Peak-based fraction collection with the Agilent 1100 Series purification system AS – Influence of delay volumes on recovery

Technical Note

Abstract

The Agilent 1100 Series fraction collector – as part of the Agilent 1100 Series purification system\(^1\) - is designed for lowest delay volume, ensuring highest performance in fraction collection. This Technical Note explains how the delay volume is measured and how it is used to trigger fractions. It describes the influence of enhanced delay volume on peak dispersion and fraction collection. This document supplements the information provided in the quick reference card\(^2\) which is shipped with the Agilent Purification/HiThruput software.
Introduction

The Agilent 1100 Series fraction collector is available in two versions:

- Agilent 1100 Series fraction collector AS (analytical scale) for flow rates below 10 ml/min, and
- Agilent 1100 Series fraction collector PS (preparative scale) for flow rates up to 100 ml/min.

Both fraction collectors are designed for minimum delay volumes offering highest fraction collection performance, which is especially important for purification even at low flow rates. Figure 1 shows a schematic drawing of the fraction collector with its two delay volumes \( V_{D1} \) and \( V_{D2} \).

Due to the optimized design of the fraction collector — the diverter valve is mounted directly onto the fraction collector arm — the delay volume \( V_{D2} \) could be minimized to 23 µl (AS version). The fraction collector AS is shipped with a 100-cm tube (0.25 mm id) to connect the diverter valve to the detector. The tube can be shortened after the 1100 Series stack is set up. The tubing volume together with the detector cell volume gives \( V_{D1} \). After setting up the 1100 Series stack and the tubing connections, the delay volumes are constants, and therefore the delay times \( t_{D1} \) and \( t_{D2} \) can be calculated for each flow rate using

\[
V_{D1} \text{ [ml]} = \dot{V} \text{ [ml/min]} \times t_{D1} \text{ [min]}
\]

\[
V_{D2} \text{ [ml]} = \dot{V} \text{ [ml/min]} \times t_{D2} \text{ [min]}
\]

where \( \dot{V} \) = flow rate

When a peak is detected the fraction collector must wait until the peak has traveled from the detector flow cell to the diverter valve before switching to collect the fraction. Therefore, the delay time \( t_{D1} \) is added to the start time \( t_0 \) of the peak (figure 2). To make sure that all of the peak is collected the fraction collector switches back to waste at the end time of the peak \( t_E \) plus the delay times \( t_{D1} \) and \( t_{D2} \). Then the end of the peak has reached the tip of the fraction collection needle.

![Figure 1](image1.png)

**Figure 1**
Schematic drawing of a fraction collector for a UV-based purification system.

![Figure 2](image2.png)

**Figure 2**
Fraction collection timing.
Influence of delay volume $V_{D1}$ on recovery

When a peak travels through a capillary, dispersion occurs due to different velocities of the mobile phase over the cross-section of the capillary. This is caused by interaction of the mobile phase with the capillary wall. Therefore, dispersion depends on the length and the inner diameter of the capillary. Figure 4 shows the dispersion of a peak travelling from the detector to the fraction collector with increasing delay volume $V_{D1}$. To increase $V_{D1}$ the standard capillary of the fraction collector (0.25 mm id) was enhanced with capillaries of different lengths but with the same inner diameter.

Equipment

All experiments were performed on an Agilent 1100 Series purification system AS consisting of the following modules:

- Agilent 1100 Series quaternary pump with degasser,
- Agilent 1100 Series well plate autosampler,
- Agilent 1100 Series thermostatted column compartment,
- Agilent 1100 Series diode array detector, and
- Agilent 1100 Series fraction collector AS.

The system was controlled using the Agilent ChemStation (rev. A.09.01) and the Agilent Purification/HiThruput SW module (rev. A.01.01).

Results

Influence of delay volume $V_{D2}$

When a fraction is triggered by the detector the fraction collector switches to the collect position as soon as the beginning of the peak reaches the diverter valve. The liquid that remains in $V_{D2}$ is also flushed out and dilutes or, in the worst case, contaminates the fraction (figure 3).

To avoid contamination of a fraction it is possible to rinse $V_{D2}$ between collecting fractions. Therefore, the needle moves to the fraction delay sensor injection port and $V_{D2}$ is flushed with mobile phase coming from the column. The problem with flushing $V_{D2}$ is timing. If a fraction is triggered while the needle is flushed it must move to the next available fraction position before the peak has reached the diverter valve. Whether this can be achieved in time depends on $t_{D1}$ and therefore on the delay volume $V_{D1}$ and the flow rate.

Another possibility to minimize contamination and dilution of a fraction is to minimize the delay volume $V_{D2}$. This was done for the Agilent 1100 Series AS fraction collector. By mounting the diverter valve directly onto the fraction collection arm it was possible to minimize $V_{D2}$ to 23 µl.

Influence of delay volume $V_{D1}$ on recovery

When a peak travels through a capillary, dispersion occurs due to different velocities of the mobile phase over the cross-section of the capillary. This is caused by interaction of the mobile phase with the capillary wall. Therefore, dispersion depends on the length and the inner diameter of the capillary. Figure 4 shows the dispersion of a peak travelling from the detector to the fraction collector with increasing delay volume $V_{D1}$. To increase $V_{D1}$ the standard capillary of the fraction collector (0.25 mm id) was enhanced with capillaries of different lengths but with the same inner diameter.
Triggering of fractions is based on the detector signal, which means the width of the fraction is determined by the width of the peak. When the peak reaches the fraction collector it is broadened due to dispersion, however the diverter valve switches to the collect position only for the time window determined by the peak width in the detector. This results in a loss of compound at the beginning and at end of the peak. The higher the delay volume $V_{D1}$, the more compound is lost. The Agilent 1100 Series fraction collector minimizes this effect, as it was designed for smallest delay volume $V_{D1}$ that can further be minimized by cutting the inlet capillary of the fraction collector after setting up the 1100 Series stack.

