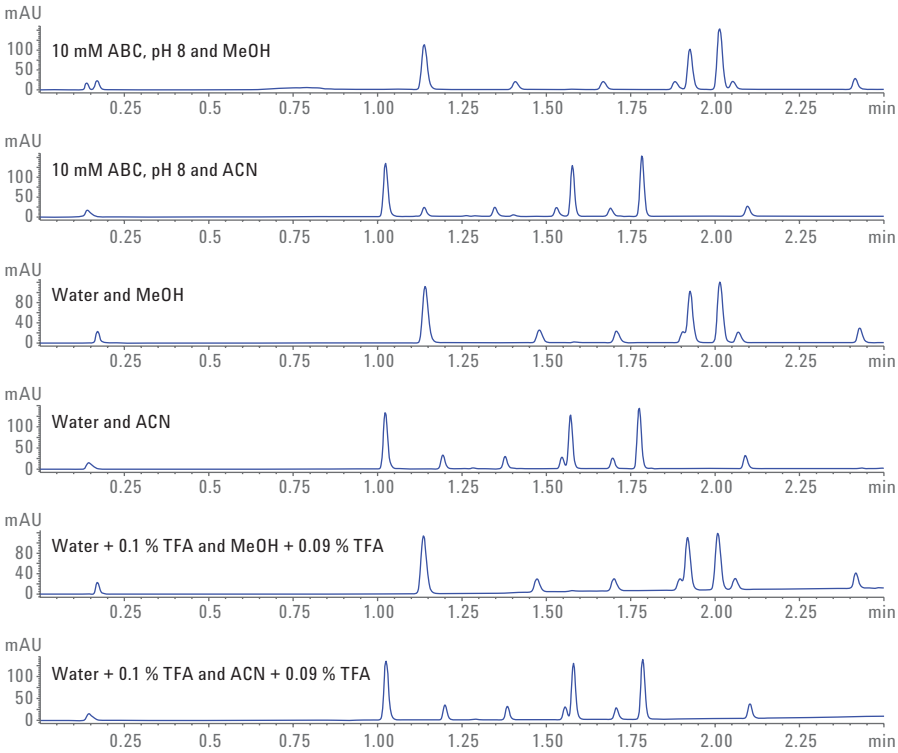


Figure 12. Solvent scouting on an Agilent ZORBAX Eclipse Plus C18 column at 40 °C.

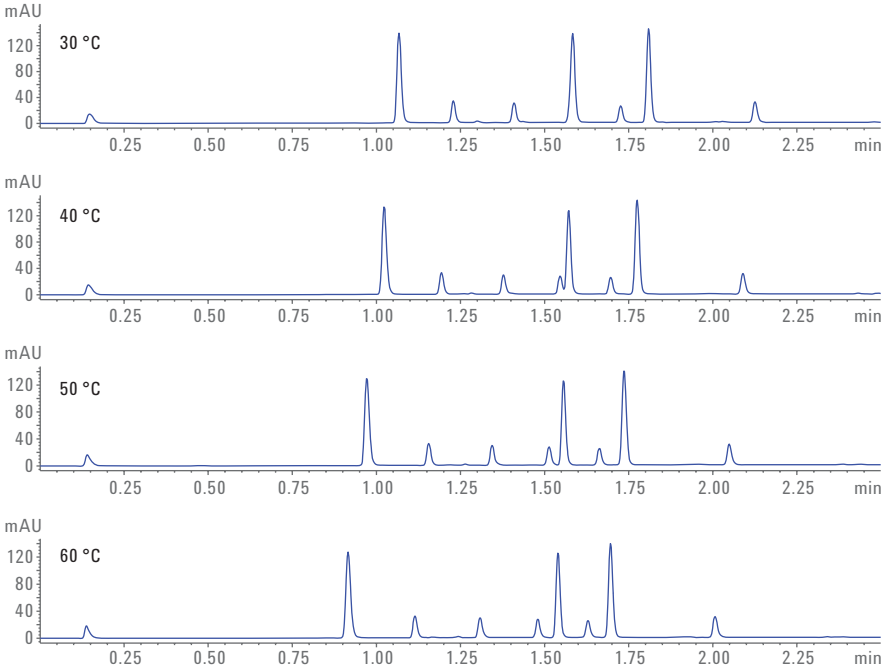


Figure 13. Temperature scouting to evaluate temperature effects on selectivity using an Agilent ZORBAX Eclipse Plus C18 column with water and acetonitrile.

To facilitate data analysis, especially when running large campaigns containing many sample injections, preconfigured report templates can be used with the Intelligent Reporting tool of the Agilent OpenLAB CDS ChemStation Edition. These reports list the chromatographic runs either by detected peaks, or resolution. In addition, the report creates a bubble plot, enabling the user to quickly identify the most promising results, and facilitate the search for the optimal separation. Figure 14 and Figure 15 show bubble plots for all injections at 50 °C, displaying the maximal number of peaks found (Figure 14) as well as the summed resolution (Figure 15). The size of the bubbles represents the number of peaks found (Figure 14) or the amount of summed resolution (Figure 15). Both bubble plots are displayed as maximal retention time on the Y-axis and injections on the X-axis.

