New Features of the Agilent Method Scouting Wizard for Automated Method Development of Complex Samples

Analysis of Large Data Sets by Method Scouting Reports and Automated Adjustment of Flow Rates and Gradient Times According to Column Dimensions

Technical Overview

Author

Edgar Naegele
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

This Technical Overview demonstrates the use of the Agilent Method Scouting Wizard for scouting of several columns, solvents, gradients, and column temperatures to identify a chromatographic method for the separation of many compounds in a complex sample. This study describes the automated adjustment of flow rates and gradient times according to columns with different dimensions in the Method Scouting Wizard. The results and analysis of the obtained data are shown and discussed. The instrumental and method setup through the Method Scouting Wizard is described in detail. The handling of the large amount of data by the built-in Agilent OpenLAB CDS ChemStation Method Development Reports is described.

Agilent Technologies

Introduction

The development of chromatographic methods is a time-consuming task, but has to be done in many areas such as pharmaceutical development, food and environmental analysis, as well as in the chemical industry. With increasing complexity of the separation problem and the number of compounds that have to be separated, the effort of method development increases dramatically. To help the analytical chemist, the Agilent Method Scouting Wizard is able to create methods for many combinations of columns, solvents, gradients, and column temperatures. The methods are organized in a sequence with automatically created methods for system flush, column equilibration, and column storage when solvents, column types, and column temperatures are automatically exchanged. The resulting sequence table can be processed on the Agilent 1290 Infinity II Method Development Solution as well as on the Agilent 1260 Infinity II Method Development Solution.

This Technical Overview demonstrates the features of the Method Scouting Wizard to adapt flow rates and gradient times to columns of different dimensions. This approach is used for the development of chromatographic methods for the separation of a complex sample. To achieve this goal, several columns with different stationary phases, inner diameters, and length are used. In the same experimental setup, different solvents, gradients, and column temperatures are used to identify the best possible separation method. The used test sample comprises 16 pesticide compounds with several possibilities for coelution and dependences on column temperatures. The optimum separation conditions are identified from a large set of data by the available Agilent Method Scouting Reports. Finally, optimum separation is confirmed by manual inspection of the data.

Experimental

Instrumentation

The Agilent 1290 Infinity II Method Development Solution used for method development comprises the following modules (Figure 1):

- Agilent 1290 Infinity II Multisampler (G7167B)

- Agilent 1290 Infinity II Flexible Pump (G7104A)

- Two Agilent 1290 Infinity II Multicolumn Thermostats (G7116B) with valve drive (Option #058), one is equipped with an Agilent Quick Change eight-column selector valve (G4239C) including capillary kit (Option #005)

- Agilent 1290 Infinity II Diode Array Detector (G7117B)

- Agilent 1290 Infinity Valve Drive (G1170A) with Agilent Quick Change 12-position/13-port solvent selection valve (G4235A)

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, Rev. C.01.07 with Agilent ChemStation Method Scouting Wizard, Version A02.06.

Columns

- Agilent ZORBAX SB C18, 4.6 × 50 mm, 3.5 µm, p/n 835975-902

- Agilent ZORBAX SB C8, 4.6 × 50 mm, 5 µm, p/n 846975-906

- Agilent ZORBAX Bonus-RP, 4.6 × 50 mm, 3.5 µm, p/n 835668-901

- Agilent ZORBAX Eclipse Plus C8 RRHD, 2.1 × 150 mm, 1.8 µm, p/n 959759-906

- Agilent ZORBAX SB-C8 RRHD, 2.1 × 100 mm, 1.8 µm, p/n 858700-906

- Agilent ZORBAX SB-C18 RRHD, 2.1 × 100 mm, 1.8 µm, p/n 858700-902

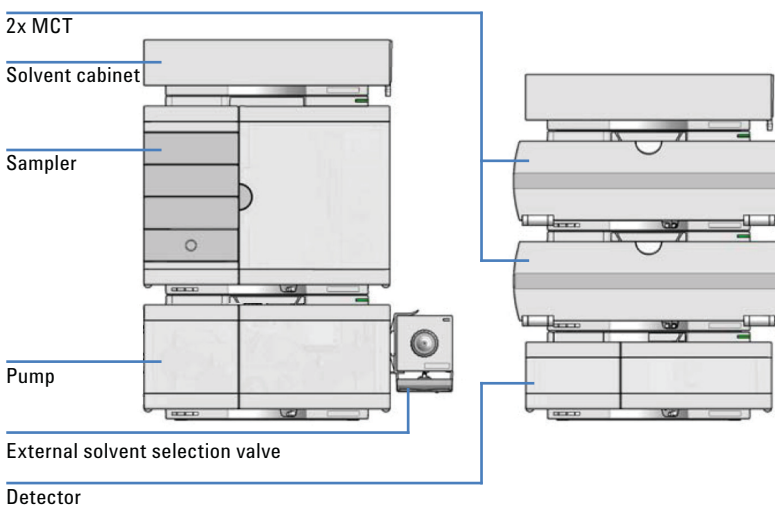- Agilent ZORBAX Bonus-RP RRHD, 2.1 × 100 mm, 1.8 µm, p/n 858768-901

2x MCT
Solvent cabinet
Sampler
Pump
External solvent selection valve
Detector

Figure 1. Setup of the Agilent 1290 Infinity II Method Development Solution as used for this Technical Overview.

Instrument setup[1,2]

The seven columns used for all applications were introduced in the columns list in the Agilent OpenLAB CDS ChemStation. For the columns, a description, geometric data, particle size, and limitations such as pH, pressure, and temperature can also be given. The column list is directly connected to the column assignment for the two clustered Multicolumn Thermostats (MCTs) (Figure 2). The two MCTs were used to house a larger number of columns, longer than 100 mm. Typically, a single MCT can house up to eight columns with a length up to 100 mm, or up to four columns with a length of 150 mm or longer. In the column assignment, the position of the column on the right or left side of the MCTs can be chosen and associated with a color code. The column data from the column list can be selected in the Column Tag Information table and associated to a column position and color code. The column used for the individual method can be selected in the Method tab of the Valve-Thermostat-Cluster (VTC). The appropriate column can be selected either by the pull-down menu, which shows all assigned columns, or by clicking the column with the right color code in the image of the VTC. 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 description is shown in the Agilent OpenLAB CDS ChemStation user interface (UI).

