Ultra-fast liquid chromatography using the Agilent 1100 Series HPLC system and 1.8-µm ZORBAX SB C18 Rapid Resolution HT columns

Technical Overview

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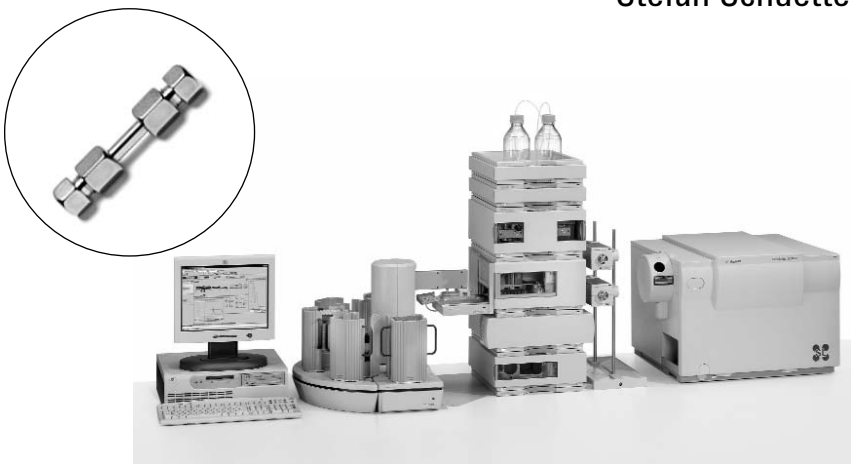

Abstract

In recent years sample generation has rapidly increased both in chemical and pharmaceutical laboratories. Therefore, high-throughput analytical approaches are needed, not only in the discovery and development process but during the entire life cycle of a product. Purity, chemical and metabolic stability data as well as quality control data are primarily gained by means of liquid chromatography (LC). In order to get conclusive LC analytical data swiftly, it is necessary to perform faster LC analyses in a reliable manner without losing chromatographic performance. In this publication we describe the recommended system configuration to achieve very fast analysis and cycle times, with and without alternating column regeneration by using ZORBAX 1.8-µm rapid resolution high throughput (RRHT) columns on the Agilent 1100 Series liquid chromatograph. Performance and robustness of the system is exemplified by the analysis of a set of alkylphenones. We illustrate the baseline separation of 9 compounds within 29 seconds, achieving very sharp peaks of 0.5 seconds as well as outstanding resolution at backpressures of only 300 bar. Furthermore, performing a long-term study using alternating column regeneration with 8000 injections (4000 injections per column) we demonstrate the extremely long lifetime of our columns and reliability of the entire system.

Agilent Technologies

Introduction

High throughput analysis in liquid chromatography is mainly determined by the instrument speed and the instrument capacity. In this publication we focus on instrument speed and describe how to reduce analysis time to seconds and therefore increase sample throughput without compromising resolution and chromatographic performance. The minimum configuration necessary for ultra-fast analysis consists of the following modules.

Agilent 1100 Series binary pump – offering
- high speed and selectivity in fast gradient runs with minimized delay volume (as low as 300 µL, if the instrument is set up and configured accordingly), and
- superior gradient performance and retention time precision with high pressure gradient mixing and variable stroke technology.

The pump is combined with a vacuum degasser to assure minimal detector noise. In order to decrease dwell volume an Upchurch filter (80 µL) instead of a solvent mixer is used.

Agilent 1100 Series well-plate autosampler (WPS) – provides increased sample injection speed of < 30 seconds including inner and outer needle wash and injection loop wash. The injection speed of the 1100 Series well-plate autosampler is more than twice as fast as the 1100 Series standard autosampler. The 1100 Series WPS allows optimization of gradient analysis by using the automatic delay volume reduction (ADVR) option to reduce delay volume to 6 µL. To further decrease cycle times, the overlapped injections (OI) option can be used, which allows the next sample to be drawn while the present sample is being analyzed.

Agilent 1100 Series thermostatted column compartment – offering Peltier heating and cooling for convenient and stable operation above, at and below ambient temperatures. Optional column switching valve for column selection and column regeneration (see item 6).

Agilent ZORBAX 1.8-µm rapid resolution high throughput (RRHT) columns – decreasing analysis time and solvent usage by using these short columns packed with sub-2 micron (STM) particles, while resolution is maintained[1-3].

Agilent 1100 Series diode-array detector – equipped with a 500-nL flow cell (for reduced dwell volume and highest resolution) or with a standard flow cell (for highest sensitivity). This detector provides fast 20 Hz multi-signal and full spectra data acquisition, a programmable slit for easy optimization of sensitivity and spectral selectivity and a dual lamp design for highest sensitivity over the complete wavelength range from 190 to 950 nm.

Optional:
Agilent 1100 Series valve solutions – for alternating column regeneration. Using two identical columns in the system and operating them alternately using a 2-position 10-port valve further reduces cycle time by saving the time needed for column flushing and regeneration[4]. In this case a second pump is required.

Agilent 1100 Series sample capacity extension (SCE) – a flexible and scalable robotic plate loading system. It expands the capacity from 2 to 80 well plates and 24 vial plates, offering a maximum number of 30,720 samples and 1296 2-mL vials. In addition, the 1100 SCE enables walk-up and mixed format operation, barcode driven execution and LIMS integration.

Instrumentation

Recommended system for ultra-fast LC-UV without alternating column regeneration.

Figure 1 illustrates the system configuration:
- Agilent 1100 Series binary pump (G1312A) without standard mixer and with Upchurch filter (80 µL, part number 5064-8273)
- Agilent 1100 Series vacuum degasser (G1322A)
- Agilent 1100 Series well-plate autosampler (G1367A)
- Agilent 1100 Series thermostatted column compartment (G1316A)
- Agilent 1100 Series DAD UV detector (G1315B) equipped with a 500-nL flow cell (G1315-60014) with 0.125-mm ID inlet (G1375-87328) and 0.125-mm ID outlet capillary (G1375-87329) or with a standard flow cell with 0.17-mm ID inlet and outlet capillaries (G1315-60012)
- Connecting capillaries (figure 1).

Note: In the standard configuration the 500-nL flow cell is equipped with 0.1-mm ID inlet and outlet capillaries. However,

to reduce backpressure we recommend using 0.125-mm ID inlet and outlet capillaries for optimized performance. For information, the backpressure for the original and the modified flow cell has been calculated for water at a flow rate of 2.5 mL/min (see Appendix).

Recommended system for ultra-fast LC-UV with alternating column regeneration.

