TECHNICAL REPORT

MODIFYING AGILENT® 1100 HPLC SYSTEMS TO ACHIEVE "UHPLC-LIKE" PERFORMANCE WITH HALO® FUSED-CORE® COLUMNS

Agilent 1100 HPLC system

With a few modifications, an Agilent 1100 HPLC can produce UHPLC-like performance using HALO Fused-Core columns.

Introduction

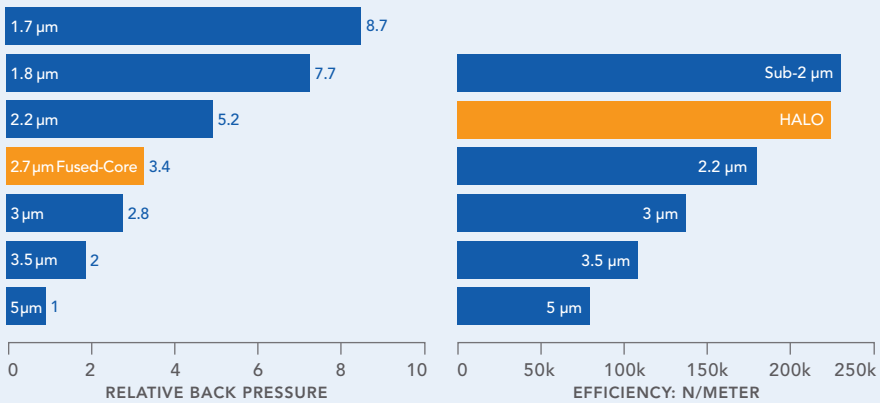
The Agilent 1100 HPLC systems are found in many labs, and their performance in their as-shipped configuration is excellent when used with conventional HPLC columns. However, the recent introduction of columns packed with Fused-Core particles offers Agilent 1100 users the opportunity to obtain even higher performance from their existing HPLC equipment. HALO columns packed with Fused-Core particles deliver resolution and speed similar to sub-2-µm UHPLC columns, but generate only 40% to 50% of the back pressure produced by sub-2-µm columns. (See Figure 1.) The high efficiency and lower back pressure of HALO columns make it possible to obtain "UHPLC-like" performance from conventional 400-bar equipment, like the Agilent 1100.

HALO columns, like their sub-2-µm column counterparts, produce smaller peak volumes (50% to 75% smaller) than conventional columns of the same dimensions. Because of the smaller peak volumes, it is important to keep extra column band broadening appropriate for the particular column geometry used. How your Agilent 1100 instrument is configured, and the method settings you choose will determine the amount of extra column band broadening, and, ultimately the efficiency and resolution you can obtain with a given HALO column geometry. Many HALO columns can be used quite effectively with the Agilent 1100 in its standard configuration, but much more performance can be obtained by modifying the equipment and settings for optimum results. For example, a 4.6 x 100 mm HALO column can reduce analysis time by 67% compared to a 4.6 x 250 mm, 5 µm conventional column using a standard Agilent 1100 quaternary system under identical conditions. However, by making a few modifications to the Agilent 1100, the same HALO column will yield 46% more plates. The moderate back pressure and high flow velocity characteristics of HALO columns facilitate a 85% reduction in analysis time compared to a conventional column, while maintaining resolution. (*see Figure 2*).

In HPLC, peak volume decreases as column ID or length decreases. Small peak volumes are more vulnerable to extra column band broadening, thus preventing maximum column efficiency from being achieved. While very good results can be obtained with many of the larger volume HALO columns using the Agilent 1100 in its standard configuration, you will likely find it worthwhile to reduce the amount of extra column volume and adjust detector settings to achieve much better results with some of the smaller volume HALO columns.

