High throughput HPLC — Alternating column regeneration with the Agilent 1100 Series valve solutions

Application

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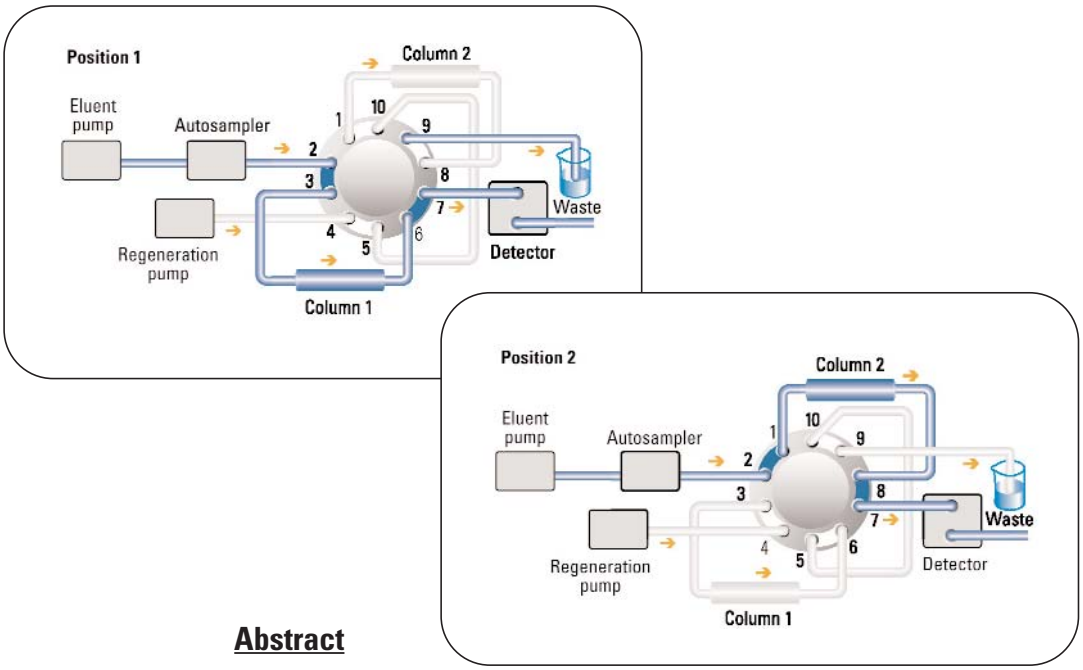

Abstract

High sample throughput analysis and purification are important tasks in the pharmaceutical industry. With the increasing number of samples synthesized – for example, by combinatorial chemistry – HPLC should not be the bottleneck in drug discovery and development. The throughput on an HPLC system can be increased by optimizing the chromatographic method[1,2,3] and by optimizing the instrument hardware[4,5].

In this Application Note we show how the throughput can be increased even further by using two identical columns in the system and operating them alternately using one of the Agilent 1100 Series valve solutions[6].

Agilent 1200 Series Verified

This application has been verified using an Agilent 1200 Series LC system, and showed comparable or even better performance.

Application Area

- High throughput analytical HPLC

Agilent Technologies

Introduction

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 eluent 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 only column equilibration 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 this Application Note we describe the method setup for the two pumps and the 2-port/10-position valve built into the 1100 Series thermostatted column compartment. How to optimize the analytical method and the Agilent 1100 Series system in general is described elsewhere[1-5].

Equipment

All experiments were performed on an Agilent 1100 Series system containing the following modules:

- Agilent 1100 Series quaternary pump with degasser
- Agilent 1100 Series well-plate autosampler
- Agilent 1100 Series thermostatted column compartment with 2-position/10-port valve
- Agilent 1100 Series isocratic or quaternary pump as regeneration pump

The system was controlled using the Agilent ChemStation (rev. A.09.03).