Figure 2 illustrates the system configuration:

- Agilent 1100 Series binary pump (G1312A) without standard mixer and with Upchurch filter (80 µL, part number 5064-8273)
- Agilent 1100 Series vacuum degasser (G1322A)
- Agilent 1100 Series well-plate autosampler (G1367A)
- Agilent 1100 Series thermostatted column compartment with integrated 2-position/10-port valve (G1316A)
- Various pump types can be used as a regeneration pump. If only column regeneration is performed, an isocratic pump (G1310A) can be used. When performing column wash and regeneration, a quaternary pump (G1354 A) or a binary pump (G1312 A) is required.
- Agilent 1100 Series diode-array detector (G1315B) equipped with a 500-nl flow cell (G1315-60014) with 0.125-mm ID inlet (G1375-87328) and 0.125-mm ID outlet capillary (G1375-87329) or with a standard flow cell with 0.17-mm ID inlet and outlet capillaries (G1315-60012)
- Capillary kit column regeneration TCC (G1316-68711), containing 12 capillaries (0.17 mm ID), tubing and fittings for the

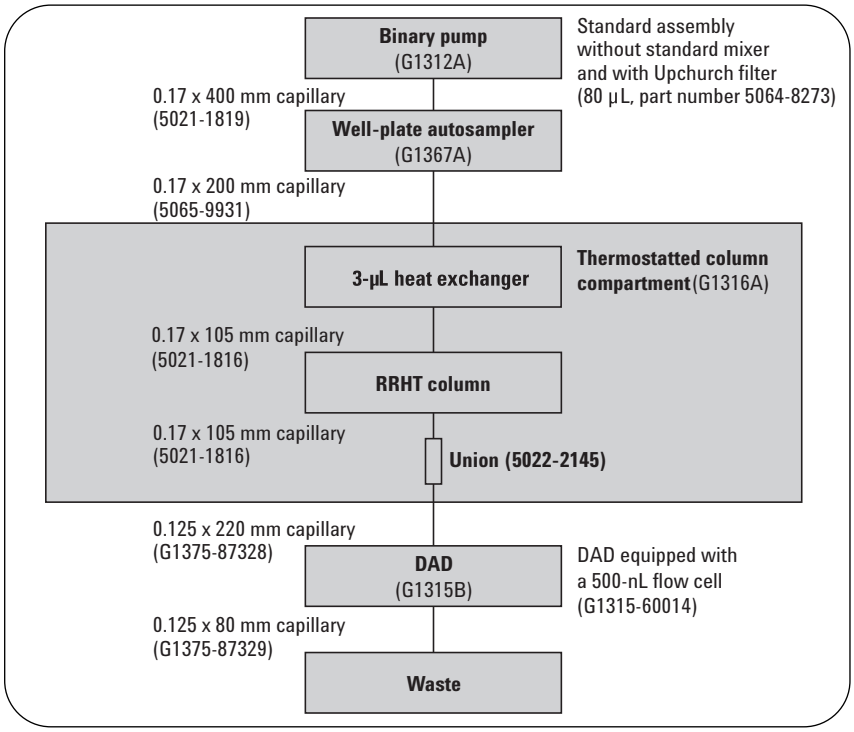

Figure 1
Agilent 1100 Series configuration, designed for ultra-fast LC analyses.

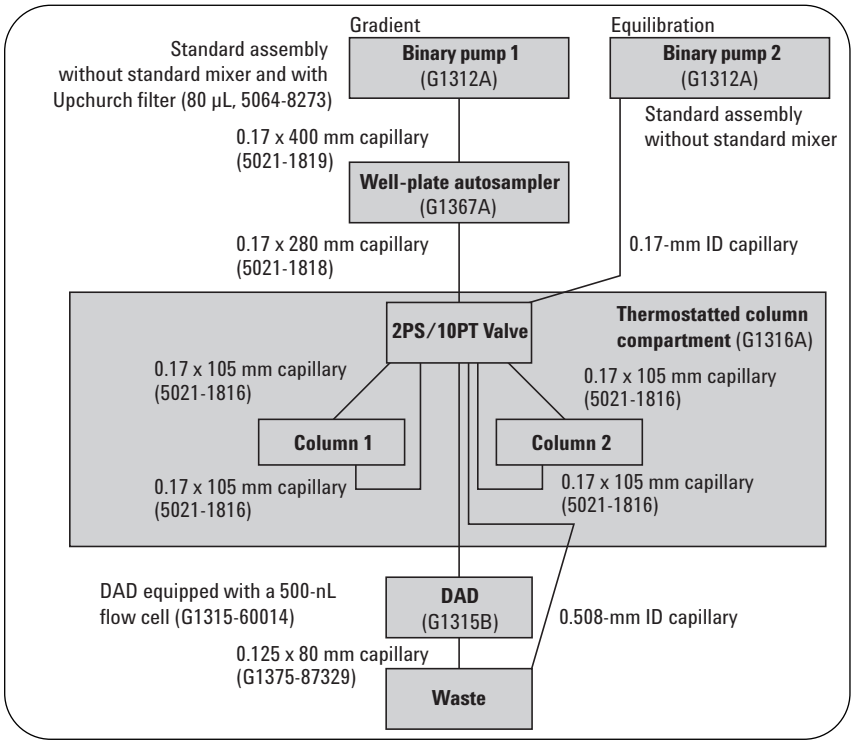

Figure 2
Agilent 1100 Series high-throughput configuration, specially designed for very fast cycle times (injection to injection times).

2-position/10-port valve. The fittings should be screwed directly into the corresponding parts, in order to prevent void volumes.

Chromatographic column
Recommended rapid resolution high throughput column:

- ZORBAX StableBond-C18 4.6 x 50 mm, 1.8 µm (822975-902)

Technical principles

Principle of automatic delay volume reduction (ADVR)
ADVR is used to switch the injector flow from mainpass (figure 3a) to bypass (figure 3b) after injection has taken place. This method allows the needle, sample loop and metering device to be excluded from the flow path thus reducing the delay volume to 6 µL. The user can specify at which time during the analyses the valve should switch to bypass (sample flush-out time t_1) using the *Sample Flush-Out Factor*, which is available in the auxiliary group (figure 3c), or choose a fixed flush-out time. When using the *Sample Flush-out Factor*, the sample flush-out time can be calculated as follows:

$$t_1 = \frac{\text{Sample flush-out factor} \times (\text{injection vol.} + \text{seat capillary vol.} + \text{valve vol.})}{\text{flow rate}}$$

where the seat capillary volume = 2.3 µL and valve volume = 1.7 µL for this instrument configuration.

ADVR can be run in either mode in conjunction with *Overlapped Injection*.

