A Multi-Tasking Software Approach to Increasing Productivity and Throughput in UHPLC

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Technical Note 172

Key Words
Chromeleon CDS, *PrepareThisInjection*, *PrepareNextInjection*, analysis time, injection preparation, injection overlap

Goal

To demonstrate how Thermo Scientific™ Dionex™ Chromeleon™ 7 Chromatography Data System (CDS) software commands can be used to integrate the entire sampler cycle time into the equilibration phase of a run and easily reduce analysis time spent per sample by 25% or more.

Introduction

With the widespread adoption of UHPLC, many laboratories are now being challenged to significantly improve sample throughput and reduce their cost per analysis. This is usually achieved by reducing run times and improving throughput on each UHPLC by using higher flow rates and shorter columns, often with smaller particles. This means either fewer instruments are needed to analyze the same number of samples or more samples can be analyzed in the same time.

An additional reduction of analysis time can be achieved by performing processes in parallel that are normally executed sequentially. Chromeleon 7 CDS software offers *PrepareThisInjection* and *PrepareNextInjection* commands that can help reduce injection cycle times by up to 25% or more simply by utilizing the advanced injection preparation procedures of the Thermo Scientific™ Dionex™ UltiMate™ 3000 or Thermo Scientific™ Vanquish™ UHPLC systems.

Here, throughput is defined as the number of samples analyzed within a certain time. In UHPLC, throughput increase can be achieved by analyzing samples in parallel, for instance by running two or more columns simultaneously, or by decreasing the analysis time of a single run. The time of a single run can be decreased by adapting the chromatographic method, operating the columns at high flow rates, or using shorter columns. System optimizations, such as reduced gradient delay volume, can also significantly contribute to throughput increase. Here we will look at how performing processes in parallel during a run will help to reduce cycle times.

A chromatographic run (cycle) starts after the *Ready* signal from the instruments and typically includes the following steps:

1. Injection preparation (sampler cycle time)
2. Actual analysis (gradient)
3. Column wash
4. Column equilibration

The latter can take place either before or after the gradient elution, as shown in Figure 1. The instrument idle time follows each run before the next injection can be started. This stage is used to finish the run in the CDS.

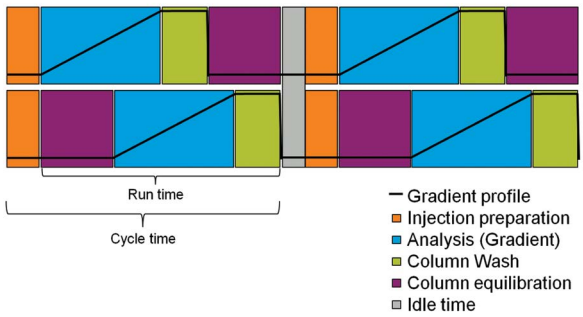

Figure 1. Sequential structure of consecutive analyses with column equilibration after separation (above) and before separation (below).

With the Chromeleon CDS features *PrepareThisInjection* and *PrepareNextInjection* (for brevity referred to as *Prepare Injection*), it is possible to reduce the overall analysis time per sample by performing the injection preparation step in parallel without any changes to the instrument or separation conditions.

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Advantages of the *Prepare Injection* Approach

The injection preparation is traditionally done by the sampler before the actual sample run and readies the sample to be injected into the flow path. In case of the Thermo Scientific Vanquish Split Sampler, the preparation includes the following steps:

1. The rack or well plate is transported to the injection position, e.g. by moving the rack from the shelf in an attached rack loader, like the Thermo Scientific Vanquish Charger module, into the sampler and/or by rotation of the carousel.
2. The injection valve switches to *Bypass* position and the needle leaves the needle seat.
3. An optional external needle wash is performed.
4. The needle is lowered into the sample container where it draws the defined volume.
5. An external needle wash may be performed before the needle returns to the needle seat.
6. With the valve switching to Inject, the run and the data acquisition are started.

The time needed for these steps is usually additional to the programmed run time of a sample. It is dependent on several sampler method parameters, such as *Draw Speed* and *Draw Delay*, but also the amount of sample to be injected and the selected wash options. In addition, the time spent on the transfer of the rack or well plate to the sampling position has to be considered. The transfer time can vary slightly depending on the position of the rack in the sampler or charger module. This may, in turn, affect the reproducibility of the results, especially if the column equilibration phase is not long enough to ensure complete fluidic and thermal equilibration of the system.

With *Prepare Injection*, the sample preparation steps are performed in parallel with the run, which reduces the overall sample analysis time. This approach is demonstrated in Figure 2 where the injection preparation is visualized with orange bars and the actual run is indicated by blue arrows.