Results and Discussion

1. Sequential run with one pump

In a normal, sequential run all four steps are performed one after another. Therefore, all add up to the overall cycle time, as shown in figure 1. The draw and inject process took about 0.85 min (not optimized). The gradient run from 5 to 95 % B was performed over 5 minutes, then the column was washed for 1 minute at 95 % B and finally re-equilibrated at 5 % B for 3 minutes. This adds up to an overall cycle time of 9.85 minutes for a single run. The timetable for the pump was set up as follows:

Pump		
Gradient:	at 0 min 5 % B at 5 min 95 % B	} Gradient run
	at 6 min 95 % B	Column wash
Stop time:	6 min	
Post time:	3 min	Column equilibration

The resulting chromatograms for six consecutive runs are shown in figure 2.

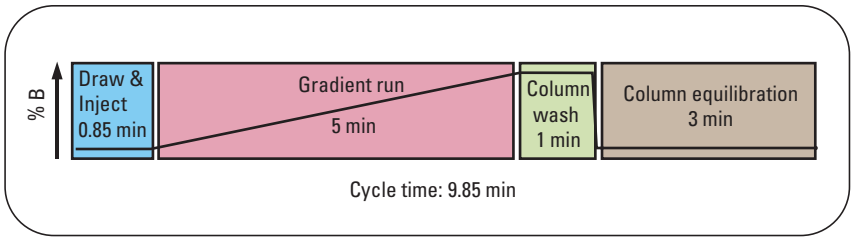

Figure 1
Sequential run

Columns	ZORBAX SB-C18 4.6 × 50 mm, 5 µm
Mobile phases:	A= water, B= acetonitrile
Flow rate:	1.5 mL/min
Injection:	5 µL
Column temp.:	40 °C
UV detector:	DAD: 220/10 nm (ref. 360/100 nm), standard flow cell (10-mm pathlength)

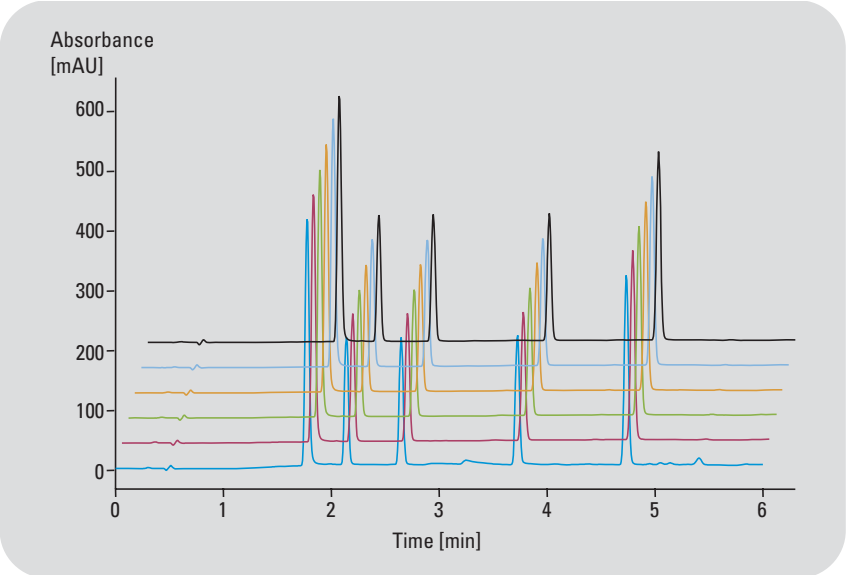

Figure 2
Chromatograms of six consecutive runs

To measure the quality of the chromatographic runs the relative standard deviation for the precision of retention times and peak areas for the last five runs were determined. The values for the precision of retention time were between 0.03 and 0.13 %, for the precision of area between 0.17 and 0.48%.

2. Alternating column regeneration with an isocratic regeneration pump

For alternating column regeneration the eluent and regeneration pump and the two columns were connected to the 2-position/10-port valve in the column compartment (see schematics on front page). At the end of each run the valve in the column compartment was switched to the next position using the *Next position* command in the *Column Thermostat Method* window as shown in figure 3.

