TELEDYNE | LABS

SFC Stacked Injection Workflow with the ACCQ*Prep*® SFC System

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Abstract

Stacked injections are commonly used for chiral purifications but are useful for achiral purifications as well. Stacked injections are useful for rapidly purifying a large quantity of compound because multiple injections of the same sample can be rapidly done. Stacked injections are always isocratic. They save time and solvent by eliminating the equilibration time between injections. In addition, the time required for the compounds to elute is "filled" by other injections. Stacked injections run faster when early or late eluting contaminates are removed. A Combi*Flash*® NextGen system is ideal to remove these compounds quickly.

Procedure

Setting up the system for multiple runs

The first step is to develop a method for an isocratic run. When the isocratic method is ready, enter the total volume of sample and injection volume for each run. To initially fine-tune the method, enter a small **Injection Volume** such as 0.2 mL (see Figure 1), as you will be able to change it later.

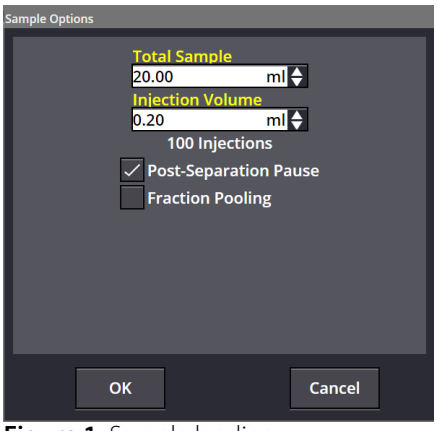

Figure 1. Sample loading.

Also, enable the **Post-Separation Pause** to pause the system between each injection so that the gradient method and injection can be fine-tuned. Collect peaks so that the system can suggest time windows that will be used for stacked injections. Do not worry that there are many injections, as this number will decrease when the sample volume is increased.

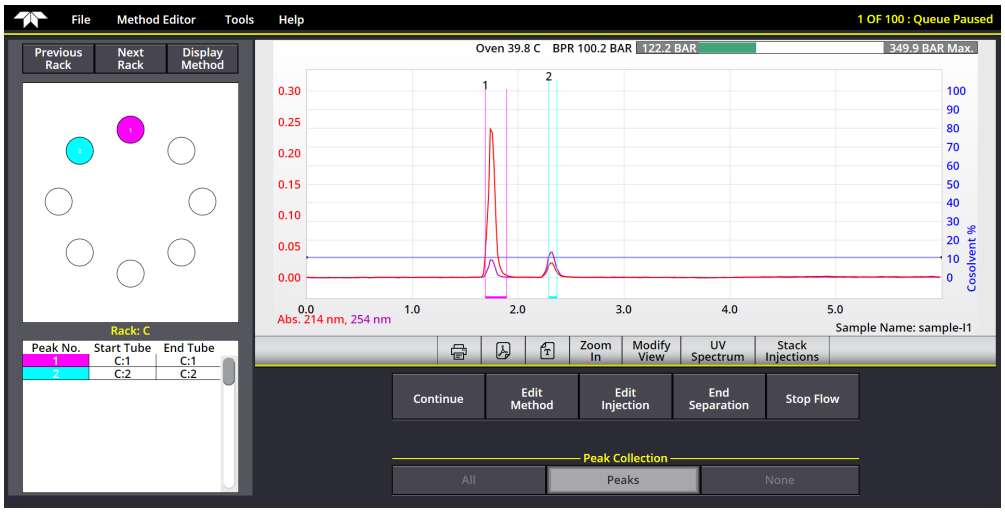

Figure 2. End of run — there is a lot of resolution, so the injection volume will be increased.

An example run is shown in Figure 2, where there is a lot of resolution between the peaks. The high resolution allows more sample to be loaded. Selecting the **Edit Injection** button allows you to change the injection volume.

The injection volume was changed to 2.0 mL for the next injection since the run suggested that there was sufficient resolution for a larger injection.

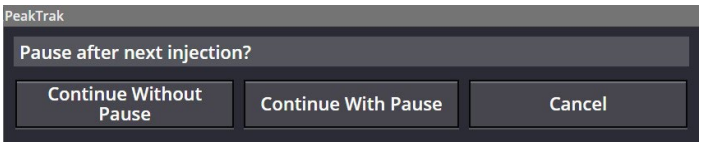

Figure 3. Choosing **Continue With Pause** will pause the run after the next injection for further adjustments and also allow a stacked injection to be performed if that injection is suitable for one.

Setting up the stacked injection

Once the run looks good, stacked injections can be run, as seen in Figure 4.

Figure 4. Higher sample loading; ready for stacked injections.