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. In the Pump-Valve-Cluster tab, the positions of the solvent selection valve can be named by the connected solvent (Figure 3). The compressibility calibration can be chosen by taking the correct solvent or type of solvent from the pull-down menu; pH and molarity values can be added. In the Pump-Valve-Cluster Method tab, the desired solvents can be chosen, and the valve will be automatically switched to the right positions. The accessible multiple solvents are sketched out in the ChemStation pump UI.
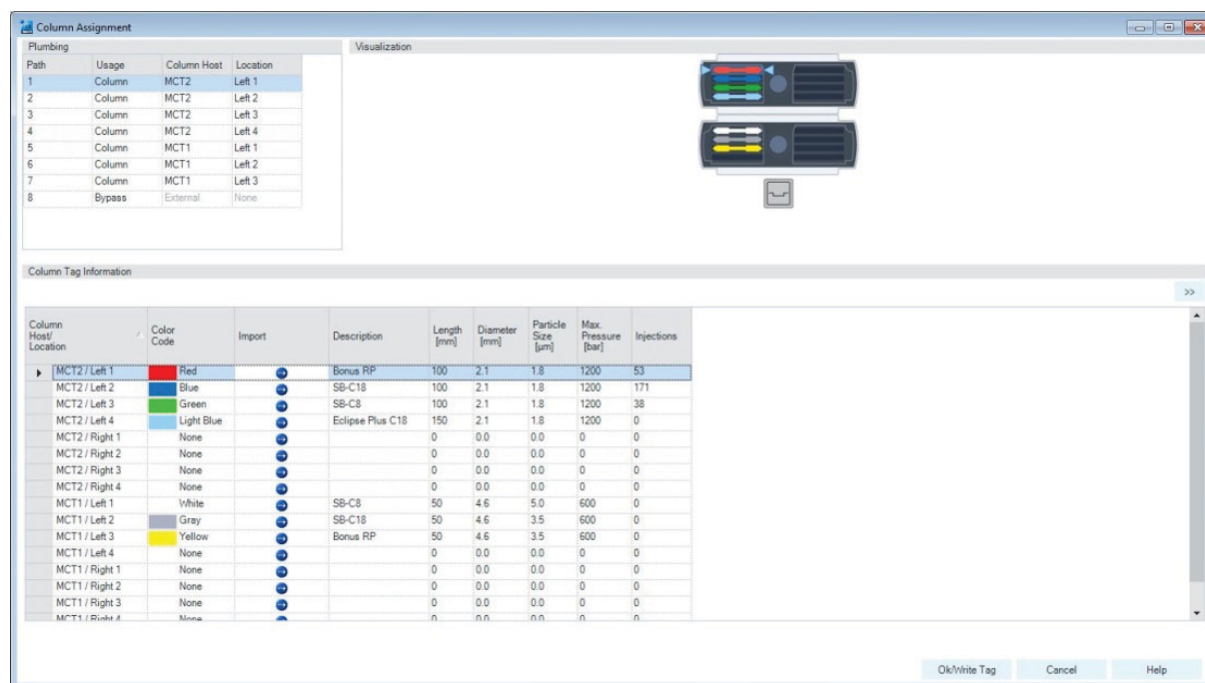

Figure 2. Column assignment to the individual positions in the MCT cluster and color codes.

Agilent Method Scouting Wizard[2]

The ChemStation Method Scouting Wizard enables the easy setup of many methods to scout different LC conditions including different columns, solvents, gradients, and temperatures. Finally, the Method Scouting Wizard automatically creates a sequence that includes solvent exchanges, re-equilibrations, and column storages when changing columns and solvents.

The columns necessary for a study can be selected in the Method Scouting Wizard's screen for column scouting (Figure 4). If columns of different inner diameter and different length are used, the flow rates are automatically calculated according to the inner diameter, and the gradient times are automatically calculated according to the length of the columns. The Agilent 1290 Infinity II MCT can host up to eight different columns up to 100 mm long or four longer columns. If more longer columns are used, they will be hosted in the second MCT included in the VTC.

Figure 3. Assignment of solvents connected to the solvent selection valve at channel B of the pump.

Figure 4. Column selection table for column scouting, and checkmarks for flow rate and gradient time adjustments (left corner at the bottom). The adjustments will be shown as Flow Factor and Time Factor in relation to the starting value, which has the factor 1.

The different solvents to be used can be set up in the window for solvent scouting (Figure 5). 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 to create a binary gradient. The solvent-selection valve that delivers the organic solvent for the binary gradient is connected to channel B.

The final sequence was created including all necessary solvent exchanges, column equilibration, and column storage methods as separate sequence lines without injection (Figure 6).

Sample

Agilent Pesticide Solution, containing 16 pesticide compounds at a concentration of 1 mg/mL each in acetonitrile/acetone (4:1); the inherent compounds can be found in the information supplied with the sample (p/n 5190-6895).
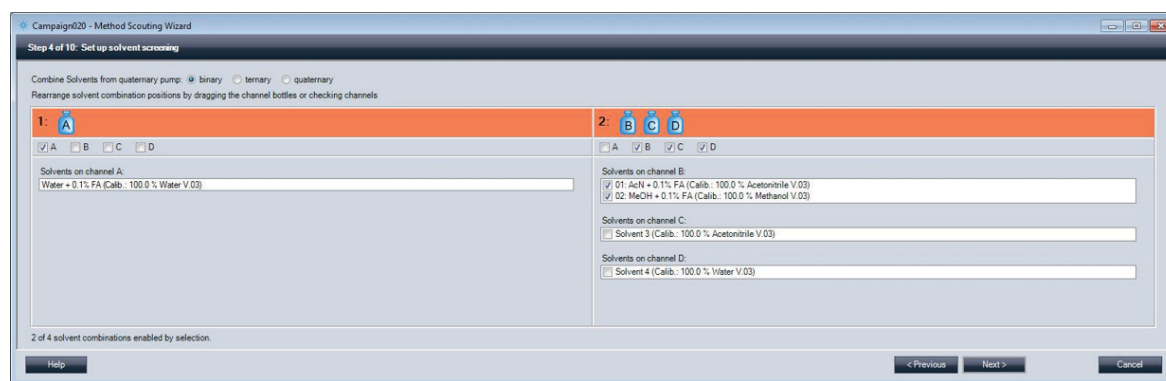
Figure 5. Solvent selection with a quaternary pump, which was used in binary mode to combine the solvent at channel A and one of the solvents at channel B.