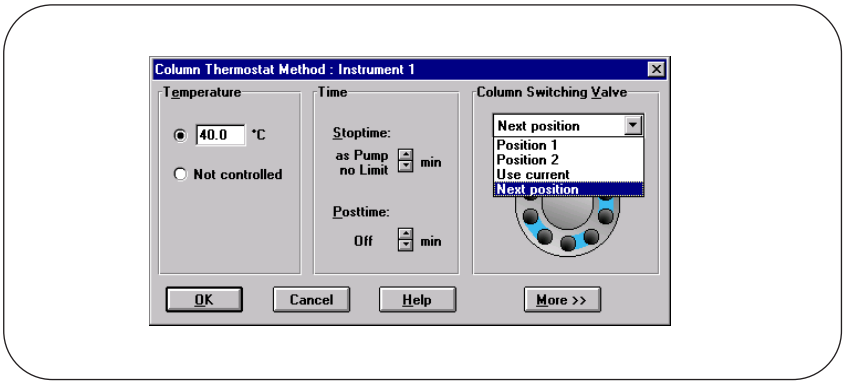

Figure 3
***Column Thermostat Method* window**

Using this command the valve remains in the position, in which it was left after the previous run was finished. At the end of the current run the valve switches to the next position. Since the regeneration pump is an isocratic pump, only column equilibration can be done while the next run is executed (figure 4) The cycle time decreased from 9.85 to 7.65 minutes, which is a time saving of about 20 %. The timetables for the pumps were set up as follows:

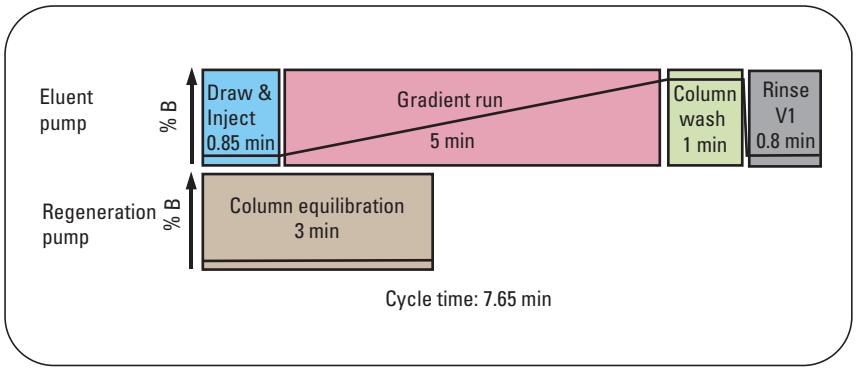

Figure 4
Run with isocratic regeneration pump

Eluent pump

Gradient:	at 0 min 5 % B	} Gradient run
	at 5 min 95 % B	
	at 6 min 95 % B	Column wash
	at 6.01 min 5 % B	} Rinse V1
	at 6.8 min 5 % B	
Stop time:	6.8 min	
Post time:	off	