Selecting the **Stack Injections** button (found just below the chromatogram) opens the window shown in Figure 5, which shows a simulation of three stacked injections. The various time windows were determined from the fraction collection and were used to determine the cycle time. The up and down arrows near the **Start** and **End** settings allow fine tuning of the time window without entering values. The different colors (green and brown) represent a different stacked injection. The crosshatch patterns represent different time windows within an injection.

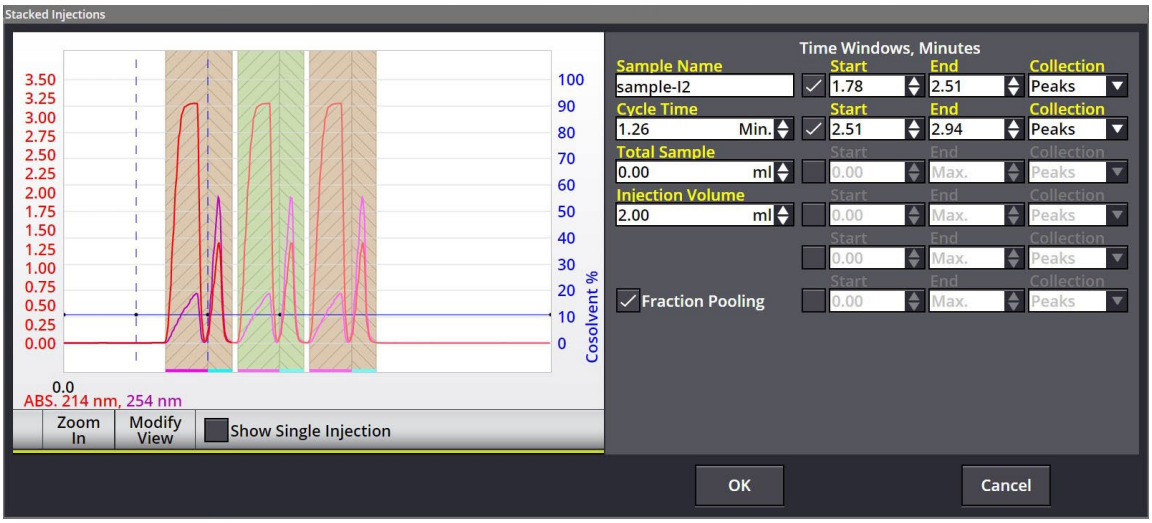

Figure 5. Simulated stacked injections for the run in Figure 4.

Depending on the complexity of the chromatogram, it may be easier to adjust the time windows within the context of a single injection. Checking **Show Single Injection** limits the view to just one injection, as seen in Figure 6.

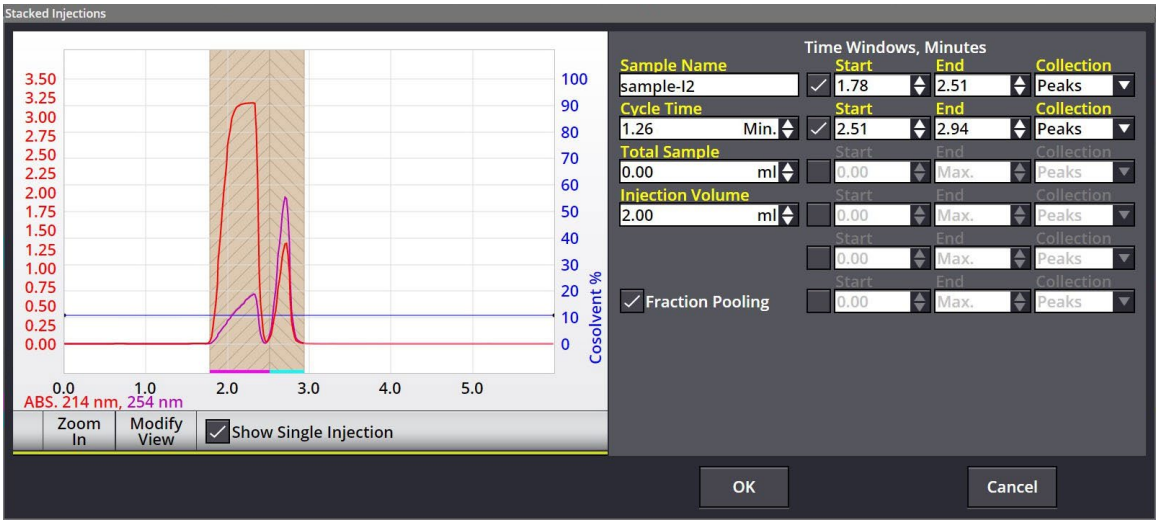

Figure 6. The single injection view.

Using the single injection view, it is easy to add a third time window if there is concern about mixing the two compounds (Figure 7). This time window can be added at the end of the list, and the time windows can be adjusted to accommodate the new one.

If no fractions were collected during the previous steps, **Show Single Injection** also permits easier manual time window settings.