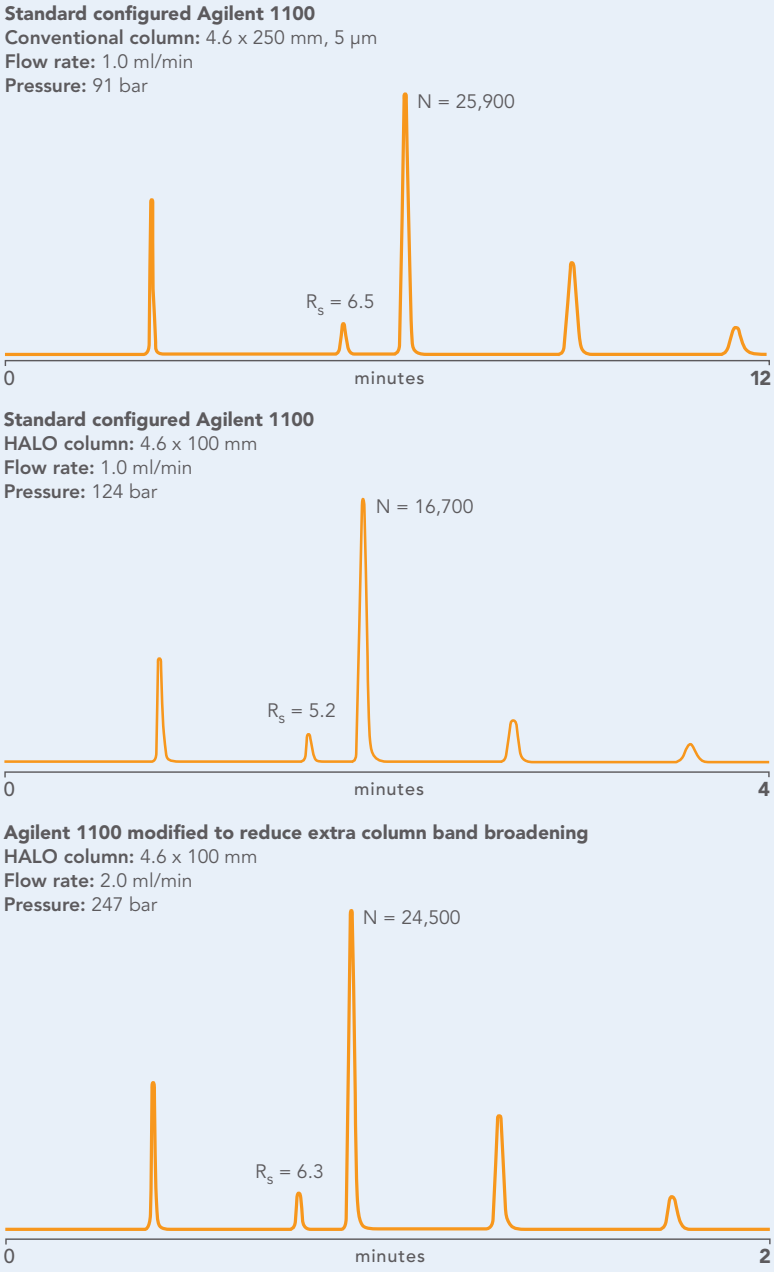
In this technical report we first discuss extra column band broadening and the instrument parameters that contribute to it. Next, we recommend how an Agilent 1100 can be configured to get the most performance from specific HALO columns. Finally, we provide step-by-step instructions for making those recommended modifications to the Agilent 1100.

FIGURE 1: HALO columns exhibit UHPLC-like performance at conventional HPLC pressure



HALO columns packed with Fused-Core particles provide over 80% of the efficiency (theoretical plates, N) and 90% of the resolving power of sub-2-µm columns, but require less than half the back pressure. This lower pressure permits HALO columns to be used with conventional 400 bar-limit HPLC equipment and achieve speed and resolution very similar to UHPLC.

FIGURE 2: An Agilent 1100 modified to reduce extra column band broadening permits significantly better performance to be obtained from HALO columns.



The HALO column delivers a much faster separation compared to a conventional column when using a standard configured Agilent 1100, but the resolution is less than expected. When the Agilent 1100 is modified to reduce extra column band broadening, 46% more plates and higher resolution are obtained from the same HALO column. The Fused-Core particles packed in the HALO column permit a higher flow velocity to be used without sacrificing resolution or exceeding the pressure limit of the Agilent 1100. The result is a reduction in analysis time of 85% while maintaining similar resolution compared to the conventional column.

