Number: SHT-A2PREP-3
Date: October 23, 2013
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SERVICE How To

Model Number:	Originator:	Topic
A2PREP	**Petro van Poppel**	**Delay Calibrations**

Delay Volume Calibration between UV and FC

You will need:

- Solvent A: 99.9% water, 0.1% formic acid
- Solvent B: 99.9% ACN, 0.1% formic acid
- Make-up Solvent: 74% methanol, 25% ACN, 0.9% water, 0.1% formic acid
- Needle Purge Solution: 50% ACN
- Needle Wash Solution: 50%ACN
- Preparative Column: SB-C18 21.2x50mm, 5µm

Procedure:

1. Install the preparative column, the above mentioned solvents and solutions and make sure that the system has been setup as per SHT_A2PREP_02.

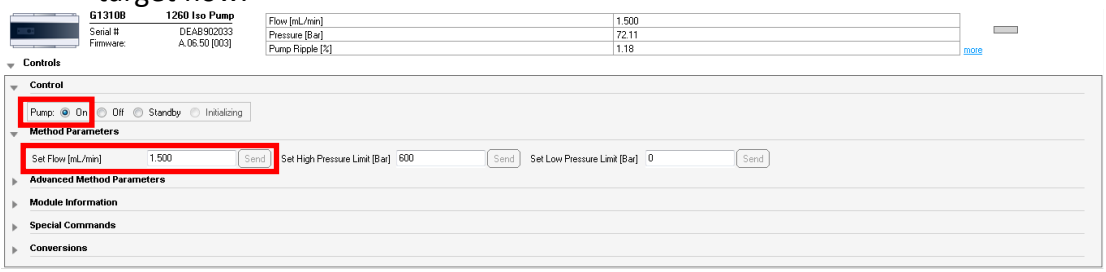
2. If using MSD, remove **remote start cable** from LC stack.

3. Remove all trays from FC.

4. Make sure that both standard and Delay Sensor waste tubings of FC go to waste container.

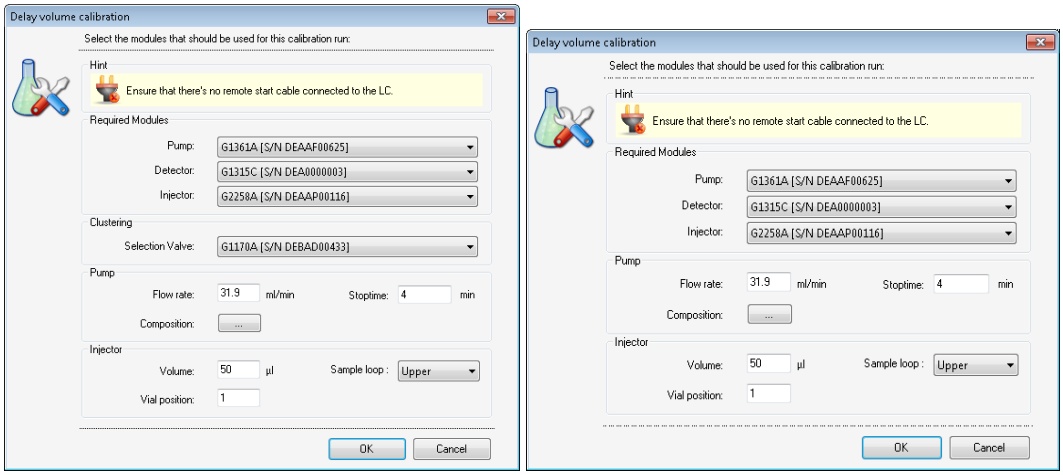
5. Place at least 500µL of Delay time calibration solution to the injector in a 6mL or 2mL vial and start LabAdvisor.

6. If the make-up pump target flow is not set yet, go to **Instrument Control** and click on **Controls** *of the make-up pump*. Start the make-up pump and set its target flow.



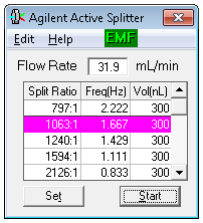
7. Navigate to Service & Diagnostics, select the target FC and start Delay Volume Calibration.