**Influence of delay volume $V_{D1}$ on resolution**

The influence of dispersion on the re-mixing of peaks that were separated on a column is also an important aspect. A parameter to measure the separation of two peaks is the resolution calculated using the 5-sigma method (figure 5). This method was selected because it accounts for the peak width at 5 sigma height (4.4% of peak height), which is close to the baseline of the peak.

\[ R = \frac{2.5(T_{R2} - T_{R1})}{W_{4.4(2)} + W_{4.4(1)}} \]

**Figure 4**

Increasing dispersion with increasing capillary volume

**Figure 5**

Resolution calculated according to the 5-sigma method
To show the influence of enhanced delay volume $V_{D1}$ on the resolution, two peaks were measured in the detector and also in the fraction collector (figure 6). The delay volume was increased by adding tubing with two different inner diameters (0.25 mm and 0.8 mm). Then the resolution of the peaks in the fraction collector and the detector was compared to give the relative resolution. Figure 7 shows that the relative resolution decreases with increasing delay volume $V_{D1}$. It also shows that the relative resolution for the same delay volume greatly decreases with increasing inner diameter of the capillary. This is due to the influence of the capillary cross-section on the resolution in which the radius is squared.

**Detector delay**

In analytical HPLC it is common to filter the raw signal coming from the detector to improve peak shape and signal-to-noise level. Therefore, different filter settings can be applied in the detector setup of the ChemStation. Filter levels are set according to the expected peak widths to make sure that two peaks are not averaged, giving a single peak after filtering. The higher the expected peak width and the peak width setting, the higher the level of filtering. The result of the filtering calculations, performed by the
Figure 8 shows the delay time $t_{D1}$ of different flow rates for two different delay volumes (50 µl and 100 µl). The horizontal lines represent the internal signal delay times for an MWD or DAD at different peak width settings. The data point for a given delay volume and flow rate must be above the signal delay time for a peak width setting to ensure that a peak can be triggered properly. We recommend setting the peak width to $> 0.01$ for the DAD and MWD or to $> 0.005$ for the VWD.

**Automatic calibration of delay volume $V_{D1}$**

The Agilent 1100 Series fraction collector AS is equipped with a fraction delay sensor (FDS). It consists of a LED lamp (GaAs Red LED Lamp, $\lambda_{max} = 654$ nm) and a Si Photo detector ($\lambda = 580–700$ nm) and is connected to an injection port inside the fraction collector (figure 9).

<table>
<thead>
<tr>
<th>Peak width [min]</th>
<th>Response time [sec]</th>
<th>Signal delay [sec]</th>
</tr>
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<tr>
<td>$&lt; 0.01$</td>
<td>$0.1$</td>
<td>$0.05$</td>
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<tr>
<td>$&gt; 0.01$</td>
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<tr>
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<td>$1.25$</td>
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<tr>
<td>$&gt; 0.10$</td>
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<td>$&gt; 0.20$</td>
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<tr>
<td>$&gt; 0.40$</td>
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<tr>
<td>$&gt; 0.85$</td>
<td>$16.0$</td>
<td>$23.9$</td>
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</table>

Table 1
**Internal signal delay DAD/MWD**

<table>
<thead>
<tr>
<th>Peak width [min]</th>
<th>Response time [sec]</th>
<th>Signal delay [sec]</th>
</tr>
</thead>
<tbody>
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<tr>
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<td>$4$</td>
<td>$5.97$</td>
</tr>
<tr>
<td>$&gt; 0.4$</td>
<td>$8$</td>
<td>$12.3$</td>
</tr>
</tbody>
</table>

Table 2
**Internal signal delay VWD**

![Figure 8: Delay time and signal delay time for different peak width settings](image)

![Figure 9: Fraction delay sensor](image)
For the delay calibration a blue dye (delay calibrant, part number G1946-85020) is injected into the system after removing the column. The dye gives a signal in the detector and in the fraction delay sensor (figure 10).

Since the delay volumes $V_{D2}$ and $V_{D3}$ are fixed, $V_{D1}$ can be calculated for any given flow rate using the following equation:

$$V_{D1} = (\dot{v} \times t_D) - V_{D2} - V_{D3}$$

where $\dot{v}$ [ml/min] = flow rate

This is done automatically by the ChemStation software and the result is automatically entered in the fraction collector configuration. The complete delay calibration process is described in the User’s Guide\(^3\) shipped with the Agilent Purification/HiThruput software.

**Conclusion**

This Technical Note showed how peak-based fraction collection is performed using the Agilent 1100 Series purification system. We showed the influence of the different delay volumes, detector signal delays and how the delay volume can be measured automatically.

We explained how:
- the different delay volumes are used to trigger peaks for maximum recovery,
- the delay volume $V_{D2}$ was minimized to avoid dilution and cross-contamination of fractions,
- the delay volume $V_{D1}$ was minimized to avoid low recovery or re-mixing of separated peaks due to dispersion,
- the peak width has to be set in the ChemStation software to optimize the detector signal delay to avoid losing compound, and
- how easy delay volume calibration is with the fraction delay sensor of the Agilent 1100 Series fraction collector.

**References**

1. “New perspectives in purification with HPLC and HPLC/MS” Agilent Technologies Brochure, 2001, publication number 5988-3673EN.