Extra Column Band Broadening

In liquid chromatography the observed peak width (w_{obs}) is comprised of the dispersion (or band broadening) from the chromatographic process itself (w_{col}) and from dispersion elsewhere in the HPLC system (w_{ec}). The relationship can be described by this equation:

$$w^2_{obs} = w^2_{col} + w^2_{ec}$$

The observed, column-related, and extra column-related variances are related similarly,

$$\sigma^2_{obs} = \sigma^2_{col} + \sigma^2_{ec}$$

where $w = 4\sigma$, σ is the standard deviation of the peak and σ^2 is the peak variance.

The total extra column variance (σ^2_{ec}) is equal to the sum of the variances from the various contributors to band broadening (assuming all variables are independent):

$$\sigma^2_{ec} = \sigma^2_{injection} + \sigma^2_{tubing} + \sigma^2_{flow\ cell} + \textbf{variance from detector response time and data rate}$$

The importance of extra column band broadening can be assessed relative to the peak width or volume at the retention time or volume for each analyte. In general, the contributions to band broadening and peak volume are inversely proportional to the retention time or volume of the analyte, so that extra column band broadening is most important for the least retained analytes in a separation.

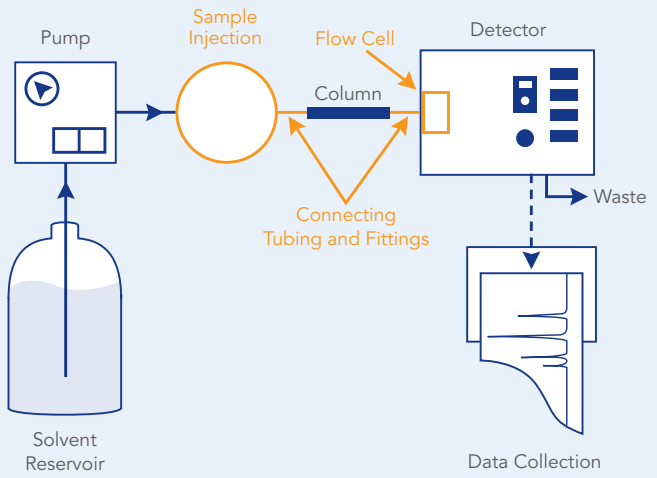
For a given HPLC system configuration, the extra column volume, or ECV, is the total volume outside of the column that contributes to the observed peak volume. For an Agilent 1100 the ECV includes the volumes associated with the following items:

- the injection volume,
- the flow path in the autosampler and valve,
- the tubing from the autosampler to the precolumn heat exchanger,
- the precolumn heat exchanger,
- the tubing from precolumn heat exchanger to the column,
- the tubing from the column to the detector flow cell,
- the detector flow cell, and
- any volume added by in-line filters, unions, guard columns, etc.

NOTE: For simplicity, any contributions to band broadening due to fittings and connections are considered to be negligible with proper choice of hardware and good practices, and will be ignored.

The total extra column band broadening also includes the dispersion due to electronic signal filtering from the detector. With Agilent UV-VIS detectors the ChemStation® software Peak Width setting is directly related to detector response time, and inversely related to data acquisition rate for a particular detector type and model. Typically, you can find the relationships among Peak Width setting, response time, and data rate summarized in a table in the appropriate detector manual. The larger the Peak Width setting is, the slower the data rate, and the greater the amount of signal filtering. Conversely, smaller Peak Width settings are associated with faster data rates, less signal filtering, and more short-term noise. A general recommendation is to

FIGURE 3: Extra Column Volume, ECV



In this schematic of an HPLC system, the gold color indicates where extra column volume, ECV, is located.

set the detector Peak Width setting to be equal to or slightly less than the half-height width of the narrowest peak of interest in the chromatogram. In practice, it is wise to compare performance (peak widths, tailing factors, signal-to-noise ratios) from chromatograms acquired with several detector Peak Width settings above and below the value for the narrowest peak, in order to choose the best setting for your needs. If the peak width setting is too small, the observed peak widths will be minimized and peak heights maximized, but the signal-to-noise ratio will suffer due to increased baseline noise. If the peak width setting is too large, baseline noise will be reduced, but the peaks will be shorter and broader and may begin to tail and merge.