Figure 6. Sequence table including the methods created by the Agilent Method Scouting Wizard for running the samples, flushing, and column re-equilibration. In the lower left corner, the number of injections, equilibrations, and flushings together with the calculated total run time of the campaign is displayed.

Solvents

All solvents were HPLC grade and 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).

The initial method contained a flow rate of 2.0 mL/min, which fit to the 4.6-mm id columns. The flow rate for the 2.1-mm id columns was calculated and applied (0.417 mL/min). The initial gradient time was set to 30 minutes, and applied to the longest column (150 mm). The shorter run times for the 100-mm and 50-mm columns were calculated by the Method Scouting Wizard and were applied (20 and 10 minutes, respectively).

Results and Discussion

To create a chromatographic separation method for the given complex sample comprising 16 pesticide compounds, several chromatographic parameters were varied, and the respective methods were generat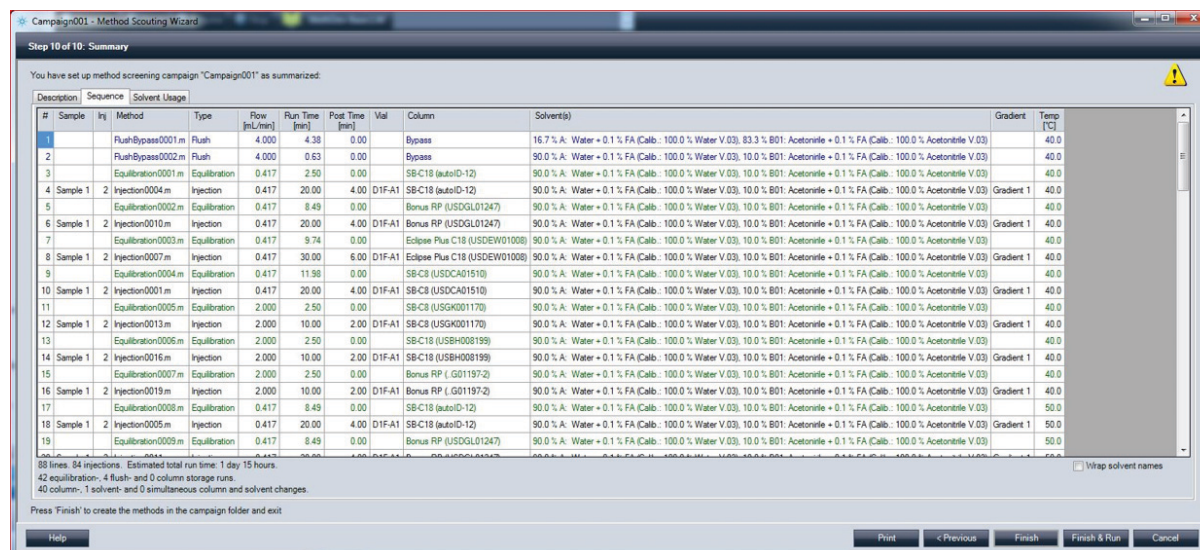ed by the Method Scouting Wizard. For the complete method development matrix, two solvent combinations (water-acetonitrile and water-methanol), seven columns, and three column temperatures (40, 50, and 60 °C) were used. Together with the initial gradient, this comprised 42 analytical methods. The optimization of the gradient was planned for a second step, if necessary. Together with the automatically created system flushing and column equilibration methods, a total number of 88 methods were generated. All sample injections were done in duplicate, consuming a total run time of 1 day and 15 hours, and creating 84 sample data files.

Initial method for the Agilent Method Scouting Wizard

Parameter	Description
Flow rate	2 mL/min
Solvent A	Water + 0.1 % formic acid
Solvent B	Methanol + 0.1 % formic acid, Acetonitrile + 0.1 % formic acid.
Gradient	10 %B at 0 minutes 70 %B at 30 minutes
Stop time	30 minutes
Post time	6 minutes
Injection volume	0.5 μL
Needle wash	6 seconds in methanol
Colum temperature	40 °C
Detection	254/4 nm, reference 360/100 nm; slit 4 nm; data rate 20 Hz

Analysis of method development data by the Method Development Reports including bubble charts

The higher the number of runs, the more complex and time-consuming is the manual analysis of the data and the identification of the best separations. To overcome this situation, the different Method Development Reports available in Agilent OpenLAB CDS ChemStation could be used. Typically, the so-called bubble-chart reports are used. They display charts in which the maximum number of peaks or the resolution sum is displayed as bubbles of variable size versus the retention time of the last eluting peak for each run. As a first approach, the bubble chart showing the maximum resolution sum was created for all used columns, the two solvent combinations used, and a column temperature of 40 °C (Figure 7).
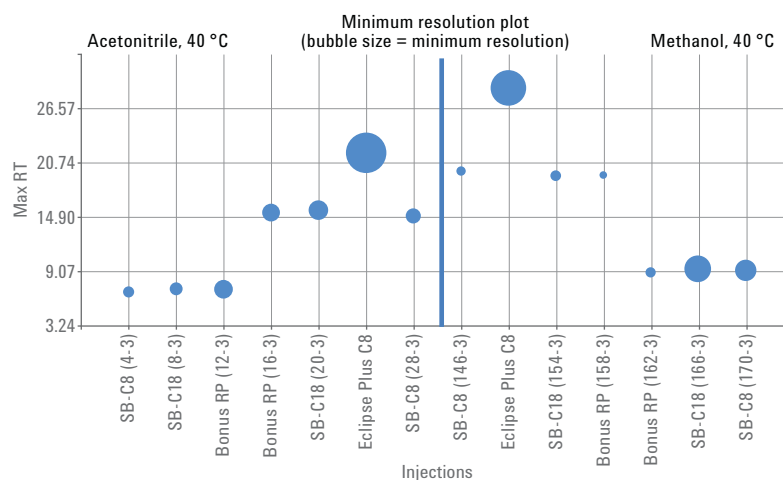

Figure 7. Bubble chart showing the maximum resolution sum displayed by the bubble area for the separation of the 16 pesticide compounds on different columns. The chart also enables a comparison for the two different solvent combinations at 40 °C.

