Agilent 1200 Series Rapid Resolution LC and Rapid Resolution LC/MS Optimization Guide

Agilent Technologies

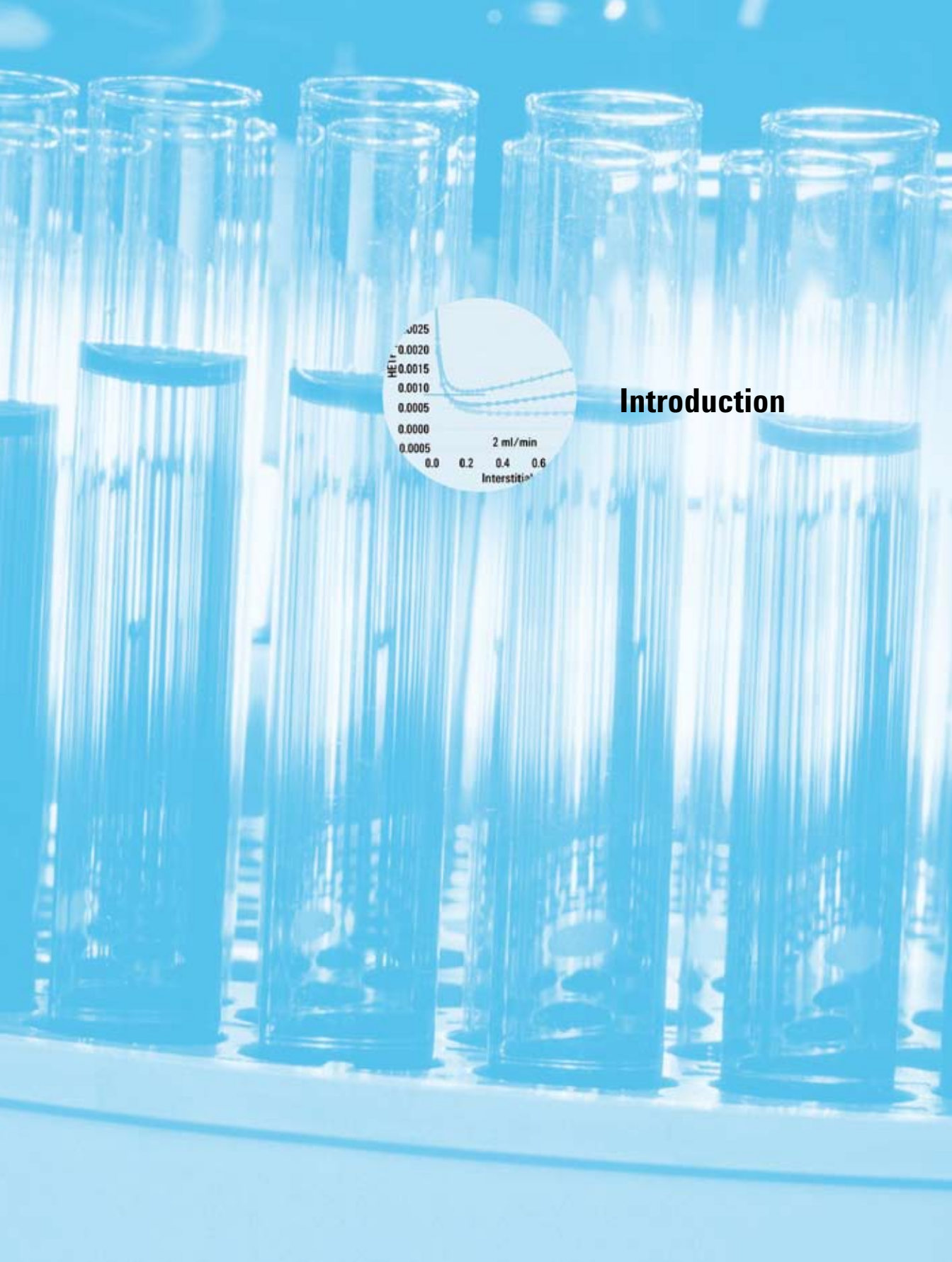
Authors:

Angelika Gratzfeld-Huesgen,

Michael Frank,

Christian Gotenfels

Table of Contents



Introduction

1

1. Theory of using smaller particles in liquid chromatography

In 2003 Agilent introduced the first commercially available porous silica columns with 1.8 µm particles. In combination with the 1200 Series Rapid Resolution LC system these RRHT columns (1.8 µm) can be used for two main objectives:

1. Faster Chromatography

   Short columns with sub-2-micron particles offer the opportunity to dramatically reduce analysis time by increasing the flow rate without losing separation performance.

2. Higher Resolution

   Long columns with sub-2-micron provide higher efficiency and therefore higher resolution, which is required for the separation of complex samples.

Separation efficiency in HPLC can be described by the van Deemter Equation (Figure 1 on page 7). This results from the plate height model used to measure the dispersion of analytes as they move down the column. H is the Height Equivalent to a Theoretical Plate, d_p is the particle size of the column packing material, u_0 is the linear velocity of the mobile phase and A, B and C are constants related to the different dispersive forces. The A term relates to eddy diffusion or multiple flow paths through the column; B relates to molecular diffusion along the column axis (longitudinal); C relates to mass transfer of the analyte between the mobile and stationary phases. The separation is at its most efficient when H is at a minimum.

The effect of each individual term and the combined equation are shown in Figure 2 on page 7 where the plate height is plotted against the linear flow rate.

$$H = A\,(d_p) + B/u_o + C u_o\,(d_p^2)$$

Figure 1 Van Deemter Equation

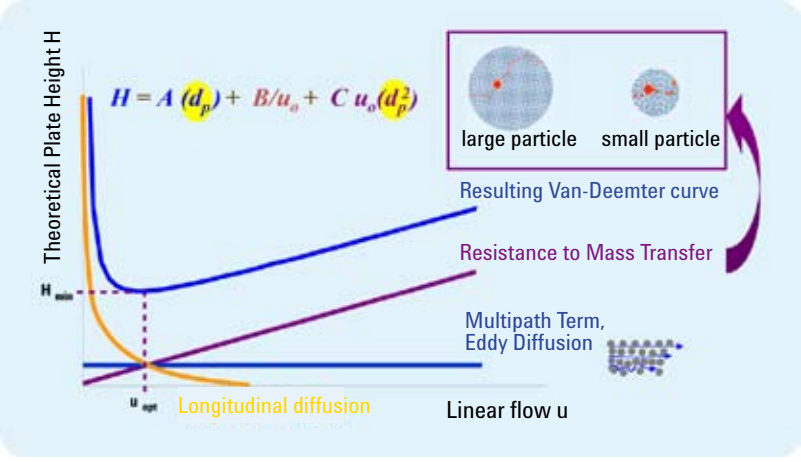


Figure 2 A hypothetical Van Deemter curve

The van Deemter plots in Figure 3 on page 8 show that reducing particle size increases efficiency. Switching from commonly used 3.5 µm and 5.0 µm particle sizes to 1.8 µm particles offers significant performance improvements. The 1.8 µm particles give two to three times lower plate height values and proportionately higher efficiencies. This allows a shorter column to be used without sacrificing resolution and hence the analysis time is also reduced by a factor of two to three.

The increased efficiency is derived to a large extent from the reduction in multiple flow paths as a result of the smaller particles - this leads to a smaller A term (eddy diffusion). In

addition, smaller particles mean shorter mass transfer times, reducing the C term, and hence there is a much reduced loss of efficiency as the flow rate increases. This means the separation can be further accelerated by increasing the flow rates without significantly reducing efficiency.

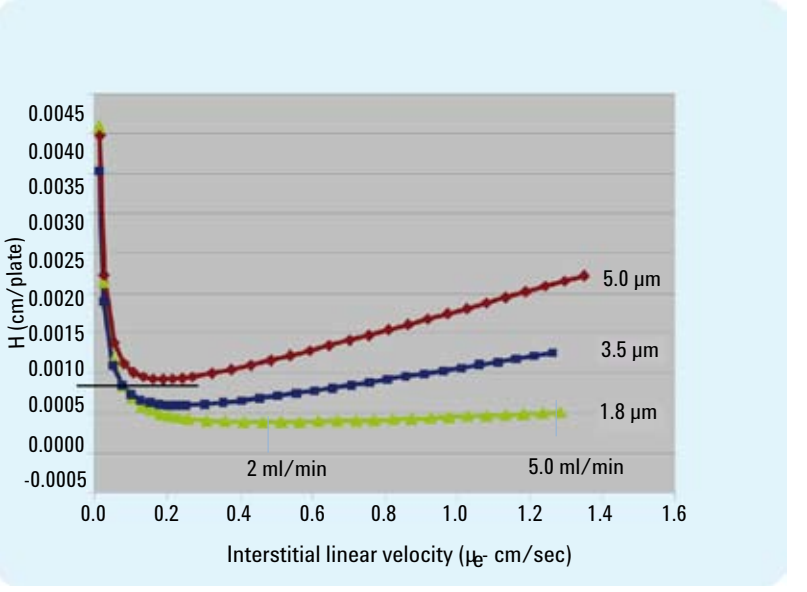


Figure 3 Van Deemter curve for different particle sizes

A chromatographic separation can be optimized based on physical parameters of the HPLC column such as particle size, pore size, morphology of the particles, the length and diameter of the column, the solvent velocity, and the temperature. In addition, the thermodynamics of a separation can be considered; the properties of the solute and the stationary and mobile phases (percentage of organic solvent, ion strength, and pH) can be manipulated to achieve the shortest possible retention and highest selectivity[2].

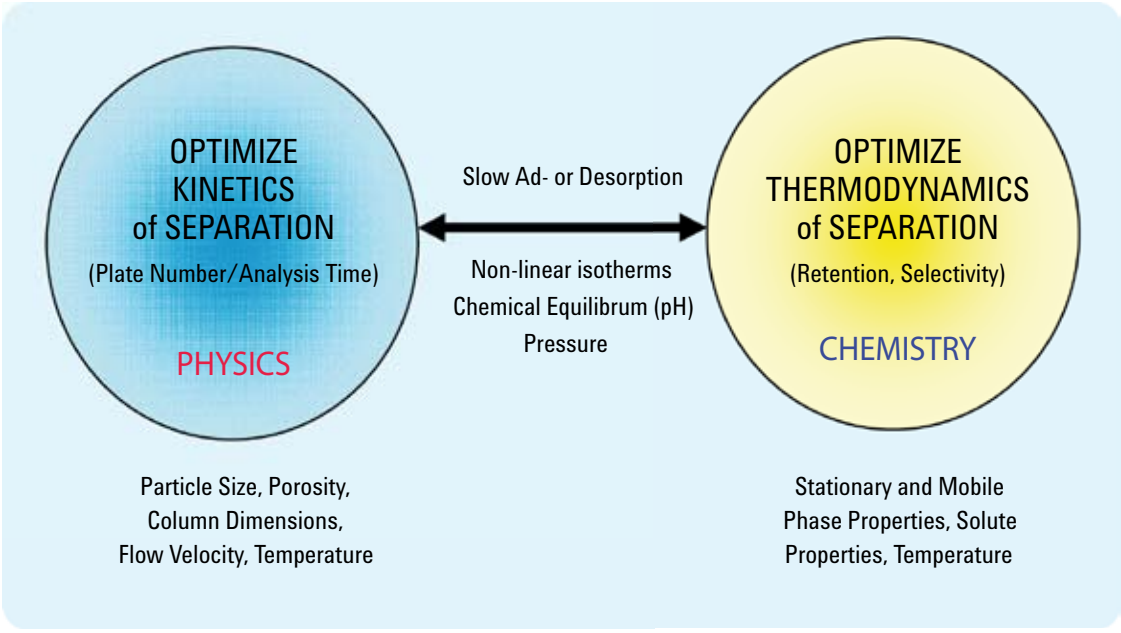


Figure 4 Selecting optimal conditions for HPLC

Nevertheless resolution is function of three parameters:

- column efficiency or theoretical plates (N),
- selectivity (α),
- retention factor (k).

According to the resolution equation (Figure 5 on page 10), the selectivity has the biggest impact on resolution (Figure 6 on page 10). This means that the selection of appropriate mobile and stationary phase properties and temperatures is critical for successful separation.

$$R_s = \frac{\sqrt{N}}{4} \cdot \left[\frac{\alpha - 1}{\alpha}\right] \cdot \left[\frac{k_2'}{k_2' + 1}\right]$$

Figure 5 Resolution equation

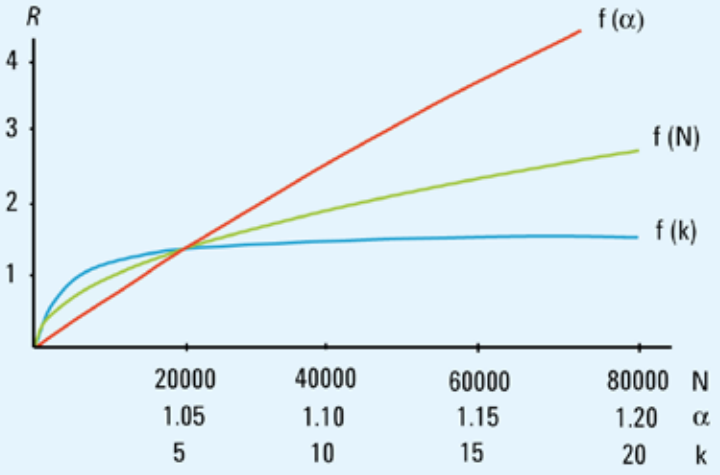


Figure 6 Effect of plate number, separation factor and retention factor on R

No matter whether the rapid resolution separation method is being newly developed or transferred from an existing conventional method, it is clearly beneficial to have a wide choice of stationary phase chemistries available in a range of column formats.

Agilent offers more than 140 ZORBAX 1.8 µm Rapid Resolution High Throughput (RRHT) columns in 14 selectivity choices, from 15 to 150 mm in length, and with internal diameters from 2.1 to 4.6 mm. This enables the optimum stationary phase to be selected so that the selectivity is maximized. The resolution, flow rate and analysis time can be optimized by selecting the appropriate column length and diameter.

Many laboratories perform an extensive screening process to select the best combination of stationary phase, mobile phase and temperature for their separations. The Agilent 1200 Series RRLC Method Development Solution facilitates complete automation of this time consuming selection process – making method development and method transfer an easy and reliable task.

ZORBAX 1.8 µm RRHT columns use the same chemistry as ZORBAX columns with 3.5 and 5 µm particles. As a result, for any particular ZORBAX phase, the 5.0, 3.5 and 1.8 µm particles provide identical selectivity, which allows easy, fast and secure bidirectional method transfer between conventional LC, Rapid Resolution LC and preparative LC.

1

1.1. Benefits of columns packed with sub-2-micron particles

1.1.1. Faster chromatography

There are several advantages of having shorter run times. High Throughput labs now have higher capacity and can analyze more samples in less time. More samples in less time also means lower costs. For example, by reducing the analysis time from 20 min per sample to 5 min, the cost for 700 samples is reduced by 79 % (Table 1 on page 12).

Table 1 Time and cost savings over 700 runs

Cycle time	20 min cycle time	5 min cycle time
Runs	700	700
Approx. costs/analysis[1]	$ 10.58	$ 2.24
Approx. cost/700runs[1]	$ 7400	$ 1570
Cost savings	-	$ 5830
Time[2]	10 days	2.5 days

1 solvents = $ 27/l, disposal = $ 2/l, labor = $ 30/h

2 24 hours/day

The Agilent cost savings calculator provides an easy way to calculate the cost savings by switching from conventional HPLC to Rapid Resolution LC. The results are presented graphically and in tabular form.

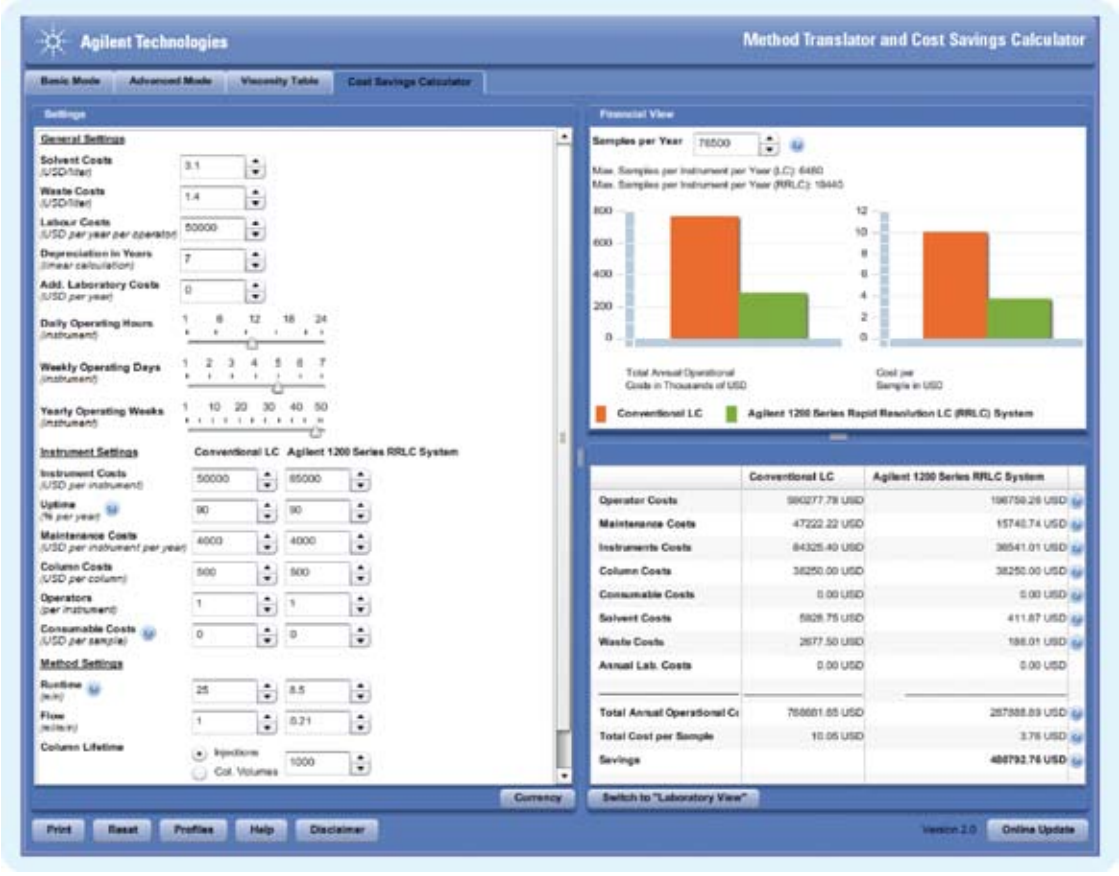


Figure 7 Cost savings calculator

Shorter run times also deliver faster answers. This is important in process control and rapid release testing. Instead of waiting hours to release a single batch of a drug, all the system suitability, calibration and sample analysis can now be done in less than an hour. Rapid answers are also important for synthetic chemists using open access LC/MS systems for compound confirmation and

reaction control. Shorter run times can also accelerate the method development process significantly.

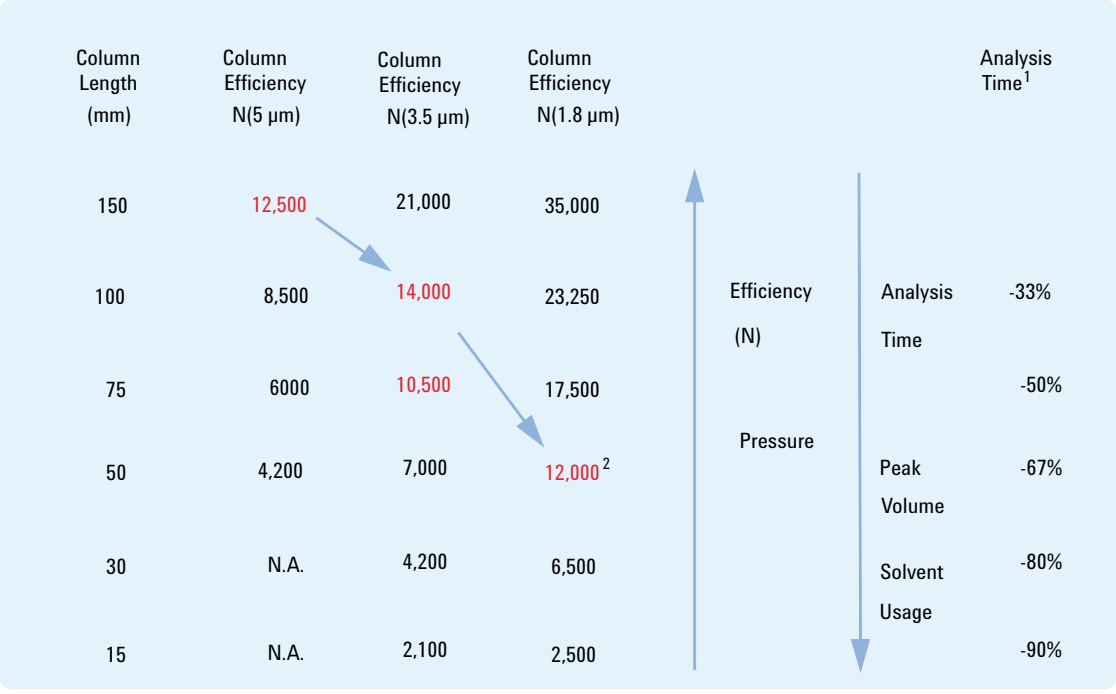


Figure 8 Relation between particle size, efficiency and analysis time

1 Reduction in analysis time compared to 150 mm column; all columns 4.6 mm i.d.

2 Shorter columns with small particles provide the efficiency of longer columns with larger particles

1.1.2. Higher resolution

Long columns packed with smaller particles result in higher efficiency and higher resolution. This is important for analysis of complex samples from metabolomics or proteomics studies. Also, applications such as impurity profiling can benefit from higher separation power. Even the LC/MS analysis of drugs in biological fluids can benefit from the higher peak capacity, because of the reduced interference from ion suppression. In general, higher separation power provides more confidence in the analytical results.

