Number: SHT-A2PREP-1
Date: October 21, 2013
Pages: 4

SERVICE How To

Model Number:	Originator:	Topic
A2PREP	**Petro van Poppel**	**Dwell Volume Determination**

Dwell Volume Determination of Standard Analytical System

You will need:

- Zero dead volume union
- Solvent A: 100% water
- Solvent B: 99.9% water 0.1% acetone
- Standard Analytical System or standard 1260 Preparative System
- Restriction Capillary (optional)

Procedure:

1. Replace the column by a zero dead volume union.

2. Degas and connect solvents A and B to the system, and purge the solvent lines.

3. Create a method with the following details:

    a. Set the detector to 263 nm wavelength with 4 nm bandwidth and without a reference.
    b. Set *auto-balance* in *Prerun*.
    c. Set *Stop Time* to *No Limit* in all modules (infinite run time).
    d. Set initial solvents composition to 10 % B.
    e. Create the following *Pump Time* table:

Time [min]	A [%]	B [%]	C [%]	D [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	90.0	10.0	0.0	0.0	0.500	600.00
1.99	90.0	10.0	0.0	0.0	---	---
2.00	10.0	90.0	0.0	0.0	---	---

4. Display the 263 nm UV profile in *Online Plot*.

5. Equilibrate the system with 10 % of solvent B with flow 1 mL/min for 2 min.

6. Set a flow in the method to 0.5 mL/min.

NOTE – In a case that the expected dwell volume of the system is below 0.2 mL use flow 0.2 mL/min or lower, but respect specifications of the given pump.

7. Check the pressure. If below 20 bar use a restriction capillary of a known volume to increase it.

8. Go to the *Sample Info* window, clear *Vial/Location* (blank run), enter a *run name* and click on *Run Method*.

9. Stop the run 2 minutes after the acetone UV signal reaches maximum absorption.

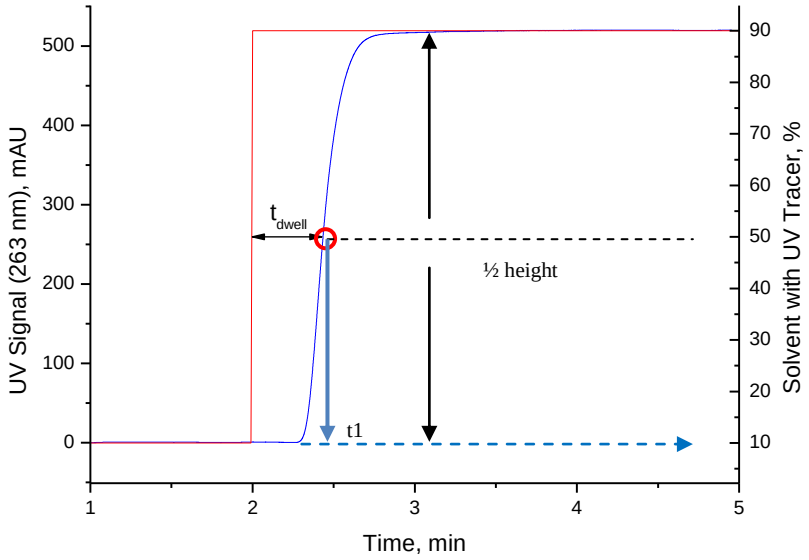

figure 1 - Example of dwell volume measurement by step boundary using 0.1% acetone as UV tracer

10. Evaluate collected data (time in min and flow in mL/min units):

    a. Determine the actual elution time of acetone, **t1**, at half of UV-signal height (blue curve in figure 1).
    b. Calculate a dwell time, **t-dwell**, as a difference between the programmed (red curve in figure 1) and **t1. t-dwell = t1 – 2**
    c. If **t-dwell** is lower than *0.3 min* then consider decreasing flow and repeating the procedure from the step 5.
    d. Calculate the dwell volume, **V-dwell**, as a product of **t-dwell** and used flow rate, **F**.
       V-dwell = t-dwell*F
    e. Subtract the *volume* of the *restriction capillary* if used.
    f. Subtract the *volume* of *capillaries* that are placed **after** the *zero dead volume union* up to the UV detector cell.

11. Repeat the procedure and check if the determined dwell volumes differ less than 5 %. If not repeat the procedure until the values are reproducible.

