Automated alternating column regeneration on the Agilent 1290 Infinity LC

Increasing throughput using two columns alternatively via an ultra-high pressure 2-position/10-port valve

Application Note

Environmental

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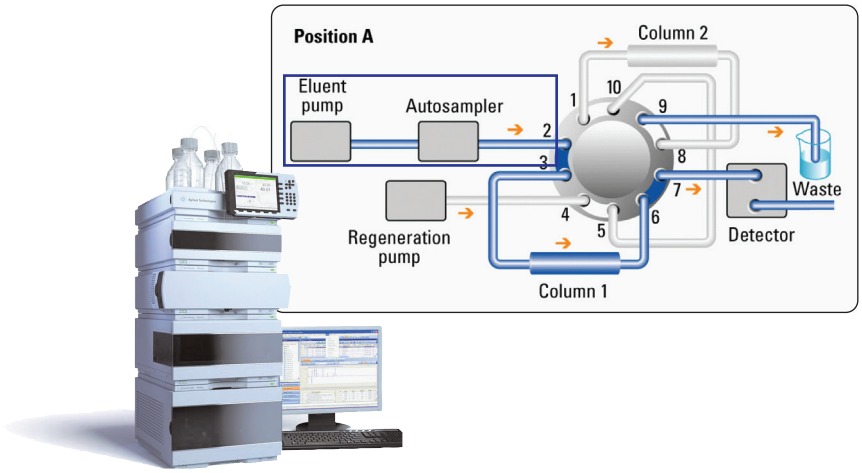

Abstract

Increasing the number of chromatographic runs per time is one demand in liquid chromatography today. This can be achieved by using shorter columns, higher flow rates and instruments that provide the lowest delay volume and allow back pressures up to 1200 bar. However, cycle times can be further reduced using automated alternating column regeneration. Using this method, about 2000 runs can be completed within 24 h, and 640 runs in 8 h. In addition, alternating column regeneration can also be used for more conventional runs. Cleaning and equilibration time can be excluded from the cycle time and 50% more runs can be performed.

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Introduction

Current technology frequently uses valves in liquid chromatography for sample enrichment, sample cleanup, and increase of sample throughput. The increase of sample throughput can be achieved by decreasing cycle times of analytical runs. For gradient analysis, the cycle time is the time needed to elute all peaks of interest. After peak elution the column should be cleaned of residues introduced by the sample matrix. Subsequently the column must be equilibrated to starting conditions of the gradient again.

In this Application Note we will demonstrate how to decrease cycle time using alternating column regeneration on the Agilent 1290 Infinity LC system. This is achieved by cleaning and equilibrating the column in parallel to the separation.

Experimental

The instrument used was an Agilent 1290 Infinity LC system, equipped with the following modules:

- Two Agilent 1290 Infinity Binary Pumps with built-in degassing units
- Agilent 1290 Infinity Autosampler
- Agilent 1290 Infinity Thermostatted Column Compartment
- Agilent 1290 Infinity DAD SL for 160 Hz operation
- Alternating ultra-high pressure Column Regeneration Valve Kit
- Agilent 6140 Single Quadrupole LC/MS System
- Agilent ZORBAX Eclipse Plus RRHD C-18 columns, packed with 1.8-µm particles

Results and Discussion

A typical HPLC run comprises four steps:

1. Sample draw and inject
2. Chromatographic run (typically a gradient run)
3. Column wash
4. Column equilibration

Usually these steps are executed sequentially. By using two identical columns and a second pump in the system, the last or the last two steps can be performed while the next analysis is already running. This procedure is called alternating column regeneration. The two columns are switched between the analytical and regeneration pump using a 2-position/10-port valve. Whether column wash, or column wash and column equilibration can be performed while the next analysis is already running depends on the type of regeneration pump used. If the regeneration pump is an isocratic pump, column equilibration only can be performed. If it is a gradient pump, both steps, column wash and equilibration, can be done. Since column wash and equilibration often require up to 50 % of the analysis time, alternating column regeneration can save a tremendous amount of time. In the following examples we used a gradient pump as the regeneration pump. Overlapped injection was used to reduce cycle time further.

