Optimizing Your Purification System for Highest Recovery

Presented by:
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- 1996 - 97 Postdoctoral fellow at the University of Hawai'i at Manoa
- Since 1997 Application Chemist with HP/Agilent
- Since 2000 Senior Application Chemist for the purification system and valve solutions

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- **Delay volume calibration**

  Fraction delay sensor

- **What is detector delay?**

  Signal filtering

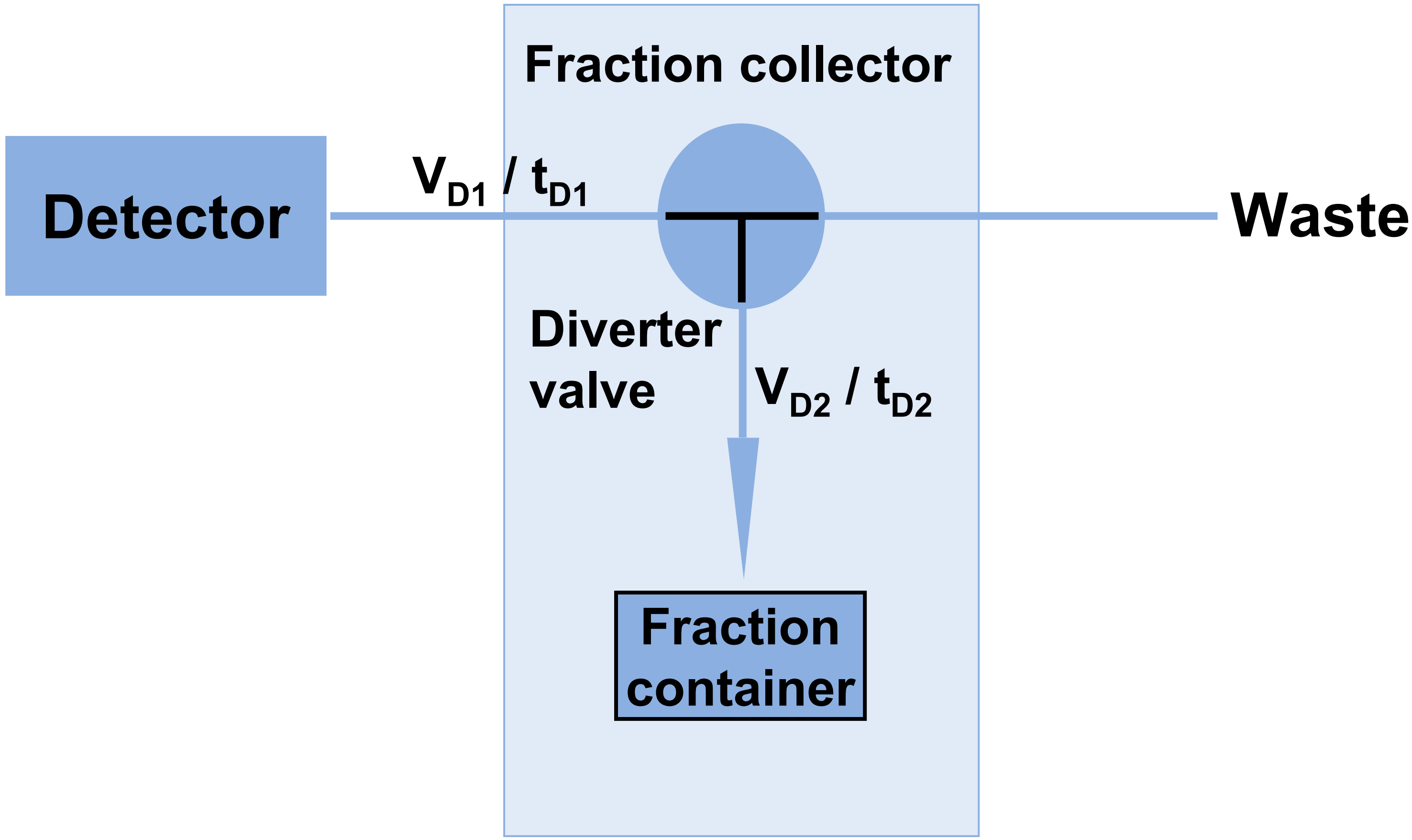
- **What is system delay?**

  The CAN network: Integrated intelligence

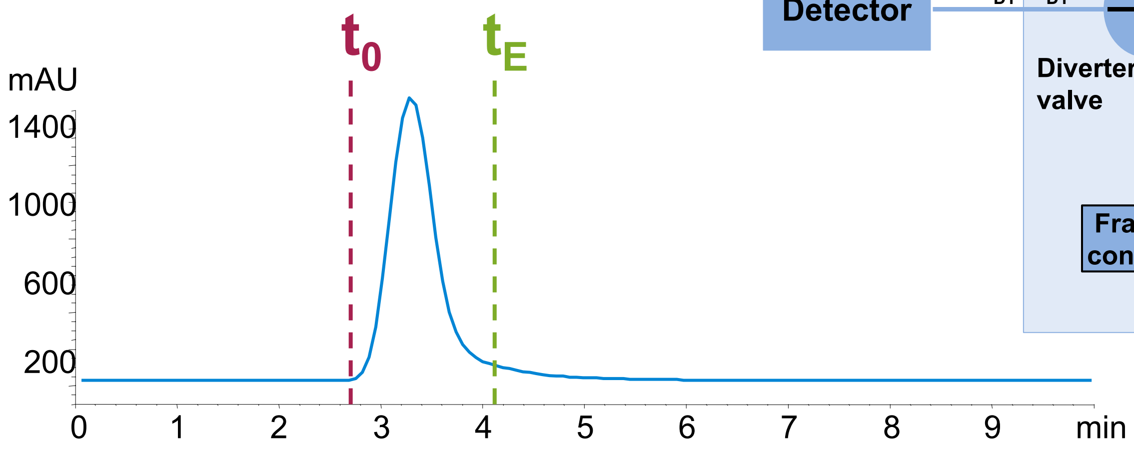
- **Influence of delay volume on recovery**