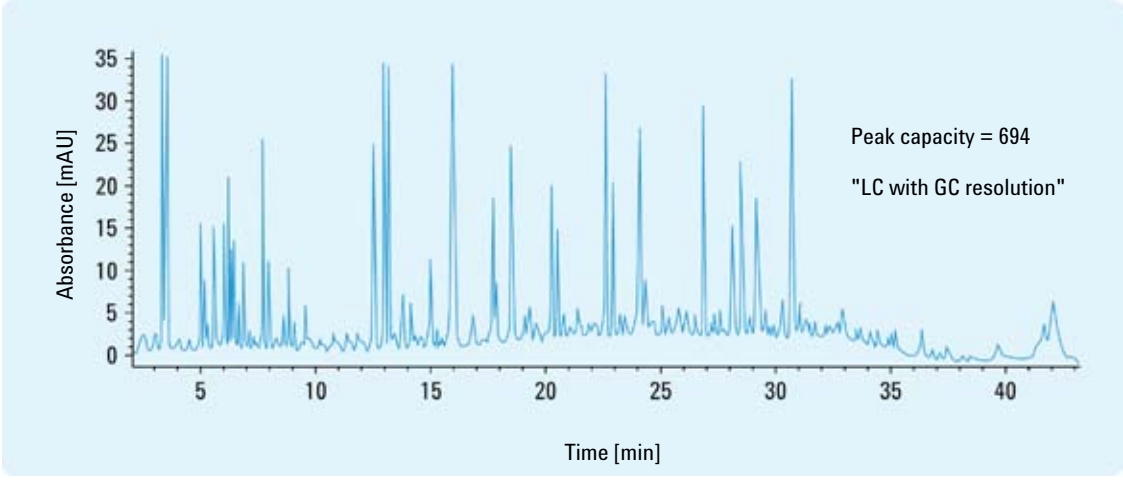


Figure 9 Peak capacities of more than 700 can be achieved using a ZORBAX RRHT SB-C18 column (2.1 x 150 mm, 1.8 µm) to analyze a tryptic digest of BSA

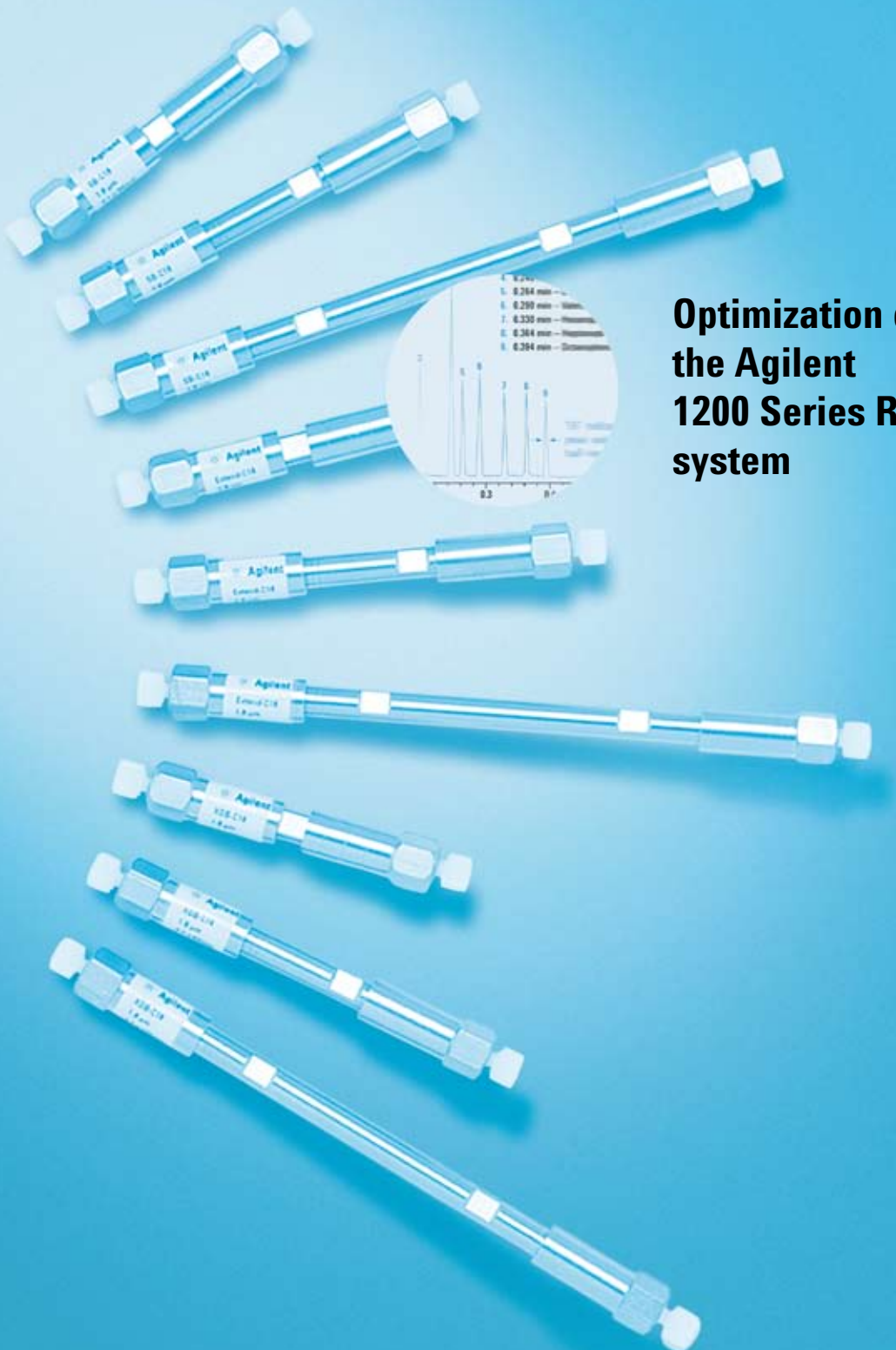
1.2. Frictional heating

Forcing mobile phase through the column at higher pressure and higher flow rates generates heat. The resulting temperature gradients (radial and longitudinal) have an impact on the column efficiency. [9, 10]

$$Power = F \cdot p$$

F	Flow rate
p	pressure

Powerful column thermostatting (for example, using a water bath) generates a strong radial temperature gradient, which leads to significant loss in column efficiency. Still-air-column thermostatting reduces the radial temperature gradient and therefore reduces the efficiency losses, but a higher column outlet temperature has to be accepted. At lower back-pressure, performance losses due to frictional heat are minimized so that 4.6/3 mm inner diameter sub-2-micron columns still deliver superior efficiencies compared with the respective 2.1 mm inner diameter columns.



Optimization of the Agilent 1200 Series RRLC system

2. Design of the Rapid Resolution LC system

The design concept of the 1200 Rapid Resolution LC (RRLC) System was to provide a liquid chromatograph offering ultra fast and high resolution separation capability and yet which retained full functionality for standard HPLC applications. This section looks at how this concept has been implemented to offer the highest flexibility with respect to column dimensions and applications.

The use of sub-two micron (STM) particles means that for high flowrates or long columns additional pressure is required to drive the mobile phase through the column. The RRLC flowpath is optimized to produce minimal backpressure and ZORBAX RRHT columns have an engineered particle size distribution that produces significantly less backpressure than other STM columns. High temperature, up to 100°C on certain columns, allows more selectivity flexibility and reduces solvent viscosity to allow even faster separation. High flow rates up to 5 ml/min can be used for ultra-fast separations. The adjustable delay volume fully supports 2.1 to 4.6 mm i.d. columns. A low dispersion tubing kit and low volume flow cells minimize peak dispersion for narrow bore columns. Detectors with high data rates preserve the resolution of very fast peaks eluting from the RRLC.

2.1. Delay volume and extra-column volume

The delay volume is defined as the system volume between the point of mixing in the pump and the top of the column. In gradient separation, this volume causes a delay in the mixture reaching the column which effectively means there is an initial isocratic segment in the gradient profile. This becomes more significant at low flow rates and can have a large impact on the transferability of gradient methods. Small delay volumes are important, therefore, for fast gradient separations, especially

with narrow bore columns (2.1 mm i.d.) as often used with mass spectrometric detection.

The extra-column volume is defined as the volume between the effective injection point and the effective detection point, excluding the volume fraction of the column containing the stationary phase. Smaller diameter columns require smaller extra-column volumes to keep peak dispersion at a minimum.

2.1.1. Optimized configuration for 2.1 mm i.d. columns

In the low delay volume configuration of the Agilent 1200 Binary Pump SL the damper and mixer are bypassed to reduce the pump delay volume to about 120 µl. Figure 10 on page 20 shows the flow path connections for this configuration. This provides the shortest gradient delay for ultra-fast gradient separations. In order to take full advantage of the electronic damping control which replaces the physical volume damping it is important to select the respective „enhanced solvent compressibility“ function in the auxiliary screen of the pump menu.

To minimize peak dispersion the low dispersion kit (**G1316-68744**) must be installed. This kit includes short 0.12 mm i.d. capillaries and low volume heat exchangers (1.6 µl and 1.5 µl) for the thermostatic column compartment (Figure 14 on page 24). To maintain resolution in the UV detector a low volume flow cell should be used (e.g., the 2 µl micro flow cell for the diode-array detector. See Figure 24 on page 46 for flow cell recommendations).

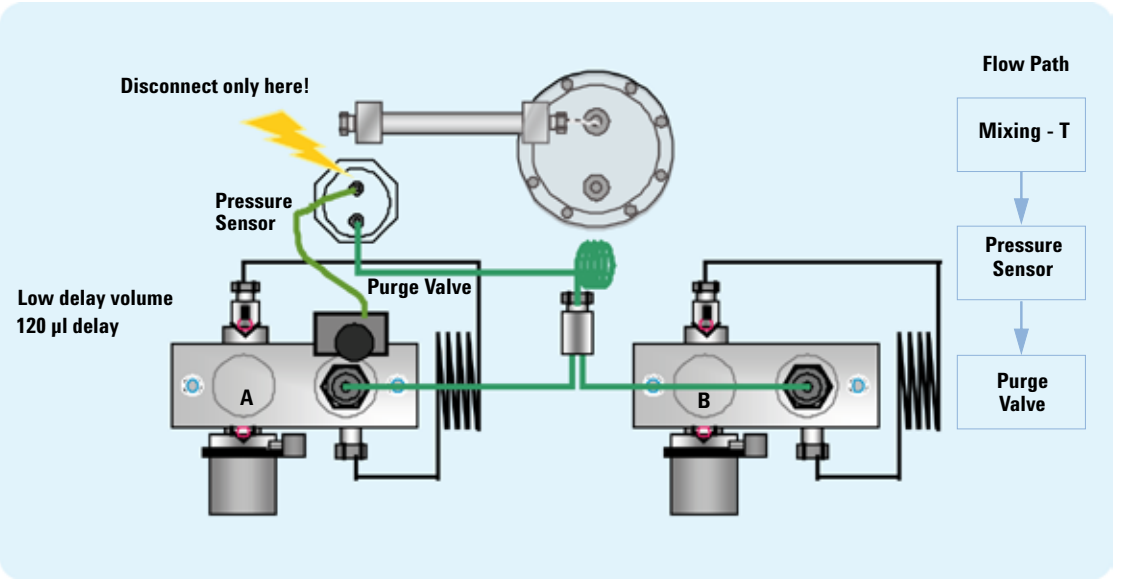


Figure 10 Low delay configuration for 2.1 mm inner diameter columns

It is important to remember to set the correct parameter in the pump auxiliary screen. This ensures that the correct compressibility values are always applied for the mobile phases used. Calibration curves are available for most common solvents.

For high sensitivity UV applications an additional 200 µl mixer (**part number 5067-1565**) can be installed to reduce any residual mixing noise. This small mixer gives the lowest UV baseline noise even under extreme gradient conditions. See Figure 11 on page 21.

The delay volume in the Agilent 1200 Series High-Performance Autosampler SL Plus can be reduced by as much as 140 µl by switching the injection valve from the mainpass position to the bypass position once the injected sample has been flushed onto the column. In practice this can be done a few seconds after injection and is activated by selecting the “Automatic Delay

Volume Reduction (ADVR)“ function in the autosampler setup menu. This functionality should not be used for carry-over sensitive applications (see page 29).

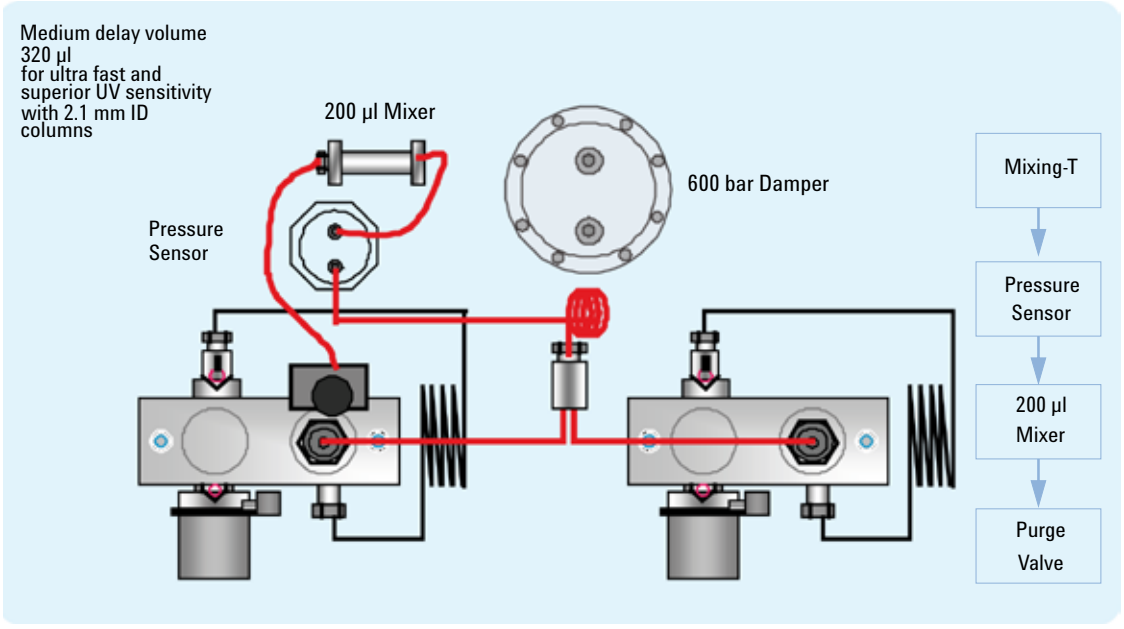


Figure 11 Medium delay volume configuration for 2.1 mm ID columns with highest UV sensitivity

2.1.2. Optimized configuration for 3 and 4.6 mm i.d. columns

The relative column volumes for 3 mm and 4.6 mm inner diameter columns are about two and five times larger respectively than for the same length 2.1 mm i.d. columns and the flow rates used are also proportionally higher. Therefore, the standard binary pump delay volume will not result in a significantly higher gradient delay.

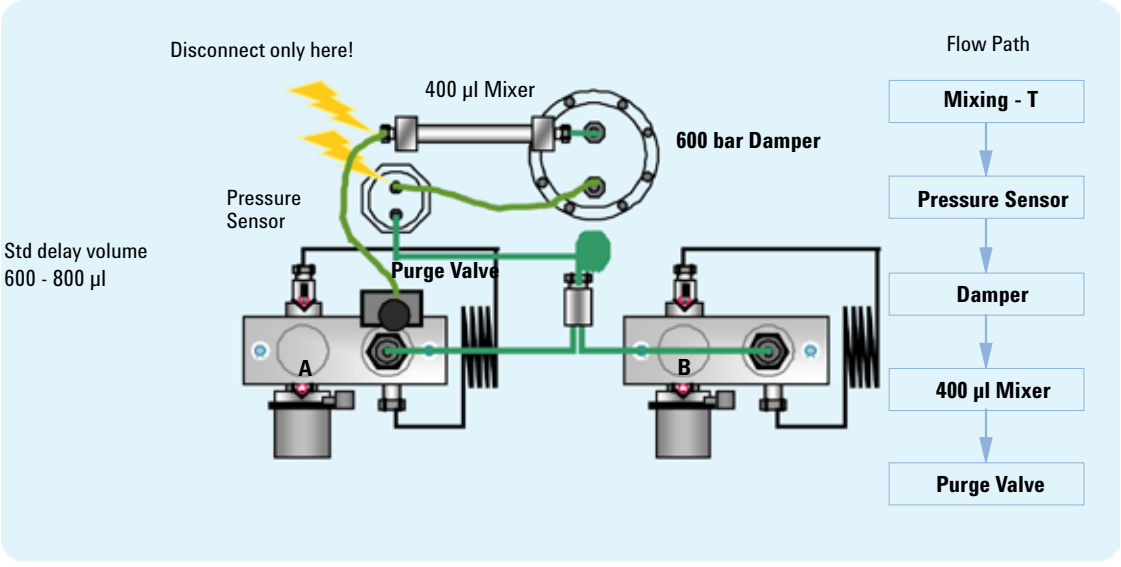


Figure 12 Standard delay volume configuration for 3 and 4.6 mm ID columns with highest UV sensitivity

The standard delay volume configuration is also the configuration which provides direct method transferability from the Agilent 1100 and 1200 Series LC system to the 1200 Series Rapid Resolution LC system or vice versa. The delay volumes are the same and so no adjustment of the gradient is necessary. This is illustrated by an example chromatogram from a transferred method in Figure 13 on page 23.

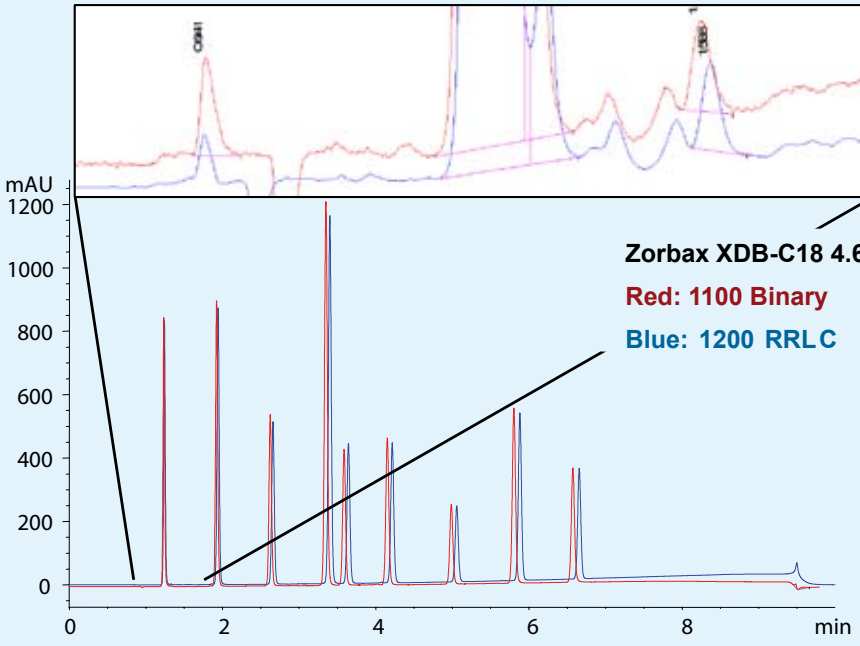


Figure 13 Comparison RRLC and standard binary LC system RRLC and HPLC on one Configuration

2.1.3. Automatic switching of delay volume

Switching between configurations can be done in two ways:

- manually, by disconnecting and reconnecting capillaries
- automatically, using a 600 bar 2PS/6PT valve (optional)

Further design features and benefits are:

- A practical flow rate range from 0.05 up to 5 ml/min up to 600 bar can be used.
- A low dispersion kit for 2.1 mm inner diameter columns is available for use with narrow-bore columns.
- The 1200 Series High Performance Autosampler SL Plus (G1367D) provides short cycle times, about 17 seconds for a 1 µl injection without a wash. The injection volume ranges from 0.1 up to 40 µl and be extended for higher volumes.
- In the 1200 Series Thermostatted Column Compartment SL (G1316B) (Figure 14 on page 24), different heater (1.6 µl) and cooling elements (1.5 µl) for low extra-column volume can be installed. The temperature is adjustable from 10 °C below ambient up to 100 °C.

Figure 14 The Thermostatted Column Compartment SL with small heater and cooler installed

Further:

- Different UV detector flow cells for use with 2.1, 3.0 and 4.6 mm inner diameter columns are available
- Fast UV and MS detectors with data rates up to 160 Hz (1200 Series VWD SL Plus), 80 Hz (1200 Series DAD SL, MWD SL) and up to 40 Hz for MS application are available
- The 1200 Series fluorescence detector (FLD) with 37 Hz data acquisition, and the 1200 Series refractive index detector (RID) are also compatible with the Agilent 1200 Series RRLC System.

A stepwise upgrade from 1100 Series to 1200 Series RRLC is possible.[3,26]

2

2.2. Shorter cycle times using a second pump and switching valve for column regeneration

Selecting a column

Shorter cycle times can be achieved by selecting a short column with good selectivity. The column dimensions are also determined by the detection system that is used. For MS applications, a good choice is a column with 2.1 mm inner diameter and a flow rate up to 1.5 ml/min. Using these conditions, no flow splitter is necessary in front of most mass spectrometers. For UV detection, 3.0 mm inner diameter columns are ideal, because here the highest linear velocities can be obtained. With 4.6 mm inner diameter columns, high linear velocities can also be reached, but the maximum flow rate is limited to 5 ml/min.

Selecting the optimum instrument configuration

The pump should be used in its standard delay volume configuration (Figure 10 on page 20) for 4.6 mm inner diameter and 3.0 mm inner diameter columns. For 2.1 mm inner diameter columns, the low delay volume configuration should be used. In addition, when using 2.1 mm inner diameter columns, the low dispersion kit should be installed to provide lowest extra-column volume. The tubing to the MS should be as short as possible, and of small internal diameter, such as that delivered with the low dispersion kit. For highest UV sensitivity, it is recommended in addition to use the short mixer. (Part number 5067-1565).

Even shorter cycle times can be achieved by using a column regeneration valve in combination with a regeneration pump, as shown in Figure 15 on page 27. Using this setup, the regeneration of the column used previously takes place during the analysis on the second column. This shortens cycle time significantly, see Figure 17 on page 29.

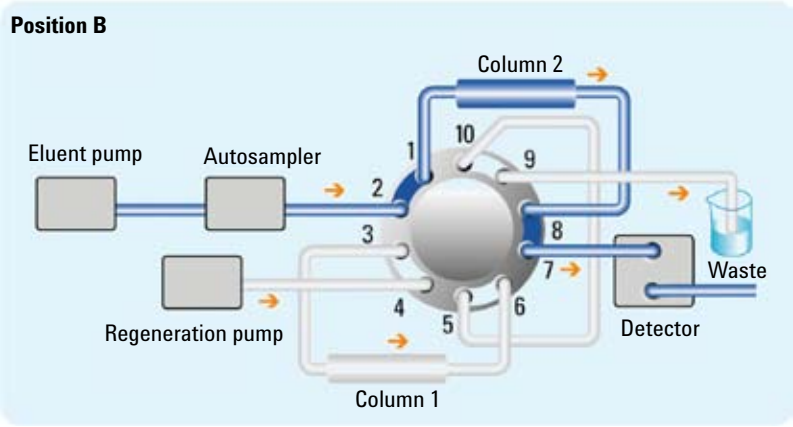


Figure 15 Alternating column regeneration using a 2-position 10-port valve and a second regeneration pump

Using two columns, two pumps and one 2-position 10-port valve allows switching between these columns for shortest cycle times from injection to injection. Typically, columns of the same chemistry and the same batch provide a retention time precision that allows data processing using the same calibration table, Figure 16 on page 28.[23].

Also, the stability over several thousand runs is maintained if precautions are taken against blockage of frits due to particles in the sample or mobile phase (algae or/and bacteria).

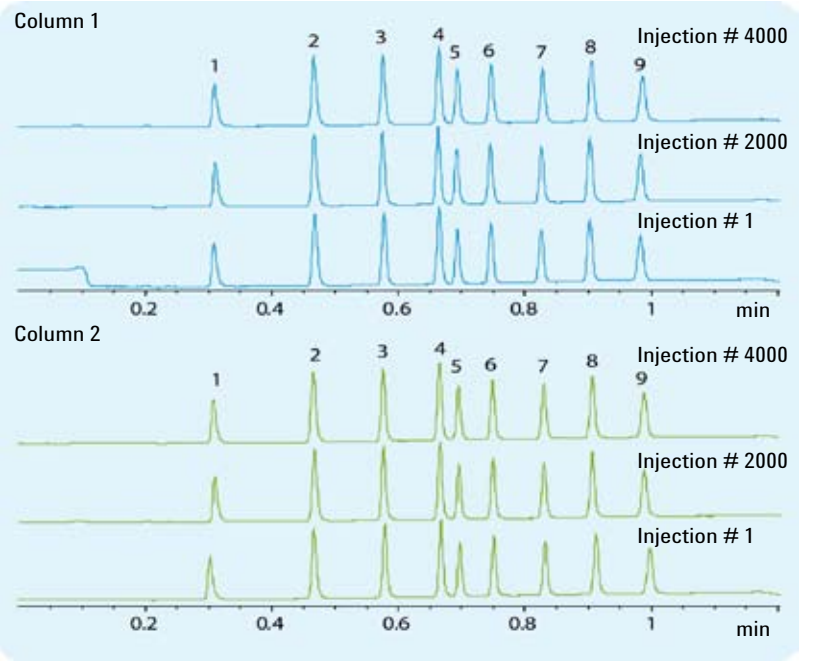


Figure 16 Comparison of the UV chromatograms at 245 nm, 2.3 ml/min and 32 °C using alternating column regeneration. The injections # 1, # 2000 and # 4000 are shown for a) column 1 and b) column 2. Compounds: Alkylphenones test mixture. System configuration with column regeneration.

Figure 17 on page 29 shows the influence of sample throughput and instrument configuration and setup. Highest throughput is achieved with automated column regeneration.

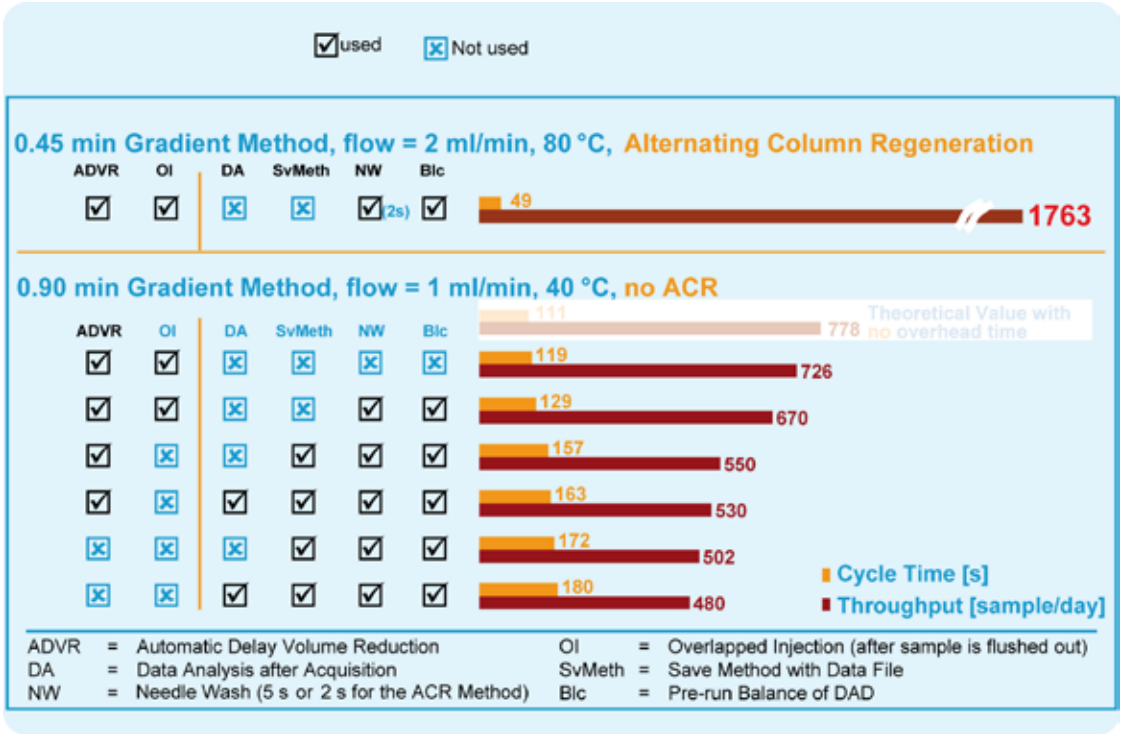


Figure 17 Cycle times using different instrument set up

The cycle time was determined as the mean of five runs from the data file creation time stamp in Windows™. Influence of “DA”(data analysis after acquisition) and “SvMeth”(save method with data file) might be dependent on the actual computer and any additional LC/MS hardware.

