Fraction collection with the Agilent 1100 Series purification system

Contents:

Handling of Delay times and volumes

Figure 1 shows a schematic drawing of the fraction-collection part of the Agilent 1100 series purification system with the two delay volumes V_{D1} and V_{D2}. For peak-based fraction collection the system delay times t_{D1} and t_{D2} can be calculated by dividing the delay volumes by the flow rate $\dot{v}$.

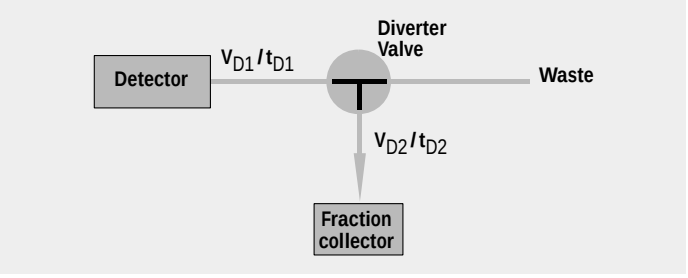

Figure 1 **Schematic drawing of the Agilent 1100 Series fraction collector**

The delay volume V_{D2} is a system constant and is 23 µl for the fraction collector AS and 120 µl for the fraction collector PS. Delay volume V_{D1}, which is displayed in the *Fraction Collector Configuration* window, is determined using the *Delay Volume Calibration* feature of the ChemStation software.

When a peak is detected during a purification run (figure 2) the diverter valve is triggered using the following delay time calculations:

Start of fraction collection: $t = t_0 + t_{D1}$
End of fraction collection: $t = t_E + t_{D1} + t_{D2}$

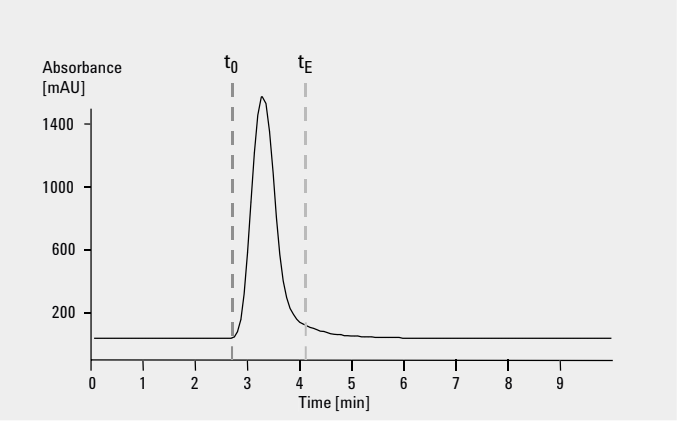

Figure 2 **Chromatogram with peak start t_0 and peak end t_E**

Detector signal delay

Every Agilent UV detector that is used for triggering fractions has an internal signal delay caused by filtering the raw data. The signal delay depends on the *Peakwidth* setting of the detector and is accounted for when the diverter valve is triggered. Tables 1 and 2 show the internal signal delay times for different *Peakwidth* settings.

Table 1 DAD/MWD

Peakwidth (min)	Response time (sec)	Signal delay (sec)
<0.01	0.1	0.05
>0.01	0.2	0.15
>0.03	0.5	0.5
>0.05	1.0	1.25
>0.10	2.0	2.75
>0.20	4.0	5.9
>0.40	8.0	11.9
>0.85	16.0	23.9

Table 2 VWD

Peakwidth (min)	Response time (sec)	Signal delay (sec)
<0.005	<0.1	0.07
>0.005	0.12	0.14
>0.01	0.25	0.29
>0.025	0.5	0.58
>0.05	1	1.31
>0.1	2	2.84
>0.02	4	5.97
>0.4	8	12.3

If the internal signal delay is longer than the delay time t_{D1} some of the peak will be lost. The maximum allowed signal delay time can be calculated using the following equation:

$$\text{Signal delay time}_{(max)} = \frac{V_{D1}}{\dot{v}} \qquad \dot{v} = \text{Flow rate}$$

After calculating the maximum signal delay time a *Peakwidth* setting can be selected that gives a signal delay time, which is shorter than the calculated maximum signal delay time. This *Peakwidth* setting should then be used for the purification run.

NOTE

- We recommend to set the *Peakwidth* always to > 0.01 for the DAD and MWD or to > 0.005 for the VWD
- If the *Peakwidth* setting cannot be reduced and the signal delay time is longer than t_{D1} it is also possible to enhance V_{D1} by adding additional tubing. However, this is not recommend because of increasing peak dispersion caused by the higher delay volume.
- The stop-time of the run in the ChemStation must be set to at least:

  Total duration of time table (time of last entry *Off*) + fraction collector delay time $(V_{D1}/\dot{v}) + 0.1\,\text{min}$

  End of last peak (t_E) + fraction collector delay time $(V_{D1}/\dot{v}) + 0.1\,\text{min}$

Rules to optimize fraction collection

Time-based fraction collection

- *Time slices* must have a length of at least 0.05 min.
- Set *# of Fractions* such that length of resulting fractions is at least 0.05 min.

Peak-based fraction collection

- Set threshold and slope values such that length of fractions is at least 0.05 min.
- Collection of fractions based on threshold only is possible by removing the values for *Up Slope* and *Down Slope* from the *Peak Detectors* table.
- Unresolved peaks can be separated using appropriate threshold and slope values. If two unresolved peaks are to be collected as one fraction, collect based on threshold only.
- When having saturated detector signals collect on threshold only. If slope values are defined, the detector may detect start and stop conditions at the top of the peaks, where the detector signal usually is very noisy.
  If you want to collect based on threshold and slope, change to a detector cell with shorter path length.
- If the baseline of the chromatogram is below or above 0 mAU, this offset is not accounted for when triggering peaks using a threshold value. The threshold value is always added to 0 mAU.

Limitations and how to avoid problems

Rinse Fraction Collection Needle

If *Rinse Fraction Collection Needle* is set to *Between fraction collection*, at least 0.1 min are required to perform this task.
When doing time-based fraction collection rinsing the needle is only possible between two time table entries, which must have a gap of at least 0.1 min.
For peak-based fraction collection a time gap of also at least 0.1 min is required. If a new peak is detected during the rinse process, it is aborted and the needle moves back to the next free fraction position. Depending on flow rate and delay volume V_{D1} the beginning of this peak may be lost.

Needle Movement

The option *into location* under *Needle Movement* in the fraction collector configuration must only be used for capped 2 or 5 ml vials or well-plates. Using other or open vials with this command can lead to a *Movement failed* error.

Replacing fraction containers

When replacing filled tubes, vials or well-plates from the fraction collector make sure to remove and re-insert the complete tray. Otherwise the fraction collector will not recognize that the fraction containers were emptied.

Recovery locations

Only the funnels of the funnel tray (G1364-84502) can be used as recovery locations.
If well-plates are configured on this tray, the funnels can only be used as recovery locations; to use the funnels as fraction locations it is necessary to configure no well-plates on the funnel tray. The needle movement from a recovery location to a fraction container takes about 0.07 min. During this time the diverter valve switches to the waste position, thus everything coming from the column will be lost.

Pooling

When pooling fractions, it is not possible to overfill fraction containers. It is the users responsibility to make sure enough fraction volume is available for the complete pooling runs. If a fraction position is full, the pump is turned off automatically.

Handheld control module

The handheld control module does not support multiple fraction collectors. When using it only one fraction collector can be configured in the system.

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