In Figures 1 and 2 an example for a workflow with sequential and alternating column regeneration with overlapped injection is shown.

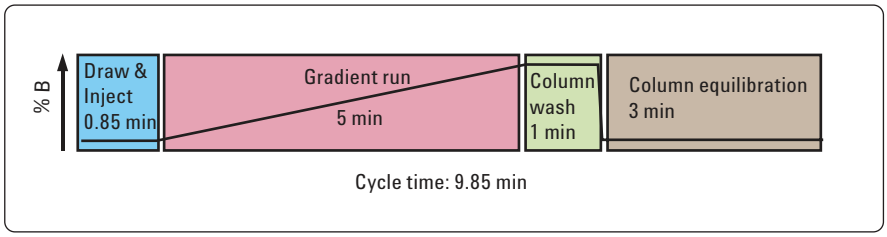

Figure 1
Sequential workflow.

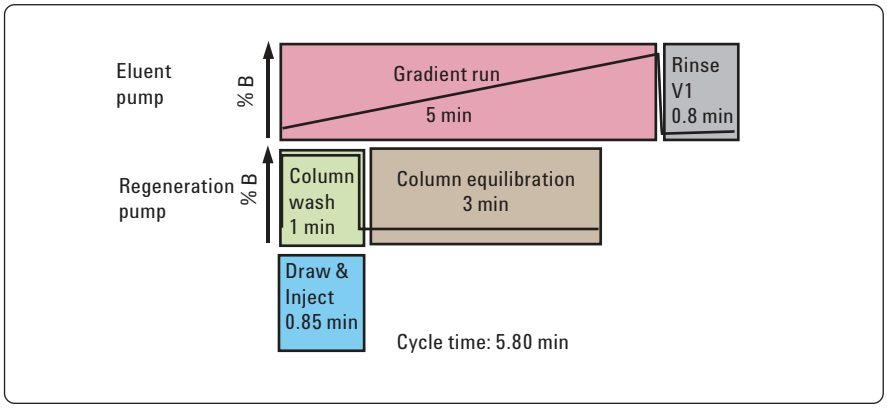

Figure 2
Alternating column regeneration with overlapped injection.

For this type of application a 2-position/10-port valve must be used. Figure 3 illustrates the plumbing for automated column regeneration. The column chemistry, length and internal diameter for columns 1 and 2 must be the same, otherwise the precision of retention time suffers and the elution series might be different. The 0.8 min, called "Rinse V1" is used to rinse volume V1 between the eluent pump and port 2 of the switching valve (Figure 2). This is a necessary step to prevent the mobile phase from the end of the previous run from remaining in V1 and running through the equilibrated column after switching the valve. Since this mobile phase contains 95% B but the column was equilibrated with 5% B the resulting chromatography would give unpredictable results.[1]