Regeneration pump

Isocratic:	5 % B	Column equilibration
Stop time:	no limit	
Post time:	off	

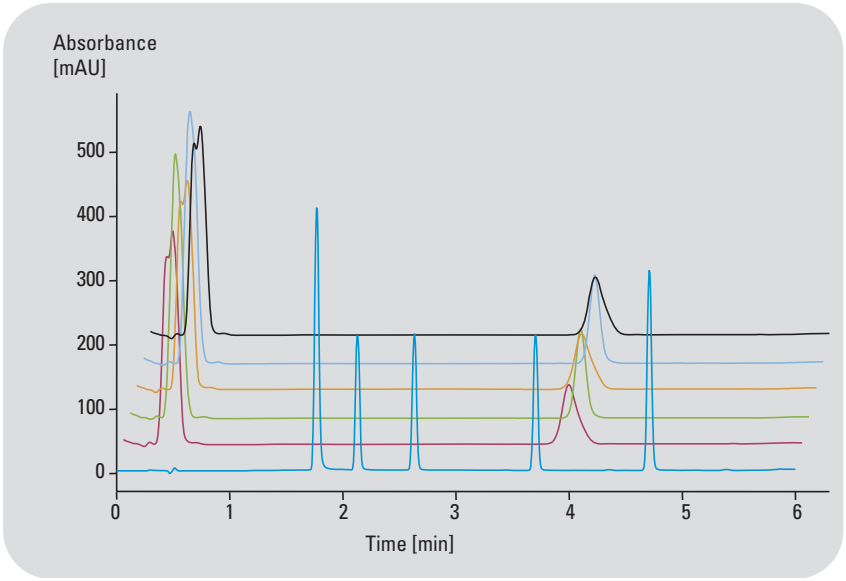

Figure 5
Result of six consecutive runs with column switching without rinsing V1

As shown in figure 4 an additional rinse time was added after gradient run and column wash are finished. This time is used to rinse the volume (V1) between the eluent pump and port 2 of the switching valve (see schematics on front page) using mobile phase of the same composition as at the start of the run. If this is not done the mobile phase from the end of the previous run remaining in V1 is applied to 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 gives unpredictable results (figure 5).

Rinsing V1 after the end of the column wash with 5 % B ensures that only mobile phase with the starting composition is applied to the equilibrated column. The time required for rinsing depends on the volume V1 and the flow rate used. For a typical analytical system rinsing V1 with 1-mL mobile phase should be sufficient. The resulting chromatograms are shown in figure 6. The precision of retention times and peak areas are comparable to those achieved without column switching.

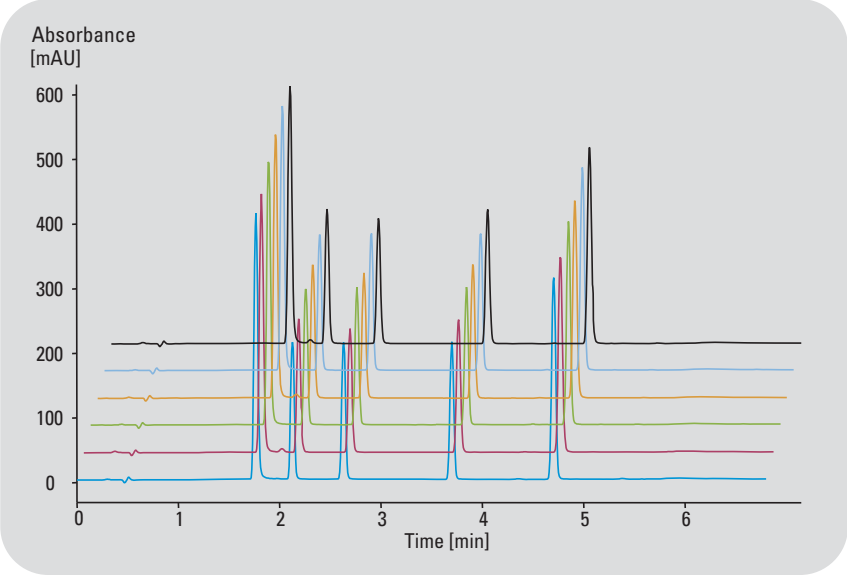

Figure 6
Result of six consecutive runs with column switching with rinsing V1

3. Alternating column regeneration with a gradient regeneration pump

When using a gradient pump as regeneration pump not only the column equilibration but also the column wash can be carried out while the next analysis has already started. This saves additional time as shown in figure 7. The time required to rinse V1 was now added directly after the end of the gradient run. The cycle time decreased from 7.65 to 6.65 minutes, which is a time saving of 30 % compared to the sequential runs. The timetables for the pumps were set up as follows:

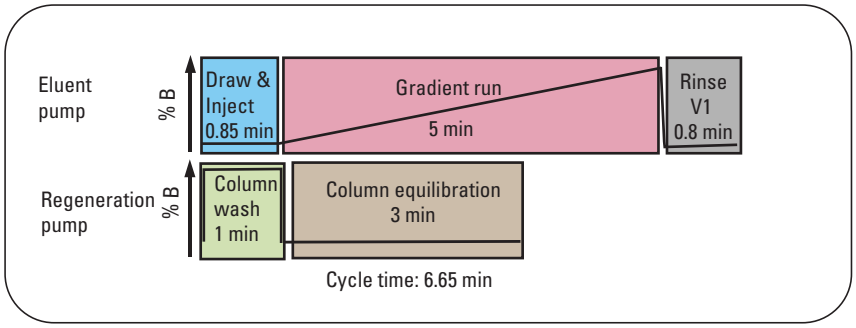

Figure 7
Run with gradient regeneration pump

Eluent pump

Gradient: at 0 min 5 % B
at 5 min 95 % B } Gradient run
at 5.01 min 5 % B
at 5.8 min 5 % B } Rinse V1
Stop time: 5.8 min
Post time: off