Several report styles (Performance, Performance and Noise, and Extended Performance) within the Data Analysis View of the ChemStation software can help you find an appropriate or optimum setting for your column and conditions. Fortunately, the data rate is automatically set when you choose the Peak Width setting as just described. Nevertheless, a good rule of thumb is to ensure that the data rate is sufficiently fast to acquire at least 20 points across the narrowest peak. As an example, for a peak width at half height of 0.05 min., the 4σ baseline width (1.7 X $PW_{½}$) is 5.1 sec., and the data rate should be at least 20 points per 5.1 seconds, or ~ 4 Hz.

The detector flow cell (volume and design), the detector response time and the data rate are typically the largest contributors to band broadening. These are the parameters you should optimize first when trying to get the most performance from HALO columns. If you are using HALO columns with ID ≤ 3.0 mm, you may also have to minimize connecting tubing volume, heat exchanger volume, and injection volume.

Recommended Instrument Configurations to Use with Specific HALO Columns

The smaller the peak volume produced by a column, the more important it is to make sure that extra column volume (ECV) does not rob you of the resolving power that the column can deliver. Depending on the dimensions of the HALO column you wish to use, three different configurations of the Agilent 1100 will be recommended; the standard configuration (as shipped from the

TABLE A: Standard configured Agilent 1100 (as typically shipped from Agilent)

Acceptable configuration for the following HALO columns: 4.6 x 150 mm, 4.6 x 100 mm, and 4.6 x 75 mm.

Description	Part No.	Volume (µL)
Needle seat capillary for standard autosampler 0.17 x 100 mm, Green	G1313-87101	2.5
Or, needle seat capillary for well-plate autosampler 0.17 x 100 mm, Green	G1367-87302	2.5
Stainless steel tubing from autosampler to column compartment heat exchanger 0.17 x 180 mm, Green	G1313-87305	4.5
Column compartment heat exchanger 3 uL		3.0
Stainless steel tubing from column compartment heat exchanger to column 0.17 x 90 mm, Green	G1316-87300	2.2
Stainless steel tubing from column to detector flow cell 0.17 x 380 mm, Green	G1315-87311	9.4
Standard flow cell for VWD 10 mm, 14 µL, 400 bar	G1314-60082	14.0
Standard flow cell for DAD/MWD 10 mm, 13 µL, 120 bar	G1315-60022	13.0
	Total ECV	**34.6/35.6**

TABLE B: Low ECV configured Agilent 1100

Acceptable configuration for the following HALO columns: 4.6 x 150 mm, 4.6 x 100 mm, 4.6 x 75 mm, 4.6 x 50 mm, 4.6 x 30 mm, 3.0 x 150 mm, 3.0 x 100 mm, and 3.0 x 75 mm.

Description	Part No.	Volume (µL)
Needle seat capillary for standard autosampler 0.12 x 100 mm, Red	G1313-87103	1.3
Or, needle seat capillary for well-plate autosampler 0.12 x 150 mm, Red	G1367-87303	1.9
Stainless steel tubing from autosampler to column compartment heat exchanger 0.12 x 180 mm, Red	G1313-87304	2.3
Column compartment heat exchanger 3 uL		3.0
Stainless steel tubing from column compartment heat exchanger to column 0.12 x 70 mm, Red	G1316-87303	0.9
Stainless steel tubing from column to detector flow cell 0.12 x 180 mm, Red	G1313-87304	2.3
Alternate longer length stainless steel tubing from column to detector flow cell 0.12 x 280 mm, Red	01090-87610	3.6
Semi-micro flow cell for VWD 6 mm, 5 µL, 120 bar	G1314-60083	5.0
Semi-micro flow cell for DAD/MWD 6 mm, 5 µL, 120 bar	G1315-60011	5.0
	Total ECV	**14.8/16.7**

