Increasing Analysis Throughput on HPLC Instruments– What is the Smartest Strategy?

Frank Steiner[1], Giovanni Maio[1], Remco Swart[2], Bas Dolman[2], Marco Karsten[2], Frank Arnold[1],
[1]Dionex Softron GmbH, Germering, Germany; [2]Dionex, Amsterdam, The Netherlands

INTRODUCTION

Maximizing sample throughput yet saving additional resources and laboratory space is a key issue in HPLC. This created demand for highly productive LC and LC-MS solutions without compromising robustness. A common approach to increase throughput requires changing key method parameters like column dimensions, column particle sizes, temperature, and flow rates. In a regulated environment this implies revalidation of methods and revision of corresponding documentation. The related workload often outweighs achievable benefits and thus prevents implementation of speed optimized methods. Hence, strategies for increasing throughput while leaving key method variables untouched are preferable.

Dual-column operation, with off-line column reconditioning (tandem operation) is such a strategy. It is applicable to gradient methods and typically increases throughput by 50–100%. Parallel chromatography with a dual-gradient HPLC system is another suitable approach. The parallel chromatography solution nearly doubles the throughput for either isocratic or gradient methods.

This presentation discusses instrument and software requirements for tandem and parallel LC operation. It introduces new hardware and software solutions that greatly facilitate the use of these techniques.

EXPERIMENTAL

The UltiMate™ 3000 ×2 Dual-Binary Micro LC system for tandem and parallel LC (Figure 1) consists of:

- Two HPG-3200M binary micro pumps
- SRD 3600 solvent rack with 6 degasser channels
- TCC-3200 thermostatted column compartment with two integrated switching valves
- WPS-3000TSL cooled well-plate autosampler
- VWD-3400 UV detector (2× for parallel LC)

The complete system is controlled using Chromeleon® Chromatography Management Software.

Optimization of the sample throughput in liquid chromatography depends on the speed of the LC instrument and the analytical method.

Figure 1. UltiMate Dual-Gradient Micro LC system for tandem LC.

INSTRUMENTATION ASPECTS

One of the instrument parameters contributing to the analysis time is gradient delay volume, in particular for low flow rate applications. In this work we have applied 2.1-mm i.d. columns and flow rates of 400 µL/min.

The HPG-3200M micro pump has been designed to have a minimal gradient delay volume of only 35 µL assuring fast gradient delivery to the column. Another important instrumental aspect concerning sample throughput is the injection speed of the autosampler. The WPS-3000 autosampler can complete an injection cycle in less than 15 s.

LC METHOD

Tandem and parallel LC were applied to the separation of a mixture of four parabenes. The experimental conditions are listed in Table I.

Table 1. Tandem and Parallel LC Method

Columns	2.1 × 100 mm, Dionex Acclaim® C18, 3 µm, 120 Å
Temperature	40 °C
Gradient	40–90% organic phase in 1.5 min, 1 min equilibration
Flow Rate	400 µL/min
Injection	10 µL
Detection	254 nm, 10 Hz
Sample	Methyl-, ethyl-, propyl- and butylparabene, 10 ppm

HIGH THROUGHPUT LC METHODS

To increase sample throughput without changing method parameters, either tandem or parallel LC can be applied.

For tandem LC the standard instrumental setup is extended with a second pump and a second (identical) column, as shown in Figure 2.

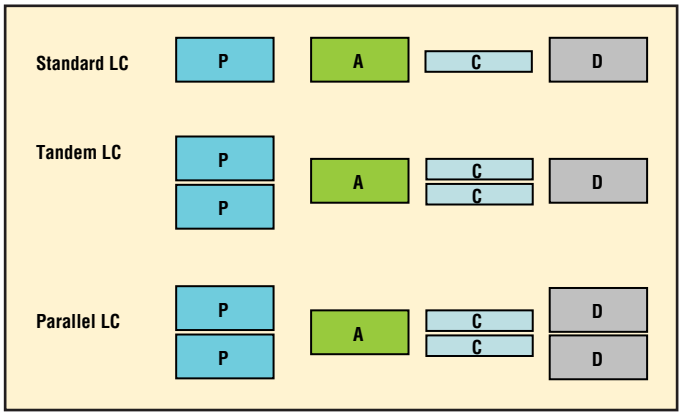

Figure 2. Instrument setup for standard, tandem, and parallel LC: pump (P), autosampler (A), column (C), and detector (D).