The Agilent 1200 Series RRLC System autosampler offers the possibility of performing overlapped injections (OI) and/or automatic delay volume reduction (ADVR). This means that the injection valve is switched out of the flow path after the sample has reached the top of the column. This reduces the delay volume significantly, see Figure 18 on page 30.

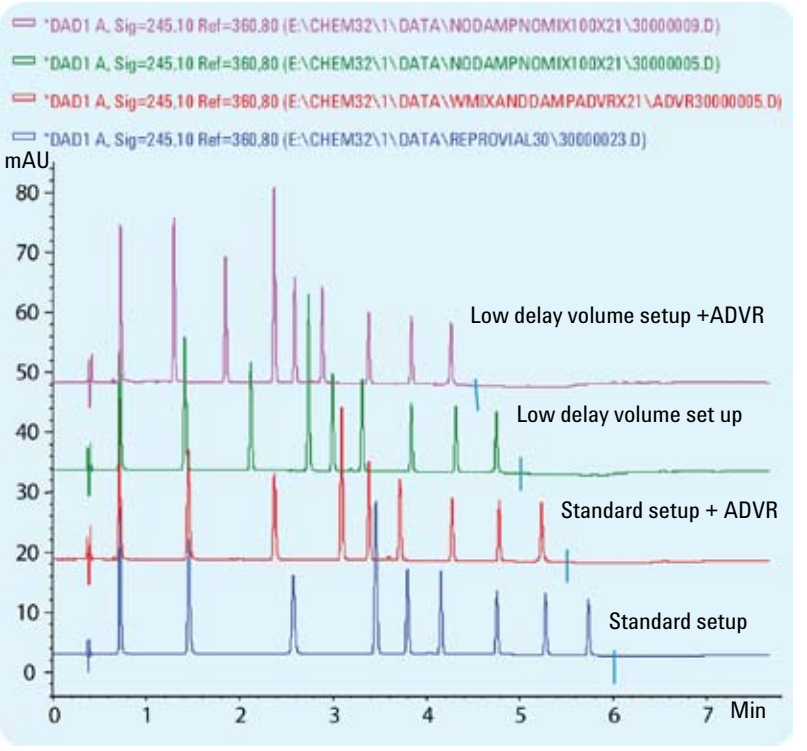


Figure 18 Reduction of the delay volume

Test Sample	Set of nine compounds, 100 ng/ul each, dissolved in Water/ Acetonitrile (65/35) **1** Acetanilide **2** Acetophenone **3** Propiophenone **4** Butyrophenone **5** Benzophenone **6** Valerophenone **7** Hexanophenone **8** Heptanophenone **9** Octanophenone
Column	100 x 2.1 mm Zorbax SB C-18, 1.8 µm for 600 bar operation
Pump	Standard and low delay volume configuration Solvent A: H2O + Solvent B: ACN Gradient: 35 to 95% B in 5 min, hold over 1 min, Stoptime 8 min , Posttime 5 min Flow Rate: 0.6 ml/min
Autosampler	Injection volume: 3 µl with and without automatic delay volume reduction (ADVR) Wash 10 sec for exterior of needle
Thermostatted Column Compartment	Temperature: 50 °C
Detector	2 µl cell, 20 Hz Data Acquisition rate, Peak width = >0.01 min, Slit: 8 nm, Signal: 245/10 nm Ref 360/80 nm

The lower the flow rate, the greater the negative impact that can be expected from delay volume. In Figure 18 on page 30, a 2.1 mm inner diameter column was used at a flow rate of 0.6 ml/min. The delay volume is reduced step by step from the lowest trace to the top trace. The influence on total run time, and the impact, especially on peak width and the heights of the first peaks, is obvious.

The drawback of overlapped injection and automatic delay volume reduction is that the autosampler is not in the flow path for the

complete run time. For very sticky compounds this could lead to higher carry-over and/or compound discrimination.

Carry-over is the percentage of compound that remains in the parts of the instrument that come into contact with the sample, and is not flushed onto the column for analysis. It also means that this percentage is lost for quantitative measurement; it is discriminated. The carry-over can be measured by injecting pure solvent after the sample run is finished. Discrimination and carry-over can become even more important if the analyte compounds are non-polar and the start of the gradient contains a high percentage of water. In the worst case, the non-polar compound precipitates at the surface of contact. Small plugs of, for example, dimethylsulfoxide before and after the sample plug can help to minimize this problem.

For overlapped injection or automated delay volume reduction, the time before the injection valve is switched to the bypass mode should be increased using the flush out factor to 20. This extends the time during which the autosampler delay volume is flushed with mobile phase.

The flow-through design of the Agilent 1200 Series autosamplers is capable of maintaining carry-over below or close to the limit of detection for many compounds. Nevertheless, some compounds lead to more carry-over than others and therefore require special precautions. Systems with PEEK tubing have demonstrated very low carry-over effects for many of these difficult compounds. These systems typically exhibit carry-over of about 0.005 %. However, PEEK has a typical upper pressure limit of 220 bars, making it unsuitable for use with sub-2-micron particle column materials that generate higher system back-pressures. Challenging carry-over applications have been addressed with the new Agilent High Performance Autosampler SL Plus (G1367D). The most significant design changes are the development of a new injection needle, needle seat, needle seat cleaning and fittings. Further, a new treatment for all flow capillaries has been developed to passivate any active sites on the inner surfaces. This autosampler offers

- significantly lower carry-over, typically < 0.001 to 0.004 % depending on the compound properties.
- significantly lower delay volume of 140 µl.
- injection volume range from 0.1 to 40 µl with enhanced precision from 1 to 5 µl.

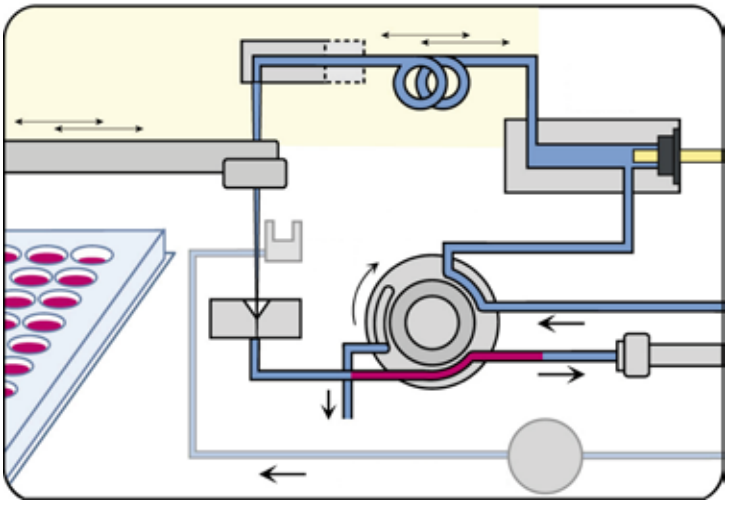


Figure 19 Autosampler Flow-Through Design - Injecting in main pass

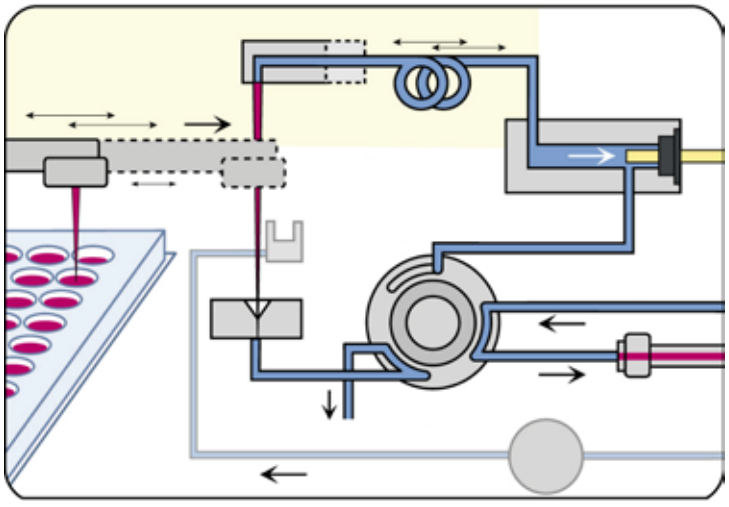


Figure 20 Autosampler Flow-Through Design,
Overlapped injection / Delay volume reduction

Flushing and cleaning of the Agilent 1200 Series Autosampler SL Plus to achieve near zero carry-over

During the injection routine of the autosampler, the sample loop, the inside of the needle, the seat capillary and the main channel of the injection valve are in the flow path, and remain there throughout the duration of the run. This means these parts are flushed continuously with mobile phase during the complete analysis. It is only during aspiration of the sample that the injection valve is switched out of the flow path. In this position, the pump effluent is led directly to the column. Prior to injection, the outside surfaces of the needle are washed with fresh solvent. This is achieved using the flush port of the autosampler, and prevents contamination of the needle seat. The flush port of the autosampler is refilled with fresh solvent by a peristaltic pump that is installed in the autosampler housing. The flush port has a volume of about 680 µl, and the pump delivers 6 ml/min. Setting the wash time to 10 seconds means that the flush port volume is refilled more than once with fresh solvent, which is sufficient in most cases to clean the outside of the needle.

Recommendations for near zero carry-over using he Agilent 1200 Series ALS SL PLUS

To ensure lowest carry-over, consider the following recommendations:

- Always use the autosampler with the injection valve in main-pass position.
- Flush the exterior of the needle with an appropriate solvent. The flush time should be a minimum of 10 s.
- If possible, reduce the draw speed to 10 µl/min.
- Use Agilent capped 2 ml vials (P/N 5182-0556).
- If the seat is contaminated, use an appropriate seat-flush procedure.
- Use flushing solvents that are capable of dissolving the sample compounds.
- Use acidic mobile phases for basic compounds.

If capacity is an issue, the HTS PAL and HTC PAL HT LC Injection Systems can be used, which is also able to wash with two solvents to keep carry-over down. The HTS PAL and HTC PAL HT LC Injection Systems are equipped with a high pressure valve and a wash station. One wash cycle takes about 15 seconds. The carry-over of the HTS PAL and HTC PAL HT LC Injection Systems are comparable to the 1200 Series High Performance Autosampler (G1367C) in mainpass.

The column compartment can be used in its standard version for 4.6 mm inner diameter columns. At flow rates above 2 ml/min, and temperatures above 60 °C, the column effluent should be cooled down to the temperature of the detector by using the 1.5 µl cooler/heater in the column compartment (Figure 14 on page 24). This ensures lowest noise level with UV detectors, even at 5 ml/min and 80°C. (Figure 21 on page 37).

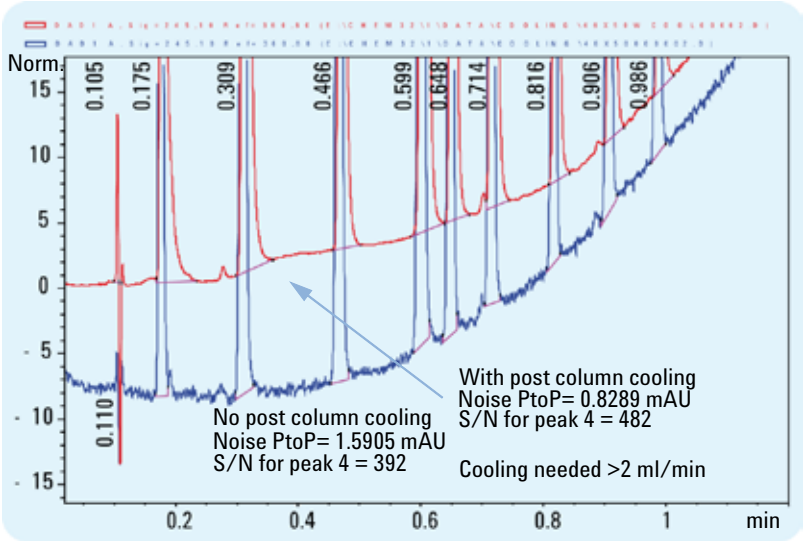


Figure 21 Influence of post column cooling (PCC) on baseline noise

Test Sample	Set of 9 compounds, 100 ng/ul each, dissolved in Water/Acetonitrile (65/35) **1** Acetanilide **2** Acetophenone **3** Propiophenone **4** Butyrophenone **5** Benzophenone **6** Valerophenone **7** Hexanophenone **8** Heptanophenone **9** Octanophenone
Column	50 x 4.6 mm Zorbax SB C-18, 1.8 µm for 600 bar operation
Pump	Solvent A: H2O + Solvent B: ACN Gradient: 35-95%B in 2 min, 0.1 min hold, 0.9 min re-equil Flow Rate: 5 ml/min
Autosampler	Injection volume 3 µl Wash 10 sec for exterior of needle
Thermostatted Column Compartment	Temperature: 80 °C for the heater on the left side, 34 °C for the cooler on the right side of the column compartment
Detectors	13 µl cell, 80 Hz Data Acquisition rate, Slit: 8 nm, Signal: 245/10 nm Ref 450/100 nm

If 2.1 mm inner diameter columns are used at low flow rates, the small heating device should be used for lowest extra-column volume, see Figure 14 on page 24.

If a UV detector is used, the data rate setting must be selected appropriately. Selecting a data rate that is too low results in increased peak width and lower resolution,
see Figure 22 on page 39.

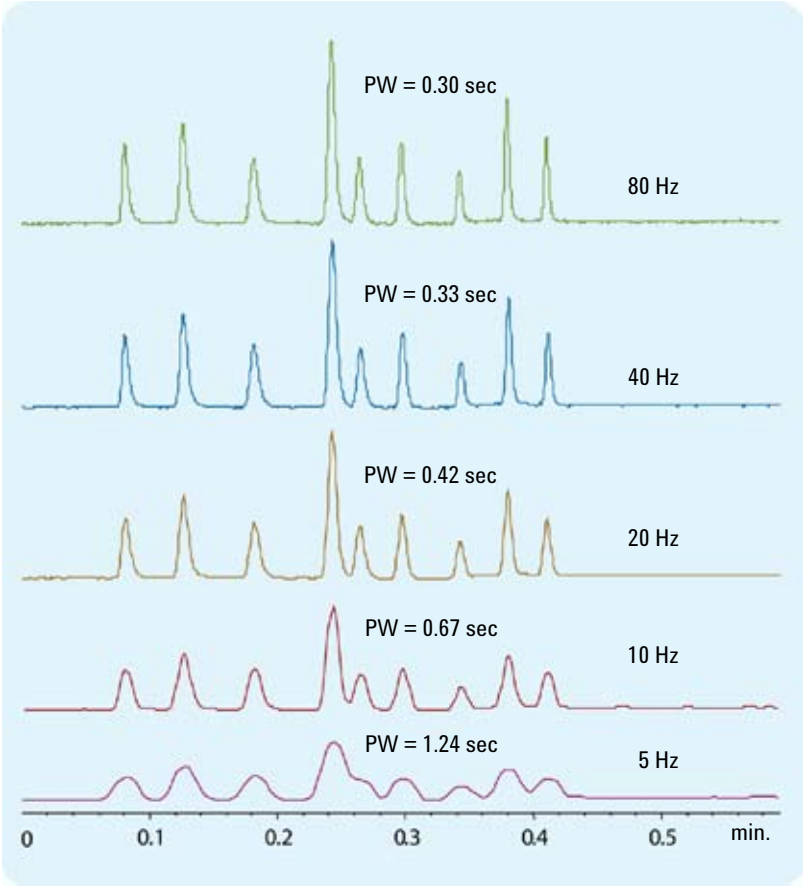


Figure 22 Peak width vs, UV data Rate

In Table 2 on page 40, peak width, resolution and peak capacity are dependent on the data rate. For fast and ultra-fast LC, high data rates are needed; otherwise, the separation that is achieved on the column is destroyed in the detector.

Table 2 Relation between data rate and chromatographic performance

Data Rate	Peak Width	Resolution	Peak Capacity
80 Hz	0.300	2.25	60
40 Hz	0.329	2.05	55
20 Hz	0.416	1.71	45
10 Hz	0.666	1.17	29
5 Hz	1.236	0.67	16

80 Hz data rate compared with 20 Hz data rate results in improvements in:

- Peak Width:
  - 30%
- Resolution:
  + 30%
- Peak Capacity:
  + 40%
- Apparent Column Efficiency:
  + 70%

If an MS detector is used, the connection between LC and MS should be as short as possible. This can be achieved by arranging the module connecting capillaries accordingly, see Figure 23 on page 41.

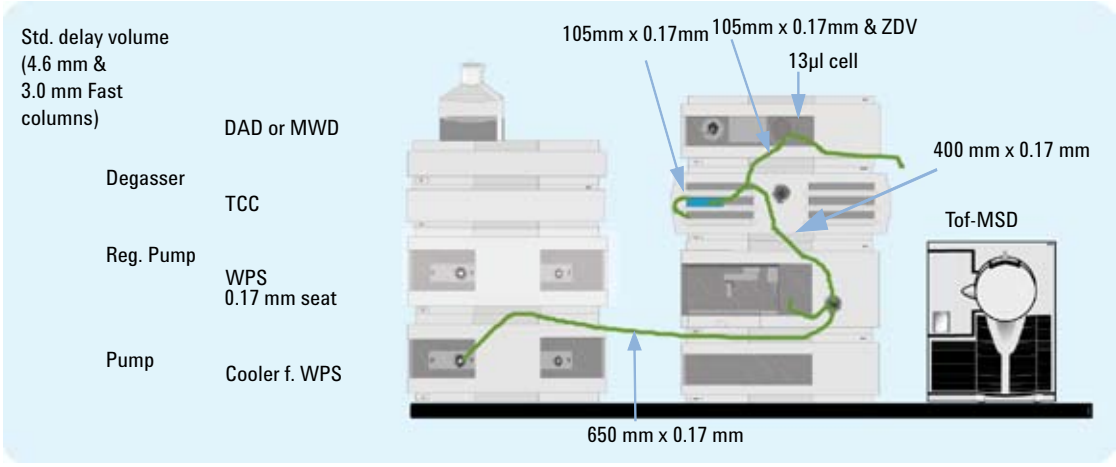


Figure 23 Optimized module setup for Agilent 1200 Series RRLC System with MS 2

The upper configuration, with 1.7 mm capillaries and a two-stack arrangement, is recommended for use with 4.6 mm inner diameter columns; the lower configuration, with 0.12 mm capillaries, small heat exchangers and a two-stack arrangement, is recommended for use with 2.1 mm inner diameter columns.

Selecting appropriate chromatographic conditions

Chromatographic conditions strongly depend on the compounds that are to be analyzed, but some rules of thumb can be used to achieve short run times:

- The flow rates should be as high as possible, depending on the required resolution, back-pressure and the detection system used.
- Steep gradients should be used.
- High column temperatures are recommended to enable high flow rates to be used, and to shorten run time even further. Zorbax SB columns can be used up to 90 °C, at low pH.

2.3. How to achieve more resolution

Selecting a column

Resolution depends on the selectivity of the column, the retention of the peak and the plate number of the column:

$$Resolution = \frac{1}{4}(a - 1)\sqrt{N}\left(\frac{k}{k+1}\right)$$

α = selectivity, N = plate number or Efficiency, k = retention factor of a compound

- The first step in improving resolution is always to test different stationary phases and to select the column with the best separation. This is the parameter that is of most importance for resolution.
- The second step is to use long columns or even coupled columns to increase the plate number.
- A third step is to shift peaks to higher retention factors. For k values of 5 to 10, the impact is significant. With higher k values, the effect is very low.

In practice, this means that longer columns with appropriate selectivity give better resolution.

Selecting the appropriate instrument configuration

To maintain the high resolution achieved on the column, the extra-column volume, especially after the column, should be as low as possible.

- For 4.6 mm inner diameter columns the standard delay configuration should be used, see Figure 10 on page 20.
- For UV detection with DAD, the 13 µl standard cell is recommended.
- For 2.1 mm inner diameter columns, the low delay configuration should be used, and the low dispersion kit should be installed. For highest UV sensitivity, the short mixer is also recommended.
- The 2 µl UV detector cell is recommended for use with the DAD, MWD and VWD with 2.1 mm inner diameter columns.
- The injection volume is also of importance, especially if the sample is dissolved in an organic solvent. In this case, the gradient should start with a low percentage of organic phase to focus the compounds at the top of the column. This avoids peaks dispersion due to the injection.

Selecting appropriate chromatographic conditions

As already stated previously, chromatographic conditions depend on the compounds to be analyzed. But here also, some rules of thumb exist.

- Moderate flow rates should be used, but recent experiments have shown that elevated flow rates can also be advantageous for improved separation[18] . For 4.6 mm inner diameter columns packed with sub-2-micron particles, a flow rate of 2 ml/min is recommended as a starting value. For 2.1 mm inner diameter sub-2-micron columns, 0.4 ml/min is a good starting point.
- Moderate gradients should be used, for example, 2 to 5% gradient change per minute.
- The column temperature is an additional parameter for optimization. Temperature can influence a separation, and should not be overlooked[19].

2.4. How to achieve higher sensitivity

The signal-to-noise ratio depends on the peak height and the noise on the baseline. Several parameters have to be optimized to reduce noise and simultaneously increase peak height.

Selection of column length and column id

Using smaller inner diameter columns will generally result in higher sensitivity and is therefore ideal for applications with limited sample amounts. If the same sample amount can be injected on a smaller i.d. column, then the dilution due to column diameter will be less and the sensitivity will increase. For example, decreasing the column i.d. from 4.6 mm down to 2.1 mm results in a theoretical gain in sensitivity of 4.7 times. This assumes that extra-column effects are minimized and there are no trade offs on the detector side. Some smaller flow cells have shorter path-length or higher detector noise and so the sensitivity gain might not quite be as great as theory indicates. However it is also important to match a small flow cell to a narrow column to preserve resolution. For a mass spectrometer detector, the lower flow rates with narrow columns can result in higher ionization efficiencies and therefore higher sensitivity.

Selecting the appropriate instrument configuration

- To achieve lowest baseline noise, the standard delay volume configuration is recommended for the 1200 Series RRLC pump module.
- Here also, the injection volume and the sample dissolution solvent are important. Care must be taken that the compounds are focused at the top of the column, to avoid peak dispersion due to the injection, which would cause a reduced peak height. In order to achieve this, the sample should be dissolved in a solvent composition of lower elution strength than the mobile phase.
- The column temperature should not be too low, to avoid long retention of the peaks on the column. This also creates peak dispersion and a lower peak height.
- Selection of the optimum detector cell depends on the id of the column being used. Typically, the longer the path length, the better the signal-to-noise ratio. The data rate of the UV detector should be selected according to the actual peak width. Higher than necessary data rates should be avoided because of higher noise levels.
- Available UV detectors are the Agilent 1200 Series Diode Array Detector SL Plus and the new Agilent 1200 Series Variable Wavelength Detector SL, with data rates of 160 Hz and significantly lower noise and drift levels. For highest sensitivity in the UV range, the new Agilent 1200 Series VWD SL Plus is the optimum choice.

Figure 24 on page 46 gives recommentations on which cell is best suited for the selected column id and flow rate.

Column ID	2.1 mm			3.0 mm		4.6 mm	
Config	No damper No mixer ++	No damper No mixer ++	No damper short mixer (5067-1565) plus firmware (A06.06) ++	Damper Mixer	Damper Mixer	Damper Mixer	Damper Mixer
Detector	VWD	DAD		VWD	DAD	VWD	DAD
Flow cell	2 µl, 3 mm #010 **G1314-60087**	2 µl, 3 mm #010 **G1315-60024** **	2 µl, 3 mm #010 **G1315-60024**	5 µl, 6 mm #016 **G1314-60083** +	5 µl, 6 mm #016 **G1315-60025** +	14 µl, 10 mm #018 **G1314-60086** +	14 µl, 10 mm #018 **G1315-60022** +
UV S/N	High	OK	Good	Highest	High	Highest	High
MS S/N	Highest	Highest	Highest				

Figure 24 Flow cell matrix for ultra-fast analysis with RRLC

** 500 nl, 10 mm could be used for UV monitoring

\+ For ultra fast analysis with step gradients the micro flow cell #010 gives the best performance

++ Note, in high resolution analysis time is not the highest priority. Higher delay volumes are accepted. Therefore we recommend to use the damper plus mixer for a highest signal to noise. If longer columns (> 50 mm) for higher resolution are used, then the next larger flow cell is the preferred choice for higher sensitivity.

The matrix shown above is somewhat simplified. In practice, there is a trade-off between dispersion (small cell with lower path length) and increased sensitivity (larger cell volume with higher path length). A rule of thumb is to calculate the expected peak volume and select the appropriate cell volume. The peak volume can be calculated using the method translator[23].

Selecting appropriate chromatographic conditions

For an optimum signal-to-noise ratio, it is of advantage if the peaks elute quickly.

- Elevated flow rates should be used to elute the peaks at low k'values.
- Faster gradients can also be applied to elute the peaks at low k'values.