TABLE C: Ultra-low ECV configured Agilent 1100

Acceptable configuration for the following HALO columns:
4.6 x 150 mm, 4.6 x 100 mm, 4.6 x 75 mm, 4.6 x 50 mm, 4.6 x 30 mm, 3.0 x 150 mm, 3.0 x 100 mm, and 3.0 x 75 mm, 3.0 x 50 mm, 3.0 x 30 mm, 2.1 x 150 mm, 2.1 x 100 mm, 2.1 x 75 mm, 2.1 x 50 mm, 2.1 x 30 mm.

Description	Part No.	Volume (µL)
Needle seat capillary for standard autosampler 0.12 x 100 mm, Red	G1313-87103	1.3
Or, needle seat capillary for well-plate autosampler 0.12 x 150 mm, Red	G1367-87303	1.9
Stainless steel tubing from autosampler to low volume heat exchanger 0.12 x 180 mm, Red	G1313-87304	2.3
Low volume heat exchanger 1.6 uL (includes connecting tubing to column)	G1316-80002	1.6
Stainless steel tubing from column to detector flow cell 0.12 x 180 mm, Red	G1313-87304	2.3
Alternate longer length stainless steel tubing from column to detector flow cell 0.12 x 280 mm, Red	01090-87610	3.6
Micro flow cell for VWD 1 µL, 6 mm 40 bar	G1314-60081	1.0
Micro flow cell for DAD/MWD 2 µL, 3 mm 40 bar	G1315-60024	2.0
	Total ECV	**8.5/11.4**

Notes:

- The ChemStation peak width setting is optimized best by carrying out several runs under method conditions using different settings. One can choose the setting that gives the best signal to noise for all analytes, consistent with observed peak width and USP tailing factors using Performance and Noise and Extended Performance reports from the Data Analysis view of the Agilent ChemStation software
- In the autosampler, replacing the 0.17 x 100 mm needle seat capillary (Agilent part number G1313-87101, 2.5 µL) with the 0.12 x 100 mm seat capillary (Agilent part number G1313-87103, 1.1 µL) will reduce ECV and improve system efficiency slightly.

TABLE D: Recommended Agilent 1100 configurations to use with specific HALO columns

HALO Column Dimensions	Agilent 1100 Configuration
4.6 x 150 mm	Standard, Low-ECV, Ultra-low ECV
4.6 x 100 mm	Standard, Low-ECV, Ultra-low ECV
4.6 x 75 mm	Standard, Low-ECV, Ultra-low ECV
4.6 x 50 mm	Low-ECV, Ultra-low ECV
4.6 x 30 mm	Low-ECV, Ultra-low ECV
3.0 x 150 mm	Low-ECV, Ultra-low ECV
3.0 x 100 mm	Low-ECV, Ultra-low ECV
3.0 x 75 mm	Low-ECV, Ultra-low ECV
3.0 x 50 mm	Ultra-low ECV
3.0 x 30 mm	Ultra-low ECV
2.1 x 150 mm	Ultra-low ECV
2.1 x 100 mm	Ultra-low ECV
2.1 x 75 mm	Ultra-low ECV
2.1 x 50 mm	Ultra-low ECV
2.1 x 30 mm	Ultra-low ECV

factory), a low ECV configuration, and an ultra-low ECV configuration. See Tables A, B and C for the three different Agilent 1100 configurations we recommend for specific HALO column dimensions.

Modifying Your Agilent 1100 System

Modifying your Agilent 1100 system from the standard configuration to either the low ECV or ultra-low ECV configuration is quite simple, once you have the parts on hand. The Agilent part numbers needed to make the modifications are listed in Tables A, B and C. Substitution of similar sized capillary tubing from a source other than Agilent is generally okay, but we recommend that the replacement low volume heat exchanger and detector flow cells come from Agilent to ensure compatibility.