The two columns are switched back and forth between two flow paths, the analysis flow path and the regeneration flow path. While a sample is analyzed on one column, the other column is washed and equilibrated. As soon as all analytes of interest have eluted from the column in the analysis flow path, the columns are switched and the next sample is injected on the reequilibrated second column. This technique shortens analysis cycles by the time it takes to wash and reequilibrate a column. In practice, this is 20–50% of the total cycle time. Accordingly, tandem LC increases sample throughput by 25–100%.

In parallel LC, the standard instrument setup is extended with a second pump, a second column, and a second detector (Figure 2). A parallel LC system configuration can, therefore, be considered two separate systems sharing an autosampler and a column compartment. The autosampler injects alternately into both flow paths. This configuration allows for simultaneous acquisition of two identical or different analyses. Parallel LC typically increases sample throughput by close to 100% for isocratic and for gradient applications. More information on the operation of tandem and parallel LC is given below.

RESULTS AND DISCUSSION

Tandem LC

In fast gradient methods, the column wash and equilibration time can significantly limit the sample throughput. To optimize the sample throughput under these circumstances, the application of overlapping injections in a tandem LC configuration can be attractive. A 2-position 10-port switching valve is used, allowing two columns to work in parallel. The benefit is that during analysis on one column, the other column can be regenerated. The instrument setup for tandem LC is shown in Figure 3.

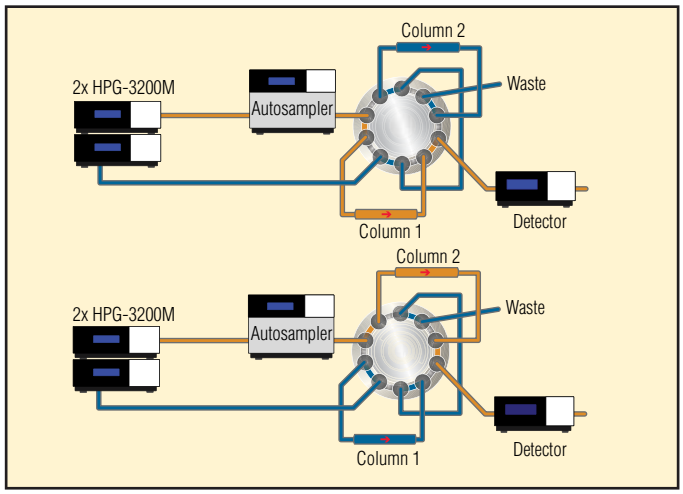

Figure 3. Instrument setup of UltiMate 3000 Dual-Binary systems for tandem LC.

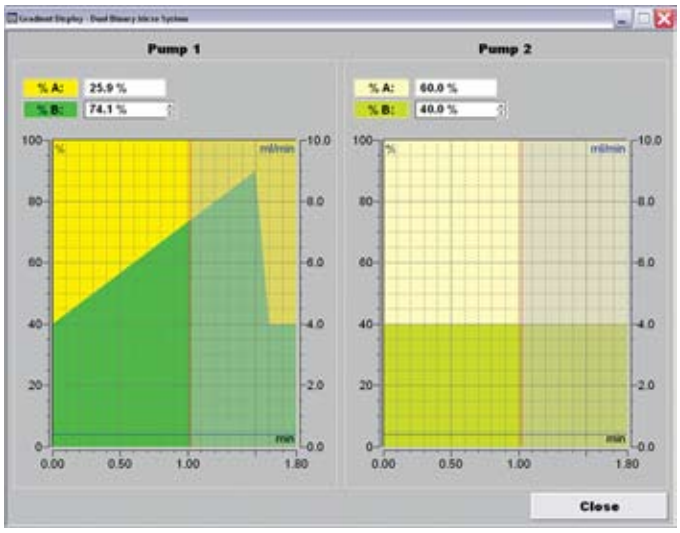

Figure 4. Solvent programs for tandem LC; pump 1 (analysis) and pump 2 (regeneration).