Regeneration pump

Gradient: at 0 min 5 % B
at 0.1 min 95 % B
at 1.1 min 95 % B } Column wash
at 1.2 min 5 % B Column equilibration
Stop time: no limit
Post time: off

Figure 8 shows the resulting chromatograms for six consecutive runs. The precision of retention times and peak areas are again comparable to those achieved without column switching.

4. Alternating column regeneration with a gradient regeneration pump and overlapped injections

With the Agilent 1100 Series autosamplers it is possible to perform overlapped injections automatically. This means that during the analysis run the next sample is already drawn into the sample loop and held there until the run is finished. At the beginning of the next run it can be injected immediately by simply switching the injection valve of the autosampler. This leads to additional time-saving because the draw and inject time only adds to the cycle time for the very first run and not to the additional runs. This is illustrated in figure 9. With overlapped injections the cycle time for all runs, except the first one, could be further reduced to 5.8 minutes. This is an overall time-saving of about 40 % over the sequential runs.

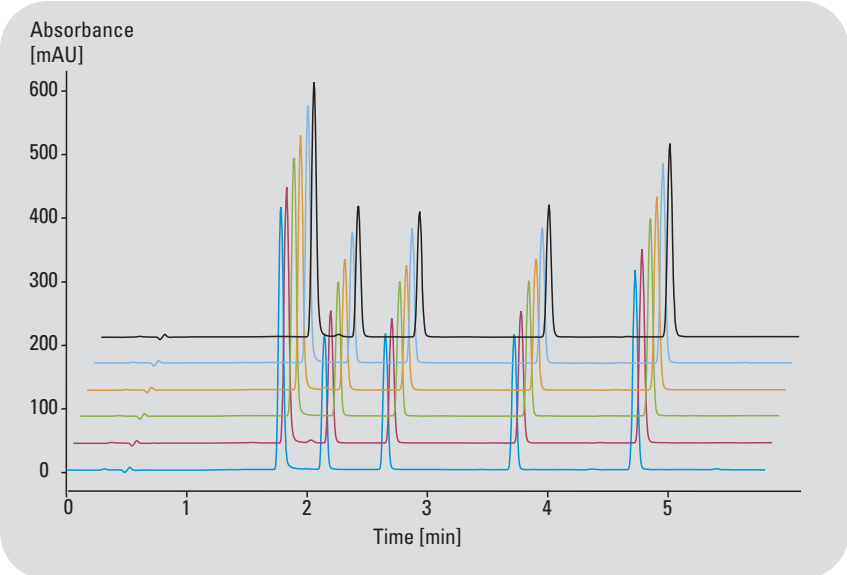

Figure 8
Result of six consecutive runs

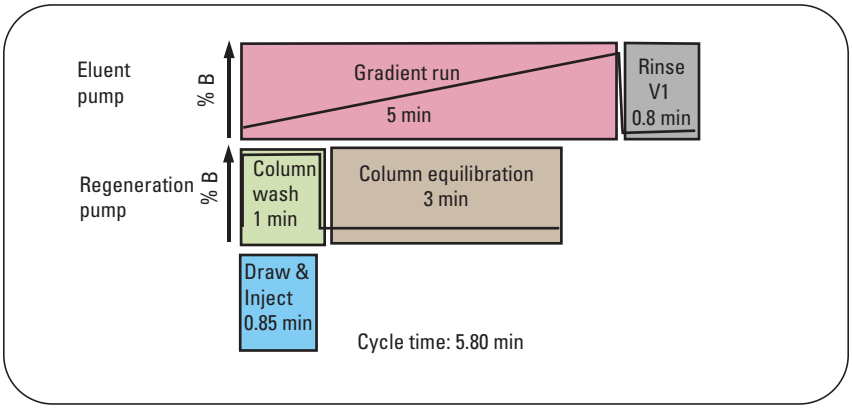

Figure 9
Run with gradient regeneration pump with overlapped injections

The timetables for the pumps and the autosamplers were set up as follows:

Eluent pump

Gradient: at 0 min 5 % B
at 5 min 95 % B } Gradient run
at 5.01 min 5 % B
at 5.8 min 5 % B } Rinse V1
Stop time: 5.8 min
Post time: off

Regeneration pump

Gradient: at 0 min 5 % B
at 0.1 min 95 % B
at 1.1 min 95 % B } Column wash
at 1.2 min 5 % B Column equilibration
Stop time: no limit
Post time: off

Figure 10 shows the resulting chromatograms for six consecutive runs. The precision of retention times and peak areas are again comparable to those achieved without column switching.

5. Column pre- and after-care

As for normal chromatographic runs it is important to make sure to equilibrate the columns before starting the first run and to flush the columns with an appropriate solvent after the last run to store them, for example, overnight or over the weekend. For column pre-care it is usually sufficient to perform one or two blank runs on each column without sample injection. These blank runs can be set up in the sequence before the first sample run and the first sample run starts automatically after column pre-care has ended. What is necessary for column after-care depends on the mobile phase used. If the column can be stored in the mobile phase, only one or two blank runs on each column can be added at the end of the sequence. If the mobile phase used for the sample runs contains, for example, a buffer, the column should be rinsed with buffer-free solvent after the last run and should also be stored in a buffer-free solvent. If a quaternary pump or a binary pump with solvent selection valve is used as eluent pump the column after-care can be performed automatically. This can be achieved by setting up methods at the end of the sequence using solvents C and D of the quaternary pump or the second set of solvents of the binary pump to perform blank runs.

Conclusion

In this Application Note we showed how to increase sample throughput with automated alternating column regeneration. The Agilent 1100 Series system was set up with two identical columns and a 2-position/10-port valve built into in the thermostatted column compartment. Depending on whether the regeneration pump is an isocratic or gradient pump, column equilibration or column wash and column equilibration can be performed while the next analysis is already in progress. The possibility to do overlapped injections further enhances the sample throughput by already drawing the next sample while an

