

# Achieving Maximum Productivity by Combining UHPLC with Advanced Chromatographic Techniques

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## ABSTRACT

Ultra-high Pressure Liquid Chromatography (UHPLC) is a rapidly growing technique that takes advantage of widely used HPLC columns packed with sub-3 µm particles. It offers ultrahigh resolution with maximum peak capacity, and significantly accelerates analyses by using higher flow rates and/or shorter columns. However, the decreasing particle size of the packing material results in significantly increased backpressure, and imposes increased technical requirements on the LC system. Further accelerating today's complex analyses to achieve sub-minute separations may require even higher backpressures that will soon reach physical and technical limits of currently available systems. Hence, perfecting system automation and utilization time are more promising techniques for maximizing productivity.

This presentation describes some technical solutions for maximizing productivity by improving system utilization time. These approaches focus on all process times which are not connected to the analysis itself, e.g., column washing and re-equilibration. The significant enhancements in productivity are shown and discussed on two different UHPLC system configurations—Tandem and Automated Application Switching.

## EXPERIMENTAL

### System Configurations

System	Tandem	Automated Application Switching
Solvent Rack	SRD-3600 Solvent Rack with six degasser channels	
Pump	DGP-3600RS Rapid Separation Dual-Gradient Pump	
Autosampler	WPS-3000TRS Rapid Separation Well Plate Sampler, thermostatted	
Column Compartment	TCC-3000RS Rapid Separation Thermostatted Column Compartment	
Column Compartment Valving	One 2-position, 10-port valve	One 2-position, 10-port and one 2-position, 6-port valve
Detector	DAD-3000RS Rapid Separation Diode Array Detector with 2.5 µL Semi-Micro Flowcell, SST	

The complete systems are controlled using the Chromeleon® Chromatography Data System software (patent pending).

The main difference between an UltiMate 3000 Intelligent UHPLC and a standard UHPLC system is that the pump actually houses two ternary gradient pumps that can operate independently. The column oven also contains 2-position, 10-port valves to allow easy switching between columns.

## HPLC Methods

Two methods for the analysis of soft drink ingredients are described in the following application examples. The first application represents a UHPLC method for the quantification of additives in soft drinks. The second application is a conventional HPLC application for the analysis of monocarboxylic acids. Both methods use completely different parameters; one using a ternary-gradient UHPLC method and the other a conventional isocratic LC method.

Table 2. HPLC Methods for Soft Drink Analyses

	Application 1: Additives in Energy Drinks	Application 2: Monocarboxylic Acids in Energy Drinks																
Column	Acclaim® PA2, 2.2 µm (2.1 × 100 mm)	Acclaim Mixed-Mode WAX-1, 5 µm (4.6 × 150 mm)																
Mobile Phases	A) Acetonitrile B) 30 mM Phosphate buffer, pH 3.28 C) 30 mM Phosphate buffer, pH 2.54	25 mM Phosphate buffer, pH 6.0																
Gradient	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>%A</th> <th>%B</th> <th>%C</th> </tr> </thead> <tbody> <tr> <td>0.0</td> <td>0</td> <td>100</td> <td>0</td> </tr> <tr> <td>14.0</td> <td>60</td> <td>0</td> <td>40</td> </tr> <tr> <td>16.0</td> <td>60</td> <td>0</td> <td>40</td> </tr> </tbody> </table>	Time (min)	%A	%B	%C	0.0	0	100	0	14.0	60	0	40	16.0	60	0	40	Isocratic
Time (min)	%A	%B	%C															
0.0	0	100	0															
14.0	60	0	40															
16.0	60	0	40															
Flow Rate	1.0 mL/min	0.8 mL/min																
Injection Volume	0.3 µL	10 µL																
Column Temperature	30 °C	30 °C																
Detector Wavelengths	210 nm, 246 nm, 265 nm and 285 nm	210 nm																

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## RESULTS AND DISCUSSION

Tandem UHPLC System is ideal for laboratories that want to run only a single analytical method per system. In this configuration, two identical columns are switched between two flow paths, an analysis flow path and a regeneration flow path (Figure 1), to allow column washing and re-equilibration off-line. While one column is equilibrated, the system injects the next sample on the other column. This solution saves the time required to wash and equilibrate a column for the next sample, and significantly increases the sample throughput without requiring new method development.

For the first series of experiments, the chromatographic separation of additives in energy drinks was chosen and initially performed under conventional LC conditions with an Acclaim PA2, 3  $\mu\text{m}$  (4.6  $\times$  150 mm) column. The retention time of the last eluting peak was 16 min, with a total run time of 28 min. In a second step, the application was accelerated using an Acclaim RSLC PA2, 2.2  $\mu\text{m}$  (2.1  $\times$  100 mm) column under UHPLC conditions (Figure 2). The total run time was reduced to 4 min, with a retention time of the last eluting peak at 2.1 min. In a last step, the method was performed using a Tandem UHPLC system setup, where the equilibration of the column was performed off-line to further shorten the total run time to 2.5 min.