Note: If more than one FC is used and if in the last working instrument configuration there were clustered FCs, perform the calibration only with the first clustered FC (the order of FCs in the FC cluster can be found in the instrument configuration in OpenLab Control Panel software). The clustered FC has one extra section "Clustering" in the Delay Volume Calibration window (see Figure below, left picture). If FCs are clustered but not the first one FC is selected for the calibration (incorrect one), then the calibration window is the same as for FCs without clustering.



8. In the Delay Volume Calibration window set and select following:
    a. Required Modules:
        i. Pump: Preparative pump that corresponds to solvent A.
        ii. Detector: UV detector for calibration.
        iii. Injector: Autosampler for calibration.
    b. Clustering (appears only for the first FC of the FC cluster):
        i. Selection Valve: Select valve that was clustered in FC cluster.
    c. Pump:
        i. Flow rate: Set target flow of the main pump.
        ii. Stop time: 4 min.
        iii. Composition: Click on "..." button and set composition to 70 % of solvent B.
    d. Injector:
        i. Volume: 50µL.
        ii. Sample Loop: Upper loop.
        iii. Vial Position: Enter position corresponding to the vial with the Delay time calibration solution.

e. If the active splitter is used:
   i. Set Agilent active splitter to Remote.
   ii. Start Agilent Active Splitter software, enter main pump flow and set split ratio (typically around 1:1000).
   iii. Start the active splitter.



f. Press OK button to start calibration.

Note: If an active or passive flow splitter is used, then the delay calibration is valid only for the used combination of main and make-up pump flows. If one of flows is changed (or if HPLC tubing is modified), then the calibration has to be measured again. The reason is that the sample signal is split in to two independent flow paths: splitter to FC and splitter to detectors that have in general different flows. If one or both flows are changed, then the resulting delays (UV to FC and UV to MSD) cannot be simply recalculated to a new condition.

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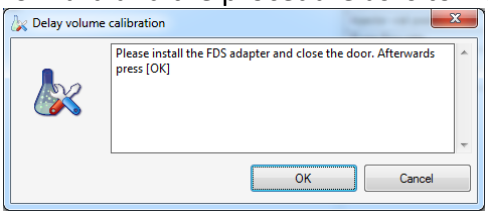
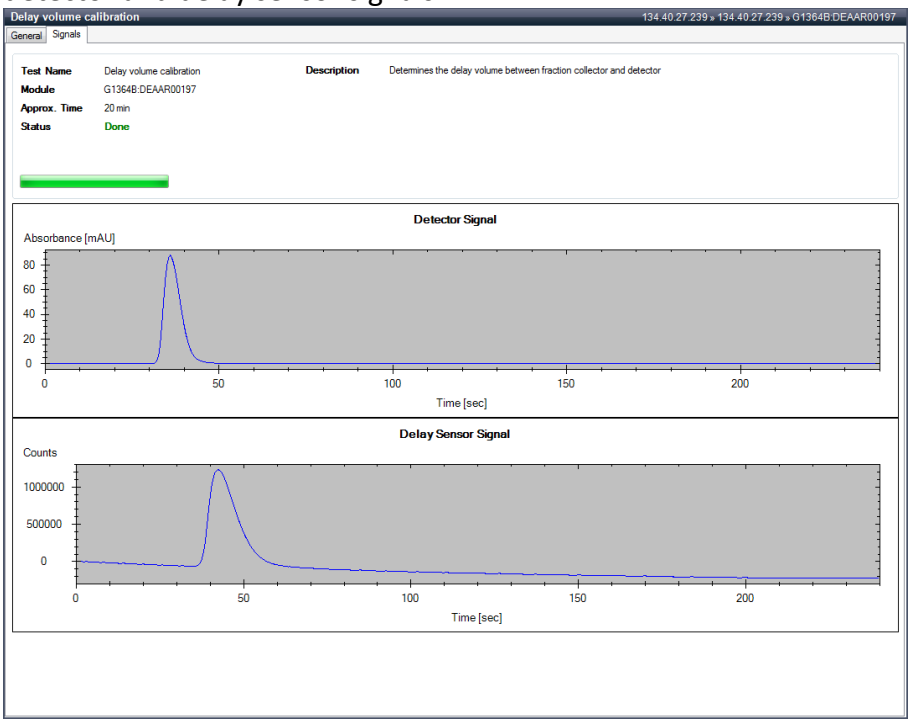
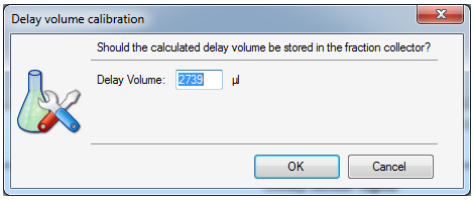
9. During initialization, which can take several minutes, the FC arm moves forward and the procedure asks to install FDS adapter and close the FC door.