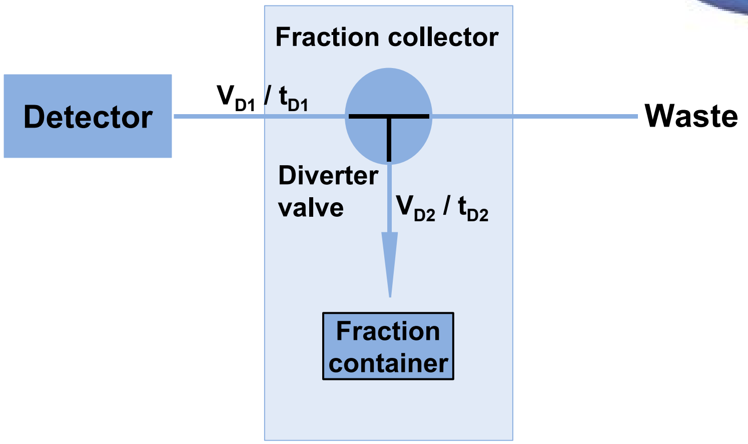
  Delay volume control with optimized design/setup

Purification system schematics



Fraction delay – UV-based fraction collection system





Start of fraction collection ⇒ when start of peak arrives at diverter valve

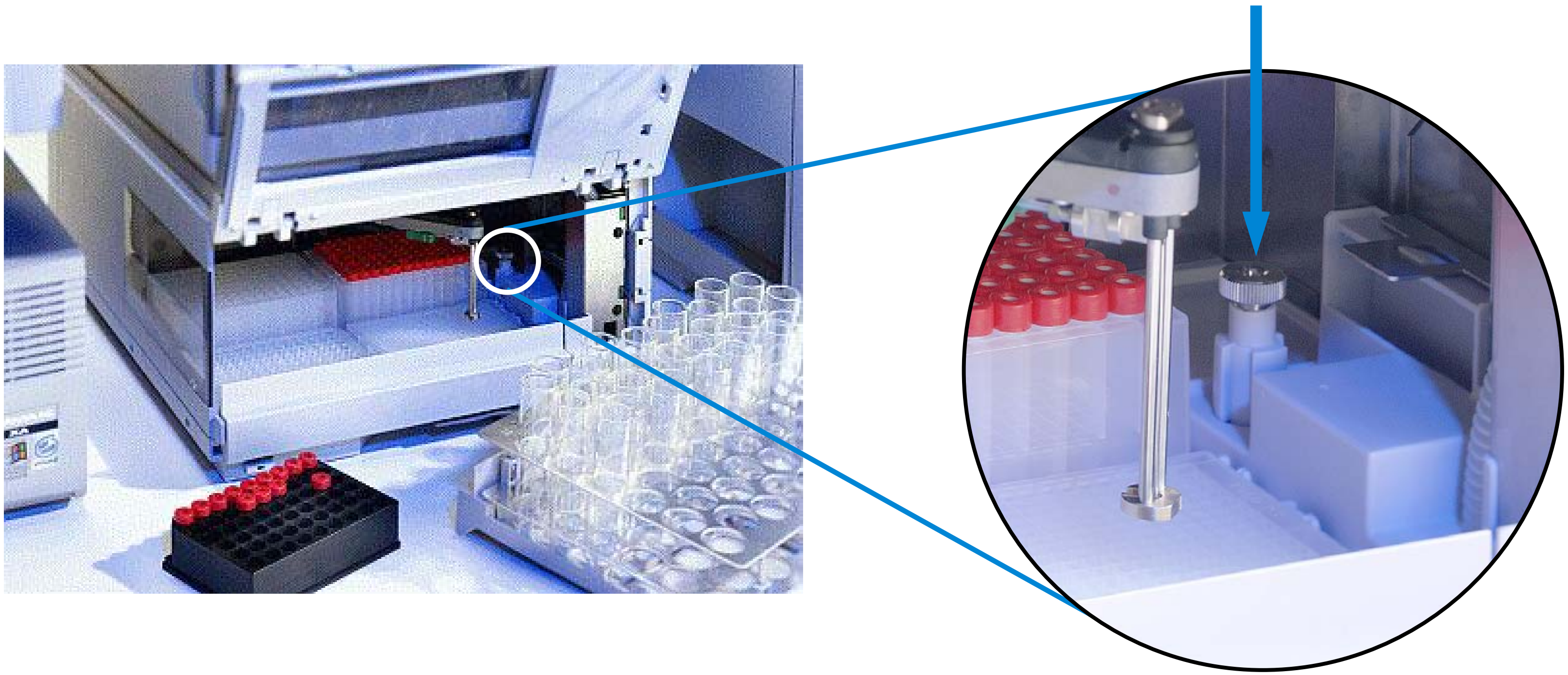
Start of fraction collection: $t_0 + t_{D1}$