2.5. How to prevent column blockages

Columns packed with sub-2-micron particles also need frits with small pore size to prevent packing material being swept out. This immediately brings increased risk of blocking these frits with particulates from the sample, mobile phase or/and from the instrument itself. To protect the column, additional small filters (Figure 25 on page 49) can be used in front of the column. It is also recommended that the sample is thoroughly filtered or/and centrifuged, and that any kind of particulate matter in the mobile phases is avoided.

To ensure best results follow these simple usage guidance:

1. Install and run the column only in the flow direction marked on the column.
2. Use only solvents that are high quality, chromatography grade.
3. Filter all aqueous buffers and all samples through an appropriate 0.2 µm filter before use.
4. Replace bottles of mobile phase buffer every 24-48 hours – do not add mobile phase to the bottle; always use a new bottle.
5. Do not use a high buffer salt mobile phase (> 50 mM) in combination with high acetonitrile concentrations due to possible precipitation.
6. An in-line filter (5067-1551 for 2.1 or 3.0 mm inner diameter columns or 5067-1553 for 4.6 mm inner diameter columns) is recommended to catch particulates and extend column life. Change the filter when the pressure increases by 10 %.

7. Purge the pumps (the connections up to the column) of any buffer containing mobile phases and flush through 5 ml of solvent before attaching the column to the instrument.
8. Flush the column with compatible mobile phase starting slowly at 0.1 ml/min for a 2.1 mm inner diameter column, 0.2 ml min for a 3.0 mm inner diameter column, and 0.4 ml/min for 4.6 mm inner diameter. Increase the flow rate to the desired flow over 5 minutes.
9. Once the pressure has stabilized, attach the column to the detector.
10. Equilibrate the column and detector with 10 column volumes of the mobile phase prior to use. (1 ml – 5 ml depending on column size.)
11. Avoid over-pressure. Check the pressure range of your gradient — which may be 100—130 bar or more — before starting any sequence.

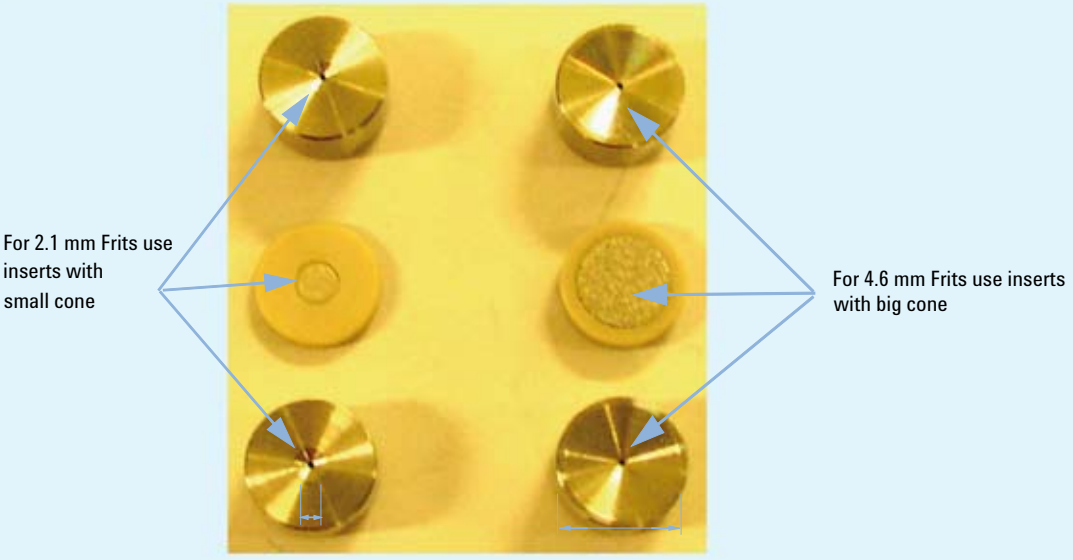


Figure 25 Protection for 4.6 and 2.1 mm id columns packed with 1.8 µm particles, inlet frit with 0.2 µm pore size


2

Optimization of the Agilent 6000 series MS instrumentation

3

3. Optimization of the Agilent 6000 series Mass Spectrometers instrumentation

Not only does the LC-part of an LCMS system need to fulfill the additional demands sub-2-micron particle columns set to a chromatographic system, but the MS detector also needs to be optimized.

If we look at the benefits of sub-2-micron particle columns the demands on the detection system become quite obvious:

Benefit	Secondary effect	Demand on MS
Speed gain	Narrow peaks	High data acquisition rate Low interscan delays Low switching times Low delay volume from column to MS source
	High throughput	Low cycle times User-friendly software
	High flow rates	Appropriate MS sources
Resolution gain	Increased peak capacity	Minimal cross talk Effective filtering software

Achieving high speed data acquisition is a very demanding task for mass spectrometry development. This is because of the additional dimensions of data information that have to be acquired and processed, in combination with complex electronics to separate the ions. To explain this, compare UV data acquisition with a rather simple single quad mass spectrometer: a diode-array detector acquires data in three dimensions (time, wavelength and intensity) as does the single quad mass detector (time, m/z ratio, intensity). In a diode-array detector, the wavelength filtering is done by an optical element, with the photons traveling at the speed of light finally hitting the detector, which is a parallel

acquisition device (an array of photo diodes, each recording at a different wavelength). In an MS detector, the situation is completely different. First, we have to deal with ions traveling at a rather slow speed through the detector, and secondly, electronics become much more complicated to create such electromagnetic fields to get ions filtered sequentially to the detector.

3.1. Single Quad Detector

The Agilent 6410 Single Quad Detector, which is one of the fastest detecting single quadrupole detectors available, is capable of acquiring 10.000 m/z per second. This means that if we consider a mass range of 100–600 Da (which covers the small-molecule application range in pharmaceutical analysis, environmental or food analysis) a data point can be acquired every 50 ms. In addition, there is an overhead time required to reset the electronics to the starting conditions ready to perform a new mass scan. With the Agilent 6140 Single Quad MS, this overhead time, or interscan delay, is very short, taking only 10ms. The resulting 60ms duty cycle and final data acquisition rate of 16.6 Hz is sufficient to cover a very narrow peak width of 1.5s. But care has to be taken with the exact conditions. The interscan delay can increase depending on the acquisition mode; for example, if positive-negative switching from one scan to another is applied, the interscan delay increases to 150-300 ms. This time is required mainly for the ion source to depolarize the inlet from about +4000 V to -4000 V and vice versa. If the Multimode Source is used to switch from APCI-only to ESI-only mode (instead of simultaneous APCI and ESI), about 50 ms interscan delay is required to depolarize the corona needle.

Under these ultra-fast scanning conditions, the rf field of the quadrupole is operated at 1.5 MHz and in the voltage range of some kilo volts. This requires very precise and sophisticated electronics. However, this is not sufficient to get good results; some tricks have to be applied to get fast data acquisition, good data quality and good sensitivity simultaneously. For example,

if we consider the standard scanning conditions of the Agilent 6140 MS, the ions are extracted with an energy of 5 eV. An ion of a mass of 100 Da then requires 65 µs to travel through the quadrupole that does the ion filtering. A heavier ion of mass 1000 Da already needs 200 µs to travel through the quadrupole. At fast scan speeds, the quadrupole settings have already changed during that time to optimize other masses; for example, an ion of 1000 Da would already experience a shift of 1 Da in the optimal filtering settings at the end of the quadrupole. This results in a reduced ion transmission and lower sensitivity. Simply increasing the extraction energy (for example, to 10 eV) the ion experiences less rf cycles, which results in reduced resolution – also not an ideal situation. So, how does the Agilent 6140 Single Quad achieve high scan speed, high resolution and high sensitivity? Firstly, the ion energy is increased to 15 eV. To compensate for reduced filtering (lower resolution) the quadrupole frequency is increased to 1.4 MHz. The step size is increased from 0.1 to 0.2 Da which finally gives a speed of 10,000 u/sec. Furthermore, different digital filtering coefficients are used, since data points are spaced further apart, and the mass filter of +/- 0.3 for normal scanning is increased to +/- 0.6 for ultra-fast scanning (seven data points). To reduce the loss in ion transmission, especially for higher masses, the Agilent 6140 Single Quad MS (and the 6130 model) uses a special hardware configuration called the “Lens2RF“. As the rf-amplitude increases to filter high masses, it becomes more and more difficult for the ions to enter the quadrupole because they are repelled by a fringing field at the outside edge of the quadrupole. Ramping the DC and RF amplitudes on lens 2 in the Lens2RF arrangement with respect to mass (see Figure 26 on page 55), and offsetting the phase relative to the quadrupole, results in an increased ion transmission into the quadrupole. This increase can be up to three times at m/z 600 and as much as ten times for m/z 3000.

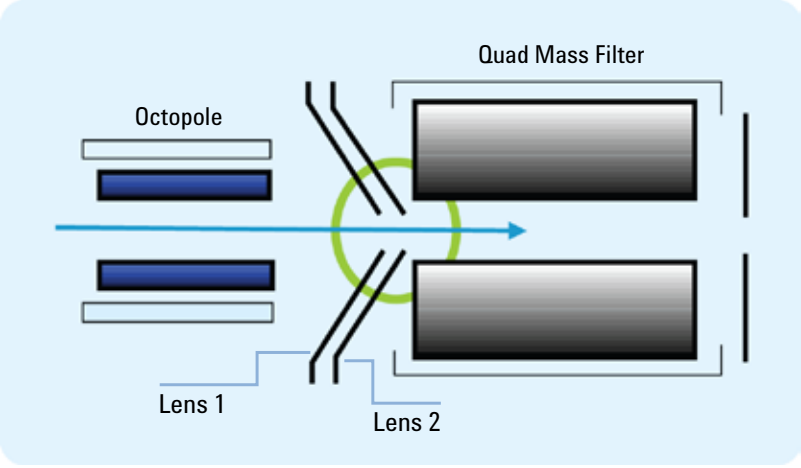


Figure 26 Lens2RF-arrangement in the 6140 (and 6130) Single quad MS giving a higher ion transmission to compensate for losses at high scan speeds

Figure 27 on page 56 shows an example of the data quality of the Agilent 6140 Single Quad MS under ultra fast scanning conditions.

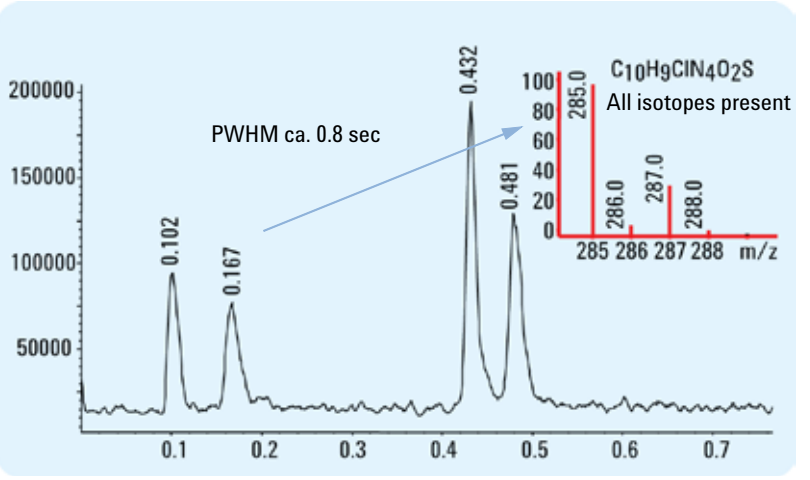


Figure 27 Spectrum of sulfachloropyridazine of the Agilent Sulfa-drug demo mix running under fast-LC conditions. The $[M+H]^+$ ion cluster is expanded.

The ^{13}C and ^{37}Cl isotopes are present and in the correct ratios.

3.2. Time of Flight Detector

Probably the most suitable MS detector for ultra fast analysis is the Agilent 6210 Time-of-Flight MS. This is because of the very high data acquisition rates of up to 40 Hz in the mass range of 100–1000 m/z (20 Hz at 100–3000 m/z), allowing a very precise capturing of even narrowest chromatographic peaks (see Figure 28 on page 57).

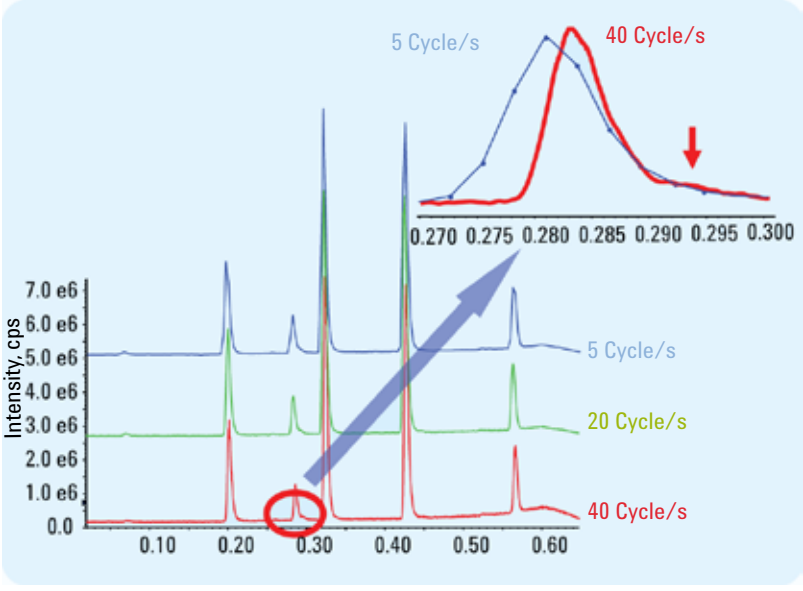


Figure 28 Better chromatographic resolution with a high data acquisition rate allows detecting a minor impurity, eluting close to one compound of interest.

How is this achieved? In contrast to a Single Quad (or Ion Trap) mass detector, the Agilent 6210 Time-of-Flight MS is not a scanning device. It extracts all generated ions into a field-free drift tube, where the ions travel with a speed related to their m/z ratio (see Figure 29 on page 59). This process, and the detection of the ions as they arrive at the detection device, is very fast compared to a single quad mass spectrometer. Ions are pulsed in the ion pulser every 100–160 µsec. An ion of m/z 118 takes 20 µsec to travel from the ion pulser through the flight tube to the detector. A heavier ion of m/z 3000 requires longer, 100 µsec, to travel through the flight tube. The detector measures every nano second; this is a 1 GHz sampling rate. But not every one of these measurements is reported to the data system; many of them are summed to achieve a statistic across the different flight times and hence mass-to-charge ratios. Finally, an average full spectrum is transferred at up to every 25 ms to the data system if highest data acquisition rate is chosen.

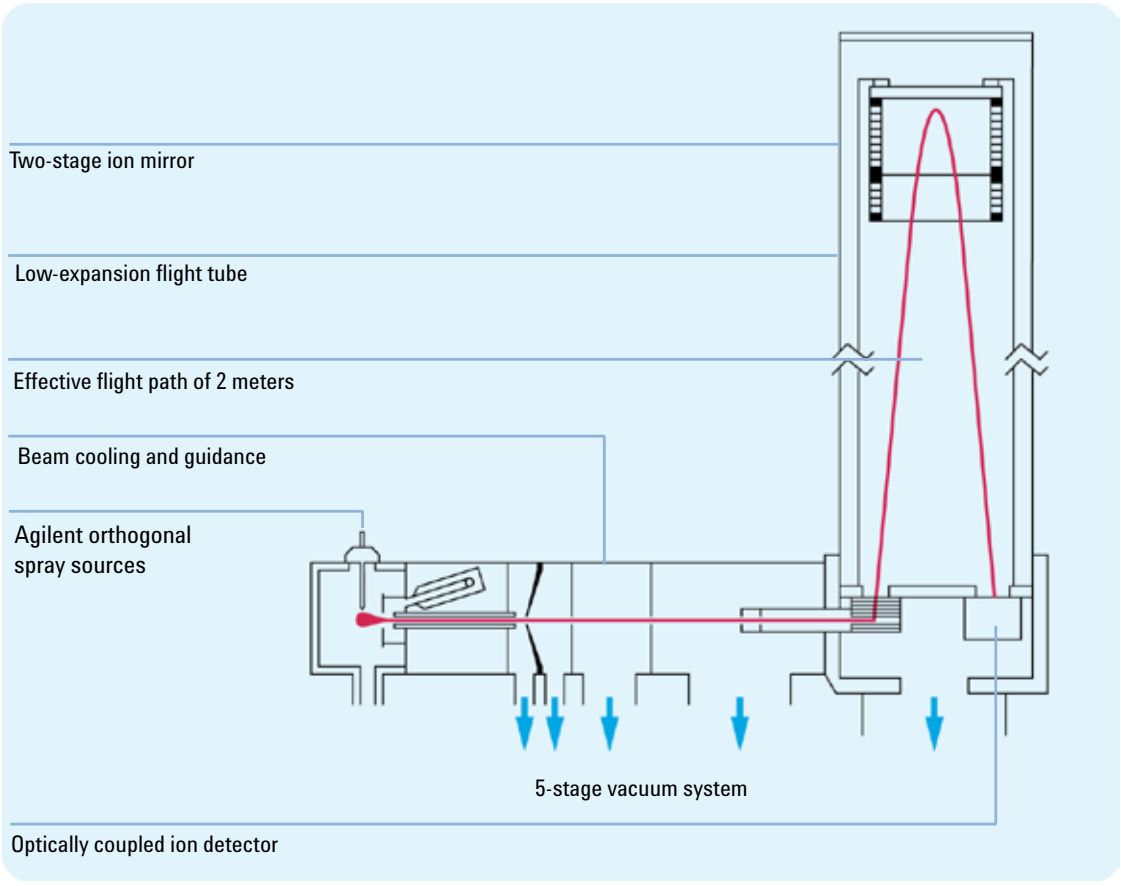


Figure 29 Schematic of the Agilent 6210 Time-of-Flight mass spectrometer

Usually, the higher the data rate chosen, the fewer the transients that are summarized, and the worse the S/N ratio becomes, the lower the mass accuracy, and the lower the resolution. But with the Agilent 6210 Time-of-Flight MS, certain design features ensure accurate mass measurements even at these high data acquisition rates. The instrument is specified to have a mass accuracy of 2 ppm for reserpine (m/z 609.2807, $[M+H]^+$). To illustrate this, 1 ppm mass difference at m/z 118 is a 10 picosecond flight time difference! This outstanding mass accuracy is achieved by an analog-to-digital (ADC) acquisition system that provides several orders of magnitude of dynamic range combined with an automated calibrant delivery system and a second nebulizer that allows the introduction of a reference mass compound at a concentration low enough that it is unlikely to interfere with analyses. Furthermore, the flight tube is made from a special, ultra low-thermal-expansion alloy that minimizes flight path changes due to temperature. Finally, mechanical and electronic temperature compensation is applied in the flight tube and electronics. In Figure 30 on page 61, an ultra fast chromatographic separation on an RRHT column, with resulting peak widths in the range of 0.34 – 0.42 s (PWHH), are shown together with the achieved mass accuracies for a mass range of 100–1000 m/z of -0.58 ppm for atenolol and -4.31 ppm for metoprolol. Note that the -4.31 ppm mass error for metoprolol is still within specification, since 2 ppm mass accuracy is defined for reserpine. A 2 ppm mass error for reserpine results in a 1.21 mDa error compared with a 1.15 mDa error detected for metoprolol in this example.

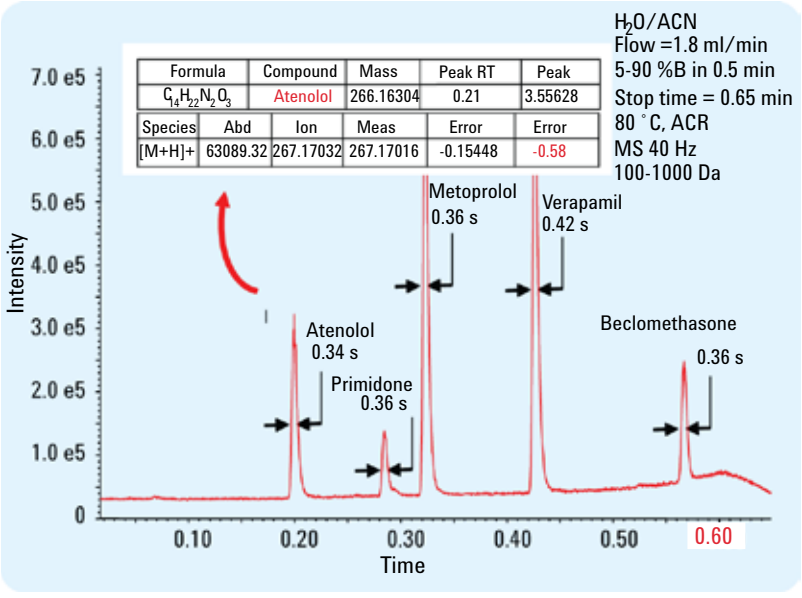


Figure 30 Achieving highest mass accuracies at highest data acquisition rates (40 Hz), mass errors for atenolol: -0.58 ppm ($[M+H]^+$ = 266.1630), primidone: -2.65 ppm ($[M+H]^+$ = 218.1055), metoprolol: -4.31 ppm ($[M+H]^+$ = 267.1834), verapamil: -0.77 ppm ($[M+H]^+$ = 454.2832), beclomethasone: 1.88 ppm ($[M+H]^+$ = 520.2228).

3.3. Triple Quadrupole Mass Spectrometer

Probably the largest application area for a triple quadrupole mass spectrometer is accurate quantitation. For this, very accurate sampling of the chromatographic peak by the mass spectrometer must be achieved; otherwise, large variations in the detected peak area can be expected. Quantitation is usually done in MRM (multiple reaction monitoring) mode, where the first quadrupole is set to transmit a characteristic precursor ion and the second to detect a product ion resulting from a collision-induced fragmentation inside the collision cell. The critical parameter here is not really the scan rate, as with the typical single quadrupole applications, but the duty cycle. The duty cycle includes the time during which representative ions of the compound of interest are counted by the system and the interscan time, during which no data are acquired while the electronics changes the parameters to detect new precursor/product ions. The Agilent 6410 Triple Quadrupole MS is capable of achieving short (5 ms) duty cycles with a minimal interscan delay of 10 ms. As can be seen in Figure 31 on page 63, the sensitivity is almost unchanged at the short dwell times needed to capture the exact peak shape, as required for ultra-fast chromatography.

Dwell	100 ms	20 ms	5 ms
Area	14860	13605	13202

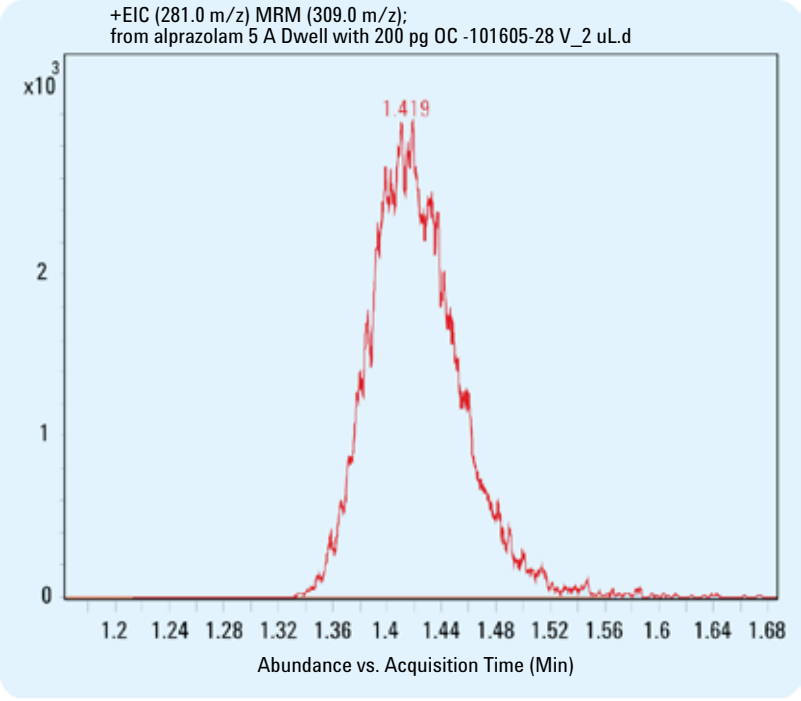


Figure 31 Extracted ion chromatogram EIC for aplazolam (281 m/z)

Another very important requirement in fragmentation experiments in combination with fast chromatography is to clear the product ions rapidly from the collision cell so that no crosstalk between different compounds occurs. To achieve this, Agilent uses a high pressure collision cell with linear axial acceleration. A small diameter hexapole with a high frequency and high speed digital electronics is used.

Figure 32 on page 64 shows that this cell is cleared within 350 µs from a low mass ion and within 600 µs from a high mass ion.