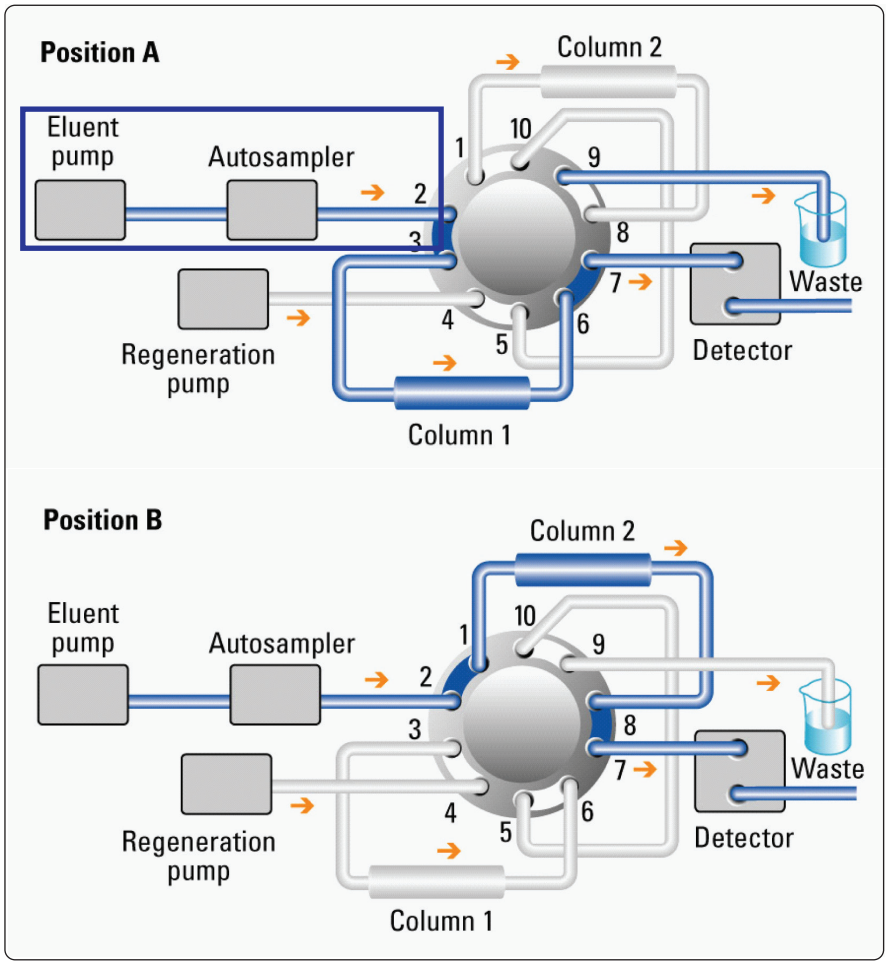

Figure 3
Plumbing for automated column regeneration.

Two application examples are discussed in the following.

Application example 1

Example 1 shows how to run about 2000 samples in 24 h or 640 samples in 8 h. The sample used contained nine compounds. Gradient time, run time and flow were optimized to achieve an injection to injection cycle time as low as 44 s. The run time was 27 s. The flow rate for the analysis was set to 2.2 mL/min. The column dimensions were 2.1 mm × 50 mm. The resulting back pressure was about 1000 bar. In Figure 4 an overlay of 10 consecutive runs on both columns is shown. The precision of retention times was typically <0.4% RSD – across two columns.

This shows two important facts. First the gradient repeatability is excellent, since the specification for the pump retention time precision is 0.07% RSD. In addition, the major effect is that the repeatability of the columns is excellent. The precision of areas for an injection volume of 0.25 µL was typically < 1.1% RSD.

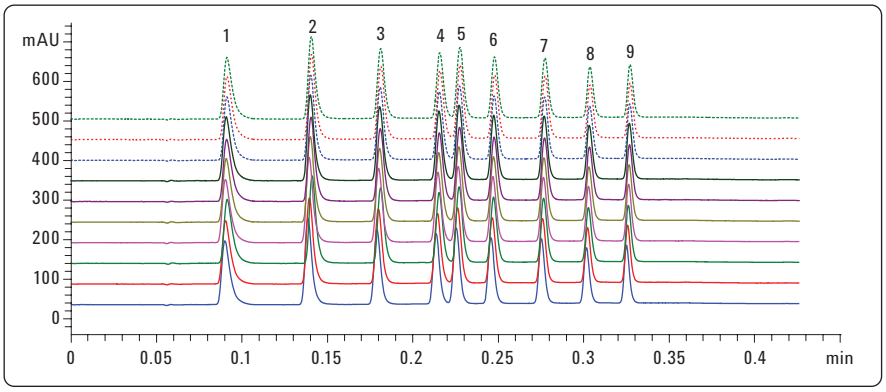

Figure 4
Overlay of ten consecutive runs on both columns.