End of fraction collection ⇒ when end of peak arrives at needle tip

End of fraction collection: $t_E + t_{D1} + t_{D2}$

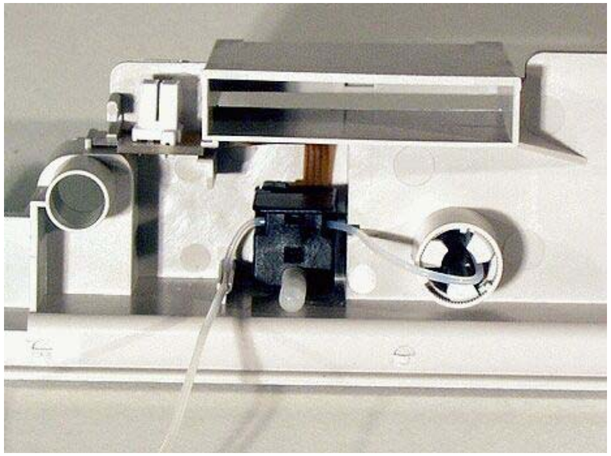
Fraction delay sensor (FDS)

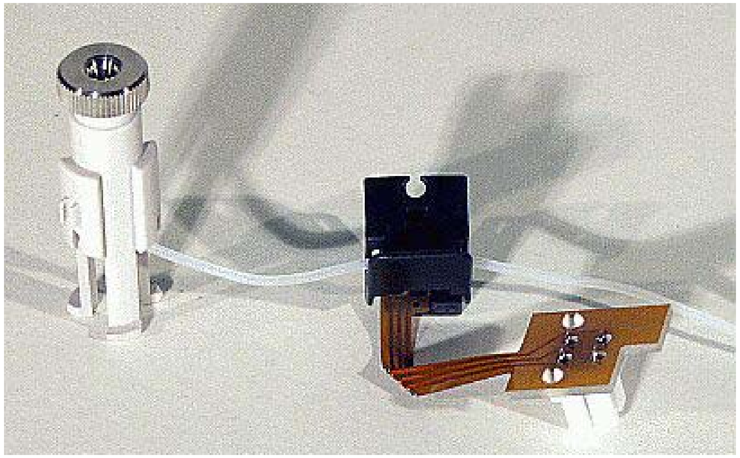
The FDS is a detector in the fraction collector. In combination with UV and MS detection of a peak, it allows accurate calculation of the time delay between peak detection and fraction collection.



Fraction delay sensor

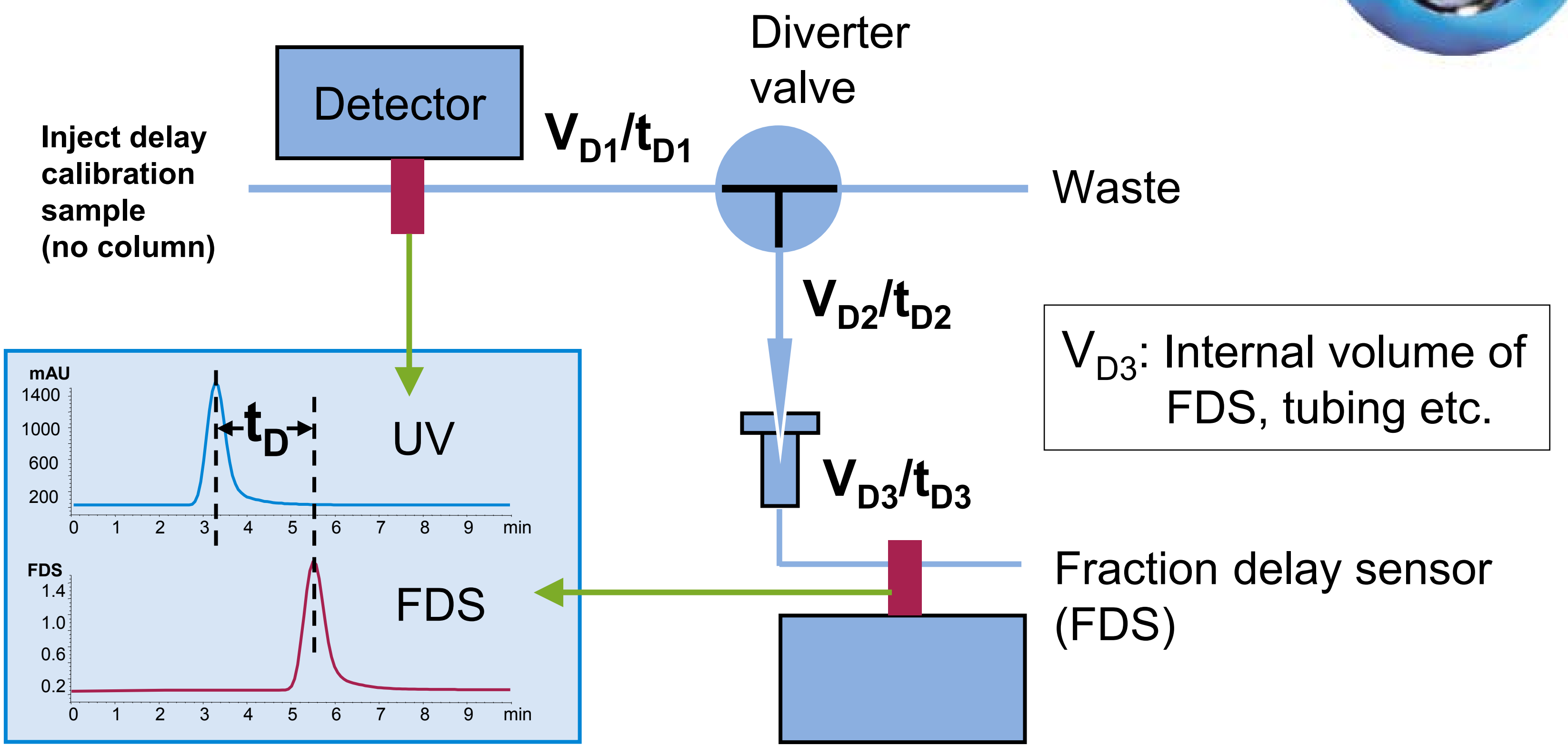
The delay calibrant contains two compounds. One is detectable by UV and by the FDS and the other is detectable by the MS. With no column in place the compounds do not separate. The time difference between their detection in the UV, FDS and MS allows accurate calculation of the delay volumes.