From the chart, it could be seen that the last peaks were eluting from the short 50-mm columns at or below nine minutes for both solvent combinations. The 100-mm columns showed elution times around 15 minutes for acetonitrile-water as eluent, and 20 minutes for methanol-water as eluent. The column that showed the largest bubbles, corresponding to the highest maximum resolution sum, was the Agilent ZORBAX Eclipse Plus C8 column with 150-mm length. The maximum elution time was 21.8 minutes for acetonitrile-water eluent, and 28.7 minutes for methanol-water eluent. The most promising separation method was the one using this column with either methanol or acetonitrile (Figure 8). The method using acetonitrile as eluent provided a shorter run time. With the method using the acetonitrile eluent (Figure 8A), all 16 compounds were separated. The three peaks that were clearly separated between 12.5 and 13.3 minutes coeluted with methanol as eluent at 20 minutes (Figure 8B).

The dependence of the elution behavior on the column temperature was examined manually by a comparison of the chromatograms acquired at 40, 50, and 60 °C for the acetonitrile and methanol elution from the 150 mm Eclipse Plus C8 column (Figures 9 and 10). If the column temperature was increased from 40 to 50 °C (Figures 9A and 9B) for the acetonitrile elution, a coelution occurred for the peaks at 12.9 and 13.3 minutes. At an increase to 60 °C, they separated again, and the peak at 13.2 minutes coeluted. Coelution behavior of other compounds could be observed at later elution times (Figure 9C). For the chromatography with methanol as eluent, no separation could be observed for the three peaks that coeluted at 20 minutes at all column temperatures (Figures 10A, 10B, and 10C). The compounds, which still eluted separately at 20.5 and 20.9 minutes at 50 °C (Figure 10B), coeluted at 19.5 minutes at a column temperature of 60 °C (Figure 10C).
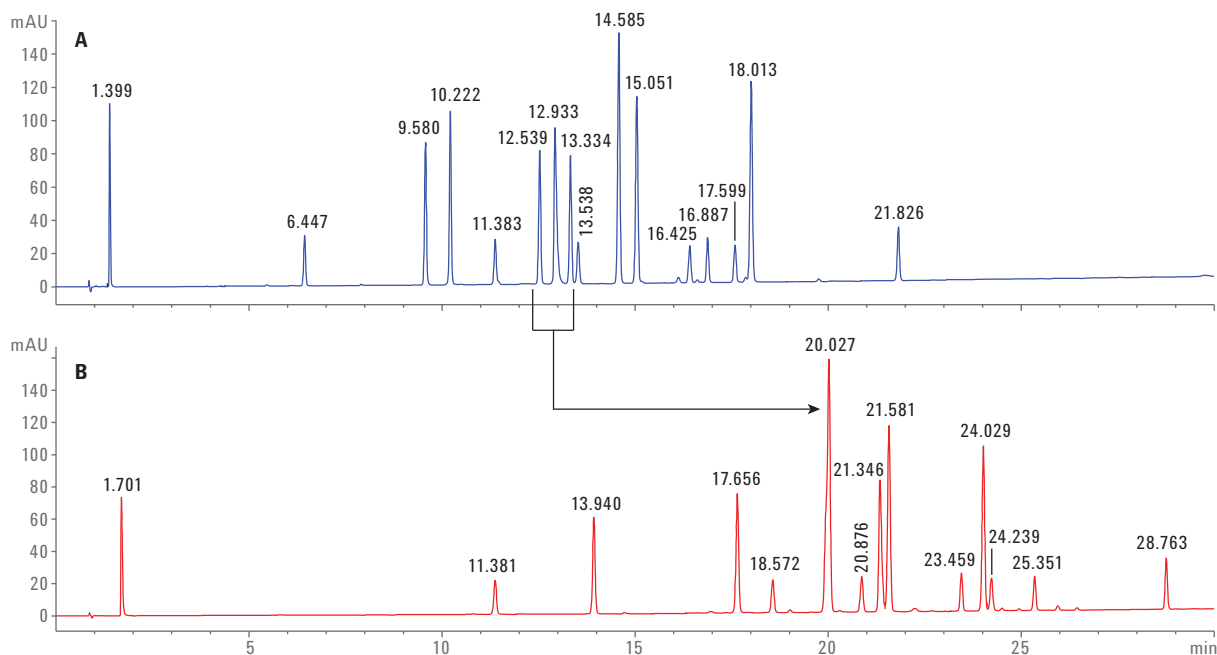
Figure 8. Separation of 16 pesticide compounds on the Agilent ZORBAX Eclipse Plus C8 column (2.1 × 150 mm, 1.8 µm) with acetonitrile (A) and methanol (B) as eluent (coelution is indicated by the arrow).