Chromatographic conditions	
Sample:	Set of 9 compounds, 100 ng/µL each, dissolved in water/ACN (65/35) 1. acetanilide, 2. acetophenone, 3. propiophenone, 4. butyrophenone (200 ng/mL), 5. benzophenone, 6. valerophenone, 7. hexanophenone, 8. heptanophenone, 9. octanophenone
Columns:	Two 2.1 mm × 50 mm Agilent ZORBAX Eclipse Plus HD C-18, 1.8 µm
Mobile phases:	A = water, B = acetonitrile
Flow analytical pump:	2.2 mL/min, max pressure = 1090 bar, stop time 0.45 min, post time off
Flow regeneration pump:	2 m L/min, max pressure = 880 bar, stop time no limit
Gradient for anlytical pump:	At 0 min 35% B, at 0.3 min 95% B, at 0.37 min 95% B, at 0.38 min 35% B, V1 = cleaned
Gradient regeneration pump:	At 0 min 35% B, at 0.01 min 95% B, at 0.1 min 95% B, at 0.12 min 35% B
Column oven:	70 °C on both sides, valve switch = next run
DAD:	245/8 nm, Ref = 380/100 nm, PW >0.0016 min, 160 Hz, slit width 4 nm
Injector:	0.25 µL injection volume, overlapped injection mode = on

Application example 2

In the second example a pesticide standard was analyzed using automated column regeneration and more conventional chromatographic conditions. The Agilent 6140 Single Quadrupole LC/MS system was used as the detector in positive scan mode. Two 2.1 mm × 100 mm columns were used. The cycle time from injection to injection was 8 min, 28 s. Cleaning of the column and equilibration was done during the following analytical run using the regeneration pump. As a result 50% more runs could be performed in the same time.

In front of the MSD, an Agilent 1290 Infinity DAD was placed and both signals were acquired. In Figure 5 an overlay of the UV signal of 10 consecutive runs from both columns is shown. The sample contained 17 pesticide compounds. The precision of retention times was typically < 0.18% RSD including both columns; the precision of areas was typically < 1.9% for an injection volume of 0.25 µL.

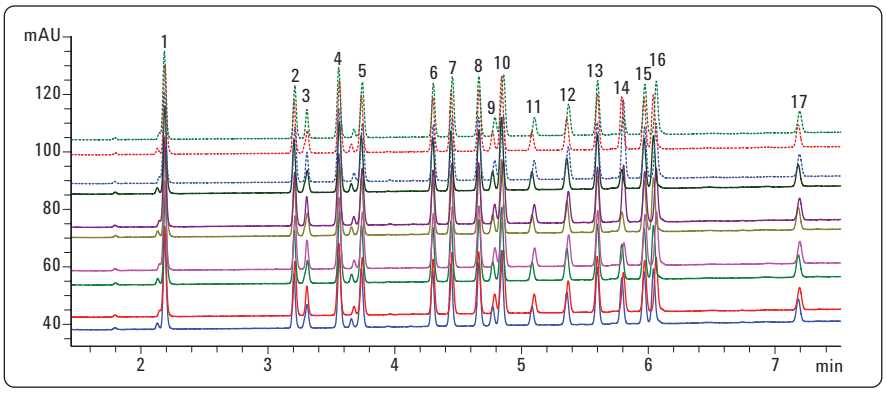

Figure 5
Overlay of ten consecutive pesticide analysis runs from both columns.

Chromatographic conditions	
Sample:	Pesticide Mix 44 from Dr. Ehrenstorfer, LA 18000044AL, 10ng/µL
Columns:	Two 2.1 mm × 100 mm Agilent ZORBAX Eclipse Plus HD C-18, 1.8 µm
Mobile phases:	A = water, B = acetonitrile
Flow analytical pump:	0.5 mL/min, stop time 8 min, post time off,
Flow regeneration pump:	0.5 mL/min, stop time no limit
Gradient for anlytical pump:	At 0 min 15% B, at 7 min 62% B, at 7.1 min 15% B,
Gradient regeneration pump:	At 0 min15% B, at 0.01 min 95% B, at 3 min 95% B, at 3.1 min 15% B
Column oven:	40 °C on both sides, valve switch = next run
DAD:	214/8 nm, Ref = 380/100 nm, PW >0.0016 min, 40 Hz, slit width 4 nm
Injector:	0.25 µL injection volume, overlapped injection mode = on at 7.5 min, needle wash for 6 s