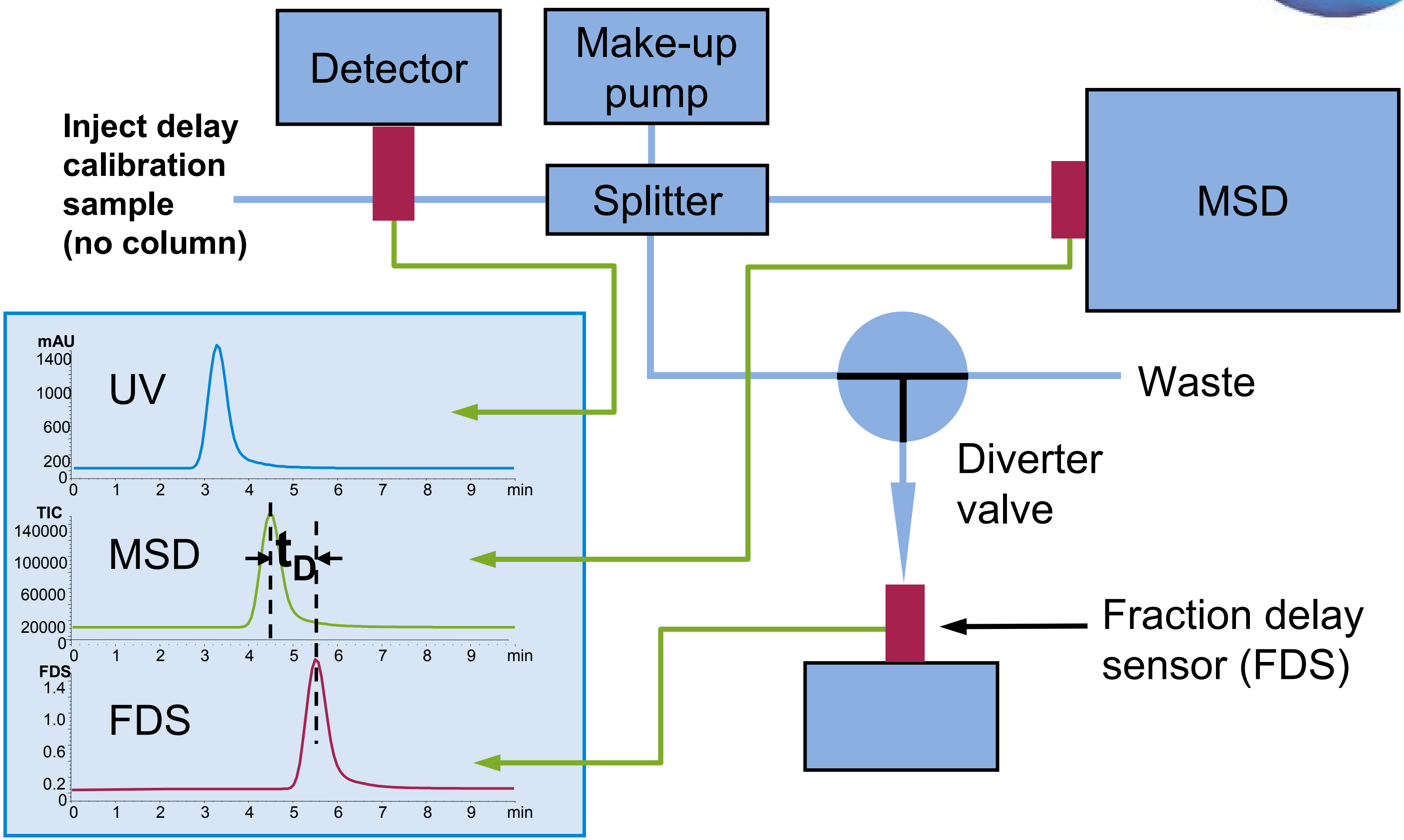
Light source:	GaAs Red LED Lamp (λ_{max} = 654 nm)
Detection:	Si Photo detector (λ = 580 - 700nm)
Delay Calibrant:	Fast Green FCF for UV detector and FDS
(G1946-85020)	Caffeine for MSD

Fraction delay – UV-based fraction collection system



$$V_{D1} = (\dot{v} \cdot t_D) - V_{D2} - V_{D3} \qquad \dot{v}\text{: flow rate}$$