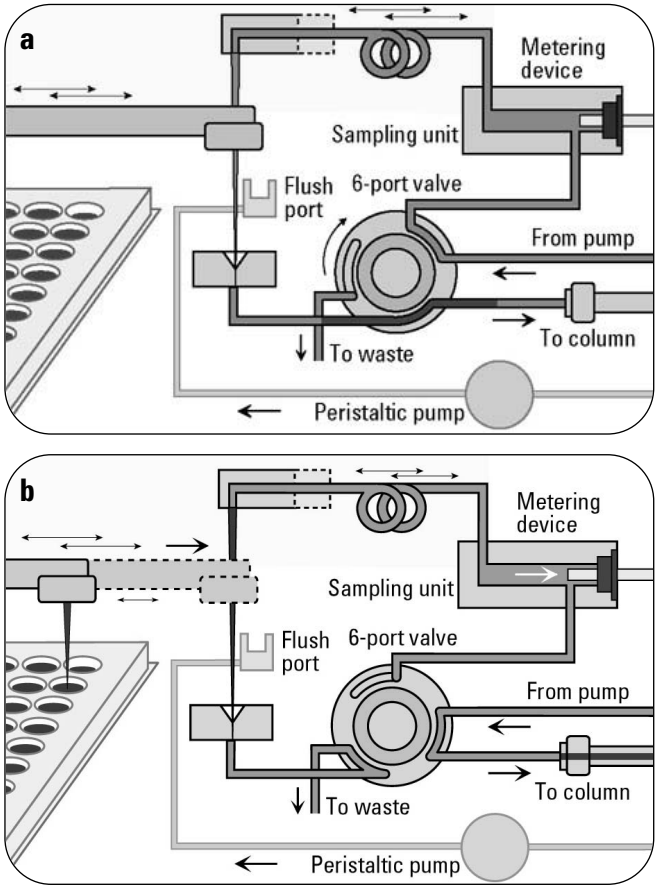

Figure 3a,b
Automatic delay volume reduction: a) mainpass mode b) bypass mode.

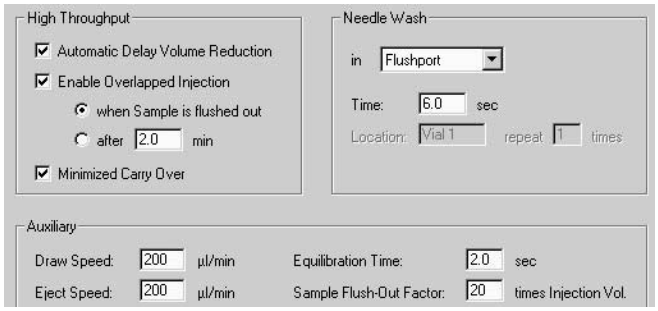

Figure 3c
ChemStation user interface for the 1100 Series well-plate autosampler.

Principle of overlapped injections (OI)
Overlapped injection (OI) provides faster throughput of samples by allowing the preparation of the next injection while the current injection is being completed. The benefit is that more samples are analyzed during a given period of time, although the actual individual injection time remains constant.

When OI mode is in operation, the next sample is drawn into the autosampler and, optionally, an outer needle wash is performed while the first sample is being analyzed, see figure 11. The new sample may be drawn either after the flush-out time for the previous sample or after a fixed time (in minutes, from 0.0 to 100.0). Selecting *When Sample is flushed out* gives the user the option to increase or decrease the flushing time needed to clean the injector and minimize carry over by changing the *Sample Flush-out Factor*.

Principle of minimized carry over (MCO)

Selecting the *Minimized Carry Over* option, the valve is automatically switched three times (bypass – mainpass – bypass) before a new sample is drawn in overlapped injection mode to remove sticky compounds from the grooves of the injection valve. MCO is available when either ADVR or OI is selected.

Principle of alternating column regeneration[4]

The use of a 2-position/10-port valve (figure 4) allows column regeneration to achieve shorter cycle times and higher throughput. Gradient elution is widely used for fast separation of complex samples. Since gradient elution requires the column to regenerate before subsequent runs, an automated column regeneration system saves valuable cycle time. The column regeneration valve allows simultaneous analysis on one column while a second, identical column is flushed and equilibrated by an additional regeneration pump. At the end of each run the valve in the column compartment is switched to the next position using the *Next position* command in the *Column Thermostat Method* window. Depending on whether the regeneration pump is an isocratic or gradient pump, column equilibration or column wash and equilibration can be performed while the next analysis is already in progress. The 2-position/10-port valve can be integrated into the thermostatted column compartment. This offers full temperature control from 10 degrees below ambient up to 80 °C.

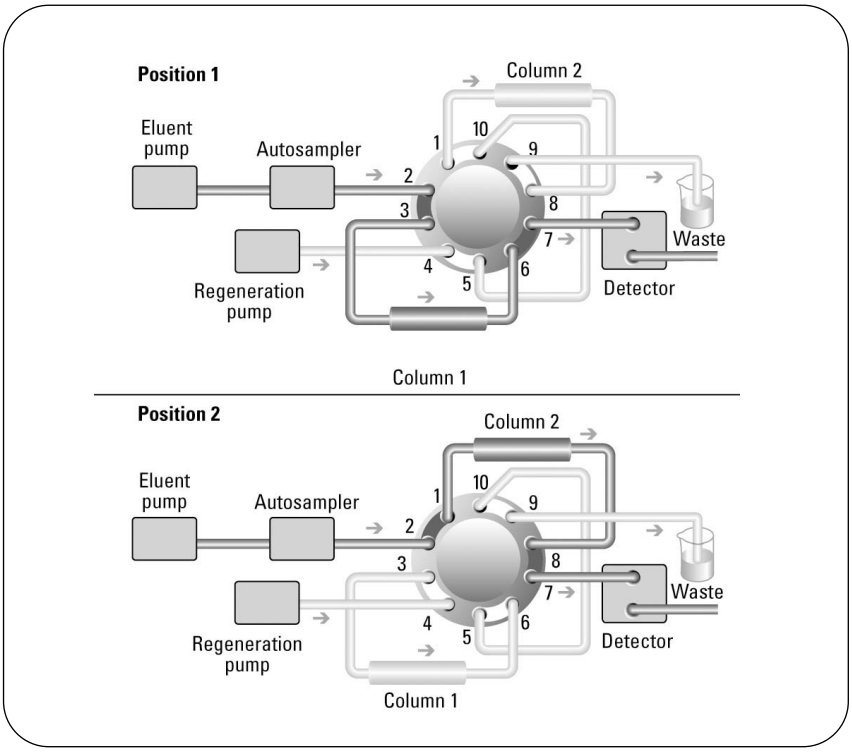

Figure 4
2-position/10-port valve for alternating column regeneration.