Step 1: Replace the standard capillary tubing (0.17 x 180 mm, green, G1313-87305) connecting the autosampler valve to the heat exchanger in the column compartment with the low volume capillary tubing (0.12 x 180 mm, red, G1313-87304). For ultra-low ECV configuration connect the outlet of the capillary tubing to the inlet union of the low volume heat exchanger (G1316-80002). Position the low volume heat exchanger in one of the wells of the column compartment heat exchanger block, and secure it using one of the column clips as needed. *See Figure 4.*

Step 2: Replace the standard capillary tubing (0.17 x 90 mm, green, G1316-87300) connecting the heat exchanger to the column with the low volume capillary tubing (0.12 x 70 mm, red, G1316-87303). For the ultra-low ECV configuration, connect the outlet tubing of the low volume heat exchanger (G1316-80002) directly to the column inlet using an appropriate stainless steel, PEEK or polyketone fitting. *See Figure 4.*

Step 3: Replace the standard capillary tubing (0.17 x 380 mm, green, G1315-87311) connecting the column to the detector flow cell with the low volume capillary tubing (0.12 x 180 mm, red, G1313-87304) or the alternate longer capillary tubing (0.12 x 280 mm, red, 01090-87610). *See Figure 4.* Note: Do not connect the tubing to the detector until the column and tubing have been flushed.

Step 4: Prime the instrument and flush the tubing and column with either 100% methanol or acetonitrile. Some people find they achieve faster equilibration by first flushing the capillary tubing before connecting the column and flushing it.

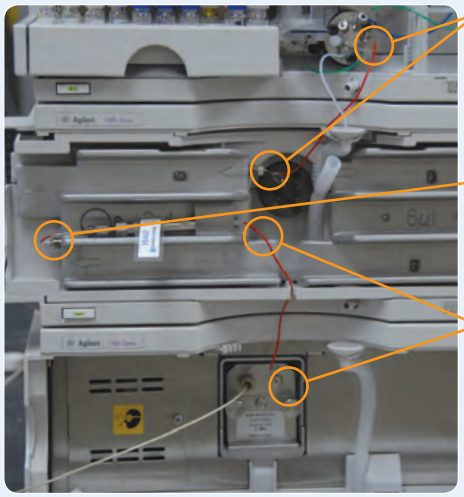
Step 5: Replace the standard detector flow cell with either a semi-micro flow cell (low ECV) or a micro flow cell (ultra-low ECV). *See Figure 5.*

Step 6: Connect the capillary tubing coming from the outlet of the HALO column to the detector flow cell. For diode-array and multiwavelength detectors the capillary tubing is normally connected to a zero dead volume union, which then is connected to the capillary tubing connected into the flow cell body. For 2.1 mm HALO columns in shorter lengths (30, 50, 75 mm) it is beneficial to connect the column directly to the capillary inlet tubing of the flow cell to minimize extracolumn volume and extra connections. It may be necessary to stack the column compartment directly above the DAD/MWD detector in such situations.

Step 7: Pump 100% methanol or acetonitrile through the tubing, column and the detector flow cell for ~ 5 minutes or until you observe stable pressure and baseline.

You should observe a flat baseline at 25-50 mAUFS with a UV detector, and can now proceed to equilibrate your column with your mobile phase and begin running samples.

FIGURE 4: Replace standard capillary tubing (0.17 mm, green) with low volume capillary tubing (0.12 mm, red).



Step 1: Replace capillary tubing from sample injector valve to heat exchanger (3 µL) in the column compartment.

Step 2: Replace capillary tubing connecting the heat exchanger to the column.

Step 3: Replace capillary tubing connecting the column to the detector flow cell.

FIGURE 5: Replace standard detector flow cell with either a semi-micro flow cell (low ECV configuration) or a micro flow cell (ultra-low ECV configuration).

Step 5: Remove standard detector flow cell and replace with a semi-micro flow cell or a micro flow cell.