Fraction delay – MS-based fraction collection system

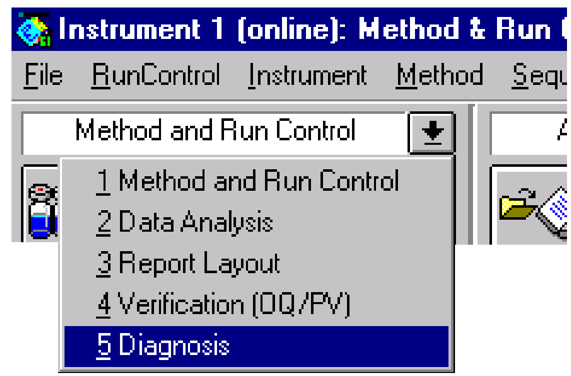


Agilent Technologies

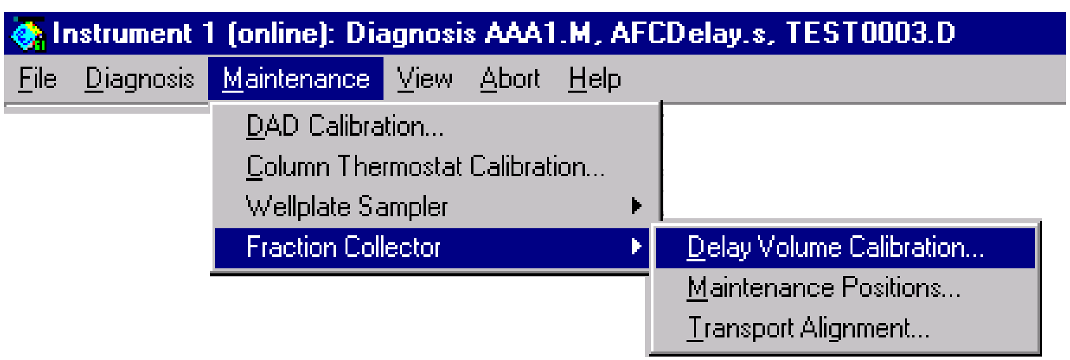
ChemStation software – delay calibration

① Set up your purification method and save it (MBFC only!)

② Go to *ChemStation Diagnosis* view

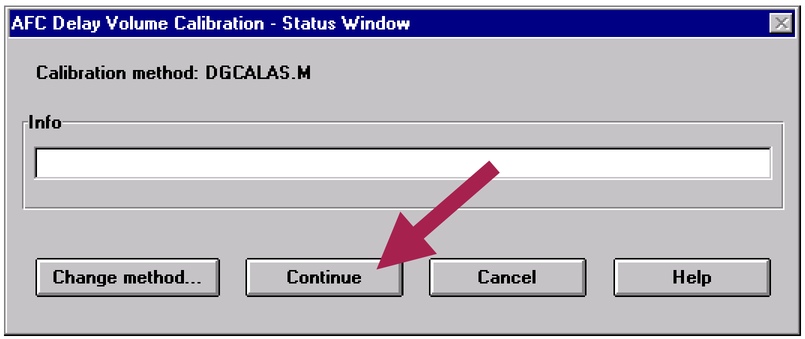


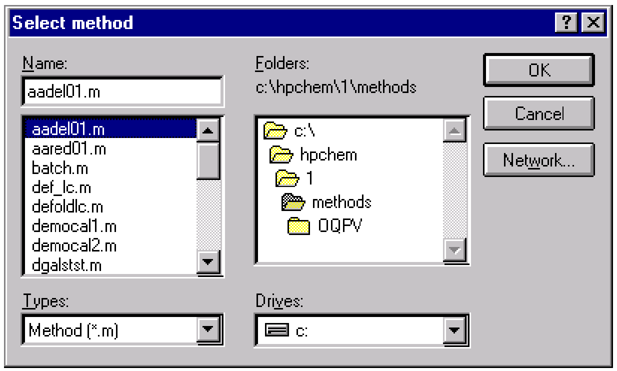
③ Start *Delay Volume Calibration*

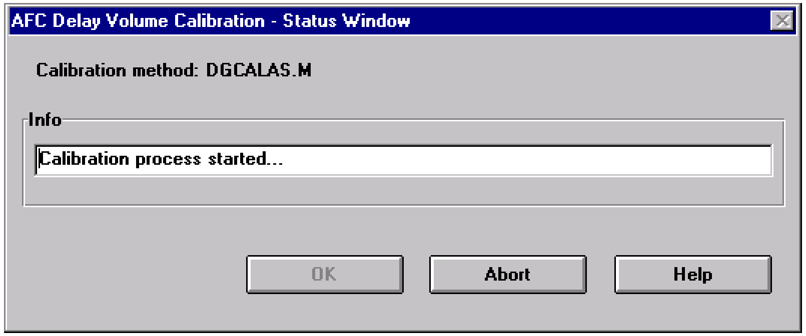


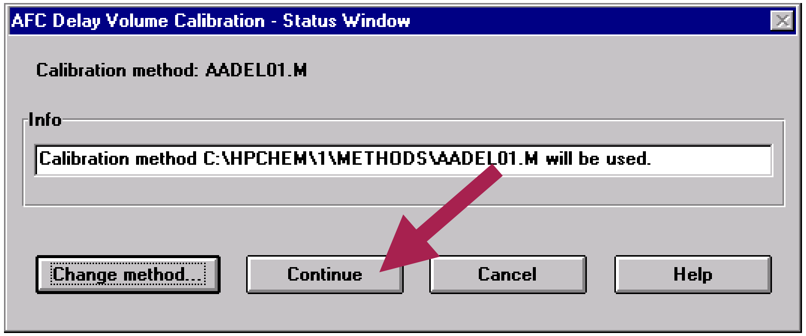
ChemStation software – delay calibration

④ Use a pre-defined calibration method (or select you own method using the *Change method* button), put the *Delay Sensor Calibrant* (Part No. G1946-85020) in vial position 1 and press *Continue*