Experimental conditions

Test mixture

A set of alkylphenones (100 ng/µL each) consisting of: 1. acetanilide, 2. acetophenone, 3. propiophenone, 4. butyrophenone, 5. benzophenone, 6. valerophenone, 7. hexanophenone, 8. heptanophenone and 9. octanophenone dissolved in acetonitrile was analyzed with the system described.

Method description

Injection:	1 µL
Separation:	Mobile phase: A: water + 0.1% HCOOH; B: acetonitrile + 0.1% HCOOH.
Gradient:	from 50% B to 100% B in 0.65 min, hold over 0.2 min. Stop time = 1.2 min.
Temperature:	32 °C
DAD detection:	UV signal = 245 nm, 10 nm Reference = 360 nm, 80 nm Slit: 8 nm Peak width (response time): < 0.01 min (0.1 sec), i.e. 20 Hz data acquisition rate.

Results and Discussion

Ultra-fast LC experiments without alternating column regeneration

The steps of a chromatographic run (gradient separation, column wash and equilibration) are executed sequentially when no alternating column regeneration is performed. Figure 5 shows the ultra-fast separation of a set of alkylphenones using the configuration without alternating column regeneration (figure 1) and a 500-nL flow cell with 0.125-mm inlet and outlet capillaries, at 3 different flow rates and at a temperature of 32 °C. The results were compared with a competitor's method[5] (table 1).

System backpressures were dependent on flow rate. The backpressure increased with increasing flow rate, achieving 383 bar at 2.90 mL/min. The influence of flow rate on chromatographic performance was determined using the following parameters (table 1): cycle time (total time injection to injection), run time, analysis time (time until the last peak elutes), retention time difference between the last and first eluting peaks (ΔRT), average 4-sigma peak width (peak width at 13.4 %, $W_{4\sigma}$), resolution between components 4 and 5 (butyrophenone and benzophenone, respectively, $R_{s\,(4,5)} = 1.176$ ($tr_{\text{peak 5}}$-$tr_{\text{peak 4}}$/ ($W_{1/2\,\text{peak 4}} + W_{1/2\,\text{peak 5}}$), backpressure and retention time precision (n = 6).

As flow rate increases the retention time difference between the last and first eluting peaks decreases. The retention time of the first and last eluting peaks (acetanilide and octanophenone, respectively) are as short as 0.233 and 0.868 min at 2.90 mL/min and 0.293 and 0.981 min at 2.3 mL/min. The average $W_{4\sigma}$ also decreases with increasing flow rate. This decrease in peak width results in an increase in peak capacity ($P = 1 + tg/W_{4\sigma}$), that is the theoretical number of peaks with a peak width ($W_{4\sigma}$) that can be separated in a given gradient time (t_g) plus one and in a constant resolution between the critical peaks 4 and 5. The constant resolution independent from the flow rate proves that increasing the flow rate on STM columns is an appropriate way to decrease analysis

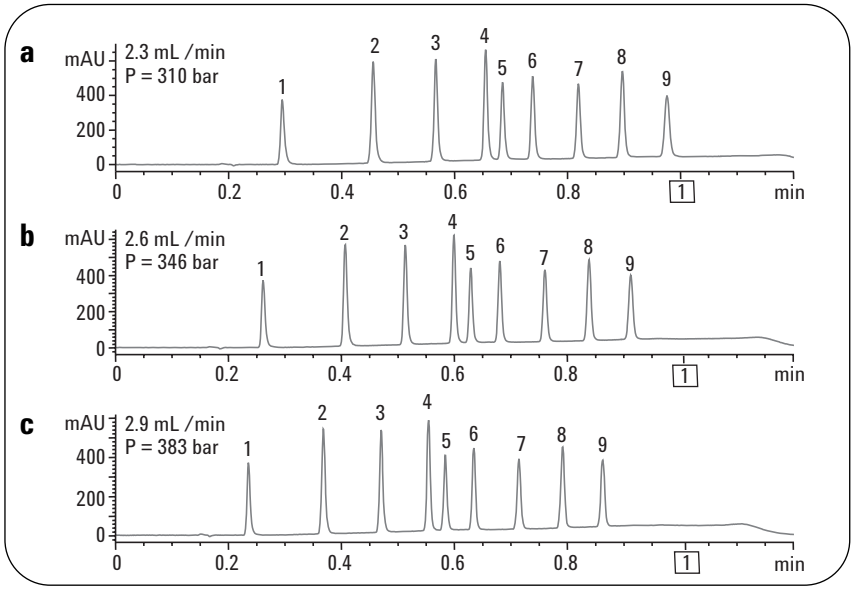

Figure 5
Comparison of 3 UV chromatograms: a) 2.3 mL/min, b) 2.6 mL/min, c) 2.9 mL/min, all at 245 nm (DAD equipped with a 500-nL flow cell). Compounds: Alkylphenones test mixture. System configuration without column regeneration.

	Flow rate 2. 3 mL/min	Flow rate 2. 6 mL/min	Flow rate 2. 9 mL/min	Competitor's performance[5] at flow rate 1 mL/min
Backpressure [bar]	310	346	383	996
Cycle time [min]	1.5	1.5	1.5	n.m.
Run time [min]	1.2	1.2	1.2	1.2
Analysis time [min]	0.976	0.912	0.862	0.914
[3] Retention time [min]	0.68	0.65	0.63	0.61
Average $W_{4\sigma}$ [sec]	0.74	0.70	0.62	n.m.
$R_{S\,(4,5)}$	2.76	2.76	2.76	n.m.
Retention time precision range [%] (n=6)	n.d.	n.d.	0.0542-0.1858	0.1824-0.2955

* : The mixture contains compounds 2 to 9 (8 phenone mix). Analysis performed at 40°C
n.m.: not mentioned n.d.: not determined

Table 1
Influence of flow rate on chromatographic performance. Comparison with a competitor's method[5]. System configuration without column regeneration.