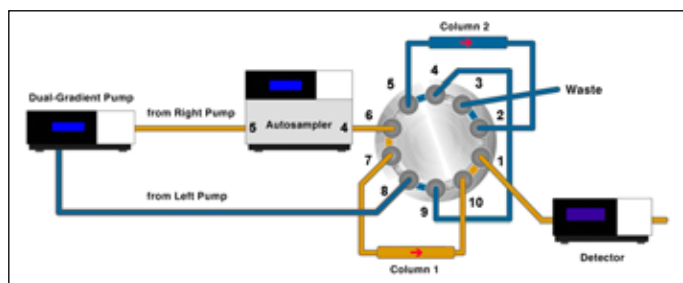


Figure 1 Flow scheme for the Tandem UHPLC system setup.

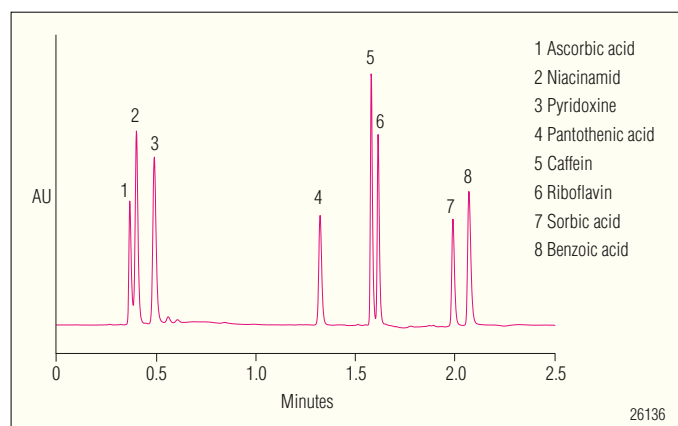


Figure 2. Application 1: Analysis of additives in an energy drink under UHPLC conditions.

## Time Saving and System Utilization Efficiency for Tandem UHPLC

In general, the cycle time of a gradient consists of three parts, the injection cycle time, the separation time, and the column- re-equilibration time. Useful data are only collected during the sample injection and the retention time of the last eluting peak. This can be defined as the utilization time for a gradient run, while the other parts of the analytical cycle do not deliver useful data (Figure 3).

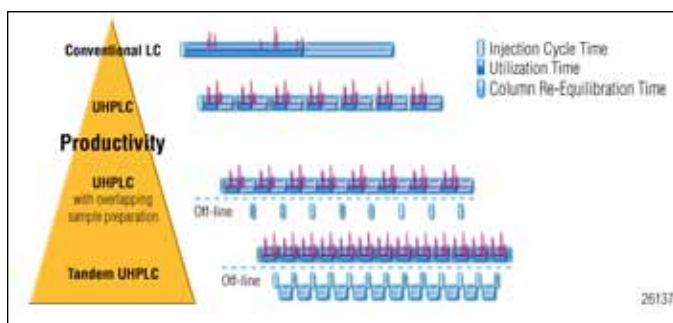


Figure 3. Comparison of injection cycle time, utilization time, and column re-equilibration time.

The utilization time for additives in energy drinks was experimentally found as 16 minutes for the conventional LC conditions and 2.1 min under UHPLC conditions, while the column reconditioning times were 12 and 1.9 min, respectively. The injection cycle time of 30 seconds was presumed. The determined total run times for the different system setups were calculated into samples per day. Table 3 shows that the change from conventional LC to common UHPLC and Tandem UHPLC increases productivity 6.4 and 11.5 times, respectively. The productivity of Tandem UHPLC is almost double (1.8 times) that of standard UHPLC.

Table 3. Overview of Total Run Times and Productivity for Different System Setups			
System Setup	Total Run Time (min)	Samples Per Day	Productivity Increase
Conventional LC	28.5	50	1
UHPLC	4.5	320	6.4 times
UHPLC with overlapping sample preparation	4.0	360	7.2 times
Tandem UHPLC	2.5	576	11.5 times (1.8 times compared to UHPLC)

The system utilization efficiency for the application is defined as the percentage of the utilization time compared to the total run time. Table 4 shows that the efficiency drops when switching from conventional LC to a standard UHPLC application. This can be explained with a more pronounced effect of the injection cycle time, which is identical for both configurations. A dramatic increase in efficiency is calculated for the Tandem UHPLC. The off-line column regeneration is shown to have the highest impact on efficiency.