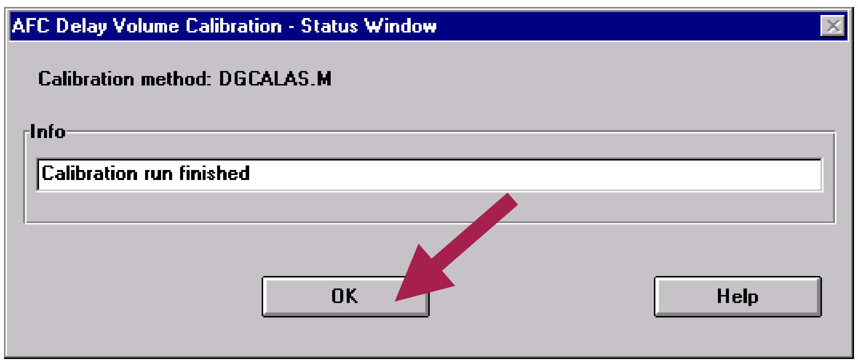


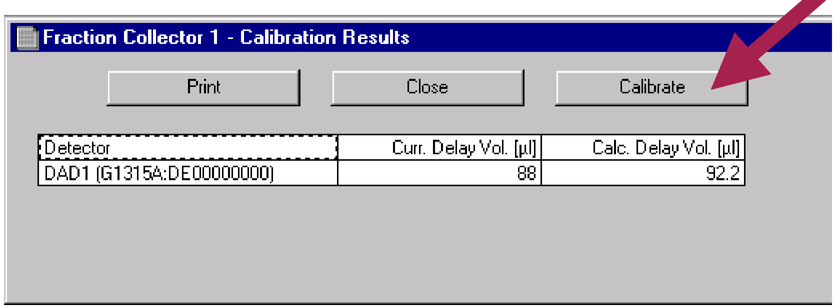


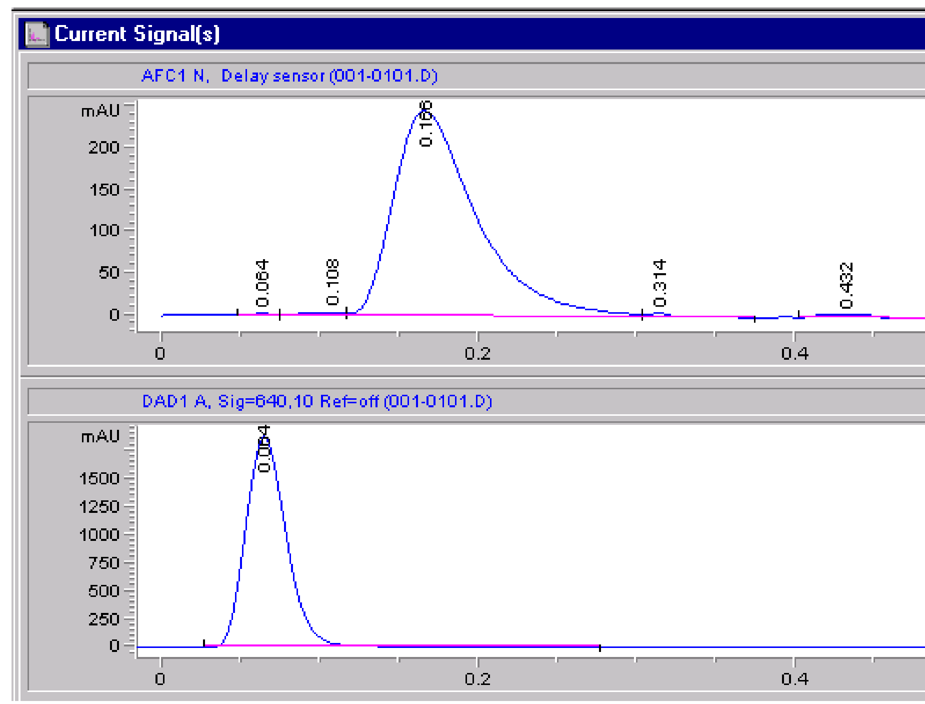


ChemStation software – delay calibration

⑤ Review the results and transfer them automatically using the *Calibrate* button

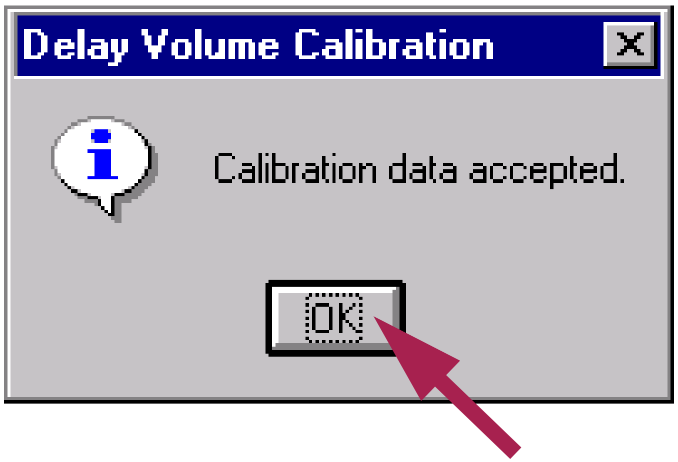


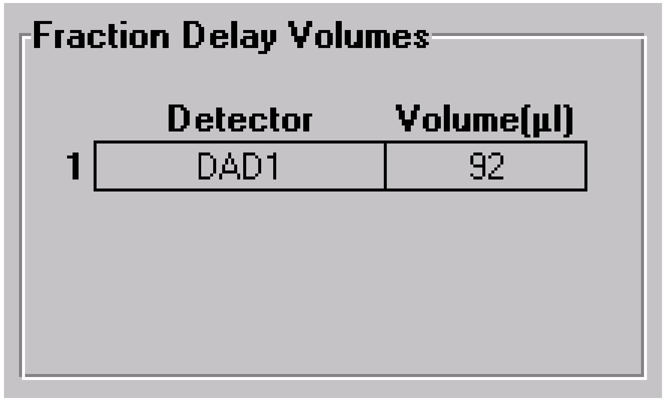




ChemStation software – delay calibration

⑥ Calibration done and result transferred to the fraction collector configuration