Figure 9. Separation of the 16 pesticides on an Agilent ZORBAX Eclipse Plus C8 column (2.1 × 150 mm, 1.8 µm) with acetonitrile as eluent at different temperatures: 40 °C (A), 50 °C (B), and 60 °C (C). The arrows indicate the peaks that shifted due to different selectivity at different temperatures.
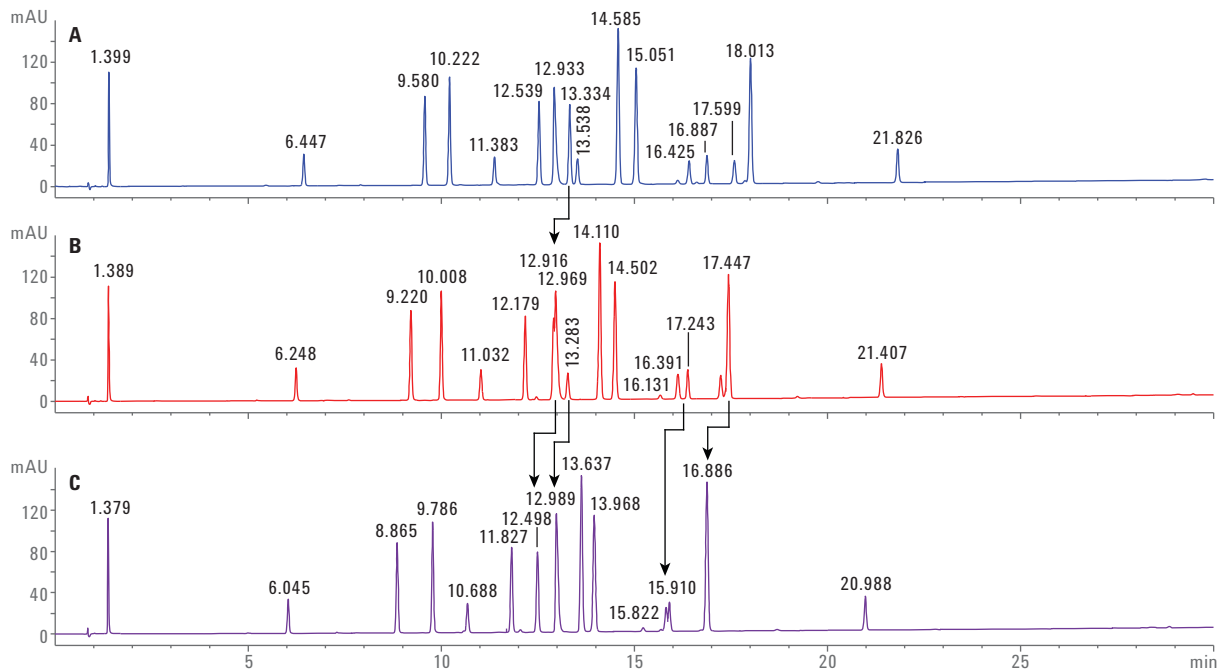

Figure 10. Separation of the 16 pesticides on an Agilent ZORBAX Eclipse Plus C8 column (2.1 × 150 mm, 1.8 µm) with methanol as eluent at different temperatures: 40 °C (A), 50 °C (B), and 60 °C (C). The arrows indicate the peaks that shifted due to different selectivity at different temperatures.
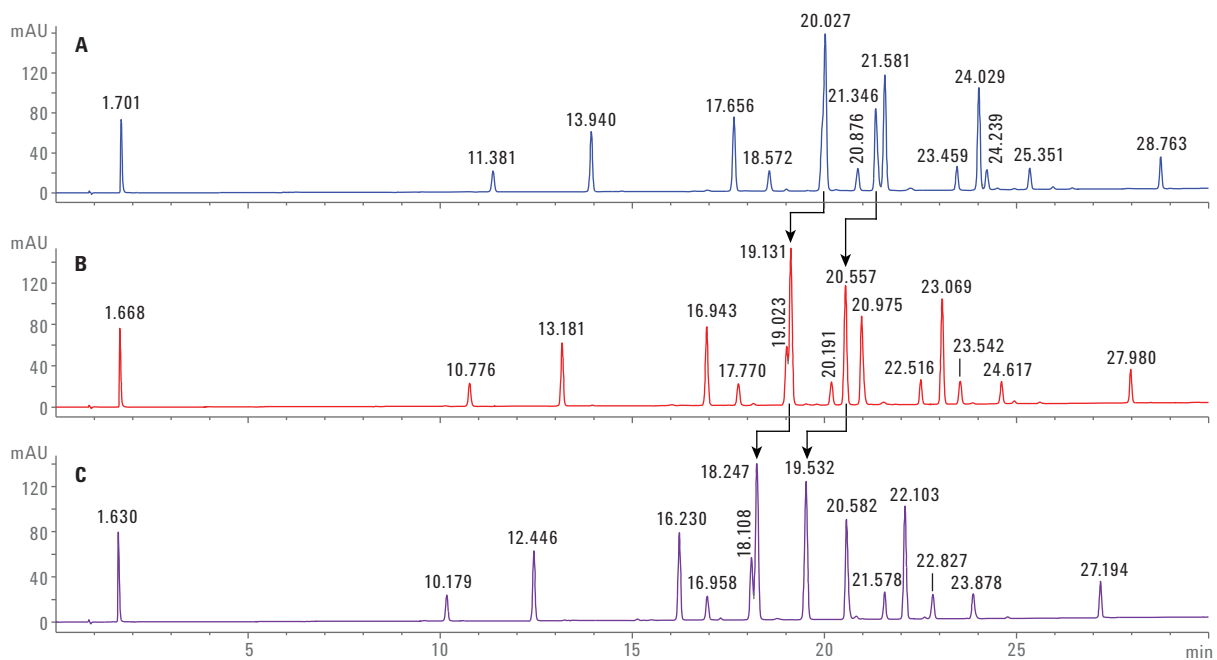
Analysis of a large set of method development data by the Summary Performance Report: highest resolution sum

Using the described approach of simple bubble charts, only a lower number of scouting runs could be handled comfortably. If many scouting runs have to be examined, other tools are needed so that possible solutions of a given separation problem are not overlooked. The Summary Performance Report can be used if such large numbers of data files have to be handled. It creates a table, showing the maximum number of peaks, the resolution sum, and the minimum resolution in the listed data files. With

this report, the best combinations could be identified easily, for instance, by exporting to Excel, and by application of the filter options and sorting functions.

In the first attempt, the table obtained from the Summary Performance Report of the 42 scouting runs was sorted from highest to lowest resolution sum. The data files belonging to the scouting runs with the six highest resolution sums were examined more closely (Table 1). It is obvious that this approach should lead to the same results as before with the bubble charts. In fact, the data files Injection 0010_2 and Injection 0031_2 were also identified with the aid of the bubble charts, and are shown

in Figures 8A and 8B, respectively. In addition, the run with methanol as the elution solvent did not give the full separation due to the lower number of identified peaks (Figure 8B). There were only 14 instead of 16, which suggested a coelution of three compounds. This also explained the misleading high number for the minimum resolution, because, these compounds showed complete overlay and were not even rudimentarily separated. The opposite could be demonstrated by data file Injection 0011_2. The maximum number of peaks was detected; the resolution sum was the highest of all, but the minimum resolution between two peaks was not sufficient for baseline separation (Figure 9B).

Table 1. Six experiments with highest resolution sum were filtered out of the Summary Performance Report from the data field of 42 scouting runs.