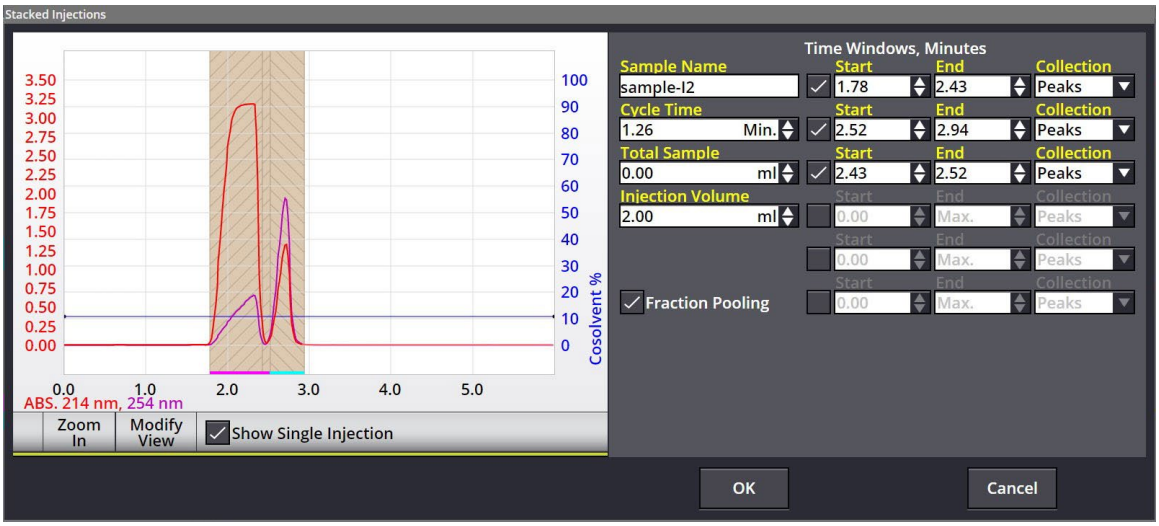

Figure 7. Adding a third time window within a single injection.

Turning off **Show Single Injection** shows a simulation of three injections with the third collection window. The effects of changing the **Cycle Time** can be most easily viewed in this mode. Although the system remembers the injection volume, enter the total volume of sample before selecting **OK**.

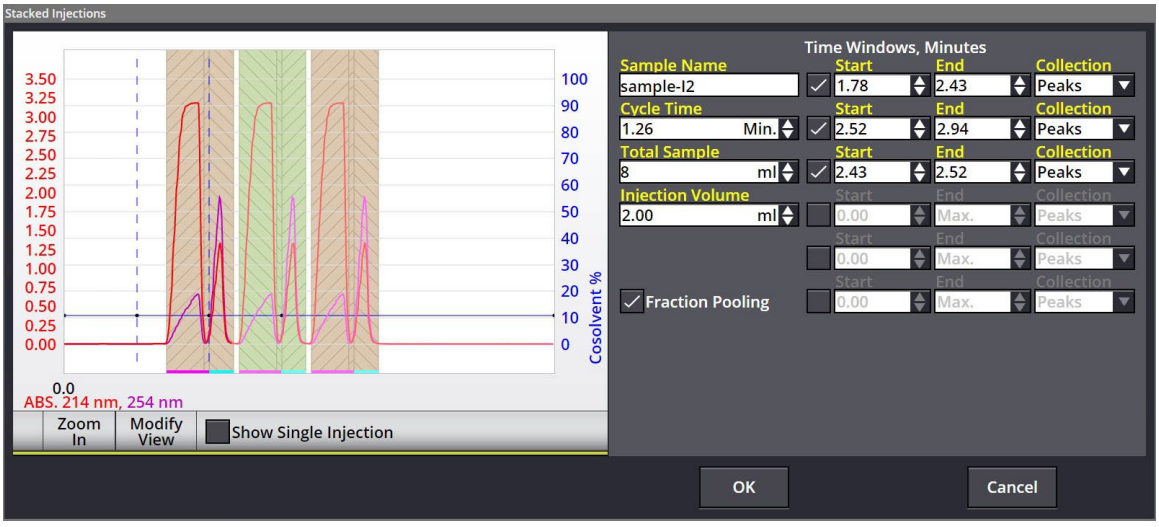

Figure 8. Simulation of three injections with a third time window

The system will start to run stacked injections.

Monitoring the injections

During the run, selecting **Stack Injections** allows all the parameters for stacked injections to be adjusted during the run if needed.

Figure 9. Four stacked injections with time windows adjusted during the run.

Conclusion

The ACCQ*Prep* SFC system allows fast and easy development of stacked injections. The fraction collection allows the system to suggest initial time windows and cycle times that easily can be adjusted by the user for their preferences. The use of time windows prevents peaks from being delivered to the wrong container, even for small injections, such as the last injection in a sequence.