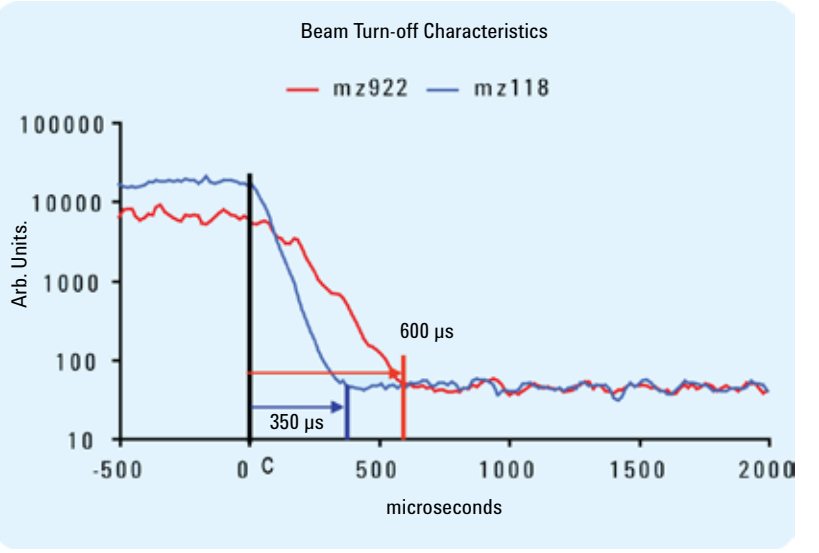


Figure 32 Beam turn-off characteristics of the Agilent 6410 QQQ collision cell.

Collision gas = 7 mTorr, 0 V collision energy, 5 V Applied Axial Potential, blue trace: ion of 118 m/z, red trace: ion of 922 m/z

Figure 33 on page 65 shows that no crosstalk occurs even under fastest sampling conditions (5 ms dwell time). Two MRM transitions have been selected here for the tuning mix which was infused into the mass spectrometer. One transition (m/z 622 -> m/z 540) results in a real ion giving a high response; the other transition (m/z 600 -> m/z 540) uses a nonexistent precursor ion and the same product ion, and shows no response. If crosstalk occurred, the second transition would show a significant response.

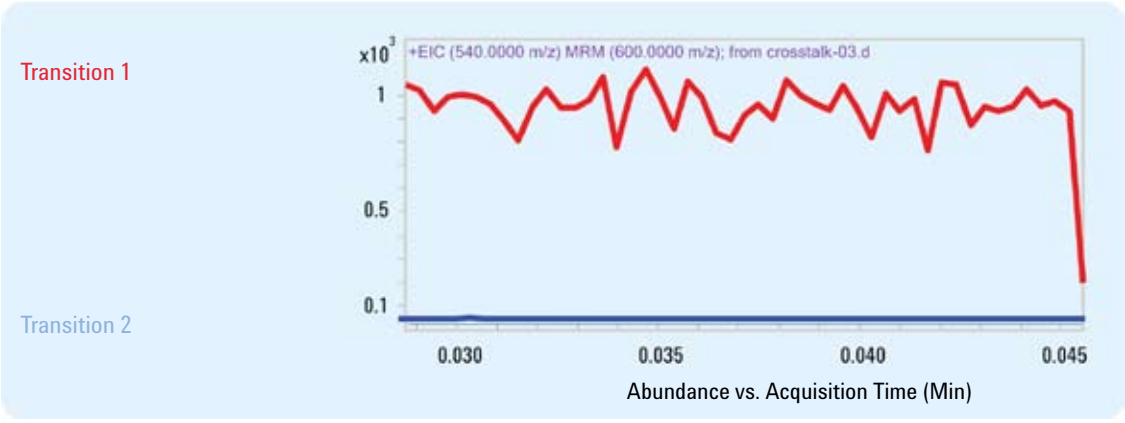


Figure 33 Infusion analysis of a compound with a622 m/z pre-cursor ion

Collision energy 30 V, axial acceleration potential 7 V, 5 ms dwell time, 5 ms interscan time; red trace: transition 622 -> 540, detecting an existing product ion; blue trace: different (nonexistent) pre-cursor ion transition to the same product ion 600 -> 540. no crosstalk between both MRMs is detectable.

Additionally, as the Agilent 6410 Triple Quadrupole MS shares many hardware parts with the Agilent single quadrupole instruments, the features explained in that section, such as the patented Lens2RF design to improve ion transmission into the quadrupoles are identical in the triple quadrupole instrument.

3.4. Quadrupole-Time-of-Flight Mass Spectrometer

The design features of the Agilent 6510 Quadrupole-Time-of-Flight Mass Spectrometer to support ultra fast analysis are very easy to explain by and examination of the schematic diagram

of the instrument (see Figure 34 on page 66). The Agilent 6510 Quadrupole-Time-of-Flight Mass Spectrometer shares most of its components with the other Agilent MS instruments. It uses the same kind of MS interfaces, the same ion guides and the same hyperpolic quadrupole as the Agilent 6410 Triple Quadrupole Mass Spectrometer or Agilent 6130/40 Single Quad Mass Spectrometer. The collision cell, with all its features for eliminating crosstalk, is identical to the 6410 Triple Quadrupole Mass Spectrometer and the Time-of-Flight instrumentation is identical to the 6210 Time-of-Flight Mass Spectrometer, with the same characteristics for mass accuracy and data acquisition.

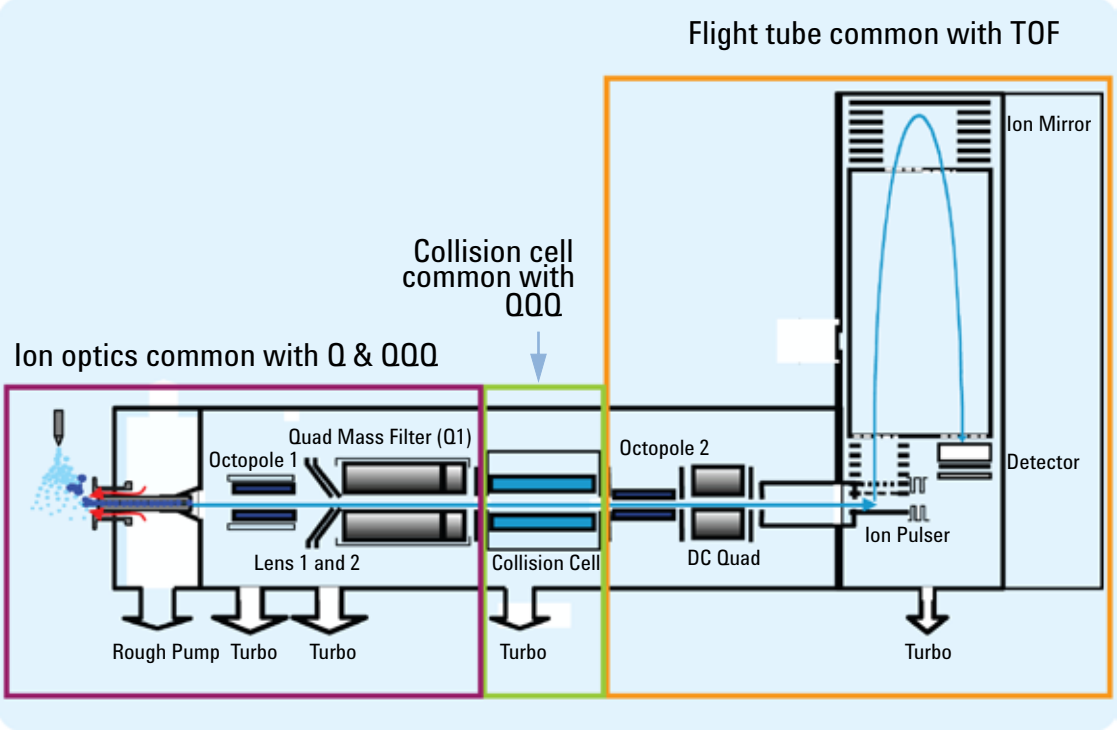


Figure 34 Assembly devices of the Agilent 6510 Quadrupole-Time-of-Flight Mass Spectrometer

3.5. MS Sources

The different available ion source (ESI, APCI, APPI, Maldi, Nano-ESI, Chip-Cube and Multimode Source) are interchangeable between different kinds of Agilent MS instruments (with few restrictions based on the production date of the MS or ion source). The interesting features with regard to supporting sub-2-micron particle columns are the allowed flow rate – this is important for ultra-fast LC with high flow rate – and the peak broadening of chromatographic peaks. Even though the ESI, APCI and APPI source are specified to a maximum flow of 1 mL/min (higher flow rates might also be tolerated if care is taken that no condensation occurs) the source of choice for such applications would be the Agilent Multimode source. This unique source allows ESI- and APCI-type ions to be generated simultaneously. This already gives a big advantage in throughput, since compounds that do not ionize in one mode but do ionize in the other mode do not need to be reanalyzed. The other big advantage lies in two powerful IR emitters (see Figure 35 on page 68), which are primarily required to de-solvate the ions for APCI-type ion generation. These IR emitters allow the complete evaporation of a stream of up to 2 ml/min of pure water, so that no flooding of the ion source occurs. The power to the IR emitters is controlled by feedback from a temperature sensor in the vapor stream exiting the source. Another advantage of these IR emitters is the fact that drying gas consumption is reduced by about 50% compared to that of a dedicated ESI source because heat is supplied by the IR emitters.

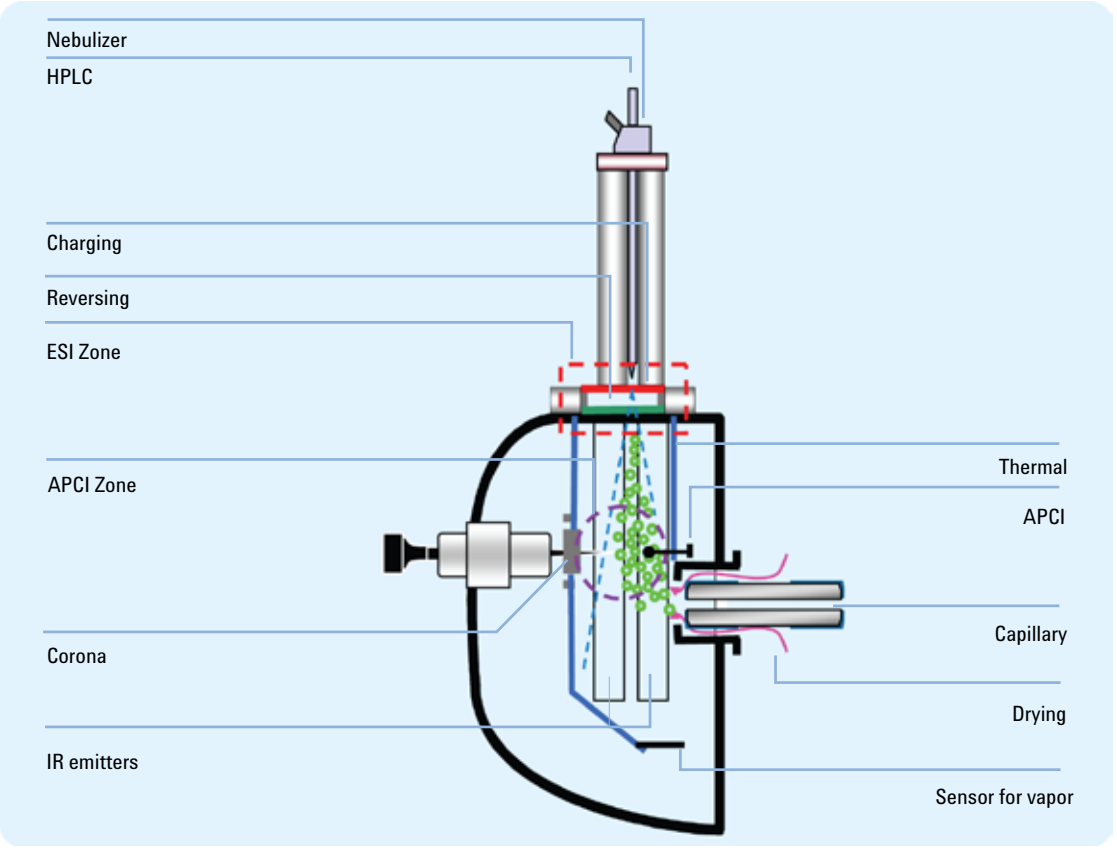


Figure 35 Schematic drawing of the Agilent Multimode source

Figure 36 on page 69 shows the comparison of a UV trace to a MS trace acquired under ultra-fast conditions on an Agilent ToF MS with an Agilent RapidResolution LC system with a DAD SL for UV detection. The ToF was equipped with a dual-ESI source, which was operated at very high flow rates (1.8 mL/min). Condensation was prevented by using highest gas temperature and gas flow rate in the source, together with alternating column regeneration,

which directs the high water content during equilibration to waste while a sample is analyzed in parallel on a second column.

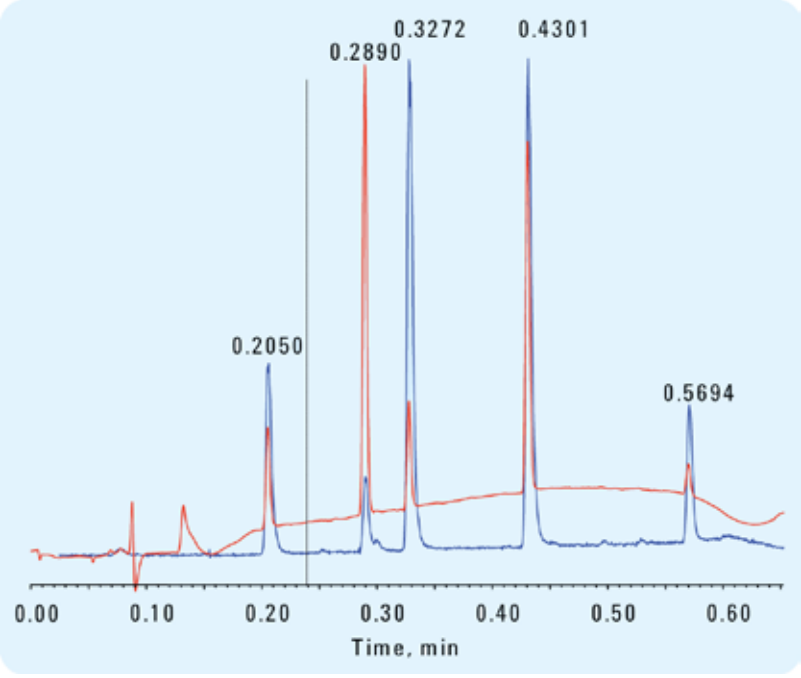


Figure 36 Comparison of a ToF-MS and a DAD-trace (red) of a ultrafast LCMS analysis

The flow rate into the ToF was 1.8 mL/min, alternating column regeneration was applied. Only a very small amount of peak broadening can be detected between the DAD and the ToF signals.

3.6. MS Software

When dealing with high throughput (that is, many samples) or high resolution (that is, many peaks) MS data, powerful software support is important to enable the tremendous amount of data generated to be handled. The high-end mass spectrometers from Agilent (ToF, QQQ and Q-ToF), which produce such a large amount of data, are controlled by the Agilent MassHunter software, which also enables qualitative and quantitative data analysis. The most obvious feature of this software is its ease of use, facilitated by a “flat” user interface. This means that all necessary data appear on one screen, which makes navigation very simple. Figure 37 on page 71 shows a screenshot of the Quantitation part of the MassHunter software; here, all samples and all compounds within one sample, together with all required data (such as retention time, target response, ISTD response, outlier flagging etc.) appear in a table. Of course the complexity of the table is user-configurable. If required, chromatograms, spectra and calibration curves for each compound can be displayed on a single screen, giving all relevant information at a glance.

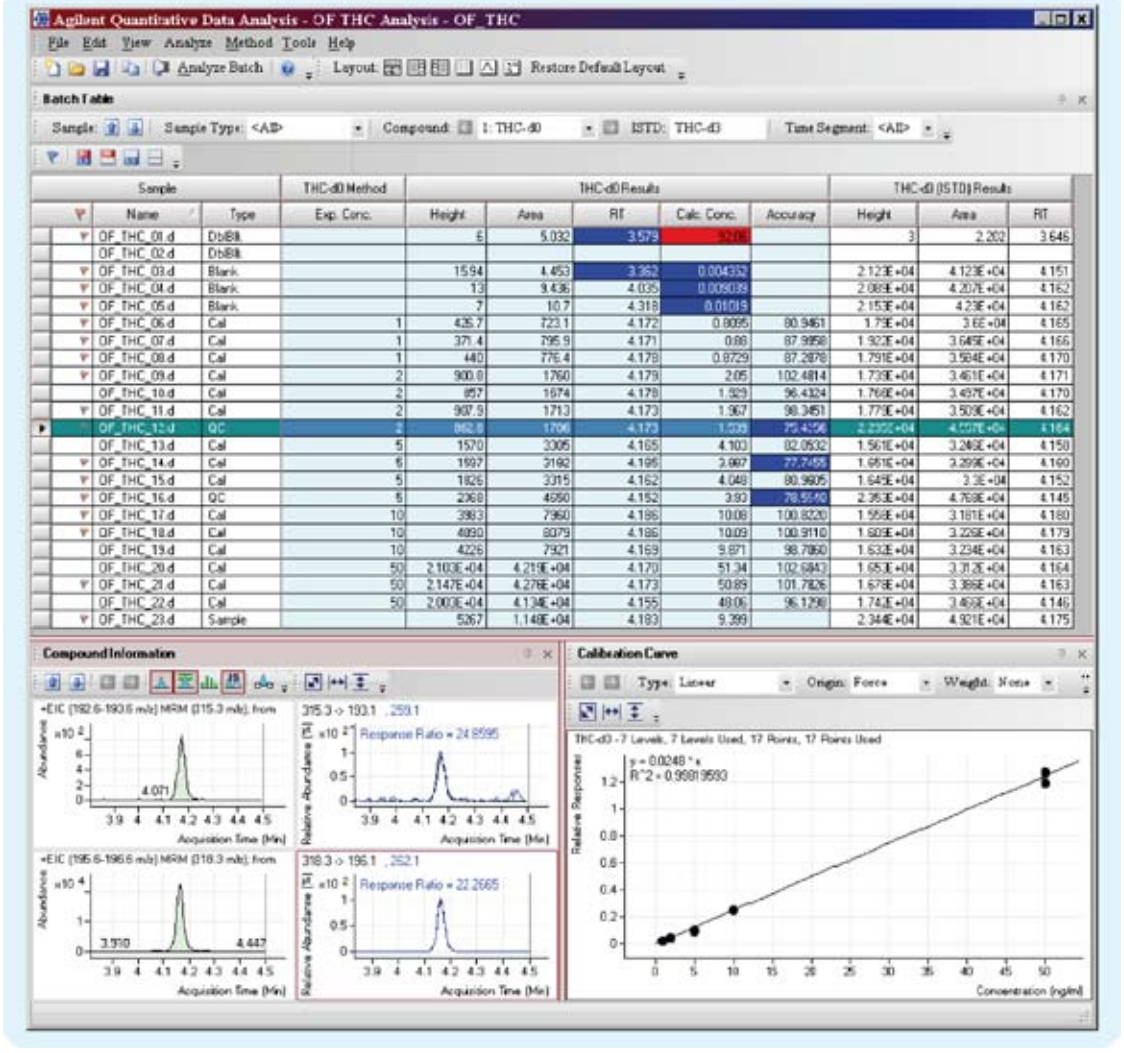


Figure 37 Screenshot of the MassHunter Quantitative Analysis software

Other noteworthy features of the MassHunter software to handle large data sets are the parameter-less integrator, curve-fit assistance for calibration curves, automation features, and customized reporting directly into Microsoft Excel data format which is easily importable into standard LIMS-systems.

For ultra-high-resolution analyses, maybe combined with a Q-ToF MS, generating very large data sets, a perfect solution to reducing the complexity is the Agilent MassProfiler software, which uses the Agilent Molecular Features Extraction (MFE) algorithm to extract compound features out of the data, and to compare them. A molecular feature is a combination of retention times and mass features that are unique to a compound, combining MS information of multiply charged ions, fragmentations and different adducts of a compound to a single data point. Figure 38 on page 73 shows an example from a trace-analysis of water. Two samples are compared down to a S/N-level of 2, and the data are compared to a target list. More than 20000 unique mass features were found, but only 6 compounds from the target list could be found in both samples, and 21 compounds were either in sample A or in sample B. By conventional means, this analysis would be extremely time consuming but the Agilent MassProfiler software reduced this task to a few mouse-clicks and a few minutes.

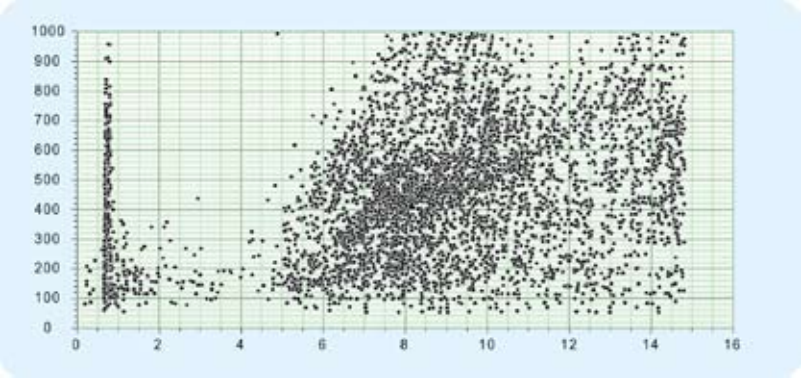


Figure 38 Summarized mass features of two waste water samples

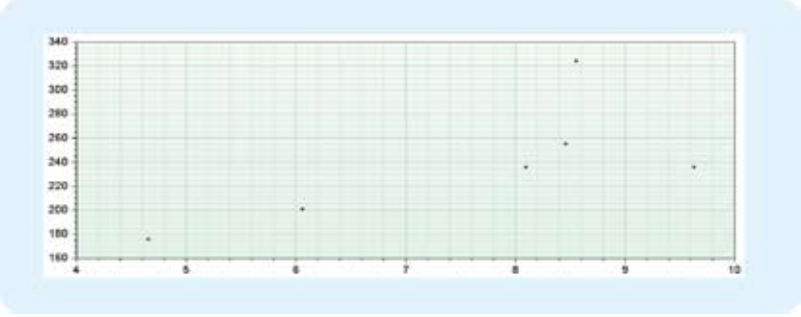


Figure 39 Six compounds from a target list are in both samples

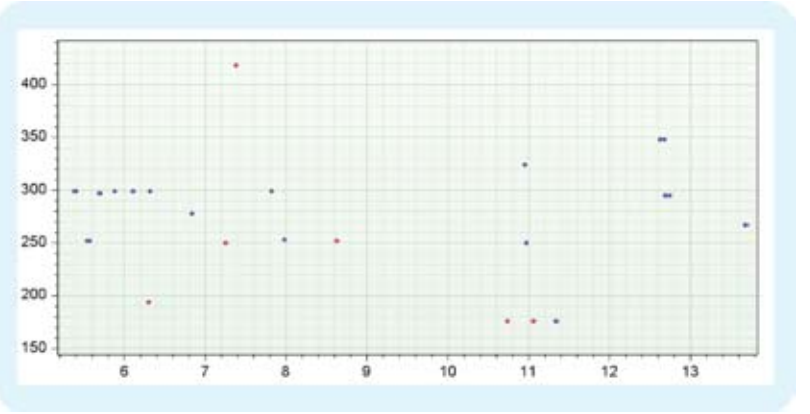
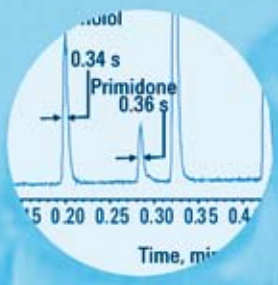


Figure 40 21 compounds from a target list are either in sample A (blue) or in sample B (red)



Application examples

4

4. Highest data content with highest throughput

4.1. Agilent 1200 Series Rapid Resolution LC system and the Agilent 6210 TOF MS

Making use of such features as alternating column regeneration (Figure 14 on page 29), overlapped injection, high temperatures and high flow rates in combinaton with with the highest data acquisition rates, the linear velocities achieved were in the range of 11 mm/s, and cycle times were as fast as 49 s for a run time of 41 s. Due to the use of columns with 1.8 µm particle size, the UV peak capacities were still about 50, and even the MS peak capacities were about 40 for a gradient time of 39 s[20]. Figure 41 on page 77)

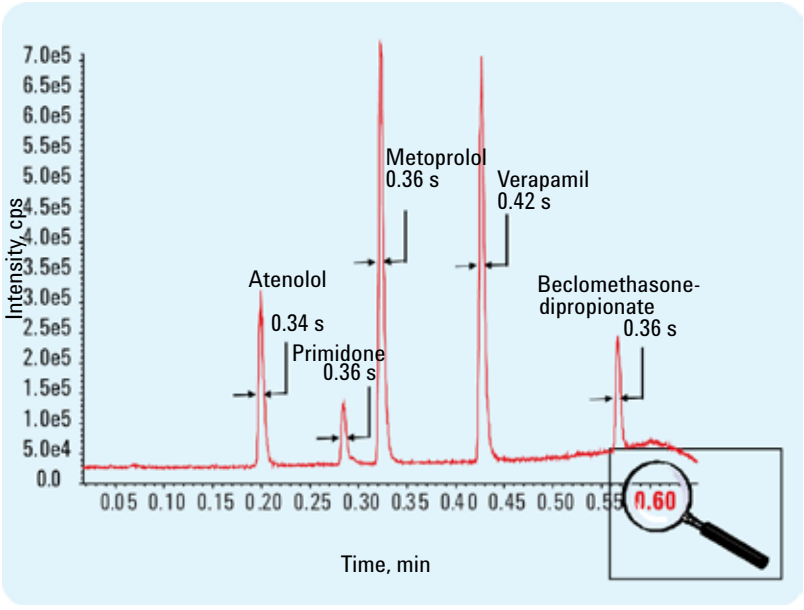


Figure 41 MS total ion chromatogram of highest speed LC-TOF-MS analysis (40 Hz TOF data acquisition rate)

Table 3 Chromatographic conditions

Solvent:	A = water (0.1% TFA), B = ACN (0.08% TFA)
Temperature:	80 °C
Flow:	1.8 mL/min
Gradient:	0.00 min 5%B
Regeneration:	0.00 min 5%B 0.50 min 90%B 0.01 min 95%B 0.51 min 5%B 0.20 min 95%B 0.65 min 5%B 0.21 min 5%B 0.65 min 5%B

Table 3 Chromatographic conditions

Stoptime:	0.65 min
Posttime:	off
DAD:	Wavelength: 210 nm (8) ref. off Peak width: >0.0025 min (0.05s responsetime), 80 Hz Spectra: no Slit: 8 nm Balance: pre-run
MS:	Scan range: 100-1000 m/z Acquisition rate: 5, 20, 30 and 40 cycles/s Data type: profile data Capillary voltage: 3000 V Fragmentor: 180 V Skimmer: 40V Gas temperature: 350 °C Gas flow: 13 L/min
Injection volume:	1 µL
Injector:	Overlapped injection, Automatic delay volume reduction, Sample flush out factor = 10
Valve position:	Next position

Table 4 on page 79 shows the cycle times and the possible daily throughput for different DAD and MS settings. Since the MS data are constantly written to the hard disc during data acquisition, whereas the UV data are buffered and added to the data file after the stop time of the method, the cycle time depends more on the UV data amount than on the MS data amount. The cycle time was calculated from the time stamp assigned to each file by the WindowsXP™ operating system when the file is closed at the end of data acquisition.

