Increasing throughput by Multiplexing Shimadzu HPLCs with Hystar on the EVOQ LC-TQ

A Shimadzu Nexera X2 duplexed HPLC system connected to Hystar was used to increase throughput and reduce LC cycle time. During the wash and re-equilibration time of the first analytical column, the next sample is injected on the second analytical column. The wash and re-equilibration is provided by a second set of LC pumps.

Introduction

In production laboratories around the world there is an increasing amount of samples to be measured with an increased pressure on turn-around time. Because of this shorter LC gradients are developed. With short gradients, the wash and re-equilibration portions of the chromatography become relatively long and this time can be better used analyzing the next sample. A duplexed HPLC System helps to overcome this issue.

Keywords: Multiplexing, Shimadzu, Hystar, TASQ, EVOQ

Authors: Louis Maljers[1], Zicheng Yang[2].
Departments of: [1]Bruker Daltonics Inc, Billerica, Massachusetts, USA; [2]Bruker Daltonics Inc, San Jose, California, USA.

With this system there are two ways to increase the throughput.

Option 1 run the batch of samples over two columns alternating from one column to the other and option 2: run two batches of different samples where each batch has its own column as long as the solvents A and B are the same, gradients and columns can be different. Option 1 one has the advantage that the batch cycle time is greatly reduced but if you are not comfortable of running the same samples over physically different columns then option 2 helps to increase throughput with different sample sets. In this study we tested option 1.

Experimental

Instruments:

- The Shimadzu Nexera X2 HPLC System used consisted of: 4 x LC-30AD pumps, 2 x DGU-20A$_{5R}$ on-line degasser, 1 x CBM-20A communications bus module, 1 x SIL-30AC MP Autosampler, 1 x CTO-30A Column oven with one FCH-32AH 10 port valve.
- 2 x Bruker intensity Trio C18 50 x 2.0 mm I.D. S-1.9µm columns.
- Bruker EVOQ Elite LC-TQ mass spectrometer.

Software:

- Bruker Hystar was used to control the complete duplex HPLC MSMS system. TASQ 1.4 (Target Analysis for Screening and Quantitation).

LC Gradient

Time (min.)	%B	Flow (µL/min.)
0.00	30	400
0.10	30	400
3.50	50	400
3.51	100	400
5.00	100	400
5.01	30	400
6.50	30	400