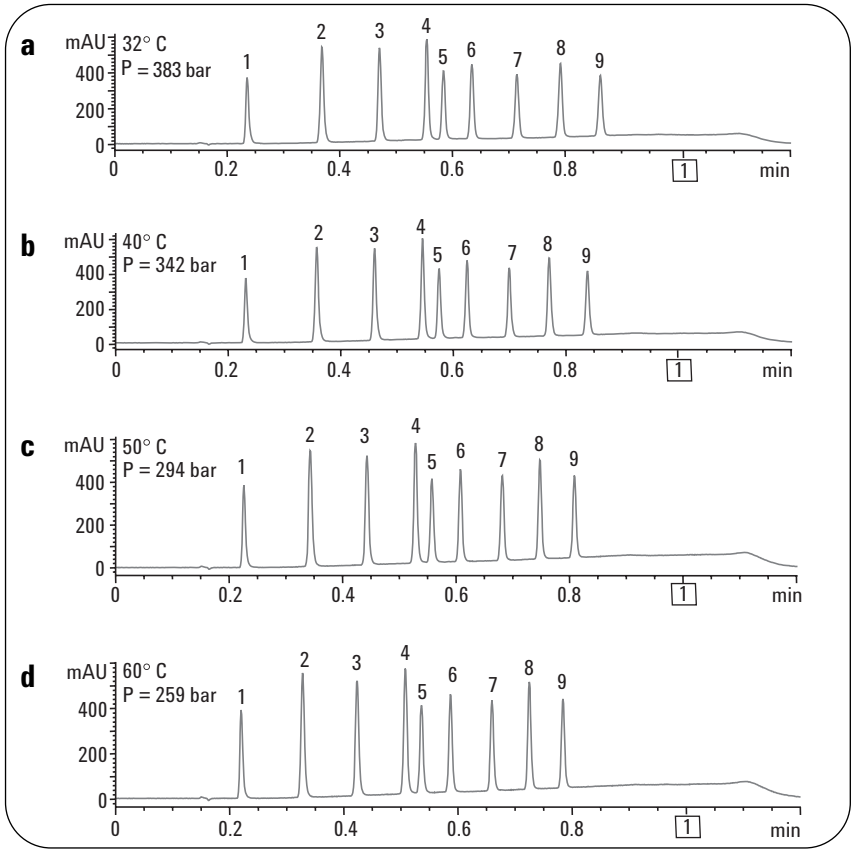

Figure 6
Comparison of 4 UV chromatograms: a) 32 °C, b) 40 °C, c) 50 °C, d) 60 °C, all at 245 nm (DAD equipped with a 500-nL flow cell) and at 2.9 mL/min. Compounds: Alkylphenones test mixture. System configuration without column regeneration.

Temperature [°C]	Flow rate [mL/min]	Backpressure [bar]	Average $W_{1/2}$ [sec]	Average $W_{4\sigma}$ [sec]	$R_{s\,4,5}$	ΔRT [min]
32	2.9	383	0.4	0.62	2.94	0.63
40	2.9	342	0.38	0.57	2.94	0.61
50	2.9	294	0.39	0.54	2.62	0.58
60	2.9	259	0.39	0.55	2.44	0.56
60	3.4	304	0.35	0.55	2.74	0.53
60	3.9	350	0.35	0.50	2.74	0.51
60	4.35	390	0.35	0.50	2.65	0.49
Competitor's method [5] (40 °C)	1.0	996	n.m.	n.m.	n.m.	0.61

n.m.: not mentioned

Table 2
Influence of temperature on chromatographic performance. System configuration without column regeneration.

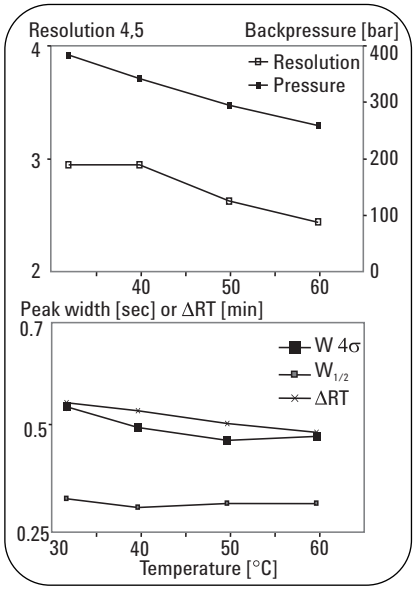

Figure 7
Influence of temperature on chromatographic performance and backpressure.

times without any loss in chromatographic performance. Compared to the competitor's method[5] (8 phenones mix, 40 °C, 996 bar) the results show slightly shorter analysis time, extremely low $W_{4\sigma}$ of 0.62 sec, an outstanding resolution of 2.76 for the critical peak pair (4,5) as well as better retention time precision at backpressures of only 310 to 383 bar and at a moderate temperature of 32 °C.

Working at temperatures higher than 32 °C and at a constant flow rate of 2.9 mL/min has a strong impact on system backpressure. Increasing the temperature from 32 °C to 60 °C results in a 32 % reduction of backpressure. Both $W_{4\sigma}$ and retention time difference between the first and last eluting peaks decrease 11 % at the expense of $R_{s\,(4,5)}$, which decreases 17 % (table 2, figure 6, 7). The decrease of backpressure at high temperatures allows working at higher

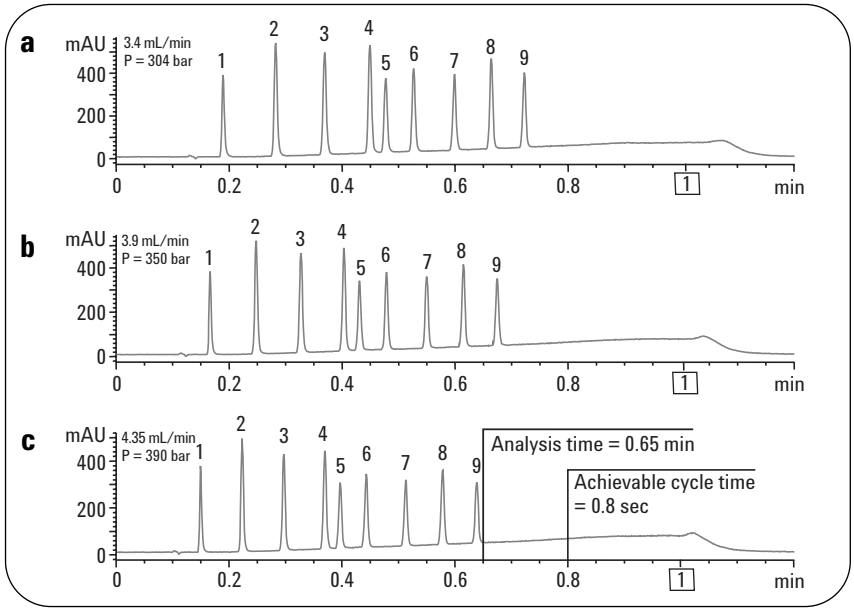

Figure 8
Comparison of 3 UV chromatograms; a) 3.4 mL/min, b) 3.9 mL/min, c) 4.35 mL/min, all at 245 nm (DAD equipped with a 500-nL flow cell) and at 60 °C Compounds: Alkylphenones test mixture. System configuration without column regeneration.