Dwell Volume Determination of combined Analytical/Preparative System

You will need:

- 2x zero dead volume union
- Solvent A: 100% water
- Solvent B: 99% water 1% (use 99.9% water 0.1% acetone if 60mm cell).
- Standard 1260 combined Analytical/Preparative System

Procedure:

1. Set a required flow path:

    a. Select the required flow path (analytical or preparative) for measurement using the 2/10 port valve.
    b. Replace the column by a zero dead volume union.
    c. If the system contains the dual loop auto sampler make sure that a set loop in a method (lower or upper) is in a main pass (right-click on an auto sampler diagram and check if a command Switch Valve to Upper or Lower Loop refers to the other loop) – if not then change the loop using this command.
    d. If the flow path consists of a splitter in front of a UV detector, bypass the splitter with a zero dead volume union that connects the HPLC stream tubing with the tubing leading to the UV detector.

NOTE – If the splitter is used in the dwell volume measurement (preparative path), then the determined value consists of an additional delay due to the splitter, which is not a real volume but a delay originating from split flow paths (main pump to splitter and splitter to detector) with different flows since the make-up flow path (splitter to detector) has typically lower flow compared to the main pump to splitter flow path.

---

2. Degas and connect solvents A and B to the system, and purge the solvent lines.

3. Create a method with the following details:

    a. Set the detector to 263 nm wavelength with 4 nm bandwidth and without a reference.
    b. Set *auto-balance* in *Prerun*.
    c. Set *Stop Time* to *No Limit* in all modules (infinite run time).
    d. Set Prep Pump Cluster/ Advanced Channel A/ Compensation to 46.
    e. Set Prep Pump Cluster/ Advanced Channel B/ Compensation to 46.
    f. Set flow to 2mL/min.
    g. Set initial solvents composition to 10 % B.

h. Create the following *Pump Time* table:

Time [min]	A [%]	B [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	90.0	10.0	2.000	400.00
1.99	90.0	10.0	---	---
2.00	10.0	90.0	---	---

4. Display the 263 nm UV profile in *Online Plot*.

5. Equilibrate the system with 10 % of solvent B with flow 2 mL/min for 4 min.

6. Go to the *Sample Info* window, clear *Vial/Location* (blank run), enter a *run name* and click on *Run Method*.

7. Stop the run 2 minutes after the acetone UV signal reaches maximum absorption.

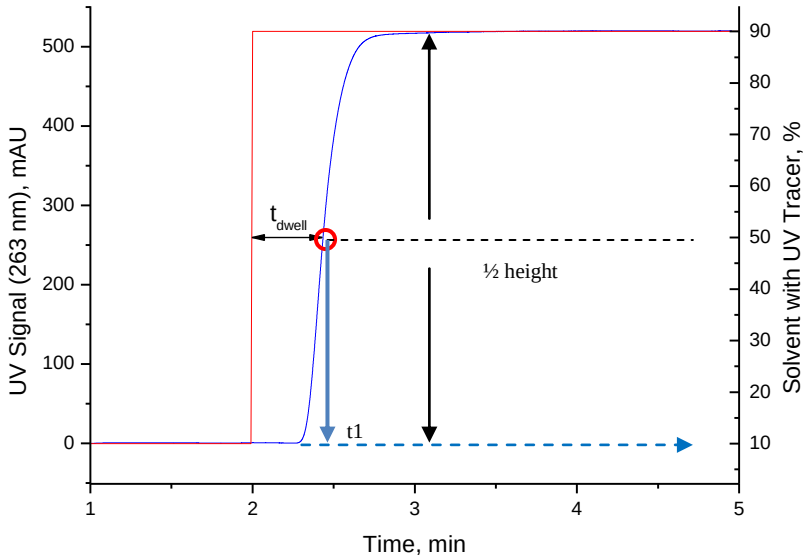

figure 2 - Example of dwell volume measurement by step boundary using 1% acetone as UV tracer

8. Evaluate collected data (time in min and flow in mL/min units):

   a. Determine the actual elution time of acetone, **t1**, at half of UV-signal height (blue curve in figure 2).
   b. Calculate a dwell time, **t-dwell**, as a difference between the programmed (red curve in figure 2) and **t1. t-dwell = t1 – 2**
   c. Calculate the dwell volume, **V-dwell**, as a product of **t-dwell** and used flow rate, **F**.
      V-dwell = t-dwell*F
   d. Subtract the *volume* of *capillaries* that are placed **after** the *zero dead volume union* up to the UV detector cell.

9. Repeat the procedure and check if the determined dwell volumes differ less than 5 %. If not repeat the procedure until the values are reproducible.