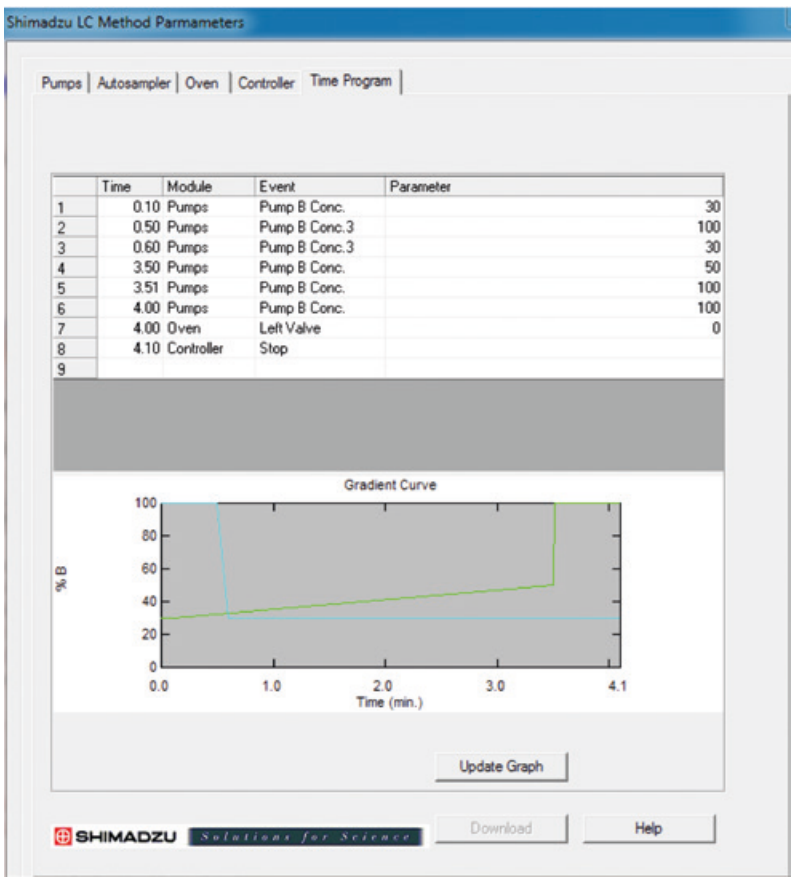

Figure 1. *Time program for both HPLC pump sets.*

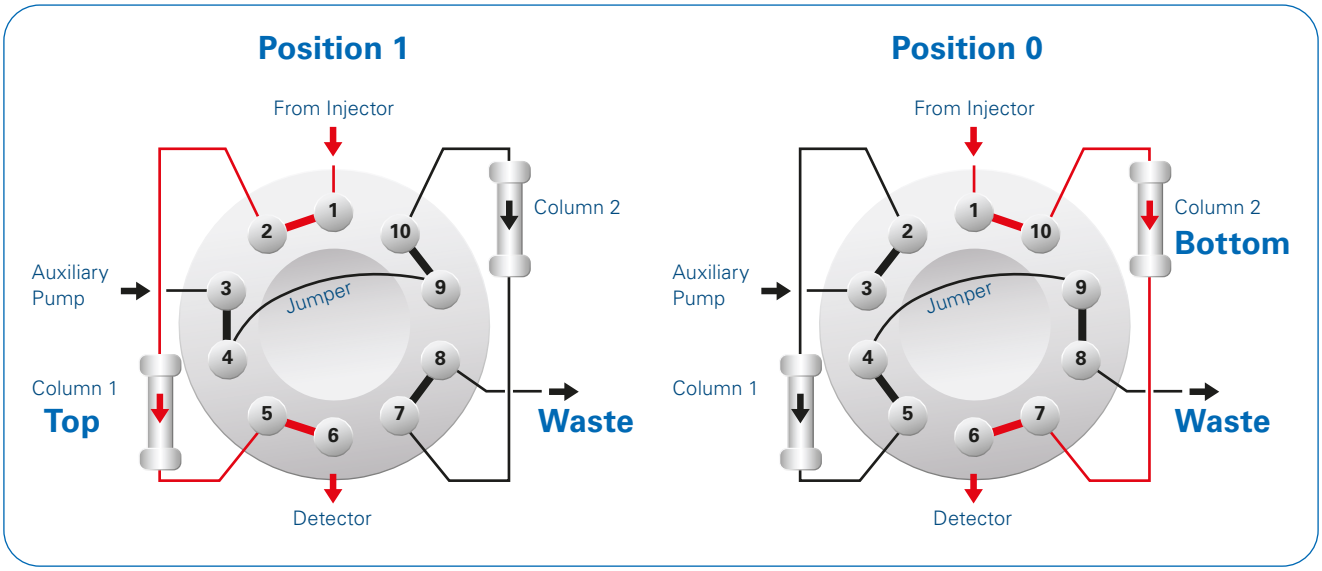

Figure 2. *10 port valve connections in the column oven to switch the pumps between the columns.*

