INTRODUCTION

Background:
Conventional liquid chromatography (LC) gradient methods consist of two segments. The first segment is the gradient, which is accountable for the separation; the second segment is the reconditioning step, which is used to wash and re-equilibrate the column. The reconditioning segment is essential for data consistency as well as column life. Depending on the gradient method, up to 60% of the total runtime is used for the reconditioning.

In various industries, analytical LC methods are constantly challenged to analyze more samples in less time. Some common practices to shorten LC analysis time include increasing flow rate, using a shorter column and 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 significantly reduced with no impact to the methods being run. 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

Method: HILIC for Water Soluble Vitamins
A single pump is used for both the gradient and reconditioning steps

1. Standard LC method:
   A single pump is used for both the gradient and reconditioning steps

   - Parallel column regeneration:
     Two pumps are used with
     Pump A: for the gradient
     Pump B: for reconditioning

SYSTEM & METHOD SETUP

System Configuration for Parallel Column Regeneration:
By adding an additional pump and utilizing the switching valves within the column manager, a standard LC system can be configured to perform parallel column regeneration.

Comparison of Standard Run with Parallel Column Regeneration:
Comparison of Column 1 & 2 in Parallel Column Regeneration:

RESULTS

Comparison of Column 1 & 2 in Parallel Column Regeneration:

- Retention time repeatability (Intra & Inter columns):

- Peak area repeatability (Intra & Inter columns):

- Peak area variability (Intra & Inter columns):

Chromatography data quality is not compromised considering column to column variability

CONCLUSION

- Parallel column regeneration increases sample throughput while maintaining high quality chromatographic results
- A HILIC method for water soluble vitamins demonstrated 47% time saving by using parallel column regeneration

References

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