PARALLEL COLUMN REGENERATION: INCREASING THROUGHPUT IN CLINICAL RESEARCH WITHOUT SUCCUMBING TO MATRIX CONTAMINATION

Heather Brown, Robert Wardle, Anahi Santoyo-Castelazo, Matthew Gill, Daniel Kenny and Lisa Calton
Waters Corporation, Wilmslow, Cheshire, UK.

INTRODUCTION

Typical gradient separation methods include a regeneration segment where the column is flushed and then equilibrated to initial conditions. Washing with 3-5 column volumes of a suitable strong solvent is usually recommended for minimization of phospholipid build-up and potential extension of LC column lifetime. Allowing sufficient re-equilibration time is important for method robustness. Reducing the time allowed for regeneration may produce acceptably performing high-throughput methods in some cases, but it comes with the risk of poor-quality data and limiting column longevity. An alternative hardware configuration, parallel column regeneration, splits the two phases over consecutive injections, allowing sufficient washing and re-equilibration to be performed on the 'passive' column, while the gradient separation occurs on the 'active' column. This has the potential to shorten the analytical run and increase sample throughput without compromising UPLC best practices.

METHODS

A Waters™ ACQUITY UPLC™ I-Class Fixed Loop Sample Manager, two Binary Solvent Managers (BSM) and a Column Manager fitted with two 6-position valves were configured as outlined in **Figure 1**. Samples were prepared from whole blood and serum following Waters application notes 720007586 and 720006320, respectively. C24:0– and C26:0-lysophosphatidylcholine (LPC) were extracted from dried blood spot disks in methanol containing $^{2}H_{4}$-labelled stable isotope internal standards, and separated on a Waters XSelect™ Premier CSH C18, 2.1x50mm, 2.5µm UPLC column with 10mM ammonium formate, 0.1% (v/v) formic acid in (A) 60% acetonitrile (aq) or (B) 90/10 (v/v) isopropanol/acetonitrile . Analyses were conducted using a Xevo™ TQ-S micro tandem mass spectrometer.

Phosphatidylcholine (PC) accumulation was estimated with precursor ion scanning by finding the average intensity of m/z 758.7 (34:2-PC), 782.6 (36:4-PC) and 806.6 (38:6-PC) precursor ions of the 184 fragment in column eluates collected after 10 injections of prepared sample on each of the two columns.

Precision and carryover performance was determined using Waters MassTrak™ Immunosuppressant and Serum Steroid Calibrator and Quality Control sets. Method performance for lysophosphatidylcholines was determined using dried blood spots prepared by enriching washed red blood cells with 0, 1 or 5 µmol/L C24:0- and C26:0-lysophosphatidylcholine, at 50% hematocrit, before spotting onto Whatman grade 903 filter cards.

Parallel column regeneration gradients were created which split the washing and re-equilibration segments over two pumps, with one BSM performing the gradient elution at all times, and the other running the bulk of column washing and all of the regeneration. The LPC pump methods (0.4 mL/min flow rate throughout) are shown in Tables 1-3.