In Figure 6 the extracted ion chromatograms of the acquired MSD signals are shown. The system is well suited for screening pesticides within 8 min. Selecting the appropriate masses in the "Extracted ion table" enables a rapid search for specific pesticides. The MSD can typically detect 5 to 50 pg (injected amount on column) of Phenyl Urea and Triazine herbicides.[2] Information about sample preparation for pesticides using Agilent SampliQ products for SPE can be found under www.agilent.com/chem/sampliQSPE.[3]

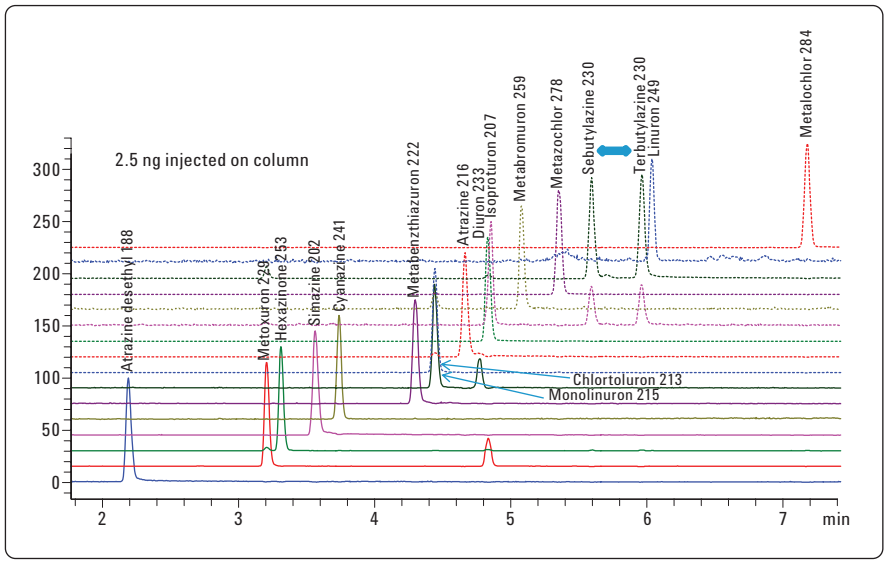

Figure 6
Extracted ion chromatograms of 17 pesticides.

Chromatographic conditions	
Sample:	Pesticide Mix 44 from Dr. Ehrenstorfer, LA 18000044AL, 10ng/µL
Columns:	Two 2.1 mm × 100 mm Agilent ZORBAX Eclipse Plus HD C-18, 1.8µm
Mobile phases:	A = water, B = acetonitrile
Flow analytical pump:	0.5 mL/min, stop time 8 min, post time off,
Flow regeneration pump:	0.5 mL/min, stop time no limit
Gradient for anlytical pump:	At 0 min 15% B, at 7 min 62% B, at 7.1 min 15% B,
Gradient regeneration pump:	At 0 min 15% B, at 0.01 min 95% B, at 3 min 95% B, at 3.1 min 15% B
Column oven:	40 °C on both sides, valve switch = next run
DAD:	214/8 nm, Ref = 380/100 nm, PW >0.0016 min, 40 Hz, slit width 4 nm
Injector:	0.25 µL injection volume, overlapped injection mode = on at 7.5 min, nedle wash for 6 s
MSD:	Peak width 0.03 min, Positive scan parameters from 120 to 400 mass range, Fragmentor = 100, Threshold = 150, Step size = 0.1 Gas temp = 350, Drying gas = 12 L/min, Neb Pres = 35 psig, V_{cap} positive = 3000 V

Conclusion

Currently, the ability to perform more chromatographic runs in a specific amount of time is one demand in liquid chromatography. This can be achieved by using shorter columns, higher flow rates, and instruments that provide lower delay volumes and allow back pressures up to 1200 bar. However, this study shows that cycle times can be further reduced by using automated alternating column regeneration. This means two columns are alternatively used via a 2-position/10-port valve. On one column the analytical run is performed, while the other column is cleaned and equilibrated using a second regeneration pump. Cycle times as low as 44 s can be achieved for highest throughput. About 2000 runs can be done within 24h or 640 runs in 8 hours. In addition, alternating column regeneration can also be used for more conventional runs. In this case cleaning time and equilibration time are excluded from the cycle time and 50% more runs can be performed.

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