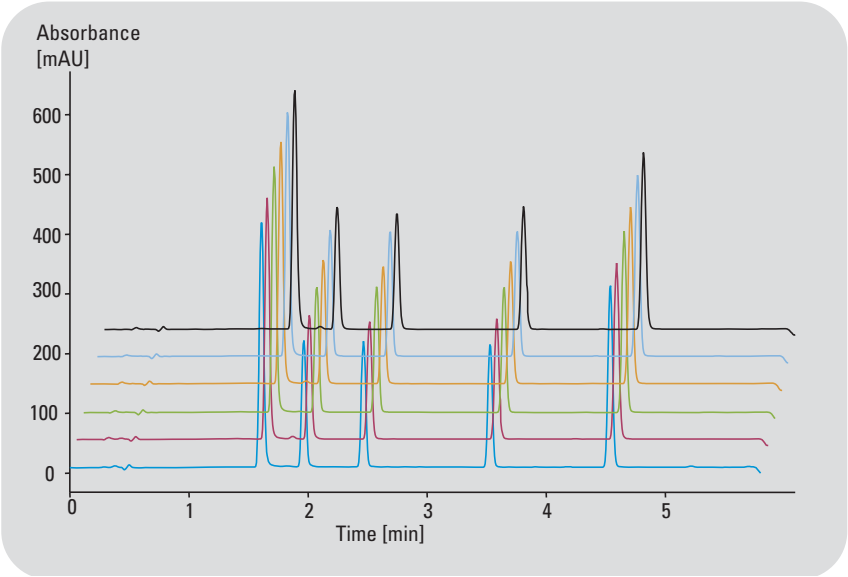

Figure 10
Result of six consecutive runs

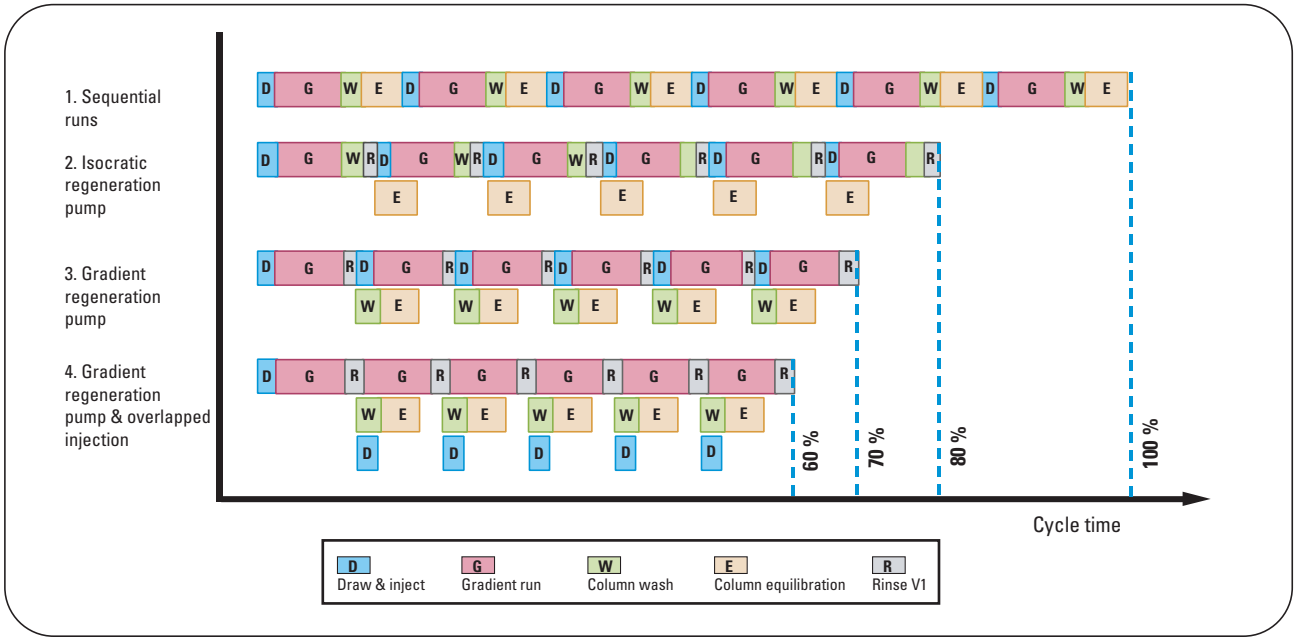

Figure 11
Comparison of the different methods and time savings achieved

analysis is running. Figure 11 compares the different methods and the time saving that is achieved. The time saving shown only applies to the method used in this Application Note. For any other method the time saved depends on the time required to perform the gradient run, column wash and column equilibration. However, the general possibilities of increasing sample throughput by alternating column regeneration using the Agilent 1100 Series valve solution can be applied to any other high throughput HPLC method.

References

1. "Optimizing the Agilent 1100 Series System for High Sample Throughput", *Agilent Technologies Technical Note*, **1998**, publication number 5968-0467E.

2. "Optimizing the Agilent 1100 Series System for Higher Sample Throughput on Columns with Internal Diameters of 1 and 2 mm", *Agilent Technologies Technical Note*, **1998**, publication number 5968-3166E.

3. "Strategies for fast and low carry over LC/MS analysis of drugs on the Agilent 1100 Series LC/MS system", *Agilent Technologies Application Note*, **2001**, publication number 5988-5026E

4. "Optimizing the Agilent 1100 Series well-plate sampler", *Agilent Technologies Application Note*, **2000**, publication number 5988-1432EN.

5. "Configuring and setting up the Agilent 1100 Series well-plate sampler for optimum performance", *Agilent Technologies Technical Note*, **2000**, publication number 5988-1596EN.

6. "New dimensions for HPLC applications", *Agilent Technologies Brochure*, **2002**, publication number 5988-6707EN.

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