Minimum resolution	Resolution sum	Minimum symmetry	Peaks	Data file	Column	Solvent 1	Solvent 2	Temperature	Gradient
0.48	295.31	0.46	16	Injection 0011_2 (Figure 9B)	Agilent ZORBAX Eclipse Plus C8 2.1 × 150 mm, 1.8 µm	A) Water + 0.1 % FA	B01) AcN + 0.1 % FA	50.0 °C	Gradient 1
0.73	287.13	0.53	14	Injection 0012_2 (Figure 9C)	Agilent ZORBAX Eclipse Plus C8 2.1 × 150 mm, 1.8 µm	A) Water + 0.1 % FA	B01) AcN + 0.1 % FA	60.0 °C	Gradient 1
0.81	243.2	0.4	15	Injection 0032_2 (Figure 10B)	Agilent ZORBAX Eclipse Plus C8 2.1 × 150 mm, 1.8 µm	A) Water + 0.1 % FA	B02) MeOH + 0.1 % FA	50.0 °C	Gradient 1
1.63	239.8	0.3	14	Injection 0031_2 (Figure 10A)	Agilent ZORBAX Eclipse Plus C8 2.1 × 150 mm, 1.8 µm	A) Water + 0.1 % FA	B02) MeOH + 0.1 % FA	40.0 °C	Gradient 1
0.98	239.47	0.34	14	Injection 0033_2 (Figure 10C)	Agilent ZORBAX Eclipse Plus C8 2.1 × 150 mm, 1.8 µm	A) Water + 0.1 % FA	B02) MeOH + 0.1 % FA	60.0 °C	Gradient 1
1.87	223.67	0.46	16	Injection 0010_2 (Figure 9A)	Agilent ZORBAX Eclipse Plus C8 2.1 × 150 mm, 1.8 µm	A) Water + 0.1 % FA	B01) AcN + 0.1 % FA	40.0 °C	Gradient 1

Analysis of a large set of method development data by the Summary Performance Report: minimum resolution

Minimum resolution could also be used as a filter parameter to search for more hidden promising scouting runs. In this case, all separations with a minimum resolution greater than one were filtered out (Table 2). The highest minimum resolutions were identified for the separations on the 2.1 × 150 mm Eclipse Plus C8 column, described earlier. In the remaining cases, the obtained minimum resolutions were above one. However, a closer look into the data showed that all cases were separations on short 4.6 × 50 mm columns with high flow rates (2 mL/min), and short run times (10 minutes). The flow rates and gradient times were automatically adjusted by the Method Scouting Wizard according to column inner diameter and column length, respectively (see Experimental section). In all four cases, the separations were incomplete with a high degree of complete coelution, producing a too high minimum resolution and a lower number of peaks (Figure 11).

Table 2. Six experiments with minimum resolution greater than 1 were filtered out of the Summary Performance Report from the data field of 42 scouting runs.

| Minimum resolution	Resolution sum	Minimum symmetry	Peaks	Data file	Column	Solvent 1	Solvent 2	Temperature	Gradient
1.87	223.67	0.46	16	Injection 0010_2 (Figure 9A)	Agilent ZORBAX Eclipse Plus C8 2.1 × 150 mm, 1.8 µm	A) Water + 0.1 % FA	B01) AcN + 0.1 % FA	40.0 °C	Gradient 1
1.63	239.8	0.3	14	Injection 0031_2 (Figure 10A)	Agilent ZORBAX Eclipse Plus C8 4.6 × 150 mm, 1.8 µm	A) Water + 0.1 % FA	B02) MeOH + 0.1 % FA	40.0 °C	Gradient 1
1.18	99.55	0.6	12	Injection 0025_2 (Figure 11A)	Agilent ZORBAX SB C18 4.6 × 50 mm, 3.5 µm	A) Water + 0.1 % FA	B02) MeOH + 0.1 % FA	40.0 °C	Gradient 1
1.09	94.95	0.77	14	Injection 0021_2 (Figure 11B)	Agilent ZORBAX Bonus-RP 4.6 × 50 mm, 3.5 µm	A) Water + 0.1 % FA	B01) AcN + 0.1 % FA	60.0 °C	Gradient 1
1.08	83.01	0.73	13	Injection 0029_2 (Figure 11C)	Agilent ZORBAX SB-C8 4.6 × 50 mm, 5 µm	A) Water + 0.1 % FA	B02) MeOH + 0.1 % FA	50.0 °C	Gradient 1
1.06	98.02	0.65	13	Injection 0018_2 (Figure 11D)	Agilent ZORBAX SB-C18 4.6 × 50 mm, 3.5 µm	A) Water + 0.1 % FA	B01) AcN + 0.1 % FA	60.0 °C	Gradient 1
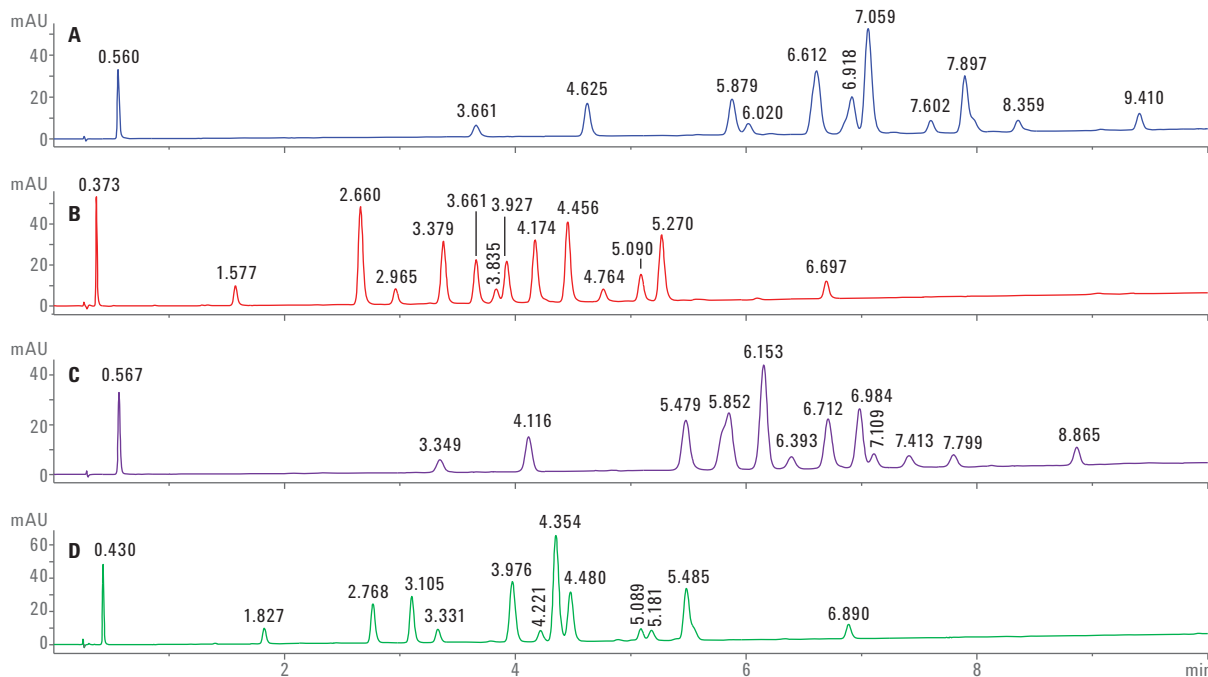
Figure 11. Separation of the 16 pesticides: A) Agilent ZORBAX SB-C18, 4.6 × 50 mm, 3.5 µm, methanol/water, 40 °C, B) Agilent ZORBAX Bonus-RP, 4.6 × 50 mm, 3.5 µm, acetonitrile/water, 60 °C, C) Agilent ZORBAX SB-C8, 4.6 × 50 mm, 5 µm, methanol/water, 50 °C, D) Agilent ZORBAX SB-C18, 4.6 × 50 mm, 3.5 µm, acetonitrile/water, 60 °C. All separations were incomplete due to coelution of some peaks, leading to a minimum resolution value greater than one, but displaying less than 16 peaks.

