INCREASING SAMPLE THROUGHPUT USING PARALLEL COLUMN REGENERATION

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INTRODUCTION

Background:

Analytical laboratories are constantly challenged to analyze more samples in less time to increase throughput. For typical gradient liquid chromatography (LC) methods, there are two segments. The first is the gradient itself during which the separation occurs. The second is the reconditioning step, also called column regeneration, which is used to wash and re-equilibrate the column. The reconditioning segment is essential for data consistency as well as increased column life. Depending on the gradient method, the duration of the reconditioning step may vary, sometimes up to 60% of the total run time.

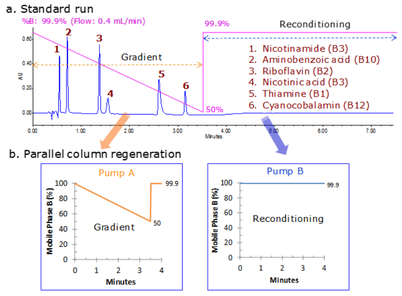
To reduce the time needed for sample analysis, some common practices include increasing the flow rate, using a shorter column and/or reducing column re-equilibration time. However, there are limits to the extent that these parameters can be modified without impacting chromatographic performance.

Parallel column regeneration is a solution where the total analysis time is reduced with no impact on the separation.[1] In this study, we will use an ACQUITY UPLC 2D (two-dimensional) LC system which is equipped with two binary pumps and switching valves within the column manager. A hydrophilic interaction liquid chromatography (HILIC) method for water soluble vitamins will be used. Using this strategy, increased sample throughput can be achieved while maintaining high quality chromatographic results.

Strategy of Parallel Column Regeneration:

The instrument used for parallel column regeneration includes:

- Two pumps (A & B): pump A is for gradient while pump B is for reconditioning
- Two identical flow paths which include column switching valves
- Two identical columns (1 & 2): while column 1 is used for the analytical separation, column 2 is being reconditioned, vice versa



EXPERIMENTAL

System Configuration:

By adding an additional pump and utilizing the switching vales within the column manager, a standard LC system can be configured to perform parallel column regeneration.

ACQUITY UPLC Systems with 2DLC Technology

Two pumps
Two identical flow paths
Two identical columns

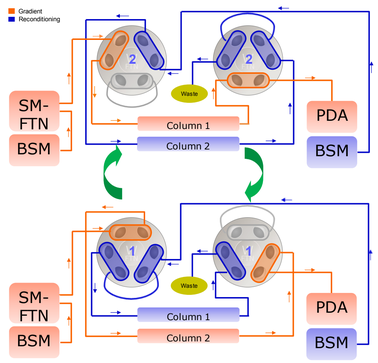
HILIC Method:

Column:	ACQUITY UPLC BEH Amide, 2.1 x 50 mm, 1.7 µm[2]
Mobile Phase A:	50/50 MeCH / 10 mM CH_3COONH_4 and NH_4OH, pH 9.0
Mobile Phase B:	90/10 MeCH / 10 mM CH_3COONH_4 and NH_4OH, pH 9.0
Column Temperature:	30 °C
Injection Volume:	1 µL
Dection:	UV @ 265nm, 20 points/sec

Gradient Pump			
Time (min)	Flow (mL/min)	% A	% B
Initial	0.4	0.1	99.9
3.50	0.4	50.0	50.0
3.51	0.4	0.1	99.9
4.00	0.4	0.1	99.9

Reconditioning Pump			
Time (min)	Flow (mL/min)	% A	% B
Initial	0.4	0.1	99.9
4.00	0.4	0.1	99.9

Work Flow :

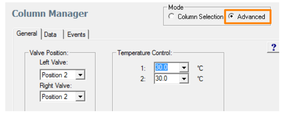


When both switching valves are at position "2", sample is injected and separated on column 1, while column 2 undergoes reconditioning.

At the end of gradient separation, the valves switches to position "1". Now, the sample is injected and separated on column 2, while column 1 undergoes reconditioning.

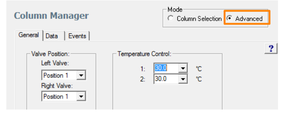
Valve Setup in Column Manager (CM) :

- Separation on column 1:



Set both left and right valves on position 2

- Separation on column 2:



Set both left and right valves on position 1

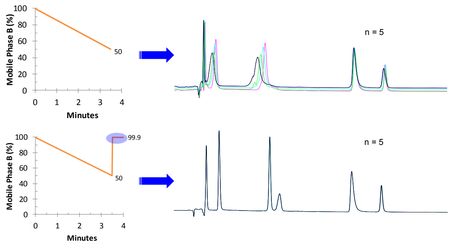
Alternating analysis on column 1 and 2 in sample set