Table 4 Dependence of the cycle time on the DAD and MS data acquisition settings, method stop-time was 0.65 min (39 s), pre-run balance was applied (ca. 2 s). The number in brackets for the DAD wavelength range stands for the scan width in nm.

DAD (80 Hz) Type	Wavelength	TOF (100 – 1000 D Centroid)	Profile	Data rate [Hz]	Cycletime [s]	Throughput [Samples/ day]
Spectral	190-900 (1)		x	20	62	1394
Spectral	190-900 (1)	x		20	62	1394
Spectral	190-400 (2)		x	20	59	1464
Spectral	190-400 (2)		x	40	59	1464
Spectral	190-400 (2)		x	30	58	1490
Signal	210/254		x	20	50	1728
Signal	210	x		30	49	1763

4.2. Transfer of conventional method

4.2.1. Rapid Screening and Analysis of Components in Nonalcoholic Drinks[21]

The classical method, using 4.6 x 250 mm 5 µm Zorbax material, takes about 35 minutes for the gradient, wash and re-equilibration steps. With benzoate and sorbate present, we need to adjust the pH carefully to ensure good separation[22]. (Figure 42 on page 80)

Conditions
ZORBAX SB-C184.6 mm x 250 mm, 5 µm

Column temp:	**30 ˚C**
Gradient:	**20 mM H_2PO_4 pH 3.65 with ammonium hydroxide, 10 % to 50 % ACN in 25 min**
Gradient slope:	**2.8% ACN/ column volume**
Analysis flow rate:	**1.41 mL/min**
Sample:	**Standards 50 µg/mL each in method/water 1/1, 15 µL injection**
Total analysis time:	**37.5 min**
Detection:	**UV 230 nm, 10-mm 13-µL flow cell filter 2 (default)**

(Datafile SDADDS000006.D

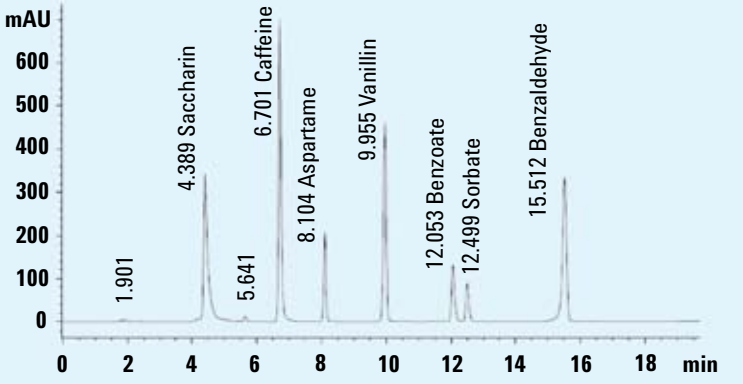


Figure 42 Analysis of Soft drink additives using 4.6 x 250 mm 5 µm in 20 minutes, with 35 minutes cycle time

Table 5 Chromatographic conditions

Sample:	sodium saccharin, caffeine, aspartame, vanillin, benzoic acid, sorbic acid, benzaldehyde, all 50 µg/ml in 1/1 methanol /water

Using a shorter column with smaller diameter and 1.8 µm particles at comparable linear velocity, the analysis was over four times faster with significantly saving in solvent. (Figure 42 on page 80)

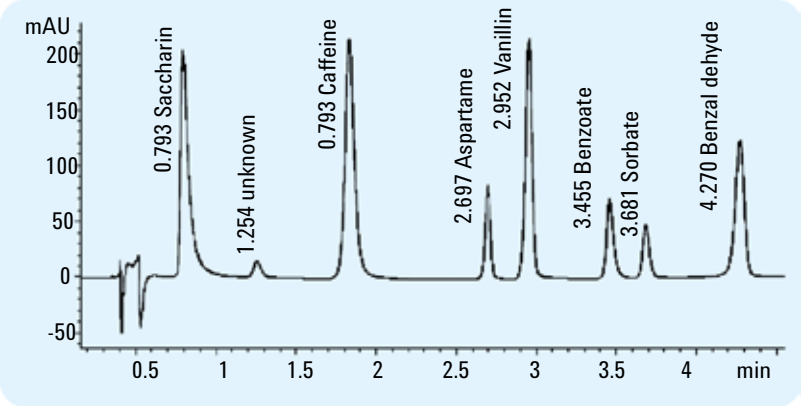


Figure 43 Analysis of soft drink additives additives on 3 x 50 mm 1.8 µm, 9 minute cycle time: 4x time saving, 7x solvent saving, improved resolution

Table 6 Chromatographic conditions

Column:	Zorbax SB, 3.0 x 50 mm, 1.8 µm
Column Temperatures:	45 °C
Gradient:	10 mM H_3PO_4, pH = 3.65 with ammonium hydroxide, 10% to 50% ACN in 5 min
Gradient slope:	2.8% ACN/ column volume
Flow rate:	0.6 ml/min
Injection volume:	2.5 µl
Total analysis time :	9 min
Detection:	UV 210 nm, 6 mm path length, 5 µl cell, filter 0.2 seconds

Increasing the linear velocity without changing the gradient allowed a further improvement: 12x faster without excessive

solvent consumption was gratifying, but then we were also able to preserve the resolution. (Figure 44 on page 82)

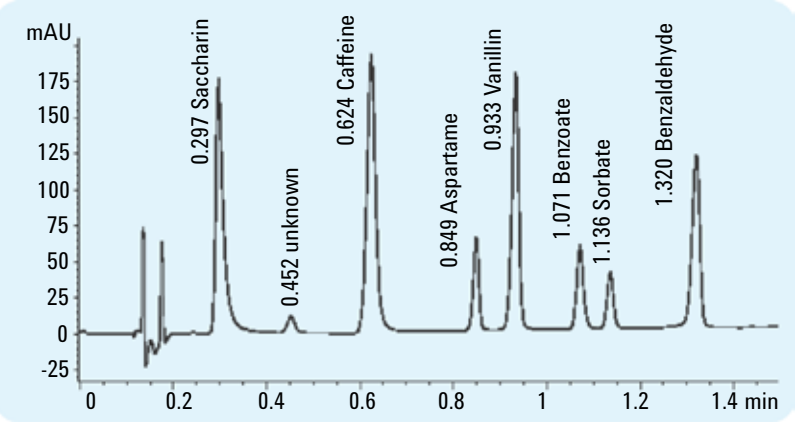


Figure 44 Analysis of soft drink additves on 3 x 50 mm 1.8 µm, 3 minute cycle time

12x time saving and 6x solvent saving, resolution preserved

Table 7 Chromatographic conditions

Column:	Zorbax SB, 3.0 x 50 mm, 1.8 µm
Column Temperatures:	45 °C
Gradient:	10 mM H_3PO_4, pH = 3.65 with ammonium hydroxide, 10% to 50% ACN in 5 min
Gradient slope:	2.8% ACN/ column volume
Flow rate:	2.0 ml/min
Injection volume:	2.5 µl
Total analysis time :	3 min
Detection:	UV 210 nm, 6 mm path length, 5 µl cell, filter 0.2 seconds

4.3. Analysis of Bisphenol A and Impurities

4.3.1. Analysis of Bisphenol A and Impurities[22]

BPA is a building block of polycarbonate and epoxy resins. It is produced using an acid-catalyzed condensation reaction of phenol with acetone. During condensation, a number of phenol-based byproducts are also formed. HPLC is used to determine the composition of many of the process streams in a commercial BPA plant.

Here, we describe the use of new HPLC column technology for the possible improvement to one of the HPLC methods used in a commercial BPA facility. This new binary gradient method is simplified, fast and has equal or better resolution than the older ternary gradient method[5]. (Figure 45 on page 83).

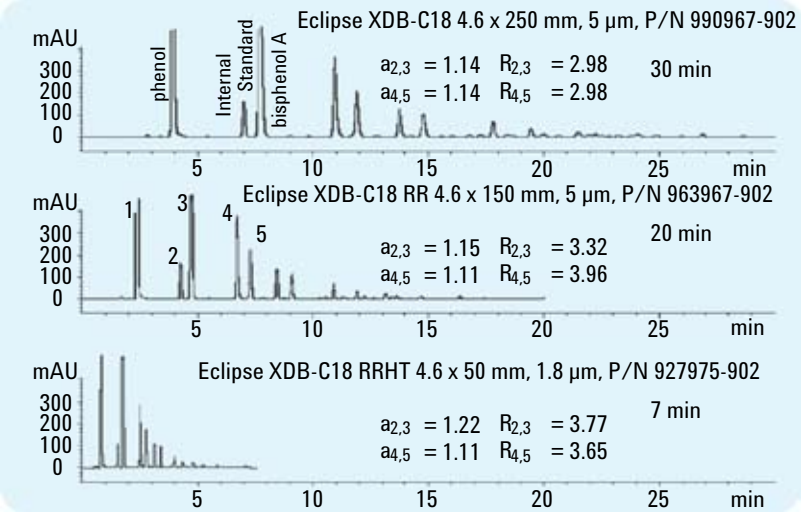


Figure 45 Bisphenol A Overall Method Improvements

Table 8 Conditions original method (upper trace)

Column:	LC-18, 4.6 x 250 mm, 5 µm
Column Temperatures:	35 °C
Mobile phase :	A= 0.025% H_2PO_4, B = ACN, C = MeOH
Gradient:	at 0 min 65%A, 25%B, 10%C At 13 min 65:.25:10 At 18 min 50:40: 10 At 23 min 50 :40:10 At 27 min 30:50:20 At 32 min 0:70:30 At 35 min 0:70:30 At 36 min 0:60:40 At 40 min 0:50:50 At 43 min 0:20:80 At 48 min 65:25:10
Flow rate:	2 ml/min
Injection volume:	20 µl
Total analysis time :	50 min

Table 9 Chromatographic conditions (lowest trace)

Column:	Zorbax XDB, 4.6 x50 mm, 1.8 µm
Column Temperatures:	25 °C
Mobile phase:	A = 0.1 formic acid, B: ACN/MeOH = 20:80
Gradient:	at 0 min 60% B At 6 min 95% B At 6.01 min 60% B At 8 min 60% B
Flow rate:	1 ml/min
Injection volume:	2 µl
Total analysis time :	8 min

4.4. Analysis of Phenolic Antioxidants and Erucamide Slip Additives

4.4.1. Using 1200 Rapid Resolution Liquid Chromatography (RRLC) with Rapid Resolution High Throughput (RRHT) Columns and Method Translator for Fast Analysis

Vitamin E (tocopherol), phenolic antioxidants and erucamide slip additives in polypropylene homopolymer formulations were resolved and detected using liquid chromatography with ultraviolet/visible detection, under guidelines suggested by ASTM Method D6042. Using the Agilent 1200 Rapid Resolution LC system with Agilent ZORBAX RRHT columns, the antioxidants could be rapidly separated with the same or improved resolution[23, 25].

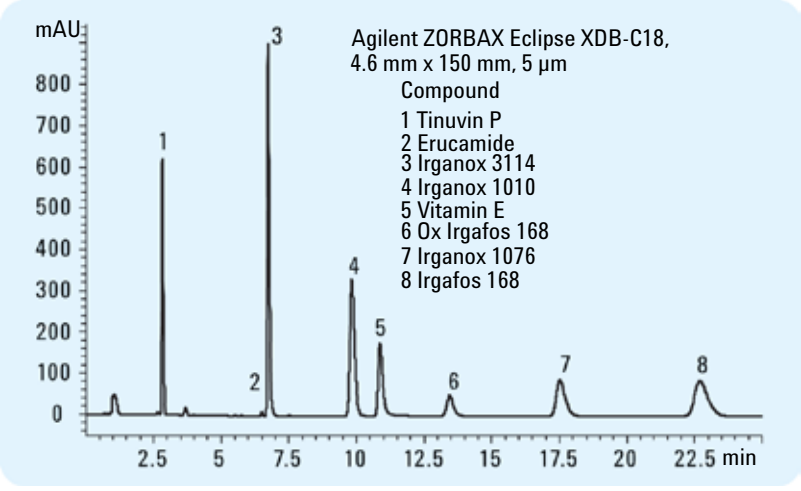


Figure 46 Analysis phenolic antioxidants and erucamide slip additives in polymer

Table 10 Chromatographic conditions: Conventional method according to ASTM D 6042

Sample:	Standard mixture, 200 µg/mL in isopropanol	ZORBAX chemistry:
Column 4.6 mm × 150 mm, 5 µm Eclipse XDB-C18	Mobile phase:	A: water; B: ACN
Flow rate:	1.5 mL/min	Gradient:
Min	%B	
0.0	75	
5	100	
25	100	
25.1	75	
30	75	Injection volume:
10 µL	Column temperature:	50 °C
Wavelength:	200 nm	

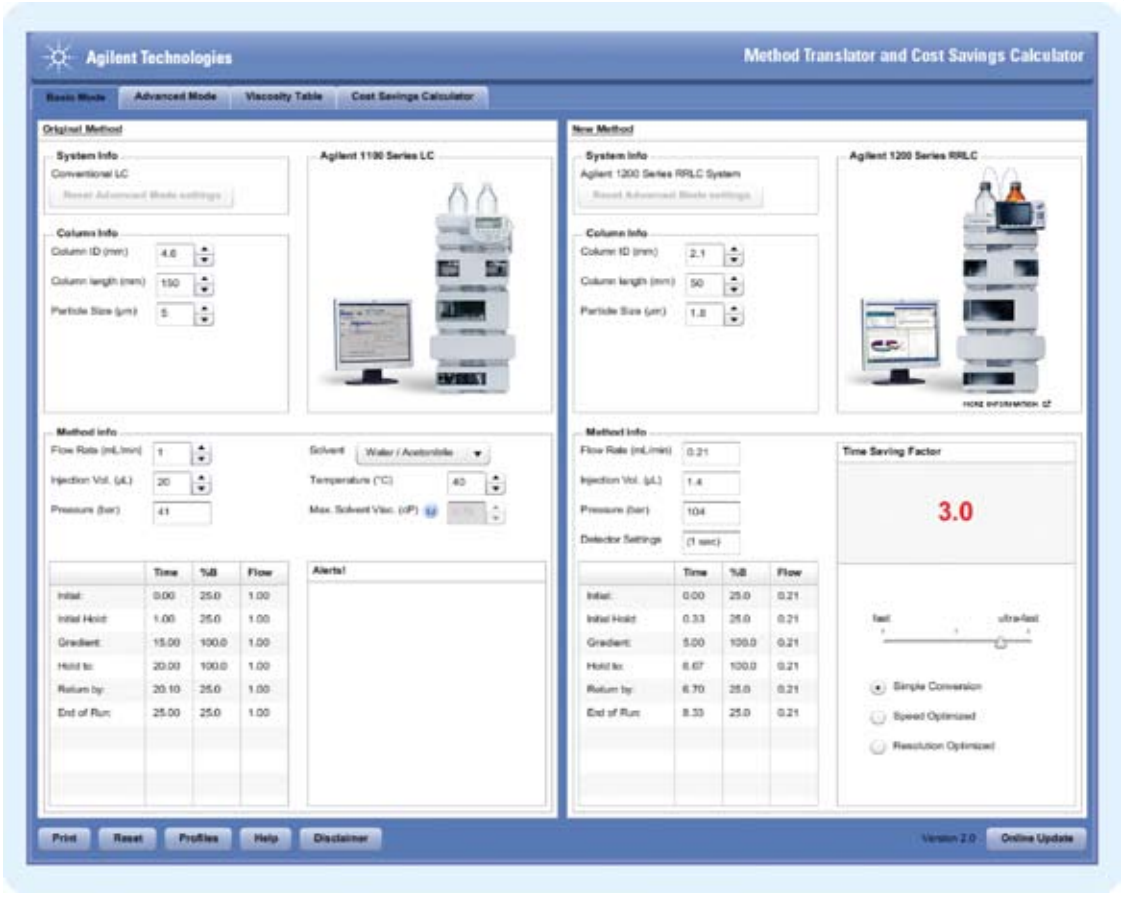


Figure 47 Method translator

The Agilent method translator[24] was used to transfer the ASTM (Figure 46 on page 85) method to new methods (Figure 48 on page 88) based on the instrument parameters, column dimensions, and particle size in three modes: simple conversion, speed-optimized and resolution-optimized methods.

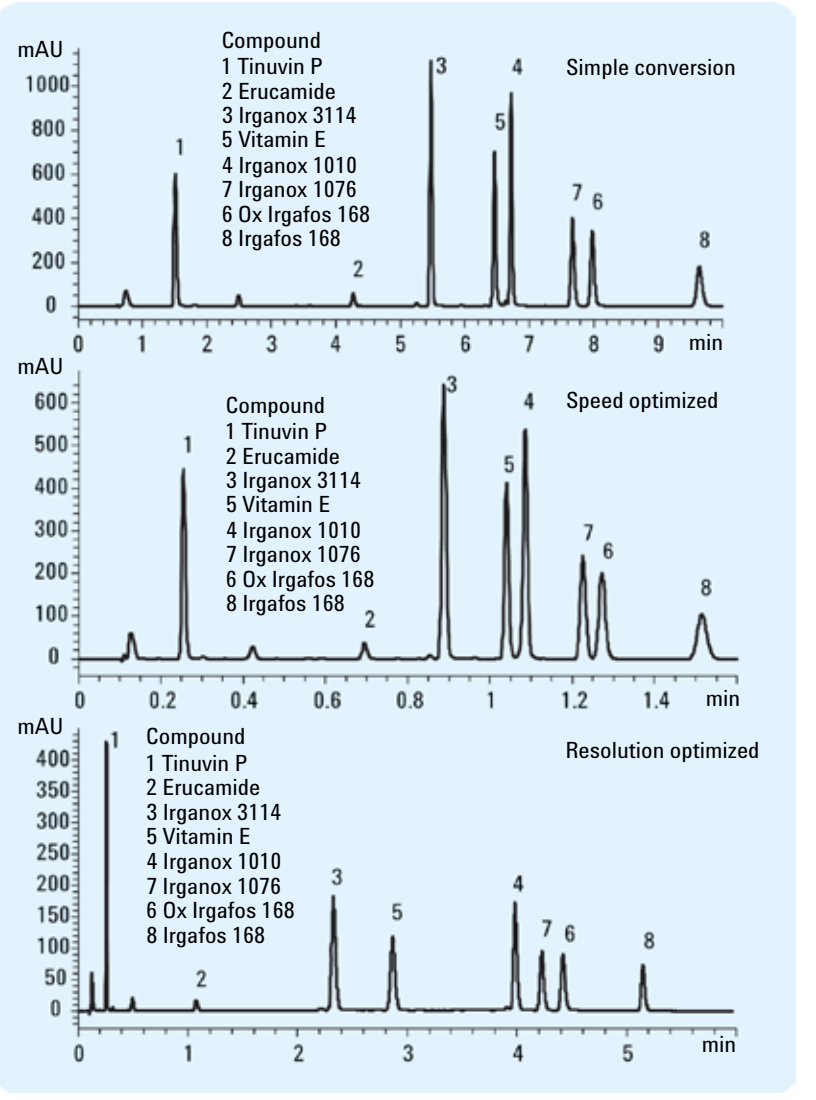


Figure 48 Separation of antioxidants on ZORBAX Eclipse XDB-C8 3.0 mm × 50 mm, 1.8 µm

Table 11 Conditions

Sample:	Standard mixture, 200 µg/mL in isopropanol	Mobile phase:	A: water; B: ACN
Temperature:	50 °C	Wavelength:	200 nm
Injection volume:	3.3 µL	Column:	ZORBAX Eclipse XDB-C8 3.0 mm × 50 mm, 1.8 µm
Mode:	Simple converted	Speed optimized	Resolution optimized
Flow rate:	0.64 mL/min	2.50 mL/min	2.50 mL/min
Pressure:	160 bar	460 bar	460 bar
Gradient slope:	3.1%	3.1%	0.8%
Analysis time:	6 min	1.4 min	3 min

A substantial improvement in analysis time and solvent reduction is achieved with only a few hours work using the 1200 SL (RRLC) system with Zorbax RRHT columns.

4.5. Validation of a fast LC method

4.5.1. Validation of method using sub-2-micron particle column

Analytical laboratories working in a regulated environment have to validate their methods to ensure that results fulfill all regulatory requirements. The validation procedure used here was based on recommendations from the U.S. Pharmacopeia (USP) and the ICH guidelines Q2B respectively. A fast LC method for one main compound and its four impurities was successfully validated[26]. (Figure 49 on page 90)

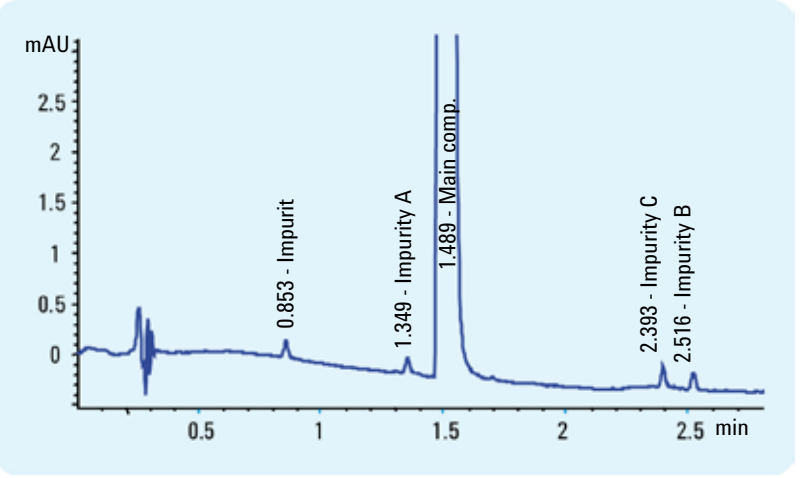


Figure 49 Fast LC method for the analysis of impurities

Column:	Zorbax SB-C18, 4.6 x 50 mm, 1.8 µm
Solvent:	A = Water (0.2 Vol-% TFA) B = acetonitrile (0.16 Vol-% TFA)
Gradient:	from 17 to 45 % B in 2.8 min 0.2 min at 45 % B
Flow:	2.2 mL/min
Temp.:	30 °C
Run-Time:	3.0 min
Post time:	1.0 min
Inj. Vol.:	5 µL
Detection:	270/ 8 nm (ref. 500/ 100 nm) flow cell 13 µL/10 mm

Having done some pre-validation experiments, the following validation protocol for the fast LC method was set up:

Validation protocol :

1. Precision of areas and RT of the main compound at 6 concentrations, 6 runs each
2. Accuracy of main compound at 6 concentrations, 6 runs each
3. Linearity of main compound at 6 concentrations, 6 runs each
4. Carry over for main compound over 3 injections of stock solution
5. Range of main compound
6. Precision of areas and RT of impurities at 7 concentrations, 6 runs each
7. Accuracy of impurities at 7 concentrations, 6 runs each
8. Linearity of impurities at 7 concentrations, 6 runs each
9. Range of impurities
10. Limit of Detection and LOQ
11. Robustness of main compound and impurities Different column temperatures, flow rates, injection volumes, TFA concentrations, gradient steepness, wavelength, users and instruments, no ruggedness tests

Example of validation results are given in Figure 50 on page 91, Figure 51 on page 92 and Figure 52 on page 92

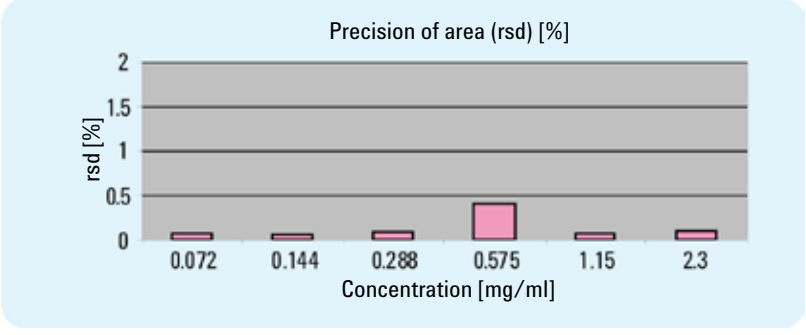


Figure 50 Precision of areas – main compound

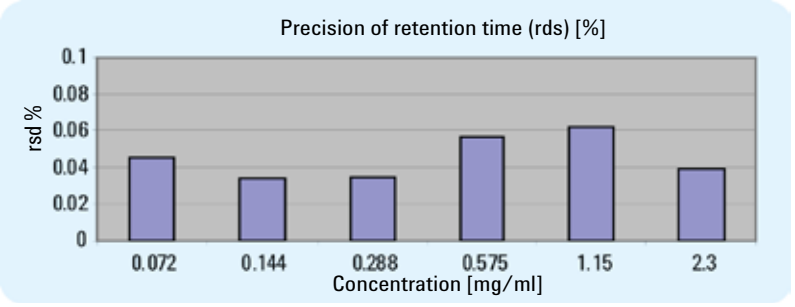


Figure 51 Precision of retention time – main compound

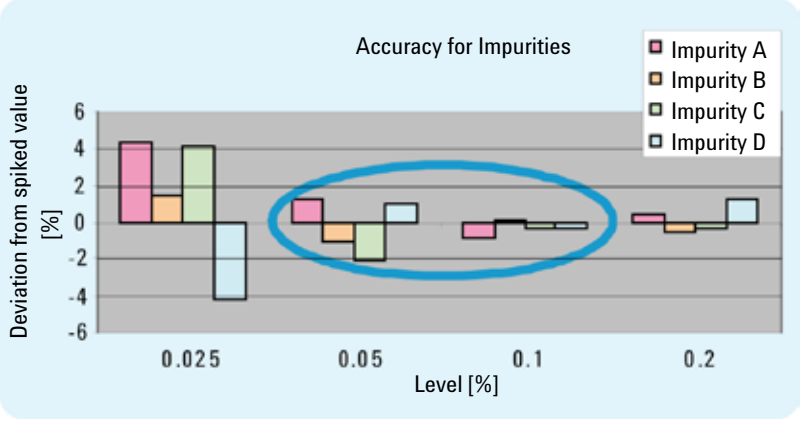


Figure 52 Accuracy – impurities The deviation from the spiked value should be less than +/- 5 % For the relevant 0.05 % level the accuracy is < 2 %

In Table 12 on page 93 the results of the method validation are summarized. The set limits are fulfilled. Special attention is required for the wavelength. The wavelength variation should

not be more than ± 1 nm. Typically, a wavelength variation of ± 3 nm is considered acceptable. In this experiment, the limits for wavelength variations are more restrictive, based on the results.