Analysis of a large set of method development data by the Summary Performance Report: number of detectable peaks

Finally, the Summary Performance Report was used to filter out all experiments that separated 16 compounds as separately detectable peaks (Table 3). It was not surprising to find the experiments discussed above (Figure 9A and 9B). In addition, four other separations showing 16 compounds were filtered out of the data. Two of them showed separations that were done on the 50 mm Bonus-RP column at different temperatures (Figure 12). Both separations were done in only 10 minutes but, unfortunately, there were partial coelutions in both cases. This also explained the lower minimum resolution values obtained for those separations. For instance, the peaks at 3.26 and 3.33 minutes were not separated at 40 °C (Figure 12A), but were separated at 50 °C (Figure 12B). The pair of peaks eluting at 5.48 and 5.60 minutes at 40 °C behaved the opposite way.

Table 3. Six experiments, showing 16 peaks of separated compounds, which were filtered out of the Summary Performance Report from the data field of 42 scouting runs.

| Minimum resolution	Resolution sum	Minimum symmetry	Peaks	Data file	Column	Solvent 1	Solvent 2	Temperature	Gradient
0.48	295.31	0.46	16	Injection 0011_2 (Figure 9B)	Agilent ZORBAX Eclipse Plus C8 2.1 × 150 mm, 1.8 µm	A) Water + 0.1 % FA	B01) AcN + 0.1 % FA	50.0 °C	Gradient 1
1.87	223.67	0.46	16	Injection 0011_2 (Figure 9B)	Agilent ZORBAX Eclipse Plus C8 2.1 × 150 mm, 1.8 µm	A) Water + 0.1 % FA	B01) AcN + 0.1 % FA	40.0 °C	Gradient 1
0.44	126.98	0.43	16	Injection 0011_2 (Figure 13A)	Agilent ZORBAX Bonus RP 2.1 × 100 mm, 1.8 µm	A) Water + 0.1 % FA	B01) AcN + 0.1 % FA	50.0 °C	Gradient 1
0.9	125.27	0.6	16	Injection 0011_2 (Figure 13B)	Agilent ZORBAX SB-C18 2.1 × 100 mm, 1.8 µm	A) Water + 0.1 % FA	B01) AcN + 0.1 % FA	40.0 °C	Gradient 1
0.58	96.76	0.82	16	Injection 0020_2 (Figure 12B)	Agilent ZORBAX Bonus RP 4.6 × 50 mm, 3.5 µm	A) Water + 0.1 % FA	B01) AcN + 0.1 % FA	50.0 °C	Gradient 1
0.84	96.59	0.58	16	Injection 0019_2 (Figure 12A)	Agilent ZORBAX Bonus RP 4.6 × 50 mm, 3.5 µm	A) Water + 0.1 % FA	B01) AcN + 0.1 % FA	40.0 °C	Gradient 1
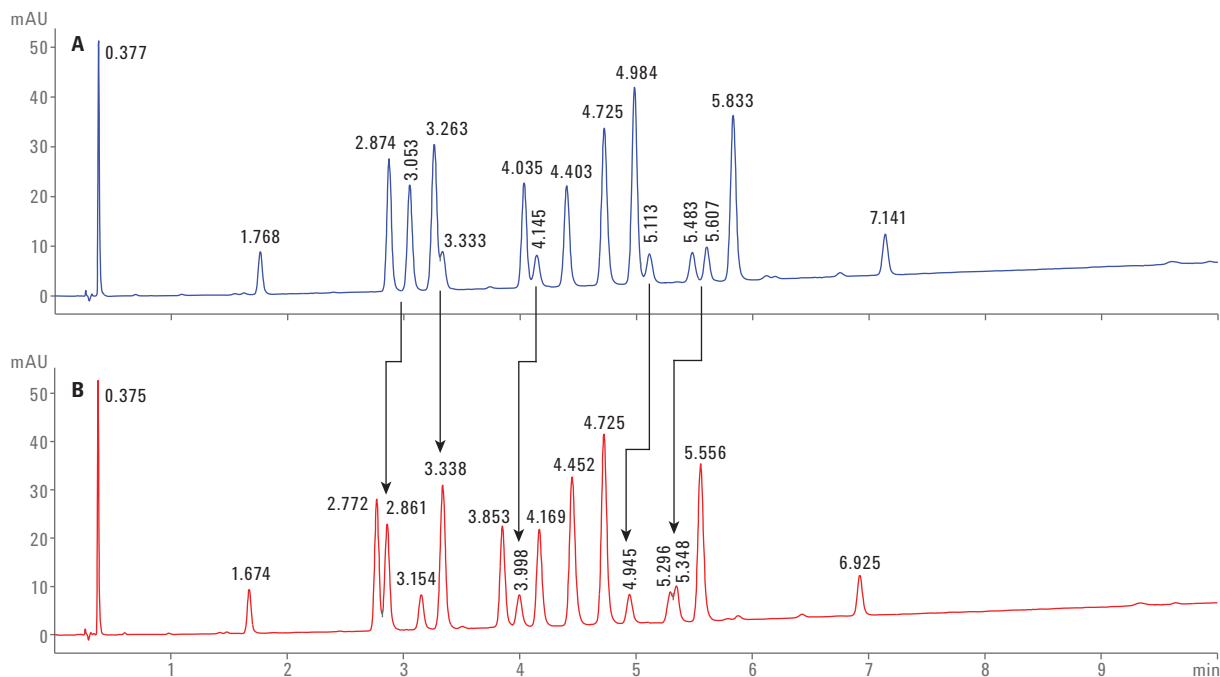
Figure 12. Separation of the 16 pesticides on an Agilent ZORBAX Bonus-RP column (4.6 × 50 mm, 3.5 µm) at a flow rate of 2.0 mL/min in 10 minutes and at column temperatures of 40 °C (A) and 50 °C (B).