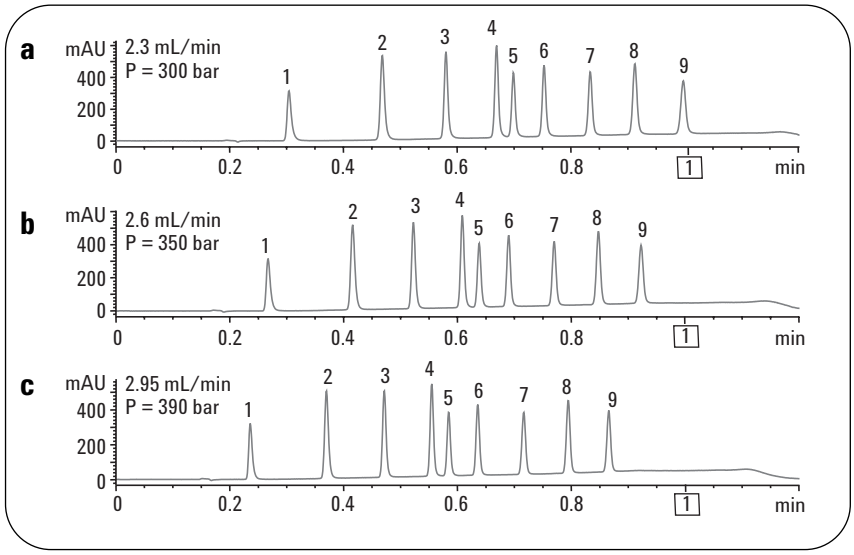

Figure 9
Comparison of 3 UV chromatograms: a) 2.3 mL/min, b) 2.6 mL/min, c) 2.95 mL/min, all at 245 nm (DAD equipped with a 500-nL flow cell). Compounds: Alkylphenones test mixture. System configuration with column regeneration.

flow rates and therefore to further reduce analysis time significantly. Working at 60 °C and 4.35 mL/min a ΔRT as short as 0.49 min can be obtained and at the same time an average $W_{4\sigma}$ of 0.5 seconds is achieved. The analysis time is reduced to 0.65 min, which facilitates cycle times of 0.8 min when using alternating column regeneration (Table 2, figure 8). Working at the same temperature as in the competitor's method (40 °C, 996 bar) we achieve the same ΔRT (0.61 min) at a backpressure of only 342 bar.

Ultra-fast LC experiments with alternating column regeneration

Shorter cycle times can be achieved by using alternating column regeneration, because the regeneration step is carried out while the next analysis is running. For the set of alkylphenones, a cycle time of 1.5 min is achieved without alternating column regeneration while with alternating column regeneration a cycle time of 1.3 min is achieved. Compared to the experiments without alternating column regeneration, the use of the 2-position/10-port valve introduces a small extra column volume in the system. Therefore, an increase on average of $W_{4\sigma}$ (9.5 % at 2.3 mL/min) and ΔRT (1.5 % at 2.3 mL/min) as well as a slight decrease in $R_{s(4,5)}$ (5.1 % at 2.3 mL/min) is aobserved. Figure 9 shows the ultra-fast separation of the set of alkylphenones using the previously described configuration (with alternating column regeneration) and using a 500-nL flow cell with 0.125 mm inlet and outlet capillaries, at 3 different flow rates and at a moderate temperature of 32 °C. As previously discussed, system

backpressures are dependent on flow rate. The backpressure increases with increasing flow rate, achieving 390 bar at 2.95 mL/min. The influence of flow rate on chromatographic performance is shown in table 3.

Carry over

When working with overlapped injections carry over can be an issue because the sample loop and needle are typically washed for a limited time (until the valve switches to bypass) with low percentage of organic only. We analyzed the set of alkylphenones and a subsequent blank run using three different injection conditions (figure 10). All experiments were performed at a flow rate of 2.3 mL/min and at 32 °C. In figure 10a the minimized carry over (MCO) option was not selected and the flush-out factor was set to 5 times injection volume (default setting). In figure 10b and c the MCO option was selected and the flush-out factor was set to 10 times and 20 times injection volume, respectively. When the MCO option is not selected and a flush-out factor of 5 is selected noticeable carry over is observed (figure 10a). The carry over is considerably reduced using the MCO option and selecting a flush-out factor of 10 (figure 10b). However, when selecting a flush-out factor of 20 times injection volume, no carry over is observed (figure 10c). It is important to recognize that no extra time is lost when using the MCO option and a flush-out factor greater than 5 (default setting), because the entire process (valve switching, external wash in wash-port and needle movement to injection port) still takes part during the run time (figure 11), i.e. after the first injection has taken place and during the waiting period for the next injection.

	Flow rate 2.3 mL/min	Flow rate 2.6 mL/min	Flow rate 2.95 mL/min
Backpressure [bar]	300	350	390
Run time [min]	1.2	1.2	1.2
Cycle time [min]	1.3	1.3	1.3
Δ Retention time [min]	0.69	0.66	0.63
Average $W_{4\sigma}$ [sec]	0.81	0.79	0.71
$R_{S\ (4,5)}$	2.62	2.62	3.06

Table 3
Influence of flow rate on chromatographic performance. System configuration with column regeneration.

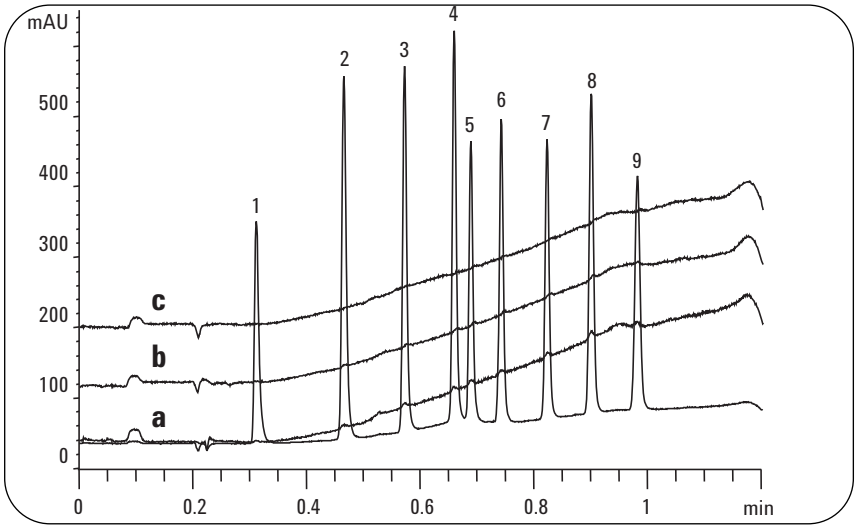

Figure 10
UV chromatogram: 2.3-mL/min, 32° C at 245 nm (DAD equipped with a 500-nL flow cell). Overlapped are the blank runs using no MCO selection and flush-out factor=5 (a), MCO selection and flush-out factor 10 (b) and MCO selection and flush-out factor 20 (c). Compounds: Alkylphenones test mixture.