Gradient Separations

All of the considerations and recommendations about reducing extra column band broadening which have been made thus far in this technical report have dealt with isocratic separations. If you are developing a new gradient method or translating an existing gradient method to a HALO column, the most important parameters are the same ones that have been discussed for isocratic separations. However, there are two major differences.

1. The extra column volume *before* the column is less important than the extra column volume *after* the column when using a gradient, and,
2. the delay volume (system volume from the point where solvents A and B are mixed up to the column itself) is important because it is a major contributor to the "hold" time at the beginning of a gradient.

Extra column volume before the column is less important for gradient separations, because there will be a refocusing of the analytes at the head of the column (unless pre-elution of polar analytes occurs) in a gradient separation. Consequently, any dispersion of the sample band after injection and before the column is usually minimized or eliminated, if the injection volume is not too large and the starting mobile phase is not too strong. However, it continues to be important to minimize extra column band broadening after the column (tubing and flow cell volume, and Chemstation Peak Width setting) for gradient separations when using low-volume, high efficiency HALO columns.

If you plan to only run gradient separations with your Agilent 1100, you may be able to skip Steps 1 and 2 when modifying the equipment.

For gradient separations the delay volume is also important because it imposes an isocratic hold to the start of the gradient program, such that the desired mobile phase composition reaches the column at a time later than programmed by a factor of V_d/F, where V_d is the delay volume and F is the volumetric flow rate. For a discussion of the translation of gradient methods please see our Quick Tips Guide http://www.mac-mod.com/QTpdf.

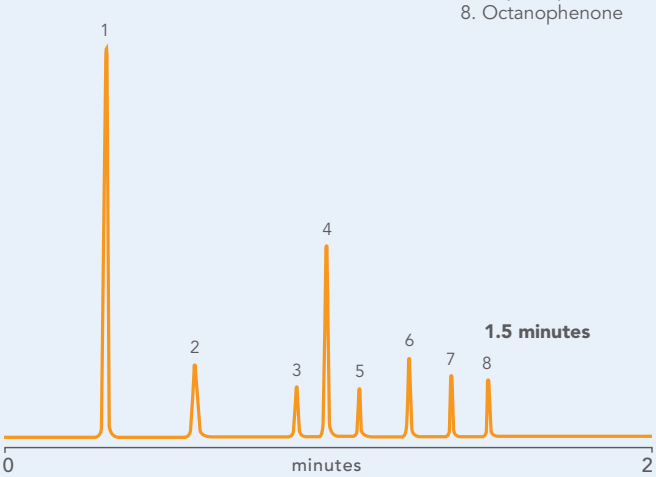
Conclusion

Figure 6 illustrates the benefits of modifying an Agilent 1100 to an ultra-low ECV configuration. Compared to a conventional column, analysis time is reduced by over 90% while resolution is maintained. This performance compares favorably with UHPLC, but is accomplished with a conventional Agilent 1100 quaternary system by making a few simple, and inexpensive, modifications to the equipment.

FIGURE 6: An ultra-low ECV configured Agilent 1100 using a HALO column provides performance that compares favorably to UHPLC systems.

Conditions
Column: HALO C18, 3.0 x 50 mm
Mobile Phase: 35% acetonitrile to 90% acetonitrile in 1 minute
Flow Rate: 2.0 mL/min.
Column Temperature: 40° C
Pressure: 304 bar —> 167 bar
Response Time: < 0.125 sec

Sample:
1. Acetophenone
2. Propiophenone
3. Butyrophenone
4. Benzophenone
5. Valerophenone
6. Hexanophenone
7. Heptanophenone
8. Octanophenone



An ultra-low ECV configured Agilent 1100 quarternary system using a high efficiency HALO column (Fused-Core particles) separates these 8 alkylphenones in 90 seconds with ample resolution between all peaks. This speed and resolution compare favorably to the performance reported by chromatographers using UHPLC equipment and sub-2 µm columns. The pressure generated by the HALO column is well within the acceptable operating limits of the Agilent 1100.

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