Table 12 Results of method validation

Precision for areas	passed
• Main compound < 2 % for all experiments	
• Impurities < 10 % at the limit of quantitation	passed
Accuracy for main compound < ± 2 %	passed
Accuracy for impurities at LOQ ± 5 %	passed
Precision of Retention times < 0.5 % within 1 series	passed
Precision of retention time < 2 % day-to-day	*passed*
Linearity > 0.999	*passed*
Resolution > 2 for all peaks	*passed*
LOD S/N > 2 at the ~ 0.007 % level for impurities	*passed*
LOQ S/N > 10 at the ~ 0.027 % level for impurities	*passed*
Range of the main compound: 2.3 to 0.073 mg/mL	*passed*
Range of the impurities: 4.9 (0.426 % level) to 0.287 µg/mL (0.025 % level)	*passed*
Robustness tests for area deviation: < 2 % for main compound	*passed*[1]
Robustness tests for area deviation: < ± 5 % for impurities at the 0.05 %level	*passed*[1]

1 wavelength variations of ± 1 nm are acceptable and should be carefully controlled

4

A fast LC method was developed for the analysis of a main compound and four impurities. The validation of this method was successful. All requirements regarding precision, linearity, accuracy and robustness were fulfilled. This signifies that the fast LC method can be used in QA/QC labs and is compliant with USP/ICH recommendations. Faster LC methods provide the same data quality and, as an additional benefit, higher sample throughput.

References

5

5. References

1 Knox, J. H.; Saleem, M. J. Chromatogr. Sci. 1969, 7, 614–622.

2 G. Rozing, LCGC LC Column Supplement April 2008

3 HPLC for Pharmaceutical Scientist, Wiley VCH, 2007, ISBN 978-0-471-68162-5 (Check if this is the right format)

4 Dao T.-T. Nguyen , Davy Guillarme, Serge Rudaz, Jean-Luc Veuthey, " Chromatographic behavior and comparison of column packed with sub-2µm stationary phases in liquid chromatography", Journal of Chromatography A, 1128, 2006, pp 105 – 113

5 Pete Broske, Robert Ricker, Bernard Permar, Wu Chen, Maureen Joseph "The influence of Sub-Two Micron Particles on HPLC Performance", Agilent application note 5988-9251EN, 2003

6 Angelika Gratzfeld-Huesgen, "Step-by-step upgrade of Agilent 1100 Series LC systems to Agilent 1200 Series Rapid Resolution LC systems - Part 2: 4.6 mm ID columns", Agilent Application note 5989-6337EN, 2007

7 Udo Huber, Michael Frank, "Rapid development of an optimized, fast LC method and seamless conversion for use on a standard LC system", Agilent Application note 5989-5949EN, 2006

8 Michael Frank, "Impurity Profiling with the Agilent 1200 Series LC System Part 3: Rapid Condition Scouting for Method Development", Agilent Application note 5989-5619EN, 2006

9 Angelika Gratzfeld-Huesgen, "More speed, better resolution and lower LOD using liquid chromatography and fluorescence detection - Comparing the 1100 Series LC to the 1200 Series Rapid Resolution system", Agilent Application note 5989-6044EN, 2006

10 Josef Kofmann, Yining Zhao, Todd Maloney, Todd Baumgartner, Robert Bujalski: "Ultra-High Performance Liquid Chromatography: Hope or Hype", American Pharm Review, Vol 9, 2006, 88-93

11 Mary J. Wirth: "Mass transport in sub-2-µm high performance liquid chromatography", Journal of chromatography A, 1148, 2007, 128-130

12 Andre de Villiers, Henk Lauer, Roman Szucs, Stuart Goodall, Pat Sandra, " Influence of frictional heating on temperature gradients in ultra-high-pressure liquid chromatography on 2.1mm id columns", Journal of chromatography A, 1113, 2006, pp 84-91

13 Georges Guiochon, Fabrice Gritti Complete Temperature Profiles in Ultra-High-Pressure Liquid Chromatography Columns, Anal. Chem. 2008, 80, 5009–5020

14 Angelika Gratzfeld-Huesgen, "Using elevated temperatures with the Agilent 1200 Series Rapid Resolution LC System for more speed, more resolution, and better peak shape in LC applications", Agilent Application note 5989-5032EN, 2006

15 Angelika Gratzfeld-Huesgen , "Evaluation of linear and step gradient performance and retention time precision of the Agilent 1200 Series Rapid Resolution LC system", Agilent application note 5989-5031EN, 2006

16 Angelika Gratzfeld-Huesgen , "Performance of the Agilent 1200 Series diode-array detector SL using different detector cells and different data rates up to 80 Hz", Agilent Application note 5989-5033EN, 2006

17 Angelika Gratzfeld-Huesgen , "Agilent 1200 Series column compartment SL with temperature control up to 100 °C and post-column cooling for lowest baseline noise", Agilent Application note 5989-5034EN, 2006

18 Angelika Gratzfeld-Huesgen ,"The Agilent 1200 Series high performance autosampler SL: Area precision, injection volume linearity, minimum accessible volume, carry over", Agilent Application note 5989-5161EN, 2006

19 Angelika Gratzfeld-Huesgen , "Performance of the Agilent 1200 Series variable wavelength detector SL using different detector cells and different data rates up to 55 Hz", Agilent Application note 5989-5361EN, 2006

20 Agilent 1200Series Rapid Resolution LC System, System manual G1312-9030017

21 Anabel Fandino, Stefan Schuette, "Ultra-fast liquid chromatography using the Agilent 1100 Series HPLC system and 1.8-um ZORBAX SB C18 Rapid Resolution HT columns", Agilent Application note 5989-1603EN, 2004

22 John V. Nightingale et al. "Resolution in gradient HPLC, fast LC and U-HPLC Part 1: The influence of peak capacity", Poster presentation, HPLC 2007 in Gent

23 John W. Henderson Jr. and William J. Long, "Tailoring Speed, Sensitivity, and Resolution in an RRHT Analysis of Cardiac Drugs", Agilent Application note 5989-5899EN, 2006

24 Michael Frank, "Agilent 1200 Series Rapid Resolution LC system and the Agilent 6210 TOF MS - Highest data content with highest throughput", Agilent Application note 5989-4505EN, 2006

25 Michael Woodman, "Rapid Screening and Analysis of Components in Nonalcoholic Drinks", Agilent Application note 5989-5178EN, 2006

26 John W. Henderson Jr , William J. Long and Stephen Fetsko, “Process Monitoring of Bisphenol-A in Industrial Feedstock using High Throughput HPLC”, Agilent Application note 5989-5231EN, 2006

27 1200 Series Rapid Resolution System - Compendium CD, 5989-5130EN, 2006, “The 1200 Series Rapid Resolution LC compendium CD is a comprehensive information package featuring the new world of rapid resolution LC and LC/MS performance. It includes application notes, brochures with detailed specifications, 3D videos and conference posters as well as a system configurator and method translator for rapid resolution LC.” Free of charge

28 Chun-Xiao Wang, Luan Wei and Michael Woodman, “Fast Analysis of Tocopherol, Phenolic Antioxidants and Erucamide Slip Additives in Polypropylene Homopolymer Formulations Using 1200 Rapid Resolution Liquid Chromatography (RRLC) with Rapid Resolution High Throughput (RRHT) Columns and Method Translator”, Agilent Application note 5989-5849EN, 2007

29 Angelika Gratzfeld-Huesgen, “Impurity Profiling with the Agilent 1200 Series LC System Part 4: Method Validation of a Fast LC Method “, Agilent Application note 5989-5620EN, 2006

30 Angelika Gratzfeld-Huesgen, “ Step-by-step upgrade of Agilent 1100 Series LC systems to Agilent 1200 Series Rapid Resolution LC systems - Part 1: 2.1 mm ID columns”, Agilent Application note 5989-6336EN, 2007

31 Angelika Gratzfeld-Huesgen, “Optimizing the Agilent 1100 series well-plate sampler”, Agilent Application note 5988-1432EN, 2003

32 Angelika Gratzfeld-Huesgen, “Optimization of the Agilent 1100 Series well-plate autosampler for lowest carry-over using an optional injector purge kit”, Agilent Application note 5989-63363357EN, 2005

33 Michael W.Dong, “ Modern HPLC for Practical Scientists,” Wiley Interscience, ISBN 0-471-72789-X, 2005

Appendices

6. Appendices

6.1. Appendix A

6.1.1. 1200 Series RRLC configuration guide

In the following typical 1200 Series RRLC instrument configurations are shown

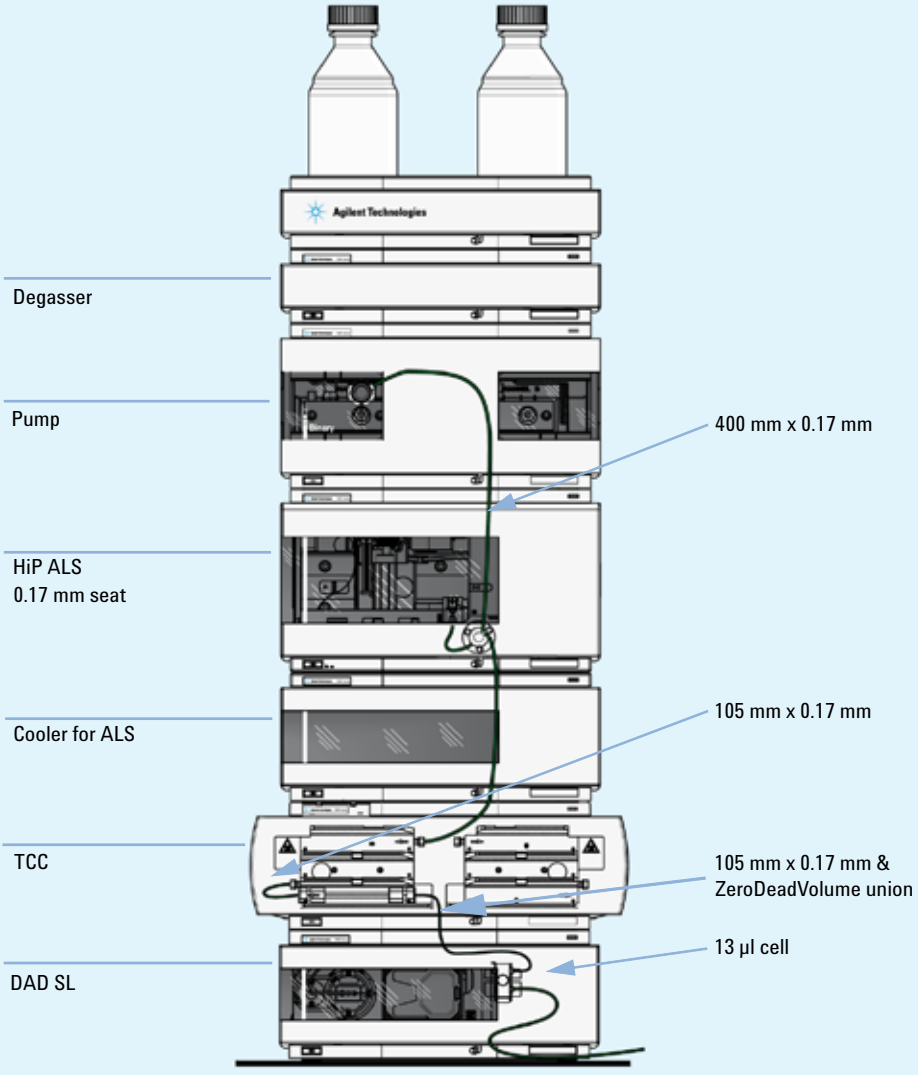


Figure 53 1200 RRLC system in standard delay volume configuration for 4.6 mm & 3.0 mm id columns

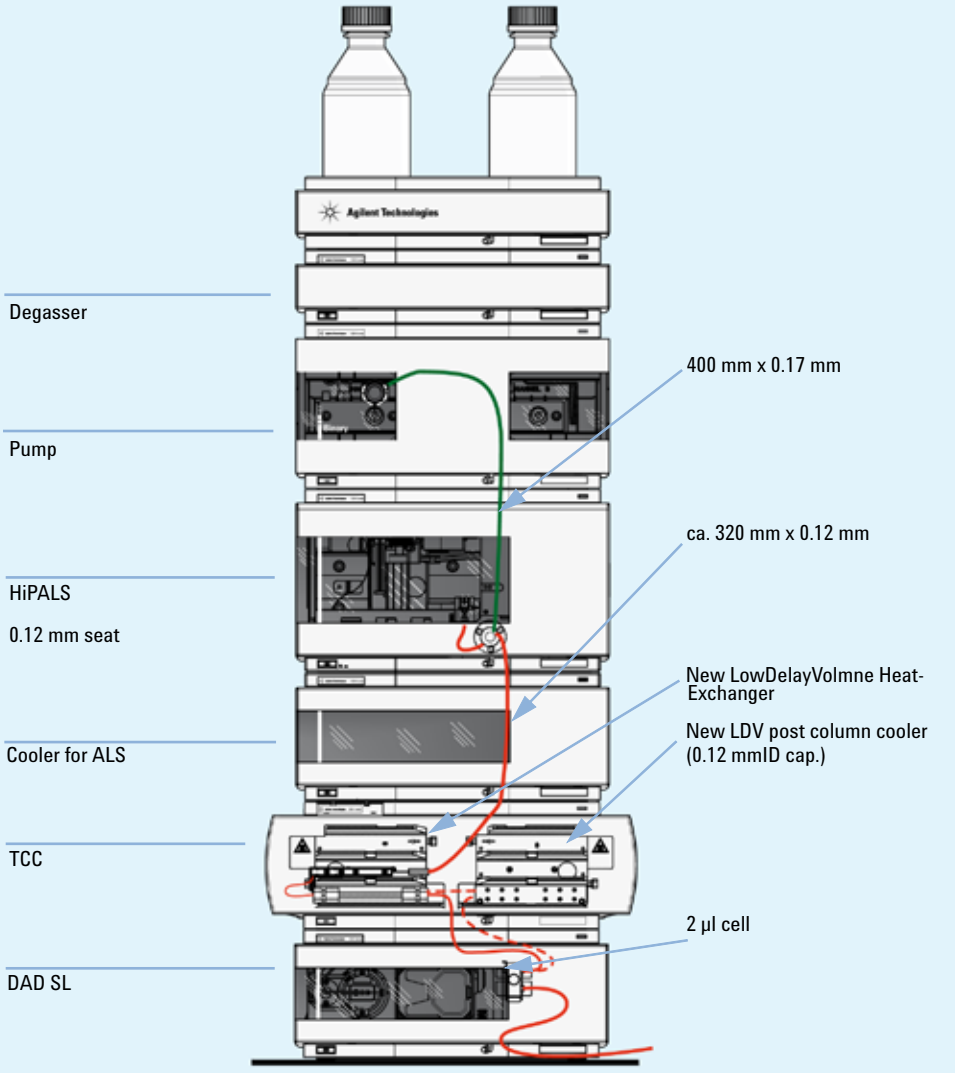


Figure 54 1200 Series RRLC System in low delay volume configuration for 2.1 mm & 3.0 mm id columns

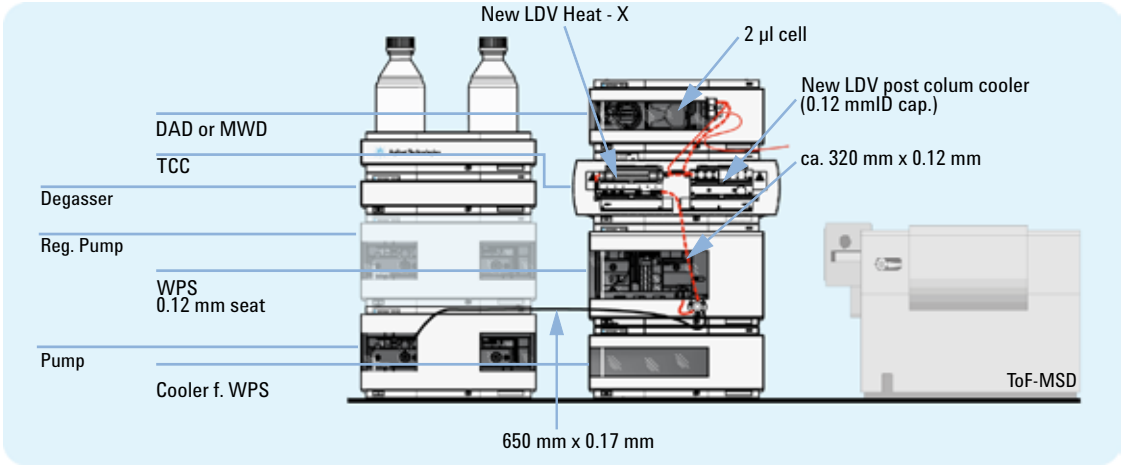


Figure 55 1200 Series RRLC System with automated column regeneration and TOF in low delay volume configuration

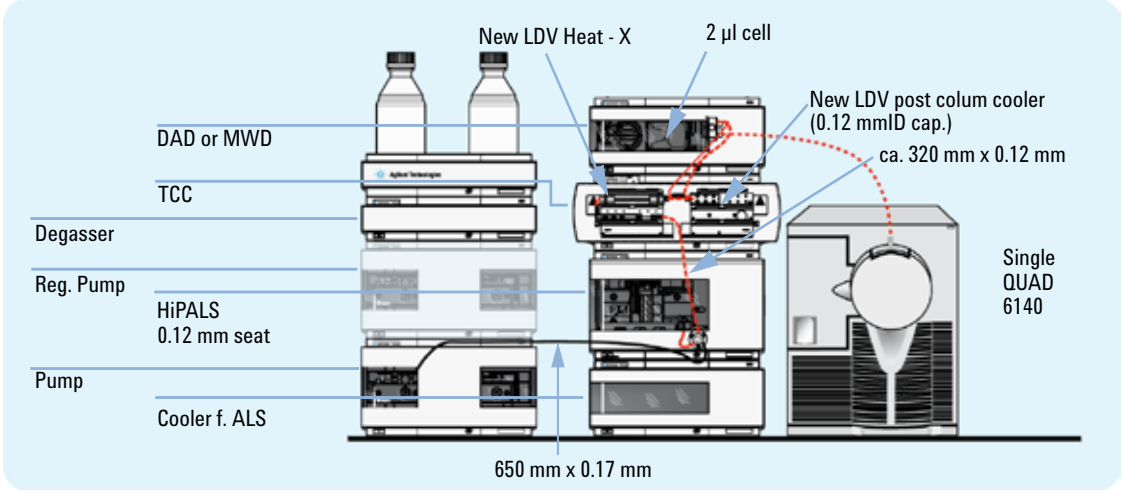


Figure 56 1200 Series RRLC System with automated coulmn regeneration and single quadrupole in low delay volume configuration for 2.1 mm & 3.0 mm id columns

6.2. Appendix B

6.2.1. Zorbax Column selection

ZORBAX Eclipse Plus – (C18 and C8) first choice for method development, industry leading peak shape performance, good for most applications, use up to 60°C. Note: L1 (C18) and L7 (C8) columns – accepted by USP

ZORBAX StableBond – good for low pH and high temperature applications, for those who want to raise temperature for faster analysis

- SB-C18 – use up to 90°C and down to pH <1 (high temp only at low pH) (L1)
- SB-C8 – use up to 80°C and down to pH 1 (L7)
- SB-Phenyl – use up to 80°C at pH 2 (L11)
- SB-CN – use up to 80°C at pH 2
- SB-AQ – use in up to 100% aqueous mobile phases for good retention – up to 60°C at pH 2

ZORBAX Extend-C18 – good at high pH – up to pH 11.5 at room temperature (L1)

ZORBAX Eclipse XDB – (C18 (L1) and C8 (L7)), general purpose bonded phase, for most types of compounds, use up to 60°C

ZORBAX Rx-Sil – for normal phase applications and “HILIC” type applications where an aqueous containing mobile phase is used on bare silica.

6.3. Appendix C

6.3.1. Specifications

Table 13 Capillaries

ID [mm]	[inch]	Color coding Peek	SS	Volume [µL/m]	Backpressure at 30 °C and 1 mL/min H2O/ACN	H2O/MeOH
0.127	0.005	Red	Red	12.67	23.8	38.4
0.178	0.007	Yellow	Green	24.88	6.2	10.0
0.254	0.010	Blue	Blue	50.67	1.5	2.4
0.508	0.020	Orange	---	202.68	0.1	0.2
0.762	0.030	Green	---	456.04	0.0	0.0

Backpressures are given for 1 m length and the maximal viscosity of the solvent mixtures at 30 °C

H_2O/ACN=9:1 (0.91 cP), H_2O/MeOH=1:1 (1.47 cP).

ACN=Acetonitrile; MeOH=Methanol

Table 14 Column void volume

Analytical ID [mm]	Length [mm]	Column volume [µL]	Preparative ID [mm]	Length [mm]	Column volume [mL]
2.1	20	48	10	20	1.10
	30	73		30	1.65
	50	121		50	2.75
	100	242		100	5.50
	150	364		150	8.25
	250	606		250	13.74
3.0	20	99	21	20	4.85
	30	148		30	7.27
	50	247		50	12.12
	100	495		100	24.25
	150	742		150	36.37
	250	1237		250	60.61
4.6	20	233	50	20	27.49
	30	349		30	41.23
	50	582		50	68.72
	100	1163		100	137.44
	150	1745		150	206.17
	250	2908		250	343.61

A proposity of 0.70 of the stationary phase was assumed.

Table 15 Buffer systems

Buffer	pKa	UV cut-off [nm]	MS-compatible
TFA	0.3	210	Yes
Phosphate	2.1; 7.2; 12.3	190	No
Citrate	3.1; 4.7; 5.4	225	No
Formate	3.8	200	Yes
Acetate	4.8	205	Yes
Carbonate	6.4; 10.3	200	Yes
TRIS	8.3	210	No
Ammonia	9.2	200	Yes
Borate	9.2	190	No
Dietylamine	10.5	235	No

Buffers are only effective within +/-1.5 pH units from their pKa value.

Source: Michael W. Dong, Modern HPLC for the Practicing Scientist, Wiley, 2006.