The remaining two separations, also showing 16 peaks, were done on a Bonus-RP column (Figure 13A) and on an SB-C18 column (Figure 13B); both had the dimensions 2.1 × 100 mm, 1.8 µm. The separation on the Bonus-RP column has clearly separated peaks and would need only minor optimization to get some better resolution of the peaks eluting at 11.7 and 11.8 minutes (Figure 13A). The separation on the SB-C18 column would need some more optimization work, because of the cluster of peaks that eluted closely between 9.3 and 9.8 minutes (Figure 13B).

The method of choice for the separation of the 16 pesticides would be the separation obtained with the Eclipse Plus C8 column with acetonitrile as eluent at a column temperature of 40 °C in 30 minutes due to the high resolution between peaks. This also leads to a method for the separation of even more pesticide compounds used in multipesticide methods. Conversely, this separation has a longer run time in comparison to the resolution obtained, for instance, on the Bonus-RP column in 20 minutes. For a complete identification of the compounds and their assignment to the respective retention

time, an Agilent single quadrupole mass spectrometer could be connected under ChemStation, running together with the Method Scouting Wizard.

Conclusion

This Technical Overview demonstrates the use of the Agilent 1290 Infinity II System for method development with the Agilent ChemStation Method Scouting Wizard. The reporting capabilities are primarily described to filter the best separation out of an acquired set of data. This study describes using the typical bubble chart reports for smaller sets of data. The advantage of tables, which can be sorted and filtered for different chromatographic parameters for larger sets of data, is shown.
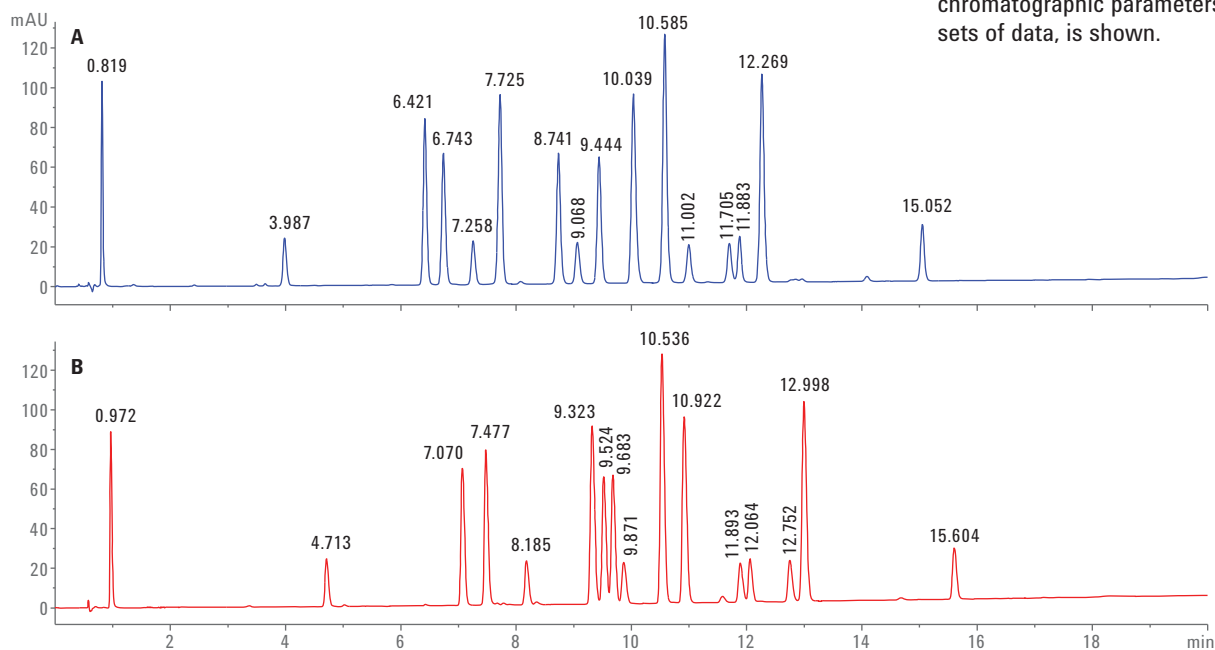

Figure 13. Separation of the 16 pesticides: A) Agilent ZORBAX Bonus-RP column (2.1 × 100 mm, 1.8 µm) at a column temperature of 50 °C, B) Agilent ZORBAX SB-C18 column (2.1 × 100 mm, 1.8 µm) at a column temperature of 40 °C. Both separations were at a flow rate of 0.417 mL/min with acetonitrile, and within a run time of 20 minutes.

References

1. Automated Scouting of Stationary and Mobile Phases Using the Agilent 1290 Infinity II Method Development Solution, *Agilent Technologies Application Note*, publication number 5991-5679EN, **2015**.

2. The Agilent 1290 Infinity II Multi-Method Solution, *Agilent Technologies Application Note*, publication number 5991-5934EN, **2015**.

Agilent Technologies