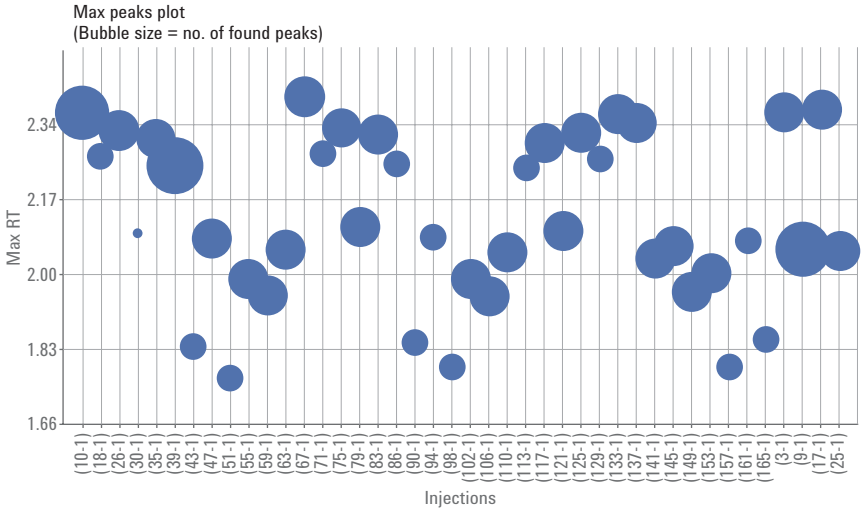


Figure 14. Maximal peak amount found per injection at 50 °C, displayed as a bubble plot.

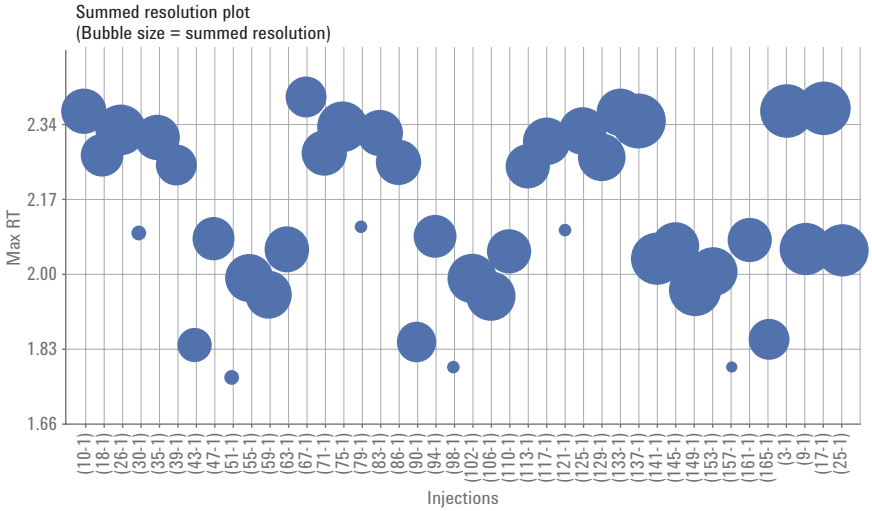


Figure 15. Maximal summed resolution found per injection at 50 °C, displayed as a bubble plot.

After complete data evaluation, the combination of the ZORBAX Eclipse Plus C18 column at 60 °C using water and acetonitrile as mobile phases was identified as one of the most optimal separation conditions (Figure 16).

Conclusion

This Technical Overview demonstrates how the combination of the Agilent 1290 Infinity II Method Development Solution and the Agilent Method Scouting Wizard facilitates automated scouting of stationary and mobile phases. The Method Scouting Wizard enables creation of column, solvent, gradient, and temperature scouting runs in any possible combination. The created scouting sequence runs all combinations, automatically including solvent flush methods, column equilibration, and column storage methods. The Agilent 1290 Infinity II Multicolumn Thermostat is part of the system, and can host up to eight columns. The Agilent 1290 Infinity II Flexible Pump, enhanced with two solvent-selection valves, can deliver up to 26 different solvents. In combination, more than 1,000 different scouting conditions are possible. Data analysis is facilitated using preconfigured report templates, accelerating the search for the optimal separation conditions.

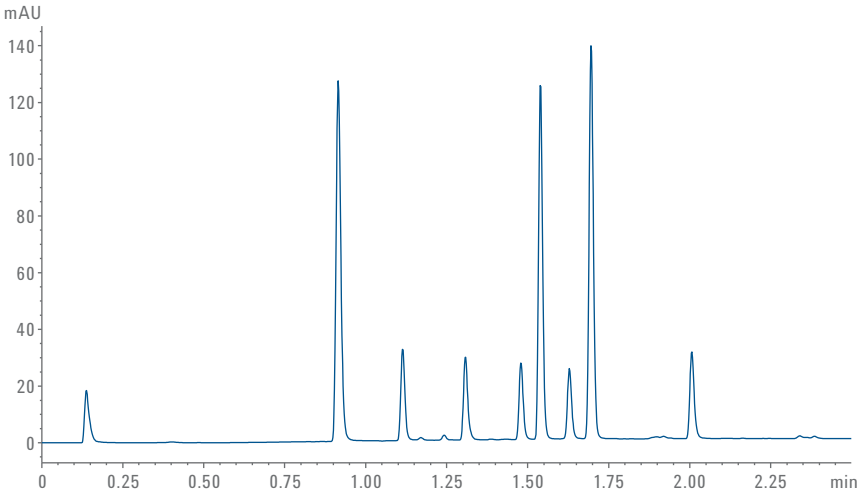


Figure 16. One of the most optimal separations found after evaluation of the scouting campaign. Using water and acetonitrile as eluents at 60 °C on an Agilent ZORBAX Eclipse Plus C18 column.

Reference

1. Schneider, S., Performance Characteristics of the Agilent 1290 Infinity II Multicolumn Thermostat, *Agilent Technologies Technical Overview*, publication number 5991-5533EN, **2015**

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Published in the USA, March 1, 2017
5991-5934EN

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