Table 16 Solvent characteristics

Solvent	Formula	Snyder polarity index	Melting point [°C]	Boiling point [°C]	Viscosity [cP]	Density [g/mL]	UV cut-off [nm]
Acetonitrile	CH_3CN	6.2	-44	82	0.37	0.782	210
Acetone	$(CH_3)_2CO$	5.4	-95	56	0.32	0.788	330
Acetic acid	CH_3COOH	6.2	17	118	1.26	1.049	---
Ethanol	CH3CH2OH	5.2	-114	78	1.20	0.789	210
Methanol	CH_3OH	6.6	-98	65	0.60	0.791	210
Propanol	CH3CH2CH2OH	4.3	-127	97	2.30	0.800	210
iso-Propanol	(CH3)2CHOH	4.3	-88	82	2.30	0.786	210
Ethylacetate	CH3COOCH2CH3	4.3	-84	77	0.45	0.901	260
THF	$(CH_2)_3$-CH_2O	4.2	-109	66	0.47	0.888	220
DMF	HCON(CH3)2	6.4	-60	153	0.91	0.945	268
DMSO	$(CH_3)_2SO$	6.5	19	189	2.24	1.096	---
Hexane	CH_3-$(CH_2)_4$-H_3	0.0	-95	69	0.98	0.659	200
cyclo-Hexane	$(CH_2)_6$	0.0	6	81	1.00	0.778	200
Toluene	C_6H_5-CH_3	2.3	-95	111	0.59	0.867	285
Water	H_2O	9.0	0	100	1.00	0.998	180

Several sources, mainly from Friedrich W. Küster, „Rechentafeln für die chemische Analytik“, de Gruyter 1982, ISBN 3-11-006653-X.

6.4. Appendix D

Important formula and relationships in LC and MS

6.4.1. Height equivalent of a theoretical plate H

$$H = \frac{L_c}{N}$$

L_c: column length

N: plate count

6.4.2. Reduced plate height h

$$h = \frac{H}{d_p} = \frac{L_c}{N \cdot d_p}$$

d_p: particle size

6.4.3. Number of theoretical plates, efficiency N

$$N = \left(\frac{t_R}{\sigma}\right)^2 = 16\left(\frac{t_R}{w_{4\sigma}}\right)^2 = 5.54\left(\frac{t_R}{w_{1/2}}\right)^2; \quad N = \frac{L_c}{h \cdot d_p}$$

σ: peak standard deviation

w: peak width

t_R: retention time

6.4.4. Linear velocity u

$$u = \frac{L_c}{t_0}; \quad u_i = \frac{4 \cdot F}{\varepsilon_i \cdot \pi d_c^{\,2}}$$

(interstitial velocity of a compound that is excluded from the pores)

ε_i: interstitial porosity

d_c: column diameter

F: flow

6.4.5. Reduced velocity v

$$\nu = \frac{u \cdot d_p}{D_M} = \frac{L_c \cdot d_p}{t_0 \cdot D_M}$$

D_M: diffusion coefficient in the mobile phase

6.4.6. Van-Deemter equation:

$$H = A + \frac{B}{u} + C \cdot u; \qquad H = A \cdot d_p + B \cdot \frac{D_M}{u} + C \frac{d_p^2}{D_M} \cdot u$$

6.4.7. Knox equation

$$h = A \cdot \nu^{\frac{1}{3}} + \frac{B}{\nu} + C \cdot \nu$$

6.4.8. Retention factor k

$$k = \frac{t_R - t_0}{t_0}$$

k (isocratic)

$$k^* = \frac{t_g \cdot F}{1.15 \cdot S \cdot \Delta\Phi \cdot V_M}$$

(average k* under gradient conditions)

t_g: gradient time

S constant

$\Delta\Phi$: gradient slope

V_M: column void column

6.4.9. Selectivity α

$$\alpha = \frac{k_2}{k_1}$$

6.4.10. Resolution equation

$$R_S = \frac{1}{4}\frac{(\alpha - 1)}{\alpha} \cdot \sqrt{N} \cdot \frac{k}{1+k}$$

6.4.11. Resolution R_S

$$R_S = 2\frac{t_{R2} - t_{R1}}{w_1 + w_2} = 1.18\frac{t_{R2} - t_{R1}}{w_{1/2_1} + w_{1/2_2}}$$

6.4.12. Peak capacity n_C

$$n_c = 1 + \frac{t_g}{w_b \cdot R_S}$$

(gradient)

$$n_c = 1 + \frac{\sqrt{N}}{4 \cdot R_S} \cdot ln(1 + k_{max})$$

(isocratic)

t_g: duration of the linear solvent strength gradient

6.4.13. Porosity ε

$$\varepsilon_t = \frac{V_o + V_p}{V_c} = \frac{V_M}{V_c}$$

ε_t(total porosity)

V_c: geometrical volume of column

V_o: volume of flowing mobile phase

V_p: volume of mobile phase in the particles

V_M: total volume of mobile phase in the column:for NP-silcia ε ca. 0.8, for RP-materials ca. 0.65, for non-porous particles ca. 0.4

$$\varepsilon_i = \frac{V_o}{V_c}$$

(interstitial or interparticle porosity)

6.4.14. Permeability B

$$B = \frac{u \cdot L_c}{\Delta p};\ B_0 = \frac{4 \cdot F \cdot \eta \cdot L_c}{\pi d_c^2 \cdot \Delta p};\ B_0 = \frac{1}{180} \cdot \frac{\varepsilon_i^3}{(1-\varepsilon_i)^2} \cdot d_p^2;\ B_0 \cong \frac{d_p^2}{1000}$$

B: permeability

B_0: specific permeability

Δp: column backpressure

η: viscosity

Kozeny-Carman equation

6.4.15. Flow resistance factor Φ

$$\Phi = \frac{d_p^2}{B_0}$$

6.4.16. Separation impedance E

$$E = \frac{t_0 \cdot \Delta p}{N^2 \cdot \eta} = \varepsilon_t \cdot h^2 \cdot \Phi$$

E is around 5000 for a good column, but can be as low as 3000

6.4.17. Pressure-particle size dependence

$$\Delta p \propto \frac{1}{d_p^2}; \qquad \Delta p = 1000 \frac{4 \cdot F \cdot L_c \cdot \eta}{\pi d_c^2 \cdot d_p^2} = \frac{\Phi \cdot \eta \cdot L_c \cdot \nu \cdot D_M}{d_p^3}$$

6.4.18. Pressure-capillary diameter dependence

$$\Delta p \propto \frac{1}{d_{cap}^4}; \quad \Delta p = \frac{128 \cdot F \cdot L_{cap} \cdot \eta}{\pi d_{cap}^4}$$

d_{cap}: capillary diameter

L_{cap}: capillary length

6.4.19. Viscosity-temperature dependence

$$\ln \eta \propto \frac{1}{T} \; ; \eta = \eta_0 e^{\frac{E_A}{R \cdot T}}$$

(for Newtonian fluids, Arrhenius-Andradeequation)

η_0: solvent constant

E_A: activation energy

R: universal gas constant

6.4.20. Probability of chromatographic separation P

$$P = \left(1 - \frac{m-1}{n_c - 1}\right)^{m-2}$$

(resolution of 1 assumed)

m: number of compounds present

n_c: peak capacity

6.4.21. Extinction E

$$E = log \frac{I_0}{I} = \varepsilon \cdot c \cdot d$$

(Lambert-Beer law)

ε: molar coefficient of extinction

c: concentration

d: path length

I: intensity

6.4.22. Mean free path L

$$L = \frac{k_B \cdot T}{\sqrt{2} \cdot p \cdot \sigma}$$

k_B: Boltzmann constant

T: temperature

p: pressure

σ: collision cross section

6.4.23. Mass resolution of MS

$$R = \frac{m}{\Delta m}$$

6.4.24. Mass accuracy of MS

$$\Delta m = \frac{m_{th} - m_{exp}}{m_{th}} \cdot 10^6 \ [ppm]$$

6.5. Appendix E

6.5.1. Common parts for the 1200 RRLC System

Description	Part number
PTFE frit for the purge valve	01018-22707
Gold seal for PFTE frit	5001-3707
Capillary: pump to injector	G1312-87303
Capillary: pump to thermo ALS	G1312-87304
Needle assay For G1367B/C	G1367-87201
Needle seat for G1367C	G1367-87104
Seat capillary 150 x 0.12 mm	G1367-87303

Description	Part number
Seat capillary 150 x 0.1 7mm	G1367-87302
3 groove Rotor seal for max 600 bar	0101-1409
Capillary kit	G1316-68711
2ml screw glass vial amber	5183-2069
Septa blue rubber	5182-0717
Low dispersion kit	G1316-68744
Rotor seal Injector PEEK	0101-1416
Capillary: sampler to column 380 x 0.17 mm	01090-87306
Capillary: sampler to column 250 x 0.17 mm	G1367-87304
Tray for 2 plates	G2258-60011
Vial plate 54 x 2 ml	5022-6539
Micro column regeneration capillary kit	G1316-68721
Standard flow cell assembly 10 mm, 13 µl	G1315-60022
Semi-micro flow cell assembly 6 mm, 5 µl	G1315-60025
Micro flow cell assembly 3 mm, 2 µl	G1315-60024
Nano flow cell kit 500 nl, 10 mm	G1315-68724
special SS hex nut fitting, PEEK ferrule, 6/pk for 600 Bar non-Agilent and Agilent columns	5067-1540
Black ferrules for connecting columns to small heaters and cooler	5067-1547
Ferrules 1/16" fittings +ferrules 10/pk	5062-2418
Polyketone fittings	5042-8957
200 µl mixer for SL pump in low delay configuration	5067-1565
Isocratic standard	01080-68704
RRLC Check out sample, 1 mL ampoule contains 8 phenones & acetanilide, 100 µg/mL in Water/ Acetonitrile 65:35	5188-6529

6.6. Appendix F

6.6.1. Literature list

For more recent applications go to our web site http://www.chem.agilent.com/

Table 17 Literature Agilent 1200 Series Rapid Resolution System

Literature type	Title	Publication number
Brochures		
Brochure	Agilent 1200 Series Rapid Resolution LC System	5989-8206EN
Data sheet	Increased Sample-Handling Capacity for High-Throughput LC/MS Applications	5989-5800EN
Data sheet	Agilent 1200 Series Pumping Systems	5989-4332EN
Data sheet	Agilent 1200 Series Injection Systems	5989-8082EN
Data sheet	Agilent 1200 Series Thermostatted Column Compartments and Valve Solutions	5989-4334EN
Data sheet	Agilent 1200 Series UV-visible Detectors	5989-8187EN
Data sheet	Increased Sample-Handling Capacity for High-Throughput LC/MS Applications	5989-5800EN
Columns		
Selection Guide	Agilent ZORBAX Column Selection Guide for HPLC	5989-5992EN
Brochure	Agilent's newly expanded ZORBAX Rapid Resolution High Throughput (RRHT) and Rapid Resolution (RR) HPLC Column Portfolio	5989-6010EN
Data sheet	Agilent ZORBAX Rx-Sil RRHT Threaded Column Datasheet	

Table 17 Literature Agilent 1200 Series Rapid Resolution System

Literature type	Title	Publication number
Data sheet	Agilent ZORBAX SB-AQ RRHT Threaded Column Datasheet	
Data sheet	Agilent ZORBAX Eclipse Plus C8 Rapid Resolution HT Threaded Column Datasheet	
Data sheet	Agilent ZORBAX SB-Phenyl RRHT Threaded Column Datasheet	
Data sheet	Agilent ZORBAX Eclipse Plus C18 Rapid Resolution HT Threaded Column	
Data sheet	Agilent ZORBAX SB-CN RRHT Threaded Column Datasheet	
Data sheet	Agilent ZORBAX SB-C8 RRHT Threaded Column Datasheet	
Data sheet	Agilent ZORBAX Eclipse XDB-C8 RRHT Threaded Column Datasheet	
Data sheet	Agilent ZORBAX Extend-C18 RRHT Threaded Column Datasheet	
Data sheet	Agilent ZORBAX SB-C18 RRHT Threaded Column Datasheet	
Data sheet	Agilent ZORBAX Eclipse XDB-C18 RRHT Threaded Column Datasheet	
Multimedia		
CD	1200 Series Rapid Resolution System - Compendium CD. It includes application notes, brochures with detailed specifications, 3D videos and conference posters as well as a system configurator and method translator for rapid resolution LC	5989-5130EN
Article reprints		

Table 17 Literature Agilent 1200 Series Rapid Resolution System

Literature type	Title	Publication number
Article reprints	Ultra-High Performance Liquid Chromatography: Hope or Hype	5989-5169EN
Article reprints	High-speed analyses using rapid resolution liquid chromatography on 1.8-m porous particles	5989-6116EN
Technical Overview		
Technical Overview	Step-by-step upgrade of Agilent 1100 Series LC systems to Agilent 1200 Series Rapid Resolution LC systems - Part 2: 4.6 mm ID columns	5989-6337EN
Technical Overview	Step-by-step upgrade of Agilent 1100 Series LC systems to Agilent 1200 Series Rapid Resolution LC systems - Part 1: 2.1 mm ID columns	5989-6336EN
Technical Overview	Easy Transfer of Standard HPLC Methods to the Agilent 1200 Series Rapid Resolution LC System	5989-5873EN
Technical Overview	The Agilent 1200 Series high performance autosampler SL: Area precision, injection volume linearity, minimum accessible volume, carry-over	5989-5161EN
Technical Overview	Performance of the Agilent 1200 Series variable wavelength detector SL using different detector cells and different data rates up to 55 Hz	5989-5361EN
Technical Overview	Agilent 1200 Series column compartment SL with temperature control up to 100 C and post-column cooling for lowerst baseline noise	5989-5034EN
Technical Overview	Evaluation of linear and step gradient performance and retention time precision of the Agilent 1200 Series Rapid Resolution LC system	5989-5031EN

Table 17 Literature Agilent 1200 Series Rapid Resolution System

Literature type	Title	Publication number
Technical Overview	Performance of the Agilent 1200 Series diode-array detector SL using different detector cells and different data rates up to 80 Hz	5989-5033EN
Technical Overview	Using elevated temperatures with the Agilent 1200 Series Rapid Resolution LC system for more speed, more resolution, and better peak shape in LC applications	5989-5032EN
Technical Overview	Performance characteristics of the Agilent 1200 Series high performance autosampler SL Plus	5989-8117EN
Technical Overview	NEW Technical note VWD SL Plus	
Technical Overview	Agilent 1200 Series Rapid Resolution LC system - Switching between standard and low delay volume configurations using a software-controlled, high-pressure valve for optimized separation performance	5989-7732EN
Software control		
Third Party SW	Agilent 1200 Series Rapid Resolution LC system controlled by Chromeleon – Supported modules and tasks	5989-8347EN
Third Party SW	Enjoying the benefits of rapid resolution:Agilent 1200 Series RRLC system is fully controlled by Agilent EZChrom Elite: Supported modules and new features of EZChElite 3.3	5989-8133EN
Third Party SW	Controlling Agilent 1200 Series Rapid Resolution LC systems through Waters Empower chromatography data software	5989-9317EN

Table 17 Literature Agilent 1200 Series Rapid Resolution System

Literature type	Title	Publication number
Application Notes		
Application	Rapid Analysis of Herbicides by Rapid Resolution LC with Online Trace Enrichment	5989-5176EN
Application	More speed, better resolution and lower LOD using liquid chromatography and fluorescence detection - Comparing the 1100 Series LC to the 1200 Series Rapid Resolution system	5989-6044EN
Application	Rapid Analysis of Drugs of Abuse by LC/ Triple Quadrupole Mass Spectrometry	5989-4856EN
Application	Fast and Ultra-Fast Analysis Using the Agilent 1200 Series Rapid Resolution LC System Compared to a Conventional Agilent 1100 Series LC System	5989-5672EN
Application	Improving the Effectiveness of Method Translation for Fast and High Resolution Separations	5989-5177EN
Application	Optimization of the Agilent 1100 Series well-plate autosampler for lowest carry-over using an optional injector purge kit	5989-3357EN
Application	Fast Analysis Method for Rubber Chemical Antidegradants Using 1200 Rapid Resolution Liquid Chromatography (RRLC) Systems with Rapid Resolution High Throughput (RRHT) Columns (HPI)	5989-6011EN
Application	Fast Analysis of Tocopherol, Phenolic Antioxidants and Erucamide Slip Additives in Polypropylene Homopolymer Formulations Using 1200 Rapid Resolution Liquid Chromatography (RRLC) with Rapid Resolution High Throughput (RRHT) Columns and Method Translator	5989-5849EN

Table 17 Literature Agilent 1200 Series Rapid Resolution System

Literature type	Title	Publication number
Application	Analysis of Phenolic Antioxidant and Erucamide Slip Additives in Polymer by Rapid-Resolution LC (HPI)	5989-5850EN
Application	Exploiting RRHT Columns with Different C18 Selectivities to Quickly Develop Methods for Endocannabinoids (Pharmaceutical)	5989-6128EN
Application	Unique Selectivity and High-Throughput Applications of SB-Phenyl RRHT (Pharmaceutical)	5989-6067EN
Application	An LC/MS System Designed for Rapid High Throughput Analysis of Pharmaceutical Compounds	5989-5917EN
Application	Agilent MassHunter - Fast computer aided analysis of LC/ESI-TOF data from complex natural product extracts - Part 1: Analysis of 6210 data with the Molecular Feature Extractor in MassHunter Workstation software	5989-5928EN
Application	Rapid development of an optimized, fast LC method and seamless conversion for use on a standard LC system	5989-5949EN
Application	Agilent MassHunter - Fast computer aided analysis oif LC/ESI-TOF dta form complex natural product extracts - Part 1: Analysis of 6210 data with the Molecular Feature Extractor in MassHunter Workstation software	5989-5928EN
Application	Impurity Profiling with the Agilent 1200 Series LC System Part 2: Isolation of Impurities with Preparative HPLC	5989-5618EN
Application	Impurity Profiling with the Agilent 1200 Series LC System Part 5: QA/QC Application Example Using a Fast LC Method for Higher Sample Throughput	5989-5621EN

Table 17 Literature Agilent 1200 Series Rapid Resolution System

Literature type	Title	Publication number
Application	Impurity Profiling with the Agilent 1200 Series LC System Part 3: Rapid Condition Scouting for Method Development	5989-5619EN
Application	Impurity Profiling with the Agilent 1200 Series LC System Part 4: Method Validation of a Fast LC Method	5989-5620EN
Application	The Benefits of Achieving High Mass Accuracy at High Speed Using Agilent's TOF-MS Technology	5989-5918EN
Application	Impurity Profiling with the Agilent 1200 Series LC Systems - Compendium	5989-5841EN
Application	Tailoring Speed, Sensitivity, and Resolution in an RRHT Analysis of Cardiac Drugs	5989-5899EN
Application	Improve Peak Shape and Productivity in HPLC Analysis of Pharmaceutical Compounds with Eclipse Plus C8 Columns	5989-5803EN
Application	Agilent 1200 Series Rapid Resolution LC system and the Agilent 6210 TOF MS - Highest data content with highest throughput	5989-4505EN
Application	Analysis of a complex natural product extract from ginseng - Part III: Species differentation of ginseng plants and authentication of ginseng products by LC/MS	5989-5493EN
Application	Analysis of a complex natural product extract from ginseng - Part II: Structure elucidation of ginsenosides by high resolution ion trap LC/MS	5989-4705EN
Application	Rapid Analysis of the Beta Blocker Pindolol	5989-5098EN
Application	High Throughput HPLC Analysis of Barbiturates	5989-5092EN

Table 17 Literature Agilent 1200 Series Rapid Resolution System

Literature type	Title	Publication number
Application	Simultaneous determination of metabolic stability and identification of buspirone metabolites using multiple column fast LC/TOF mass spectrometry	5989-5110EN
Application	Scalability as a Function of Column Dimensions Using ZORBAX Rapid Resolution HT Columns for the Analysis of the Pharmaceutical Triamcinolone	5989-4878EN
Application	Achieving fastest analyses with the Agilent 1200 Series Rapid Resolution LC system and 2.1-mm id columns	5989-4502EN
Application	The High-Resolution Reversed-Phase HPLC Separation of Licorice Root Extracts using Long Rapid resolution HT 1.8-um Columns	5989-4907EN
Application	Analysis of a complex natural product extract from ginseng - Part I: Structure elucidation of ginsenosides by rapid resolution LC-ESI TOF with accurate mass measurement	5989-4506EN
Application	Impurity Profiling with the Agilent 1200 Series LC System Part 1: Structure Elucidation of Impurities with LC/MS	5989-5617EN
Application	Resolving Potentially Harmful Azo-Colorant Amines Using the Distinct Selectivities of the Agilent ZORBAX Eclipse Plus Phenyl-Hexyl and StableBond Phenyl Columns	5989-8542EN
Application	Polycyclic Aromatic Hydrocarbon (PAH) Separations Using ZORBAX Eclipse PAH Columns - Analyses from Six to 24 PAHs	5989-7968EN
Application	Robustness of Eclipse PAH Columns for HPLC Analysis of Polycyclic Aromatic Hydrocarbons	5989-7828EN

Table 17 Literature Agilent 1200 Series Rapid Resolution System

Literature type	Title	Publication number
Application	Separation of Explosives in EPA 8330: Column Choices Optimize Speed, Resolution, and Solvent Use	5989-7632EN
Application	A Total Solution for the Analysis of Melamine and Cyanuric Acid in Pet Food	5989-7546EN
Application	Determination of Buprenorphine, Norbuprenorphine, and Their Glucuronides in Urine Using LC/MS/MS	5989-7072EN
Application	The Analysis of Benzodiazepines in Hair Using RRHT LC/MS/MS	5989-7270EN
Application	Determination of Benzodiazepines in Oral Fluid Using LC/MS/MS	5989-7201EN
Application	Quantitative Analysis of Ethylglucuronide in Urine Using the Agilent 1200 RRLC and 6410 Triple Quadrupole Mass Spectrometer	5989-7000EN
Application	Improving performance, data and results through expanded dynamic range and higher resolution - Identification of pharmaceutical metabolites using the Agilent 6520 QTOF LC/MS system and MassHunter Met ID software, RRLC, rapid resolution	5989-8528EN
Application	Fast, efficient HPLC purification of peptides from solid-phase synthesis	5989-8306EN
Application	Examination of the differences in related complex samples using accurate mass data from TOF and QTOF analysis	5989-8086EN
Application	Detection of Phencyclidine in Human Oral Fluid Using Solid Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometric Detection	5989-8084EN

Table 17 Literature Agilent 1200 Series Rapid Resolution System

Literature type	Title	Publication number
Application	Fast, computer-assisted detection of degradation of products and impurities in pharmaceutical products - Identification of minor components in drug substances using the Agilent 6210 time-of-flight mass spectrometer and MassHunter Profiling software	5989-7869EN
Application	Increased Identification of Impurities in Octyl Dimethyl p-Aminobenzoic Acid Using the Agilent 6140 High Throughput LC/MS	5989-6953EN
Application	Separation of Salicylic Acid Impurities with Different Acid Mobile-Phase Modfiers	5989-7731EN
Application	Improving productivity in the determination of parameters for early in vitro ADME - Part 1	5989-7488EN
Application	Improving productivity in the determination of parameters for early in vitro ADME - Part 3 – Experimental details and results of Caco-2 permeability assay	5989-7668EN
Application	Improving productivity in the determination of parameters for early in vitro ADME - Part 4 – Experimental details and results of plasma protein binding assay	5989-7669EN
Application	Improving productivity in the determination of parameters for early in vitro ADME- Part 2 – Experimental details and results of human liver microsome stability assay	5989-7667EN
Application	Developing a fast, generic method for rapid resolution LC with quadrupole MS detection	5989-7592EN

Table 17 Literature Agilent 1200 Series Rapid Resolution System

Literature type	Title	Publication number
Application	Fast LC analysis with quadrupole MS detection – without loss of spectral quality	5989-7591EN
Application	Development of reliable quality control methods for TCM preparations using rapid resolution LC with UV and MS detection	5989-7682EN
Application	Rapid determination of the metabolic stability of pharmaceutical drug candidates by triple quadrupole mass spectrometry	5989-7195EN
Application	An interwoven, multi-algorithm approach for computer-assisted identification odf drug metabolites	5989-7375EN
Application	Rapid and selective quantification of drug compounds for cassette-dosing DMPK studies by multi-MRM triple quadrupole MS	5989-7211EN
Application	Improved Metabolite Isolation from Samples of Biological Origin Using Liquid	5989-6668EN
Application	Fast identification of main drug metabolites by quadruple time-of-flight LC/MS	5989-6759EN
Application	Success Story at Orchid Chemicals and Pharmaceuticals – Fast analysis with the Agilent 1200 Series Rapid Resolution LC system cuts method development time by 50 percent	5989-8152EN
Solution		
Solution	Food Solutions with HPLC	5989-1947EN

Table 17 Literature Agilent 1200 Series Rapid Resolution System

Literature type	Title	Publication number
Manuals		
Manual	Agilent 1200 Series Rapid Resolution LC System System Manual	G1312-90300
Others		
	Method translator & Cost savings calculator	
	http://www.chem.agilent.com/Scripts/Generic.ASP?lPage=60931	

www.agilent.com/chem/RRLC

Printed in Germany
01/2009

Agilent Technologies

G1312-90301