The command *PrepareNextInjection* (middle schematic of Figure 2) integrates the sampling of the following injection to the equilibration phase at the end of the current run. Thus, it saves the time spent on sample preparation for the consecutive run. In case the column equilibration phase is programmed to take place before the gradient, the command *PrepareThisInjection* can be used (bottom schematic). The sample preparation is thus integrated into the column equilibration phase from the first injection in a sequence.

The green triangles in Figure 2 indicate the total analysis time saved by using *Prepare Injection*. In reality, the actual saving is dependent on several method parameters as mentioned before. An average injection preparation including a routine wash step takes about 15 seconds, which, if now removed from a 1 minute cycle by using *Prepare Injection*, reduces the analysis time by 25%. The reduction can be even higher if the time spent on injection preparation is longer, e.g. because intensive wash steps are included in the instrument method or the rack needs to be transferred from an attached Charger module before injection. In addition, this approach ensures always the same and consistent state of column equilibration. Even for ultrashort run times where the equilibrium may not be fully established, this method helps to sustain a better retention time precision.

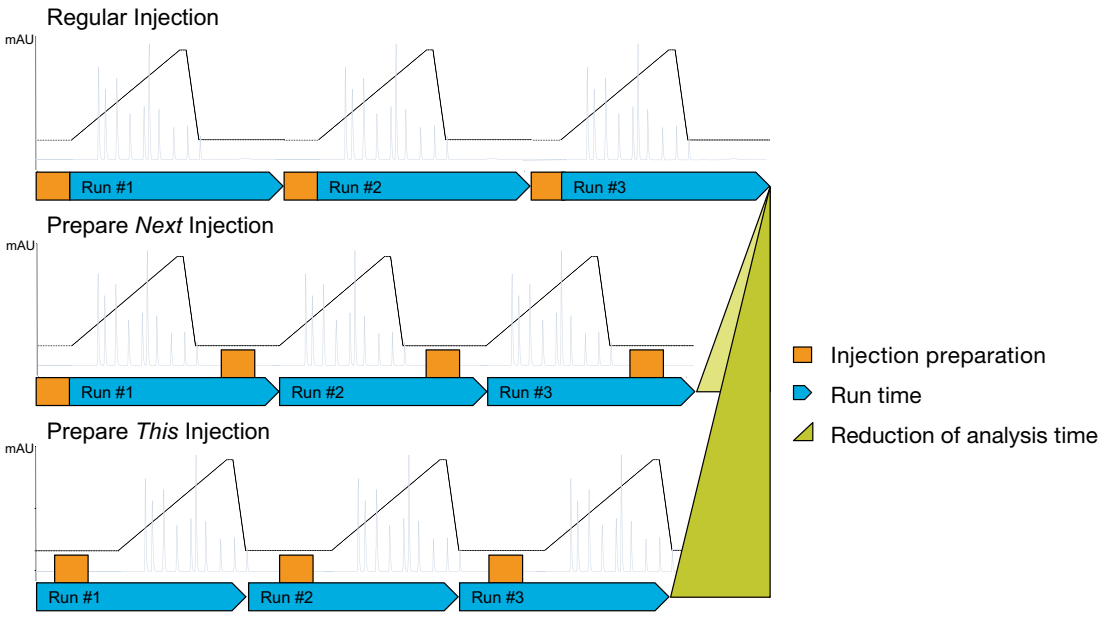

Figure 2. Reduction of analysis time with *Prepare Injection* (schematic).

Use of the Commands

The column is usually equilibrated to the starting conditions after the actual gradient run and column wash. During this step, the baseline acquisition is generally on, which enables monitoring late eluting peaks. This can be helpful in method development and when analyzing unknown samples.

The command *PrepareNextInjection* enables the autosampler to perform all preparation steps of the following run, while equilibration of the current one is still in progress.

To achieve this, the command may be easily inserted into the instrument method as demonstrated in Figure 3 for Chromeleon CDS, version 7.2. First, select the *StopRun* line in the method editor (1.) to add a time stage for the preparation procedure. To do so, click on *Time* (2.). This time represents the start of the injection preparation, which begins with the rack transport, if required, and the valve switching to *Bypass* position. The sample fluidics should be filled with mobile phase that matches the starting conditions to avoid a different solvent composition in the sample flow path affecting the separation. To ensure this, allow at least one dwell volume to flush the system after the end of the gradient before starting injection preparation.

Example: Return of mobile phase to starting conditions (= end of gradient) at 0.3 min + dwell time: 0.2 min = Start of injection preparation at 0.5 min (3.).

Note: The dwell time of a system depends on the volume of the respective system parts and capillaries, and flow rate.

Add a *Command* line (4.), type *PrepareNextInjection* (5.) and press enter. Chromeleon CDS will enter the complete command (*SamplerModule.Sampler.PrepareNextInjection*) with correct syntax automatically. Save the method (6.).

To achieve the lowest possible cycle times, make sure the time between the *Prepare Injection* command and the *Stop* command is not less than the average sampler cycle time.