While pump 1 delivers the gradient from 40–90% B in 1.5 min sequentially, pump 2 flushes the second column isocratically with the starting mobile phase. If required, the program of pump 2 can be extended with a solvent wash step. The detection system, which can be either a UV detector and/or a mass spectrometer, is switched in series with the appropriate column. From Figure 4, it is clear that the increase in sample throughput is larger for methods with equal gradient and regeneration cycle times.

Figure 5 compares standard LC mode and tandem LC mode with respect to throughput. Upon reaching the end of the gradient on column 1, the next sample is injected on column 2 while column 1 is regenerated off-line with the second pump. The presented application applies a steep wide range gradient which requires approximately the same time for column regeneration as for the separation.

In the case of the fast parabene separation shown in Figure 5, the sample throughput was increased by approximately 90% compared to the standard LC method.

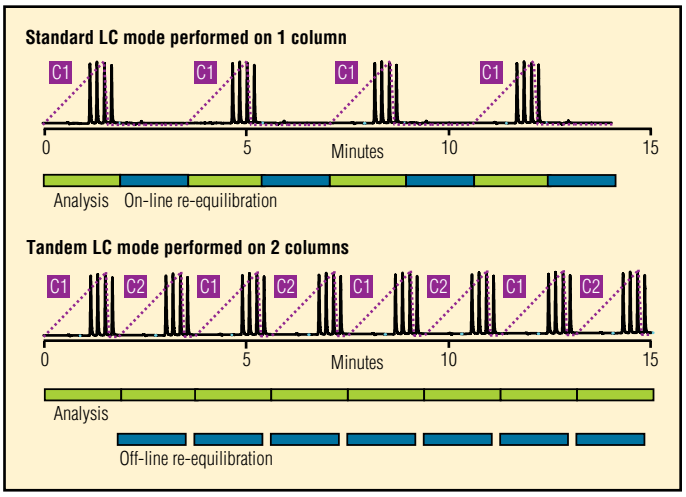

Figure 5. The separation of parabenes in tandem LC mode.

The retention time and injection precision are excellent. Repeatability data for 10 consecutive injections on two different columns are shown in Table 2.

Table 2. Retention Time and Peak Area Precision

	Repeatability (% RSD, n=10)	
	Retention Time	**Peak Area**
Methylparabene	0.10	0.54
Ethylparabene	0.12	0.55
Propylparabene	0.06	0.65
Butylparabene	0.05	0.63

To obtain high sample throughput for quantitative LC applications the system must be robust. The robustness of the system was tested by performing several sequences of over 200 sample injections. The variation in retention times was < 0.20% RSD for two combined columns, and the variation in peak area was <1.0% RSD.

Parallel LC

Another technique that can be used to increase the sample throughput is parallel LC. In this mode of operation two columns and detectors are used in parallel. A 2-position 6-port switching valve is used for injections from one autosampler on two columns. The advantage is that the autosampler and column compartment can be shared for two instrument setups, thereby increasing the productivity of instrument modules.

