INTRODUCTION

Laboratories regularly have to analyze a variety of parameters from the same sample set. Often more than one HPLC method is needed to obtain the required results. Running multiple methods can be time- and labor-intensive, and different laboratories adopt various strategies to increase their productivity.

Traditionally the analyst runs one method and manually switches to the other method. The manual method change-over makes it impossible to run the methods consecutively, unattended, over night or over the weekend. When changing from the first to the second method, typically the analyst has to flush eluent lines, wash the column at a high percentage of organic eluent, and finally equilibrate the column to the start conditions of the second method. All these tasks require frequent operator interaction.

Another approach runs each method on separate dedicated HPLC systems at the same time. This approach requires two HPLC systems and enough bench space. Both systems have to be operated simultaneously, possibly by two different operators.

Regardless of the approach, after starting the instrument basic system parameters like pressure ripple, baseline noise, and drift have to be monitored before the first sample can be injected. Monitoring these parameters is tedious and distracts from other responsibilities in the lab. This results in increased labor time, compromising productivity.

An alternative concept—Automated Application Switching (AAS)—is based on a novel Dual-Gradient system occupying the same bench space as one system. The operator sets up both methods. After the system automatically starts and equilibrates, the first method runs and then the system automatically switches to the second method without any additional operator intervention. This approach frees operator time, increases system usage time, allows automation, and thus boosts productivity.

This poster demonstrates the implementation and benefits of using automated application switching in the food and beverage market. Two typical analyses of the same sample set are used for this purpose.

EXPERIMENTAL

System Setup

The UltiMate™ 3000 x2 Dual-Ternary HPLC system for Automated Application Switching consists of:
- DGP-3600 Dual-Ternary Gradient Pump
- SRD-3600 Solvent Rack with six degasser channels
- TCC-3200 2x2P-10P Thermostatted Column Compartment with two 2-position 10-port valves
- WPS-3000TSL Thermostatted Autosampler
- PDA-3000 Photodiode Array Detector with 13 µL flowcell

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

Only the combination of the unique UltiMate 3000 system with the Chromeleon software makes Automated Application Switching possible. The two 2-position valves and the DGP-3600 pump in the system provide the instrumental feature for the switchover. Chromeleon’s advanced yet easy-to-use automation features allow analysts with minimal training to take advantage of this powerful technology.

HPLC Methods

Two typical methods for the analysis of soft drink ingredients are used in this application example.

The first application represents a straightforward method for the quantification of some of the most widespread substances in soft drinks. The second application is a USP method for the determination of some pharmacologically active compounds in drinks, including quinine as the most important analyte.

The methods used for the analysis of compounds in the beverages are completely different. The respective columns, mobile phases, detection parameters and gradients are optimized for each intended application.
Application 1: Analysis of Sweeteners and Additives in Soda

Flow: 0.8 mL/min

Gradient: Time %B
0 0
1 0
2 31
6.5 31
7.5 40
15 40
15.1 0
20 0

Column Temperature: 35 ºC

Detection Wavelengths: 210 nm, 230 nm and 262 nm

Column: Acclaim® OA, 5µm, 120 Å, 4.0 × 150 mm

Mobile Phase A: 14.2 g/L Na₂SO₄, 0.55 mL/L methanesulfonic acid (MSA) in water

Mobile Phase B: Methanol

Mobile Phase C: 70/30 (v/v) water/acetonitrile

Standards: Erythorbic acid, Citric acid, Acesulfame-K, Aspartylphenylalanine, Caffeine, Aspartame, Potassium sorbate, Benzoic acid

Samples are filtered with a 0.5-µm filter and diluted 5-fold with mobile phase A.