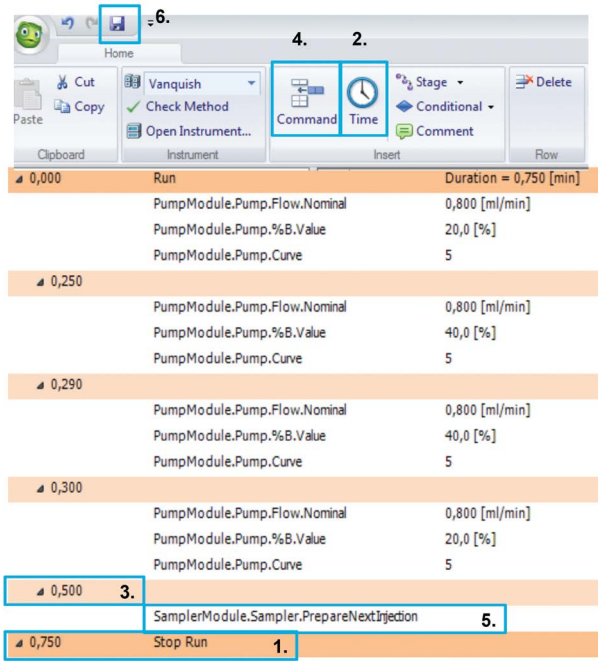

Figure 3. Setup of *PrepareNextInjection* command in Chromeleon CDS 7.2.

This command can be used with any existing method without further adaptations to the gradient or conditions. The preparation of the first injection will be processed before the actual start of the run as usual, even if the method includes a *PrepareNextInjection* command.

Depending on application requirements or personal preference, the equilibration can be also performed before the gradient. In this case, Chromeleon CDS sets a negative time stamp to initiate the equilibration before the injection. The valve switching to the *Inject* position indicates the start of the sample run and baseline acquisition. Therefore, the signal is not acquired during equilibration with this setup. Especially with well-established methods, this approach can be more convenient.

Reduction of cycle time is also achievable with this approach with the command *PrepareThisInjection*. It can be implemented in any existing method with the equilibration stage before the analytical run as described below and demonstrated in Figure 4.

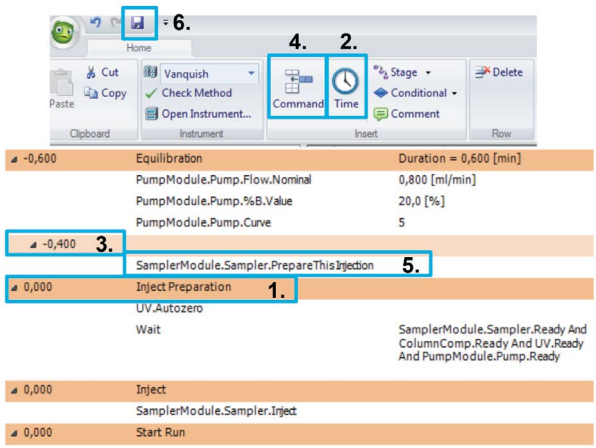

Figure 4. Setup of *PrepareThisInjection* command in Chromeleon CDS 7.2.

Select the line *Inject Preparation* (1.) and add a time step (2.). Consider the dwell time of the system to ensure the sampling path is filled with mobile phase at starting conditions (3.). This helps to avoid any effects on the separation due to deviating solvent conditions. Subsequently, add a *Command* (4.), type *PrepareThisInjection* into the line and press enter. Save the method to finish (6.). Without the *PrepareThisInjection* command, the preparation would begin after the equilibration phase but before the start of the gradient, meaning that the equilibration phase (and the overall run time) would be extended by the time needed for injection preparation.

Conclusion

Typically, the sampler cycle time is dependent on method parameters such as *injection volume* and *draw speed*, as well as *draw delay* and the chosen wash options. Also, the time for transferring the rack or well plate into sampling position has to be considered. The injection preparation time can thus vary slightly from run to run, which can have an impact on the equilibration state of both system and column.

The Chromeleon CDS commands *PrepareThisInjection* and *PrepareNextInjection* can integrate the entire sampler cycle time into the equilibration phase of a run and thus easily reduce the total cycle time by up to 25% or more as shown in the example above. This delivers an increase in productivity together with a reduced cost of analysis without the alteration of the analytical method, column, or instrumentation. In addition, the commands can help to eliminate possible variability on the chromatography due to different equilibration times, giving improved method robustness.

The implementation of the commands is easy, without the need to change any other method parameters. These features can further optimize throughput with both UltiMate 3000 and Vanquish UHPLC systems in conjunction with Chromeleon CDS.

Useful Links

AppsLab Library

The eWorkflow and the Chromeleon Backup (cmbx) file can be downloaded at AppsLab Library:
https://appslab.thermofisher.com/

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