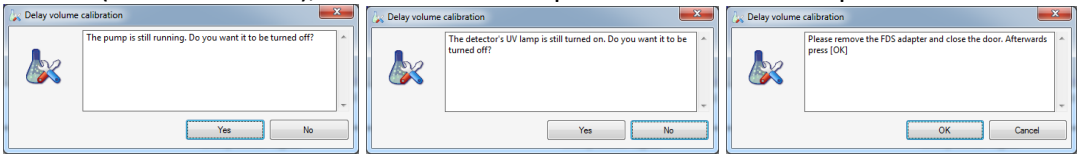
10. After the sample is injected it is possible to click on Signals tab to see UV detector and delay sensor signals.



11. Write down the Delay Volume for further use in Automated Purification Software.



12. After the end of calibration, the procedure offers to stop main flow (recommended), switch off UV lamp and remove the adapter.



13. Stop the splitter and go to Instrument Control and stop the make-up pump.

14. Connect back the remote start cable to LC stack (if used).

15. The delay volume is written to the FC firmware and the value is coupled with the used UV detector and will be used for a standard UV based fraction collections outside Automated Purification Software.

Note: To check or change the value, go to **Service & Diagnostics**, select **FC**, **Delay Volume Tool**. Here measured delay volumes are listed with the respective UV detector.

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16. Set the same delay volumes for other FCs by **Service & Diagnostics,** select the other **FCs**, **Delay Volume Tool**. In case of different lengths of capillaries between the FC and UV detector execute Delay Volume Calibration for each FC in order to calibrate them individually but if FC clustering is used, remove it first from the instrument configuration.

Delay Time Calibration between UV and MSD

You will need:

- Solvent A: 99.9% water, 0.1% formic acid
- Solvent B: 99.9% ACN, 0.1% formic acid
- Make-up Solvent: 74% methanol, 25% ACN, 0.9% water, 0.1% formic acid
- Needle Purge Solution: 50% ACN
- Needle Wash Solution: 50%ACN
- Preparative Column: SB-C18 21.2x50mm, 5µm

Procedure:

1. Install the preparative column, the above mentioned solvents and solutions and make sure that the system has been setup as per SHT_A2PREP_02.

2. Place at least 500µL of Delay time calibration solution to injector in 6mL or 2mL vial.

3. Set-up a method:
    a. Use the Standard Analytical method settings as described SHT_A2PREP_02.
    b. Set 2/10 port valve to the Preparative flow path.
    c. Set Stop Time to No Limit in all modules (infinite run time).
    d. Prep Pump Cluster:
        i. Set Solvents: 70% B.
        ii. Clear Time Table.
    e. Injection volume 50µL.
    f. If using dual loop autosampler:
        i. Set Upper Injection Loop.
        ii. Set Partial loop filling.
    g. Set UV detector to 600nm wavelength with 4 nm bandwidth and no reference.

Note: If using UV lamp only: use 280 nm wavelength with 4 nm bandwidth and no reference

    h. Set **Autobalance** in **Prerun**.
    i. Set the active splitter to Local (in order to execute external contacts timetable in the UIB method).