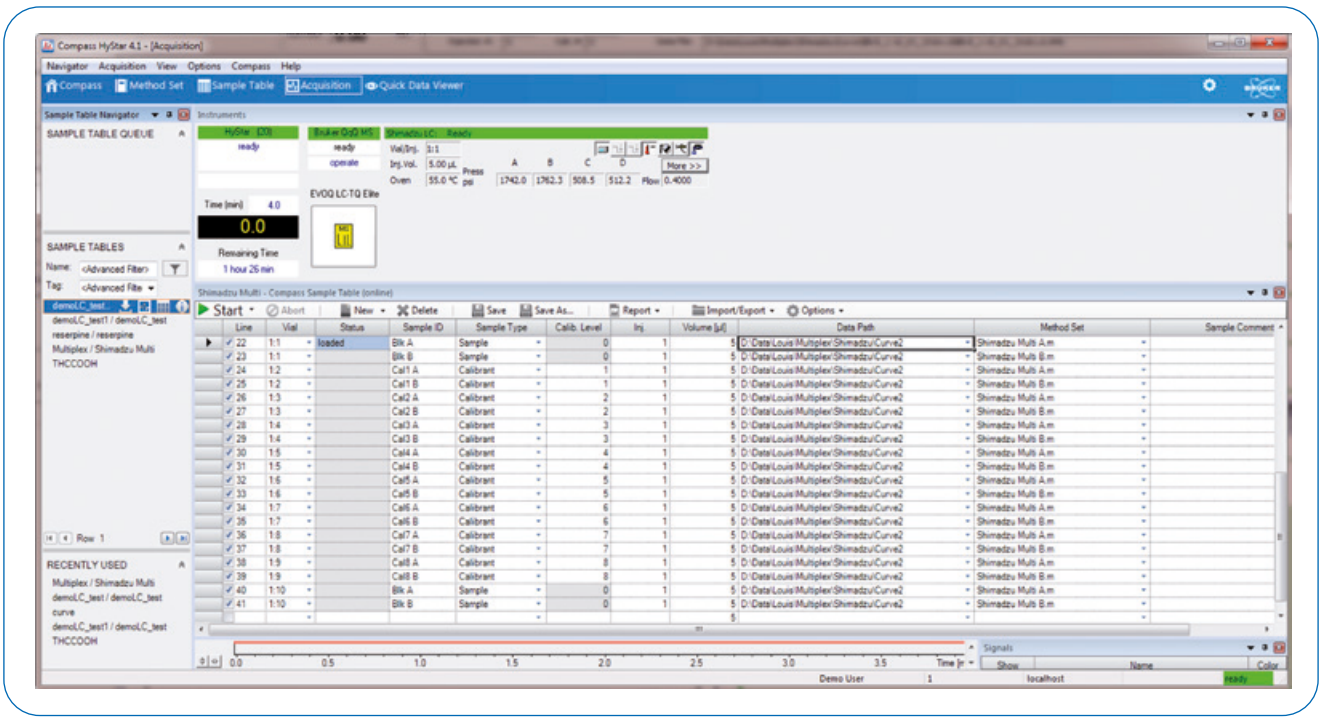

Figure 3. *Instrument connection and Sequence table in Hystar 4.1.*

We developed a simple LC-MSMS method with four compounds (verapamil, loperamide, terfenadine, and diclofenac). Oven temp. 55°C, injection volume 5 µL.

In order to facilitate the duplexing, we created two methods one for each column. The methods differ from each other only in the valve setting. In this way one pump set is used for the analytical gradient and the other for washing and equilibrating the column. After the last peak of interest has eluted from the column the valve is switched and the acquisition stopped. During the next sample run, the first column is washed and equilibrated. In our test the last compound to elute is Diclofenac at 3.2 min. in order to get some baseline after the peak we switched the valve at 3.9 min. and stopped the acquisition and started the next sample run. This workflow reduces time per sample by 40%. A typical 8 point calibration curve injected in duplicate with two blanks saves nearly 1 hour of time.

Results and Discussion

We created two methods to make the duplexing work. The methods differ only in the switching of the valve in the column oven as can be seen on line 7 in the time program. The valve connections are displayed in Figure 2. While pump set 1 is creating the analytical gradient for column 1, pump set 2 starts at high organic solvent followed by a re-equilibration step to wash and prepare column 2 for injection of the next sample.

In Figure 3 shows the connection of the duplexed LC system, the EVOQ LC-TQ, and the sequence table in Hystar. In the sequence table the two interleaved methods are specified in the method set column.

The New TASQ 1.4 software provides a turnkey solution for when there is a requirement to screen, confirm or quantify few or hundreds of compounds in a single analysis. It is user configurable as to what is visible on the screen from very user friendly with limited amount of information to a more information rich screen with lots of diagnostic data for the most demanding user. Figure 5 shows the quantitation perspective with Regression plot, a residual plot, an integration check/correction plot, a calibration/quantitation table, and a useful diagnostic data plot showing the retention time over the batch.

As we can see the duplexing didn't have any negative effect on the retention times of verapamil, one of the used test compounds.

Figure 6 shows the chromatograms view. Chromatograms view can be used to quic kly review peak integration of the whole batch.