	Plate/Well	Inj Vol (uL)	# of Injs	SampleName	Method Set / Report or Export Method	Run Time (Minutes)	
1	1:A,1	1.0	1	blank	B99_50_04mLmin_3_5m col1	4.00	Column 1
2	1:A,1	1.0	1	blank	B99_50_04mLmin_3_5m col2	4.00	Column 2
3	1:C,1	1.0	1	Sample 1	B99_50_04mLmin_3_5m col1	4.00	Column 1
4	1:C,2	1.0	1	Sample 2	B99_50_04mLmin_3_5m col2	4.00	Column 2
5	1:C,3	1.0	1	Sample 3	B99_50_04mLmin_3_5m col1	4.00	Column 1
6	1:C,4	1.0	1	Sample 4	B99_50_04mLmin_3_5m col2	4.00	Column 2

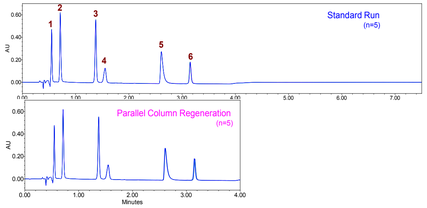
RESULTS AND DISCUSSION

Considerations When Switching Columns:

During column switching, the fluidics from the pump A to the valve needs to be flushed out to match the initial mobile phase conditions of the gradient for the next injection. This can be accomplished by adding a short segment of initial condition at the end of the gradient.



Comparison of Standard Run with Parallel Column Regeneration: (for the same column)



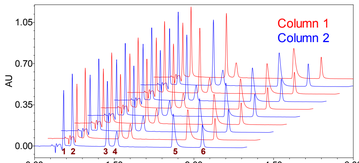
Peak Label	Standard (n=5) RT (min)	Standard (n=5) RT RSD (%)	Standard (n=5) Area RSD (%)	Column Regeneration (n=5) RT (min)	Column Regeneration (n=5) RT RSD (%)	Column Regeneration (n=5) Area RSD (%)	Abs. RT Δ (min)
1	0.550	0.00	0.58	0.547	0.10	0.70	0.003
2	0.712	0.06	0.78	0.710	0.08	1.08	0.001
3	1.378	0.08	0.11	1.372	0.10	0.08	0.006
4	1.553	0.08	0.40	1.548	0.11	0.46	0.005
5	2.610	0.03	0.62	2.609	0.11	0.27	0.002
6	3.151	0.07	0.86	3.153	0.18	0.74	0.002
Average		**0.05**	**0.56**		**0.11**	**0.56**	**0.003**

⇒ There was no significant difference in data quality observed between these two configurations.
⇒ Parallel column regeneration demonstrated the same level of reproducibility as standard runs.

References

1. Root D and Claise P. Increasing Sample Throughput Using the ACQUITY UPLC System with 2D Technology and Parallel Column Regeneration. Waters Technology Brief, 720004598en. 2013 Mar.
2. ACQUITY UPLC BEH Amide columns, Waters product solution, 720003122en, 2009 Oct.

Comparison of Column 1 & 2 in Parallel Column Regeneration:



- Retention time repeatability (Intra & Inter columns):

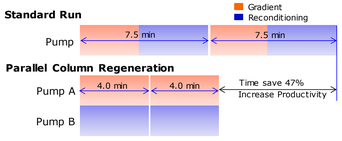
Peak Label	Column 1 (n=5) RT (min)	Column 1 (n=5) RSD (%)	Column 2 (n=5) RT (min)	Column 2 (n=5) RSD (%)	Abs. Δ (min)	Column 1 and 2 (n=10) RSD (%)
1	0.548	0.08	0.547	0.10	0.001	0.12
2	0.704	0.12	0.710	0.08	0.007	0.50
3	1.368	0.08	1.372	0.10	0.003	0.16
4	1.552	0.07	1.548	0.11	0.004	0.17
5	2.634	0.10	2.609	0.11	0.025	0.52
6	3.146	0.17	3.153	0.18	0.007	0.20
Average		**0.10**		**0.11**	**0.008**	**0.28**

- Peak area repeatability (Intra & Inter columns):

Peak Label	Column 1 (n=5) Area RSD (%)	Column 2 (n=5) Area RSD (%)	Column 1 and 2 (n=10) RSD (%)
1	0.73	0.70	1.36
2	0.06	1.08	0.72
3	0.15	0.08	0.59
4	0.45	0.46	1.61
5	0.61	0.27	0.52
6	0.48	0.74	0.61
Average	**0.41**	**0.56**	**0.90**

⇒ The chromatographic data quality across both columns is not compromised in parallel column regeneration
⇒ The low RSD of peak area demonstrates the suitability for quantitative data analysis using the parallel column regeneration setup.

Time Saving:



CONCLUSION

- Parallel column regeneration increases sample throughput while maintaining high quality chromatographic results
- ACQUITY UPLC® system with 2D technology can be configured to perform parallel column regeneration

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