4. Wash column with 70 % of solvent B (30 mL/min for 2 min).

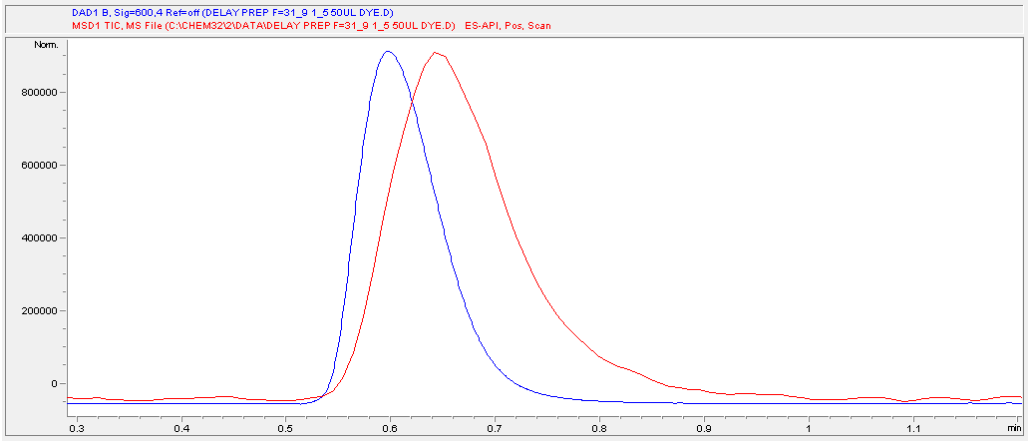
5. Set the target preparative- and make-up flow.

6. Monitor 600 nm UV signal and positive scan MSD signal in Online Plot.

7. Go to Sample Info window:
    a. Set Vial/Location of the sample vial
    b. Enter a run name.
    c. Run Method.

8. Stop the run after the delay marker peak is eluted.

9. Evaluate data:



    a. Load MSD positive scan data and read time at the peak apex (signal maximum), t_{MSD}.
    b. Load UV 600 nm data and read time at the apex of the peak, t_{UV}.
    c. UV/MSD delay time: subtract MSD and UV time, $t_{UV/MSD} = t_{MSD} - t_{UV}$.

10. Check value of MSD to FC delay time ($t_{MSD/FC}$):
    a. Recalculate UV to FC delay volume from the Lab Advisor calibration to the delay time using applied flow, F ($t_{UV/FC} = V_{UV/FC}/F$).
    b. Calculate MSD to FC delay time as difference between UV to FC and UV to MSD delays: $t_{MSD/FC} = t_{UV/FC} - t_{UV/MSD}$.
    c. The value has to be at least 0.01 min or otherwise MSD-based collection cannot be performed. If the value is less than 0.01 min see **Insufficient MSD to FC delay time** section below.

11. Clean autosampler (see SHT_A2PREP_02), or:
    a. Purge needle (right-click on autosampler diagram, Start purging..., 3x).
    b. Wash needle (right-click on autosampler diagram, Wash Needle, Flush Port, 15 s).

Insufficient MSD to FC delay time

There are several reasons why the MSD to FC delay time is too low (below 0.01 min):

- The make-up pump flow is too low for the applied main flow of the preparative cluster. There are limitations for combinations of main and make-up flows.
- The volume of tubing between the splitter and FC is too low.
- The volume of tubing between the splitter and the MSD is too high.

There are several ways for increasing the MSD/FC delay time, but each of them has a certain disadvantage so consider the most suitable one, for the given situation:

1. Decrease the main flow.
2. Increase the make-up flow (it increases speed of analytes when passing detectors, which results in narrower peaks so consider the signal response and data collection rate of MSD).

Note: If using a passive splitter, any change in main and make-up flow will result in a different split ratio of the passive splitter. Some settings of flows can even prevent splitting at all. The active splitter is independent of these changes of flows.

3. Increase the volume of tubing between the splitter and FC (use a longer delay coil or add an extra tubing).
4. Decrease the volume of tubing between the splitter and MSD.
5. After one or more solutions are applied, repeat the delay calibration from the beginning.