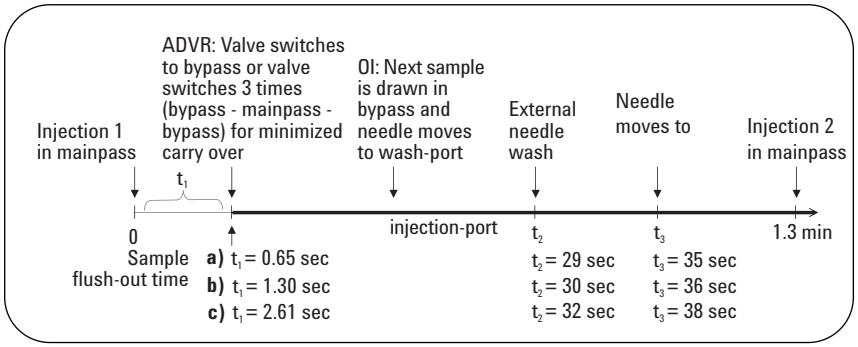

Figure 11
Illustration of cycle time (injection to injection time) including the injection steps ADVR, OI, needle movement to wash-port, external needle wash and needle movement to injection-port and next injection in mainpass. a) default setting, b) MCO+ flush-out factor = 10, c) MCO + flush-out factor = 20

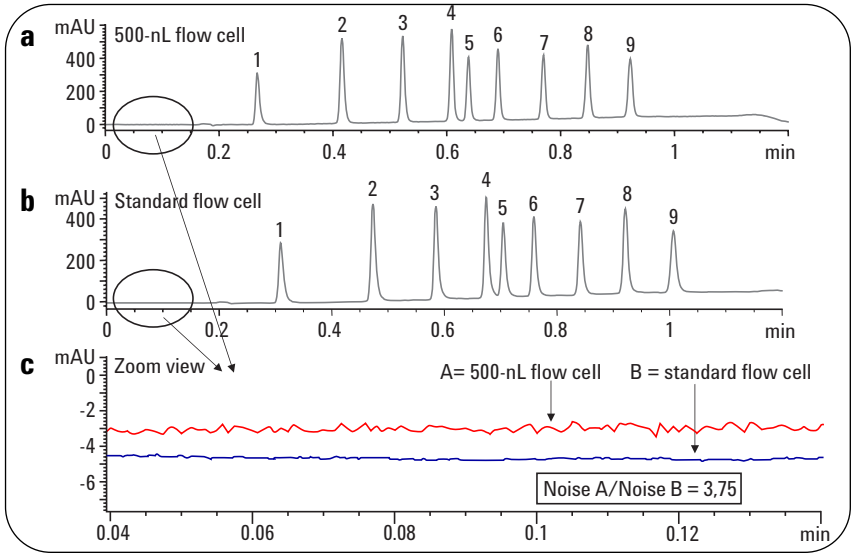

Figure 12
Comparison of the UV chromatograms at 245 nm, 2.6 mL/min and 32 °C using the 500-nL flow cell (a) and the standard flow cell (b). c) shows the noise relation between both flow cells. Compounds: Alkylphenones test mixture. System configuration with column regeneration.

	500-nL flow cell	Standard flow cell
Δ Retention time [min]	0.66	0.70
Average $W_{4\sigma}$ [sec]	0.79	0.95
Average $W_{1/2}$ [sec]	0.45	0.53
$R_{S\ (4,5)}$	2.52	2.21

Table 4
Influence of flow cell on chromatographic performance. System configuration with column regeneration.

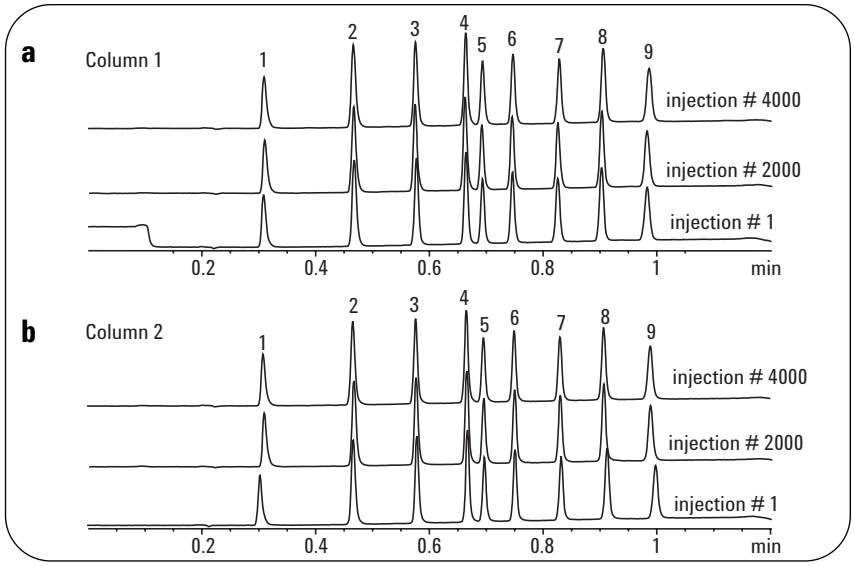

Figure 13
Comparison of the UV chromatograms at 245 nm, 2.3 mL/min and 32 °C using alternating column regeneration. The injections # 1, # 2000 and # 4000 are shown for a) column 1 and b) column 2. Compounds: Alkylphenones test mixture. System configuration with column regeneration.

Comparison between the 500-nL flow cell and the standard flow cell

Figure 12 shows the high throughput separation of the set of alkylphenones using the previously described configuration (with alternating column regeneration) and using:
a) a 500-nL flow cell with 0.125-mm inlet and outlet capillaries and b) a standard flow cell with 0.17-mm inlet and outlet capillaries, at a flow rate of 2.6 mL/min and at a temperature of 32 °C.