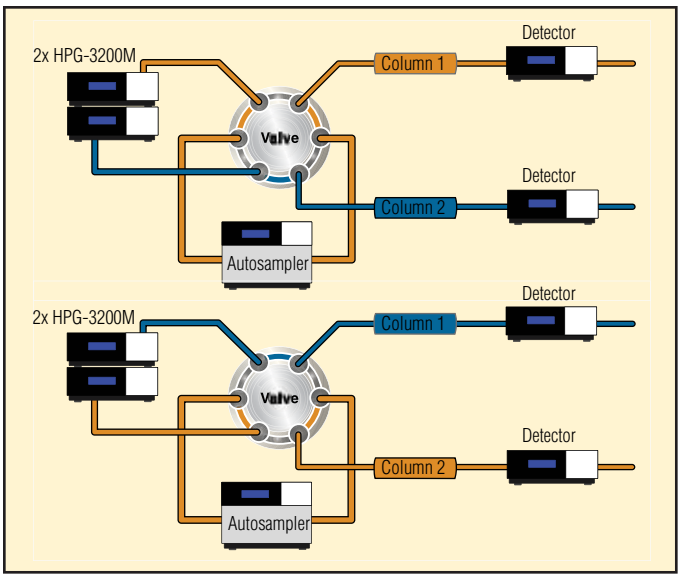

Figure 6. Instrument setup of UltiMate 3000 Dual-Binary systems for parallel LC.

Parallel LC requires that the autosampler and column compartment are shared between two LC systems, a feature that is fully supported by Chromeleon software. First, the autosampler is assigned exclusively to system 1, then, after the injection, the autosampler is assigned to system 2. The use of two detectors allows for simultaneous data acquisition. In addition, compared to tandem LC, parallel LC offers the advantage that the sample throughput can be increased up to 100% even for long shallow gradients or simple isocratic methods.

The separation of parabenes in parallel LC mode is shown in Figure 7.

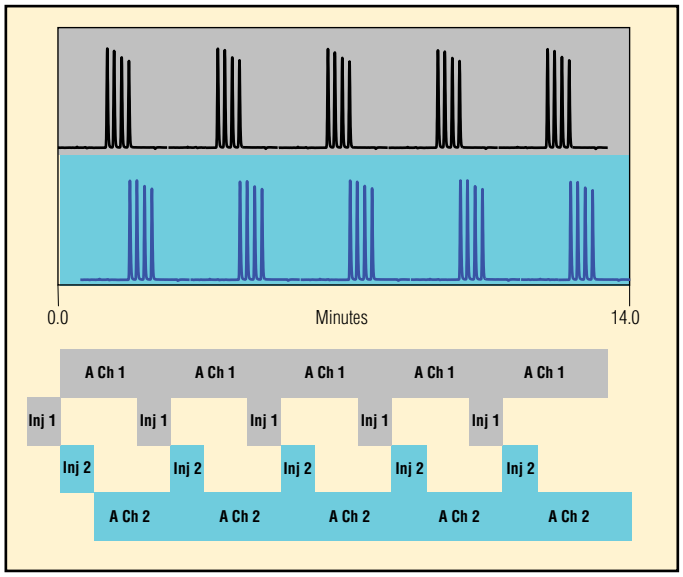

Figure 7. The separation of parabenes in parallel LC mode.

After sample injection on column 1 (I1), the 2-position valve is switched, and the gradient and data acquisition (A Ch 1) are started. Next, injection on column 2 is performed and the gradient started. In comparison to standard LC operation, the sample throughput was increased approximately twofold.

The retention time and peak area precision are excellent. Repeatability data for 10 consecutive injections are shown in Table 3. In contrast to tandem LC, parallel LC always uses individual calibration for each column/channel due to inevitable detector response differences.

Table 3. Retention Time and Peak Area Precision for Parallel LC

	Repeatability (% RSD, n=10)	
	Retention Time	Peak Area
Methylparabene	0.14	0.58
Ethylparabene	0.11	0.66
Propylparabene	0.08	0.59
Butylparabene	0.09	0.59

Tandem LC versus Parallel LC

For the exemplified parabene separation, the analysis time for 10 samples is 18 and 14 min for tandem and parallel LC, respectively. The difference between tandem and parallel LC is relatively small, but it should be noted that the advantage of parallel LC compared to tandem LC increases with increasing length of the gradient. Using a standard LC method, the analysis of 10 samples would require 28 min. The use of fast gradients is very common in bioanalysis using tandem mass spectrometric detection. Tandem LC is ideally suited for bioanalytical LC/MS analysis because the MS acquisition time is maximized.

Other LC applications, for example impurity profiling of pharmaceutical compounds with UV or fluorescence detection, typically require more shallow gradients for optimized resolution. In Table 4 a comparison between various modes of LC operation is given for these two applications.