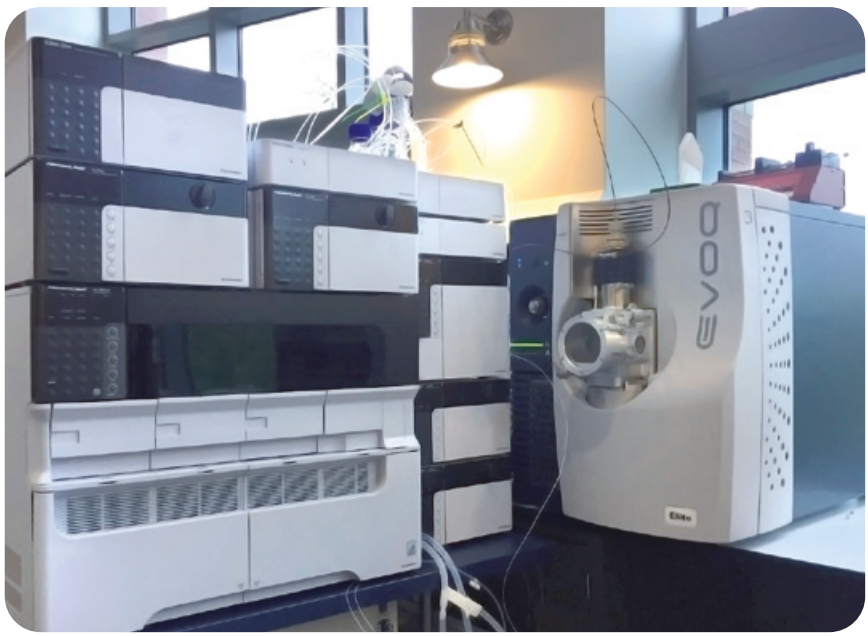

Figure 4. *Picture of the integrated system in the Billerica demonstration facility.*

Figure 5. *TASQ Quantitation Perspective.*

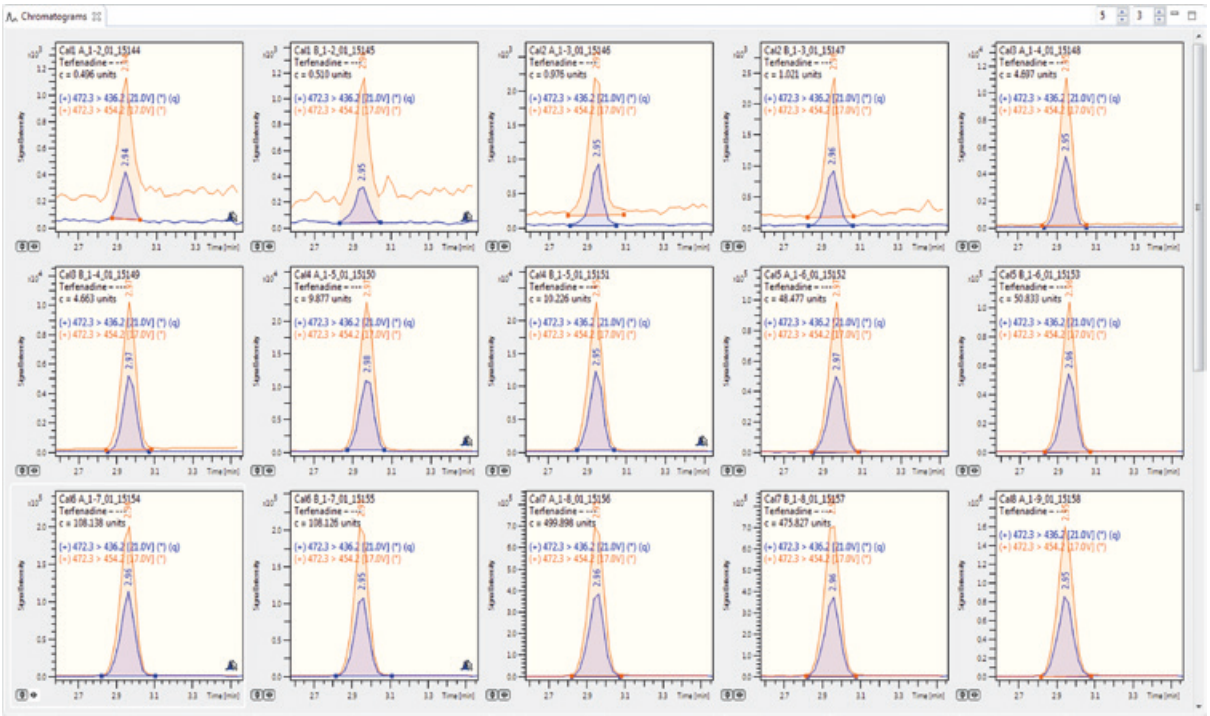

Figure 6. *TASQ Chromatograms view.*

Conclusions

It has been shown that duplexed LC systems with a single mass spectrometer can dramatically increase throughput for demanding laboratories. This shortens turn-around times and improves the laboratory effectiveness. In our experiments we were able to save as much as 40%. Even more time can be saved with shorter chromatographic runs.

- TASQ 1.4 software allows for quick and easy data processing with a good intuitive workflow.

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Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com