The influence of the used flowcell on chromatographic performance was determined by calculating the following parameters (table 4): ΔRT, average $W_{4\sigma}$ and $R_{s\ (4,5)}$. When comparing the chromatograms obtained with the 500-nL flow cell and the standard flow cell, it is evident that both flow cells yield satisfactory results. However, the 500-nL flow cell yields lower retention times, sharper peaks and better resolution than the standard flow cell. Using the standard flow cell results in a 6 % increase in retention time, a 20 % increase in average $W_{4\sigma}$ and 12 % decrease in resolution between peaks 4 and 5. On the other hand the standard flow cell shows 3.75 times lower noise than the 500-nL cell, so that the decision of what flow cell to use depends on the specific requirements of the analysis. If the priority is detection at low levels a standard flow cell may be used. If the priority is the achievement of highest peak capacity and resolution and thereby highest analysis speed a 500-nL cell should be chosen.

Long-term performance stability of the columns

The long-term performance stability of the columns was tested with alternating column regeneration at a flow rate of 2.3 mL/min, corresponding to a backpressure of 300 bar. In figure 13 the chromatograms for injection # 1, # 2000 and # 4000 for column 1 and 2, respectively, are shown. Figure 14 illustrates the long-term performance of the ZORBAX 1.8-µm RRHT columns and the stability of the entire system that makes it suitable for unattended and automated 24×7 operation.

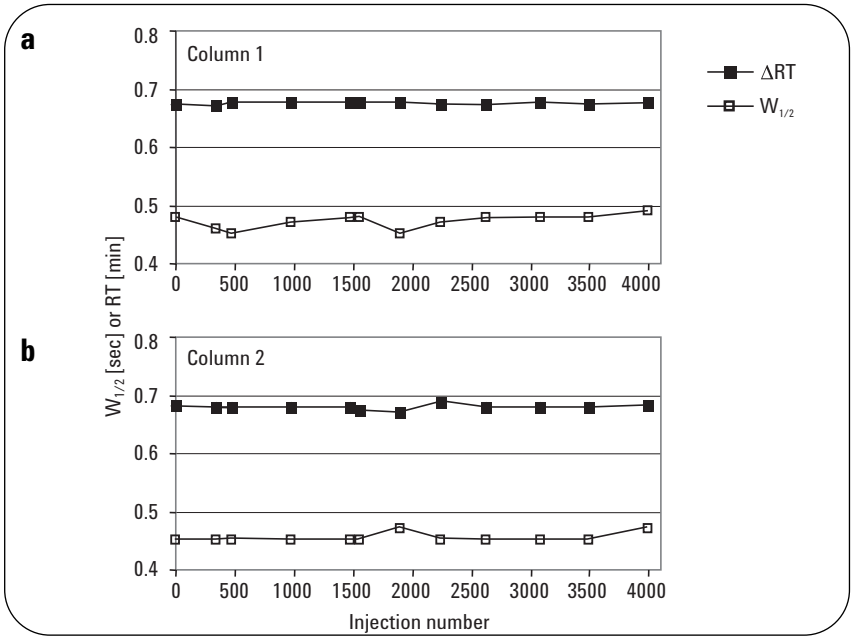

Figure 14
a) Exhibited average $W_{1/2}$ and Δ retention time of column 1 and 2 over 4000 injections each.
b) Exhibited resolution $_{4,5}$ of column 1 and 2 over 4000 injections each.

Conclusions

The speed of separation in liquid chromatography is mainly determined by the instrumentation used as well as the analysis conditions. Regarding the instrumentation, we demonstrated how the combination of the Agilent 1100 Series high pressure mixing binary pump, the Agilent Series 1100 well-plate autosampler with a valve-switching option for automatic delay volume reduction and overlapped injections, the Agilent Series 1100 thermostatted column compartment equipped with a 2-position/10-port valve, an additional pump for alternating column regeneration, rapid diode-array detection as well as the Agilent ZORBAX rapid resolution high-throughput columns with 1.8 µm particles achieve cycle times shorter than one minute for a baseline separation of 9 compounds at moderate backpressures without compromising chromatographic performance. With respect to the analysis conditions, we illustrated that the decrease of backpressure at high temperatures allows working at higher flow rates and therefore further reduces analysis time. After optimization of instrumentation and analysis conditions, 9 compounds could be baseline separated within 29 seconds, achieving an average $W_{4\sigma}$ of 0.5 seconds and a resolution of ≥ 2.65 for all compounds. Furthermore, performing more than 8,000 injections using 2 columns and alternating column regeneration, we have proven the long-term performance of our columns and reliability of the entire system.

Appendix

Pressure drop calculations

The connecting tubing's contribution to backpressure (from eluent pump to flow cell) was calculated for water at a flow rate of 2.5 mL/min with the SF Pressure Drop Version 5.0 software from Software Factory (Schifferstadt, Germany, www.software-factory.de, www.pressure-drop.com).

Connections	ID [mm]	Length [mm]	Pressure drop [bar]
Eluent pump to injection valve	0.17	400	8.147
Injection valve to 2/10 valve	0.17	280	5.703
2/10 valve to column 1	0.17	105	2.139
Column 1 to 2/10 valve	0.17	105	2.139
2/10 valve to column 2	0.17	105	2.139
Column 2 to 2/10 valve	0.17	105	2.139
2/10 valve to 2/10 valve	0.17	105	2.139
2/10 valve to standard flow cell capillary	0.17	105	2.139
Standard flow cell inlet capillary	0.17	200	4.073
Standard flow cell outlet capillary	0.17	200	4.073
Total pressure			**30.552**

Table 5
Configuration with 2-position/10-port valve and standard flow cell.

Connections	ID [mm]	Length [mm]	Pressure drop [bar]
Eluent pump toinjection valve	0.17	400	8.147
Injection valve to 2/10 valve	0.17	280	5.703
2/10 valve to column 1	0.17	105	2.139
Column 1 to 2/10 valve	0.17	105	2.139
2/10 valve to column 2	0.17	105	2.139
Column 2 to 2/10 valve	0.17	105	2.139
2/10 valve to 2/10 valve	0.17	105	2.139
2/10 valve to 500-nL flow cell inlet capillary	0.17	105	2.139
500 nL flow cell inlet capillary	0.125	220	15.328
500 nL flow cell outlet capillary	0.125	80	5.574
Total pressure using 0.125 mm ID flow cell inlet and outlet capillaries			**43.308**
500 nL flow cell inlet capillary	0.1	220	37.423
500 nL flow cell outlet capillary	0.1	80	13.608
Total pressure using 0.1 mm ID flow cell inlet and outlet capillaries			**73.437**

Table 6
Configuration with 2-position/10-port valve and 500 nL flow cell.

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