Table 4. System Utilization Efficiency			
System Configuration	Utilization Time (min)	Total Run Time (min)	System Utilization Efficiency
Conventional LC	16	28.5	56.1 %
UHPLC	2.1	4.5	46.7 %
UHPLC with overlapping sample preparation	2.1	4.0	52.5 %
Tandem UHPLC	2.1	2.5	84.0 %

### Automated Application Switching Setup

The Automated Application Switching (AAS) configuration (Figure 4) is ideal for laboratories that need to run two different analytical methods, using different mobile phases and different columns, on the same instrument. The process for changing an instrument setup from one analytical method to another is as tedious, time consuming, and error prone as starting up and equilibrating an instrument. In addition, when a sequence is started on Friday afternoon, a manual method changeover to continue with the next method would require the operator to return to the laboratory during the weekend. UltiMate 3000 Intelligent LC systems with Chromeleon Chromatography Data System software enable laboratories to automate different LC methods on one system, significantly increasing use time.

The right pump and column 1 can be used to run one application and the left pump and column 2 can be used to run a second application. This provides the analyst with a fully automated application switching setup. For this purpose, initial instrument startup and equilibration conditions, well-defined shutdown procedures, and automated switch-over steps are required.

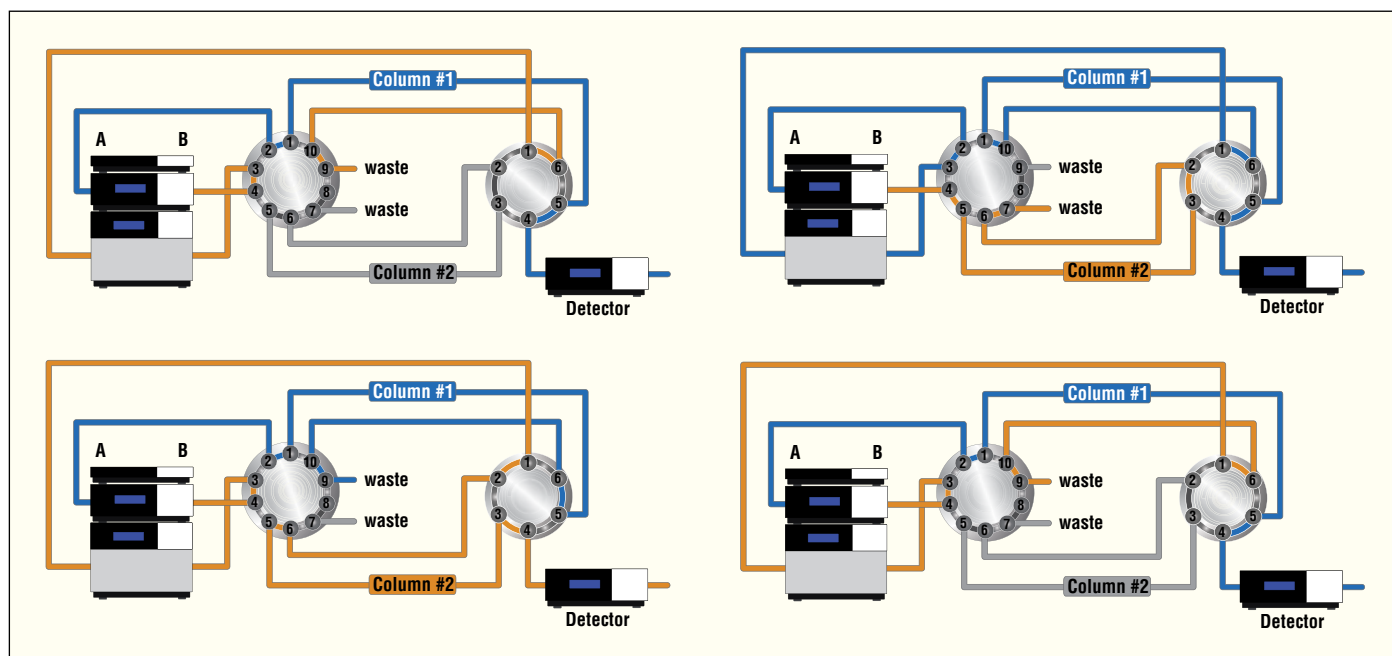


Figure 4. Automated Application Switching system configuration.

In the second series of experiments, an Automated Application Switching (AAS) setup was used to perform the above mentioned UHPLC analysis of additives in energy drinks, followed by a conventional LC analysis of monocarboxylic acids (Figure 5), all on a single UHPLC system.