Maximized throughput is most easily achieved by parallel LC. The sample throughput can be doubled independent of the LC method and gradient profile. Moreover, parallel LC can combine two different LC methods on one instrumental set-up as long as the solvents are fullty miscible. In this case, the run times must be harmonized and time windows for flushing out transferred solvent plugs must be implemented. Tandem LC is attractive for fast gradients for which the column wash and regeneration are the limiting steps.

Table 4. Sample Throughput for Various LC Modes

	Number of Analyses / Hour	
LC mode	Fast gradient[1]	Shallow gradient[2]
Standard	21	2
Tandem	33	2
Parallel	43	4

[1]Typical fast gradient: 1.5 min gradient + 1.0 min equilibration
[2]Typical shallow gradient: 25 min gradient + 5 min equilibration

Tandem LC-MS

Diuretics are used to regulate the excretion of water and salts in the treatment of several diseases. In addition, sports applications of diuretics include the induction of rapid excretion of prohibited substances taken by athletes and the rapid, temporary weight-loss attempted in certain sports to influence weight classes[1]. A tandem LC setup connected to an ion-trap mass spectrometer was used for the analysis of three diuretic compounds. Ethacrynic acid, furosemide, and bumetanide were separated on two 2.1-mm i.d. × 100 mm Acclaim C18 columns. The separation conditions were similar to those described for the parabenes. Successful quantification is even achieved in the case of coeluting compounds due to: (1) the selectivity of mass spectrometric detection and, (2) the ability to extract ion signals specific to each diuretic. The extracted ion chromatograms of the diuretic compounds are shown in Figure 8.

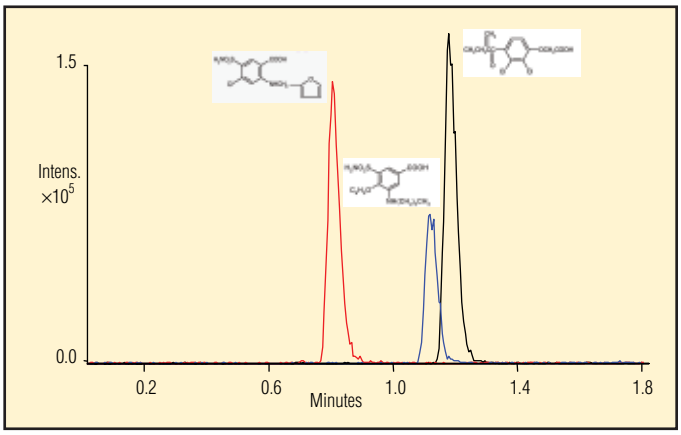

Figure 8. Tandem LC-MS analysis of furosemide (329.1 m/z*), bumetanide (363.1* m/z*), and ethacrynic acid (301.1* m/z*).*

The MS signal area repeatability for QC samples was 1.5% RSD. Calibration curves were linear in the range of 0.1–30 µg with correlation coefficients (r^2) > 0.995. No difference in peak area was observed between samples injected on two different columns. Using a 1.5 min gradient, the total number of LC-MS analyses was 31 per hour. It should be noted that an additional increase in the sample throughput is feasible by optimizing the gradient and flow rate, but this was not explored in this study.

CONCLUSIONS

- UltiMate 3000 Micro LC systems can be easily configured for tandem or parallel LC to increase sample throughput without changing LC method parameters.
- Chromeleon software features dedicated tools for tandem and parallel LC operation such as instrument sharing and direct control of switching valves.
- The retention time and injection precision of the UltiMate 3000 Micro system are excellent in both tandem and parallel LC operation.
- Both tandem and parallel LC analysis can significantly increase the sample throughput. Tandem LC is advantageous for fast gradients, whereas parallel LC can achieve a 100% increase independent of LC conditions, but requires a second detector.

REFERENCES

1. K. Deventer, F. T. Delbeke, K. Roels, and P. Van Eenoo. *Biomed. Chromatogr.* **2002**, 16, 529–535.