Table 1 Routine LC Analysis LPC

Minutes	% A	% B
Initial	50	50
1.60	24	76
2.1	1	99
3.2	50	50
5.00	50	50

Table 2 Parallel LC Gradient Pump

Minutes	% A	% B
Initial	50	50
1.60	24	76
1.70	1	99
2.35	50	50

Table 3 Parallel LC Regeneration

Minutes	% A	% B
Initial	1	99
0.75	50	50
2.30	50	50

Improving Instrument Usage Efficiency

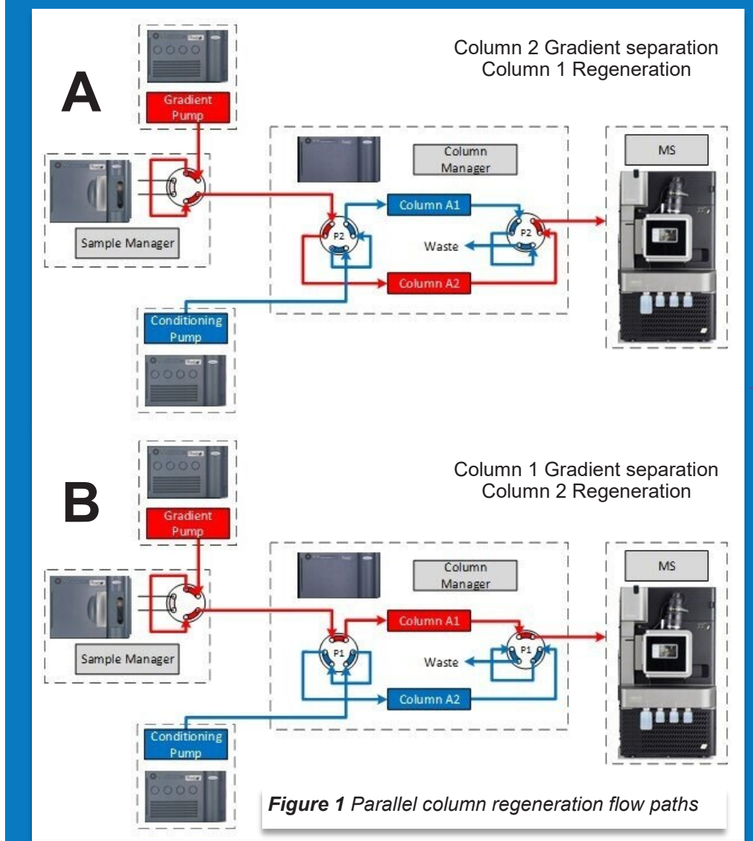
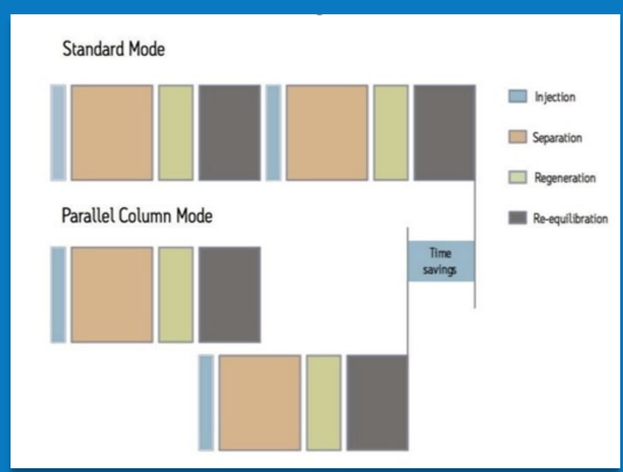


Figure 1 *Parallel column regeneration flow paths*



RESULTS

Whole Blood Immunosuppressants

	Routine	Column Regeneration
Injection cycle duration	2 min 2 sec	1 min 50 sec
Samples per hour	29.5	32.7
Washing Column Volumes	3.5	6.4
Re-equilibration Column Volumes	3.1	6.2
Wash / Re-equilibration Solvent Consumption	0.7mL	1.3mL
Phospholipid Ion Counts in Column Eluate	1.4e7	3.8e6
Maximum retention time RSD †	1.6%	1.0%
Equivalence of QC ‡	—	Yes

Serum Steroid Hormones

	Routine	Column Regeneration
Injection cycle duration	7 min 13 sec	6 min 7 sec
Samples per hour	8.3	9.8
Washing Column Volumes	1.4	8.7
Re-equilibration Column Volumes	2.6	7.0
Wash / Re-equilibration Solvent Consumption	0.7 mL	2.7 mL
Phospholipid Ion Counts in Column Eluate	4.9e6	4.8e4
Maximum retention time RSD †	0.7%	0.2%
Equivalence of QC ‡	—	Yes

Dried Blood Spot Lysophosphatidylcholine

	Routine	Column Regeneration
Injection cycle duration	5 min 43 sec	3 min 17 sec
Samples per hour	10.5	18.3
Washing Column Volumes	2.3	3.2
Re-equilibration Column Volumes	5.8	5.4
Wash/Re-equilibration Solvent Consumption	1.4 mL	1.5 mL
Phospholipid Ion Counts in Column Eluate	1.7e4	1.0e2
Maximum retention time RSD †	1.0%	0.7%
Equivalence of QC ‡	—	Yes

† 10 injections on each LC column; ‡ 95% confidence interval derived from Measurement Uncertainty with k=2 derived from 35 measurements of QC brackets the mean of the alternative inlet.

CONCLUSION

- Parallel column regeneration is easily configured using an additional pump and column manager with any conventional LC-MS/MS system commonly encountered in clinical research.
- This configuration demonstrated laboratory efficiency improvements, with up to 74% more samples analyzed per hour, without compromising analytical performance
- Thorough column washing can be employed to minimize phospholipid accumulation to improve method and column robustness

For Research Use Only. Not for use in diagnostic procedures.