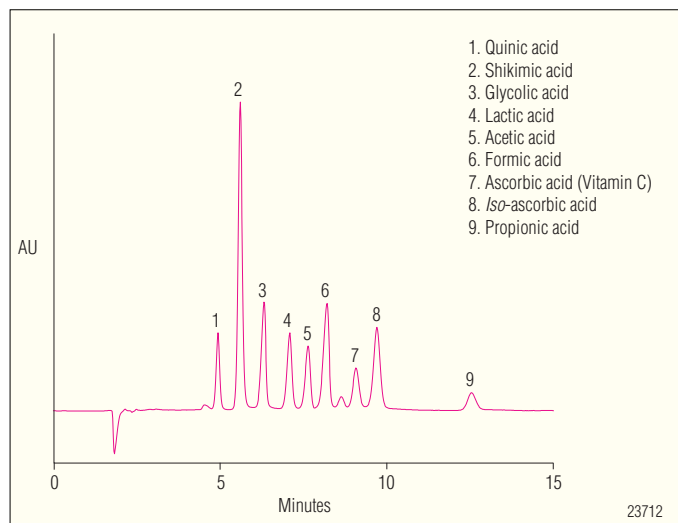


Figure 5. Application 2: Analysis of monocarboxylic acids under isocratic conventional LC conditions.

## Time Savings for Automated Application Switching

To determine the total amount of time that can be saved using AAS, it is necessary to consider two different scenarios. In one the application is started during the work week. The other occurs if the application is started on a day before a non-working day, for example before a weekend. Figure 6 illustrates the two possible situations.

The time that is gained in both scenarios is the time it takes to convert the HPLC instrument from the first to the second application, followed by the equilibration of the system. Converting and equilibrating the HPLC system can take up to 2 h of labor.

**Scenario 1:** The first application is started during the working week.

If the first application is run during the work week, it may not be finished until after hours. Without application switching, the instrument is idle for the remainder of the night. With AAS the remainder of the night is used to run the next application, resulting in gained hours of HPLC productivity time.

**Scenario 2:** The first application is started before the weekend.

In this case the first application is finished during the weekend. Without application switching, the instrument is idle over the remainder of the weekend. With AAS the remainder of the weekend is used to complete the next application. This can easily free more than a complete day of productivity time (Figure 6).

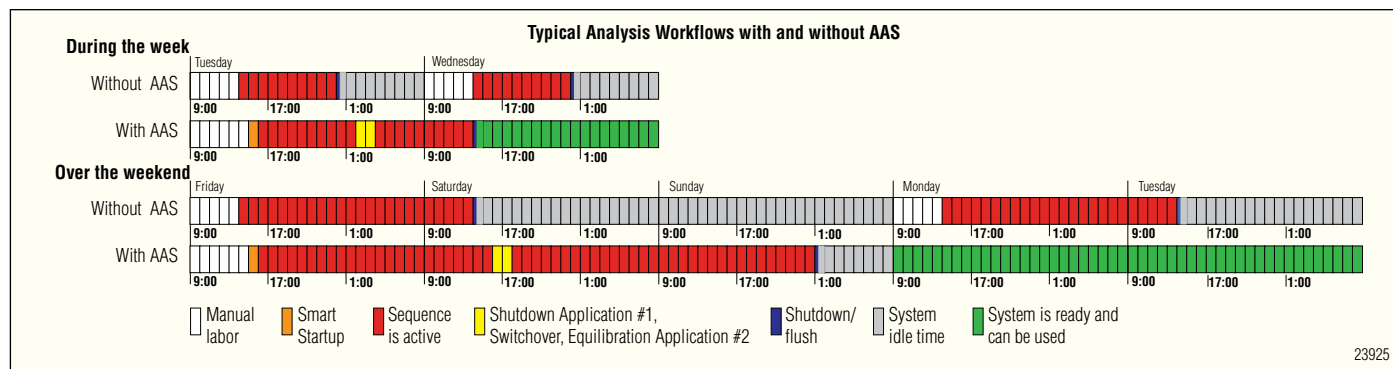


Figure 6. Comparison of analysis workflows with and without AAS.

## CONCLUSION

For systems which run only a single application, the Tandem UHPLC system setup significantly boosts lab productivity and system utilization efficiency.

Tandem UHPLC offers the following advantages over other approaches:

- Significantly improved productivity compared to UHPLC
- Highest system utilization efficiency
- Highest sample throughput
- Can be used easily with existing methods
- Lowest cost of ownership per sample

Running two applications on a single system, the UHPLC Automated Application Switching system setup showed a boost in lab productivity without the need to purchase additional LC instruments. Beside the increase in efficiency, one of the further key advantages of the AAS configuration is the high flexibility to run UHPLC and conventional LC methods on a single system, even if the conventional LC method requires injection volumes higher than 40  $\mu$ L.

AAS offers the following advantages over other approaches to improve productivity:

- Highest application flexibility
  - Injection volume of up to 100  $\mu$ L without any re-plumbing
  - UHPLC and conventional LC application on a single AAS setup
- Can be used easily with existing methods
- Frees operator time
- Minimizes errors
- Increases system use time and return on investment

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