Application 2: Analysis of Quinine in Tonic and Bitter Lemon

Flow: 1.0 mL/min

Gradient: Time %B
0 0
10.5 0
10.6 50
15 50
15.1 0
20 0

Detection Wavelength: 235 nm

Column Temperature: 35 ºC

Column: Acclaim PA C16, 5µm, 120 Å, 4.6 × 150 mm

Mobile Phase A: 896 mL H₂O, 100 mL acetonitrile, 1.4 mL MSA, 0.8 mL acetic acid, 2 mL diethylamine

Mobile Phase B: 30/70 (v/v) water/acetonitrile

Mobile Phase C: 70/30 (v/v) water/acetonitrile

Standards: Cinchonidine, Quinine, Dihydroquinine, Benzoic acid

Samples are filtered with a 0.5-µm filter and injected directly.

Figure 1. Automated Application Switching (AAS) enables running two methods in series with zero idle time in-between.
AUTOMATED APPLICATION SWITCHING PRINCIPLE

A common requirement in analytical laboratories is to run many 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 come back into the laboratory during the weekend.

Ultimate 3000 Intelligent LC systems with Chromeleon Chromatography Management Software enable laboratories to automate different LC methods on one system, significantly increasing use time.

The main difference between an Ultimate 3000 Intelligent LC and a standard HPLC system is that the pump actually houses two ternary gradient pumps that can operate independently. The column oven contains two switching valves that allow two columns to be installed and connected to the different fluidic paths of the pump. This kind of setup (Figure 2) is ideal for laboratories that want to run two different analytical methods on the same instrument. 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 start-up and equilibration conditions, well defined shutdown procedures, and automated switch-over steps are required.

Automated Instrument Start-Up and Module Check of the First Application

Ultimate 3000 Intelligent LC systems in combination with Chromeleon software offer smart routines to automate otherwise manual, time-consuming, and error prone method setup and change over steps.

SmartStartup ensures that all needed HPLC modules (in this case all modules involved to run the first application—Schematic A, Figure 2) are activated in a defined, secure, and automated way. For example, the eluent flow is turned on before heating the column or switching on the lamps. This prevents typical errors and frees operator time.

After instrument start-up, key module parameters like pump pressure ripple and detector drift are monitored. Once these checks (Figure 3) are successful the first application sequence is started.

![Figure 2. Typical automated application switching (AAS) setup.](image1)

![Figure 3. Automated monitoring of key module parameters.](image2)
Automated Shutdown of First Application and Application Switch-Over

At the end of the first sequence the SmartShutdown routine helps to place the first application on hold by reducing the flow rate (Figure 4).

At the same time, valve 1 is switched and the fluidic lines of the autosampler are flushed to waste (Schematic B, Figure 2). Only then the second valve (valve 2) is switched to allow equilibration of the second application (Schematic C, Figure 2).

Automated Instrument Start-Up and Module Check of the Second Application

SmartStartup is again used to monitor key module parameters running the second application. While the column compartment, autosampler, and detector were already in use with the first application and do not need instrument start-up, the second pump does. After successful start-up of the second pump, all key module parameters are again checked for running the second application. After successful equilibration, the second sequence is started and processed automatically.

Shutting Down the Instrument After Completion of Both Applications

At the end of the second sequence, automated software routines allow the controlled and well-defined shut down of both applications and the instrument modules (e.g., lamps are switched off, column compartment and autosampler thermostating can be deactivated).

Benefits of Automated Application Switching

Chromatographers use uptime as a reliability indicator, but uptime is not the only important requirement. Equally significant is use time to maximize instrument load and the production of useful data. Application switching leaves the original applications untouched and can be achieved with minimal effort, while fully documenting each method and transition for regulatory compliance. Figure 9 compares AAS with the traditional sequential approach.

INGREDIENT ANALYSIS OF SOFT DRINKS

Although the AAS approach relies extensively on automation and unattended operation, Figures 5 and 6 prove that this does not compromise chromatography. Both retention time and peak area show very good RSDs, below 0.031% and 0.25%, respectively.
The chromatographic separation of the analytes is also well suited for quantification, with only a simple dilution step for sample preparation. As an example, Figure 7 shows the chromatogram and the amount table for a cola-type soft drink. All analytes of interest are baseline separated. Interesting about this sample is the comparably high citric acid concentration. A complementary analysis of citric acid and phosphoric acid by ion chromatography would certainly be of interest to determine the overall acid content.

Figure 7. Ingredient analysis of a cola-type soft drink.

Figure 8 shows the chromatograms of a tonic water and a bitter lemon. While the bitter lemon contains quinine and its two common acetyl derivatives, the tonic water only contains one of the acetyl derivatives—dihydroquinine—above the LOD.

Figure 8. Ingredient analysis of a bitter lemon (left) and a tonic water (right).

**Time Savings**

About 2.5 h are needed to prepare the mobile phases, samples, and standards for each application, including the column installation. Each injection requires 22 min (application 1) or 23 min (application 2), respectively. The automated equilibration including purging requires about 60 min. Application 1 with 16 different samples takes ~24 h to complete (time includes standards for calibration and SST). Application 2 with six samples takes ~15 h to complete (time includes standards for calibration and SST).

To determine the total amount of time that can be saved by 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.

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 3 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 (Table 1).
Automated Application Switching boosts lab productivity without the need to purchase additional HPLC instruments. AAS offers the following advantages over other approaches to improve productivity:

- Can easily be used with existing methods
  - No additional method development
  - No extra validation effort
- Frees operator time
- Minimizes errors
- Increases system use time and return on investment

The Dionex UltiMate 3000 ×2 Dual-Ternary HPLC system in combination with the powerful Chromeleon Chromatography Management Software provides analysts with a robust and easy-to-use turnkey solution for Automated Application Switching.

Both techniques are initiated Friday at 12:00. Without AAS, the two applications finish early on Tuesday morning, while with AAS both applications are completed during the weekend. With AAS, the results are available much sooner and the analyst can use the HPLC system on Monday to start new applications. Therefore, the “gained HPLC productivity time” is 24 h (see Table 1).

**Table 1. Productivity Gain through AAS Using the Above Application Examples**

<table>
<thead>
<tr>
<th></th>
<th>Without AAS</th>
<th>With AAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start Time</td>
<td>Friday, 12:00</td>
<td>Friday, 12:00</td>
</tr>
<tr>
<td>Prepare mobile phases, eluents and standards, install column</td>
<td>2.5 h</td>
<td>4.5 h</td>
</tr>
<tr>
<td>Equilibrate system for Application 1</td>
<td>1 h</td>
<td>1 h</td>
</tr>
<tr>
<td>Run Application 1</td>
<td>24 h</td>
<td>24 h</td>
</tr>
<tr>
<td>Shutdown/flush Application 1</td>
<td>0.5 h</td>
<td>0.5 h</td>
</tr>
<tr>
<td>Stop time Application 1</td>
<td>Saturday, 16:00</td>
<td>Saturday, 18:00</td>
</tr>
<tr>
<td>Idle time</td>
<td>41 h</td>
<td>–</td>
</tr>
<tr>
<td>Prepare mobile phases, eluents and standards for Application 2</td>
<td>2.5 h</td>
<td>–</td>
</tr>
<tr>
<td>Switch system to Application 2 and equilibrate</td>
<td>3 h manual input required</td>
<td>1 h fully automated</td>
</tr>
<tr>
<td>Run Application 2</td>
<td>15 h</td>
<td>15 h</td>
</tr>
<tr>
<td>Shutdown/flush Application 2</td>
<td>0.5 h</td>
<td>0.5 h</td>
</tr>
<tr>
<td>Stop time</td>
<td>Tuesday, 6:00</td>
<td>Sunday, 10:30</td>
</tr>
<tr>
<td>Total time</td>
<td>90 h</td>
<td>46.5 h</td>
</tr>
<tr>
<td>Total instrument idle time</td>
<td>44 h</td>
<td>22.5 h</td>
</tr>
<tr>
<td>Time Saved</td>
<td>–</td>
<td>43.5 h or 48%</td>
</tr>
<tr>
<td>Gained HPLC Productivity Time</td>
<td>–</td>
<td>24 h</td>
</tr>
</tbody>
</table>

**Figure 9. Comparison of analysis workflows with and without AAS.**

Ultimate is a trademark and Acclaim and Chromeleon are registered trademarks of Dionex Corporation.