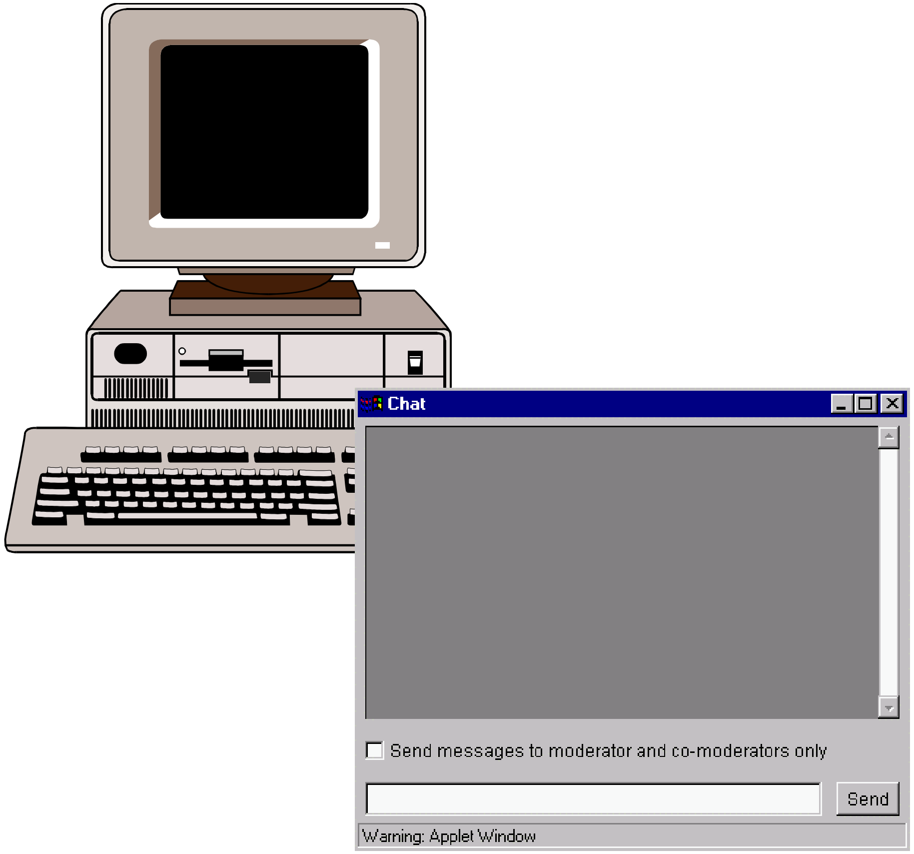
Break Number 1

Please type your

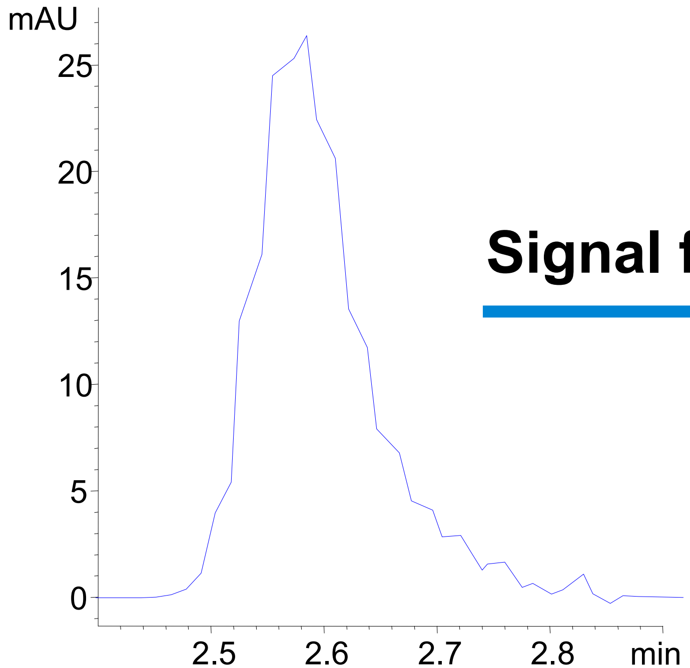
question into the

Question Box at any time

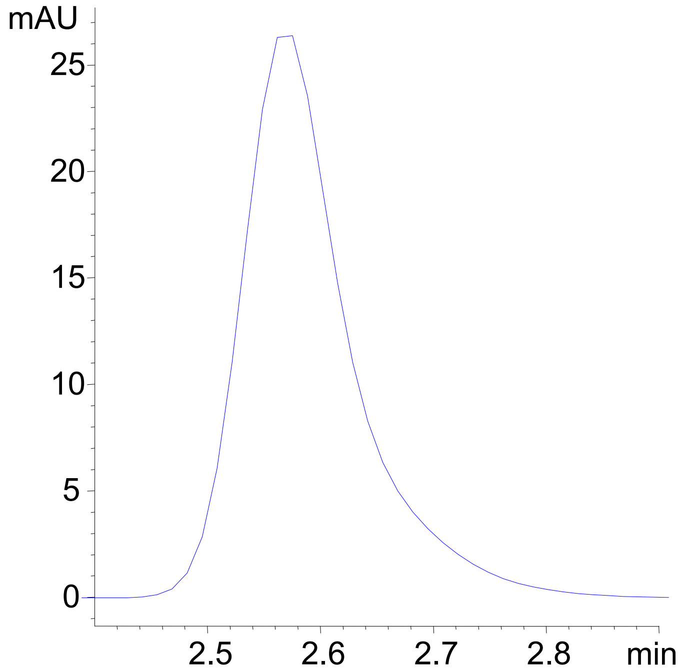
during the presentation.



What is a detector delay time?



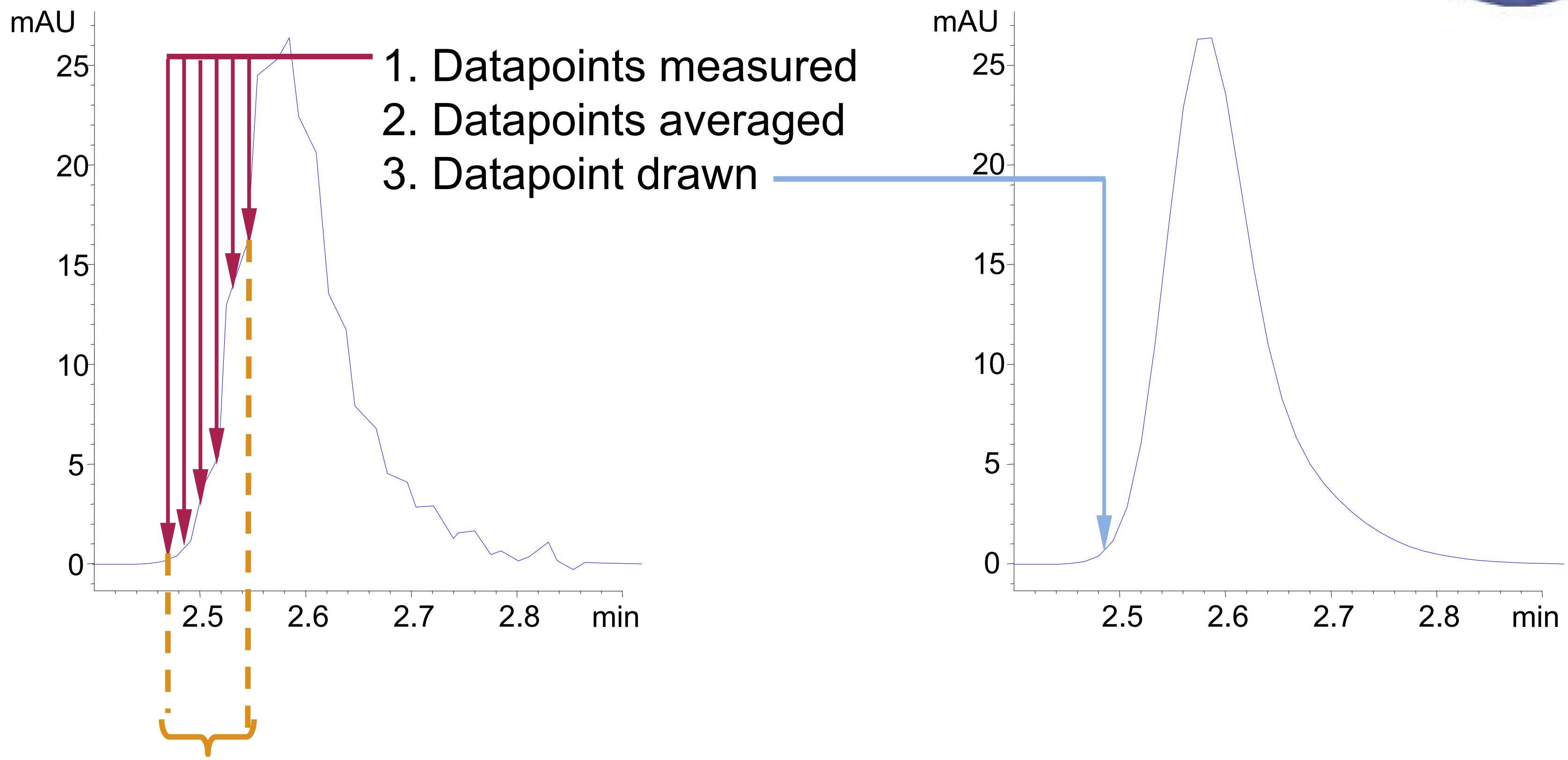
Signal filtering →



Signal measured in the detector

Signal displayed in ChemStation

What is a detector delay time?



Delay between actual measurement point and point drawn in software

$\Rightarrow$ **Detector delay**

Detector delay times for different filtering/peakwidth settings

DAD/MWD

Peakwidth [min]	Response time [s]	Signal delay [s]
< 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

VWD

Peakwidth [min]	Response time [s]	Signal delay [s]
< 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
> 0.2	4	5.97
> 0.4	8	12.3

Peakwidth (filtering) must be smaller then peakwidth of narrowest peak in chromatogram. Otherwise 2 peaks could be “filtered” together to give a single peak.

Detector delay time

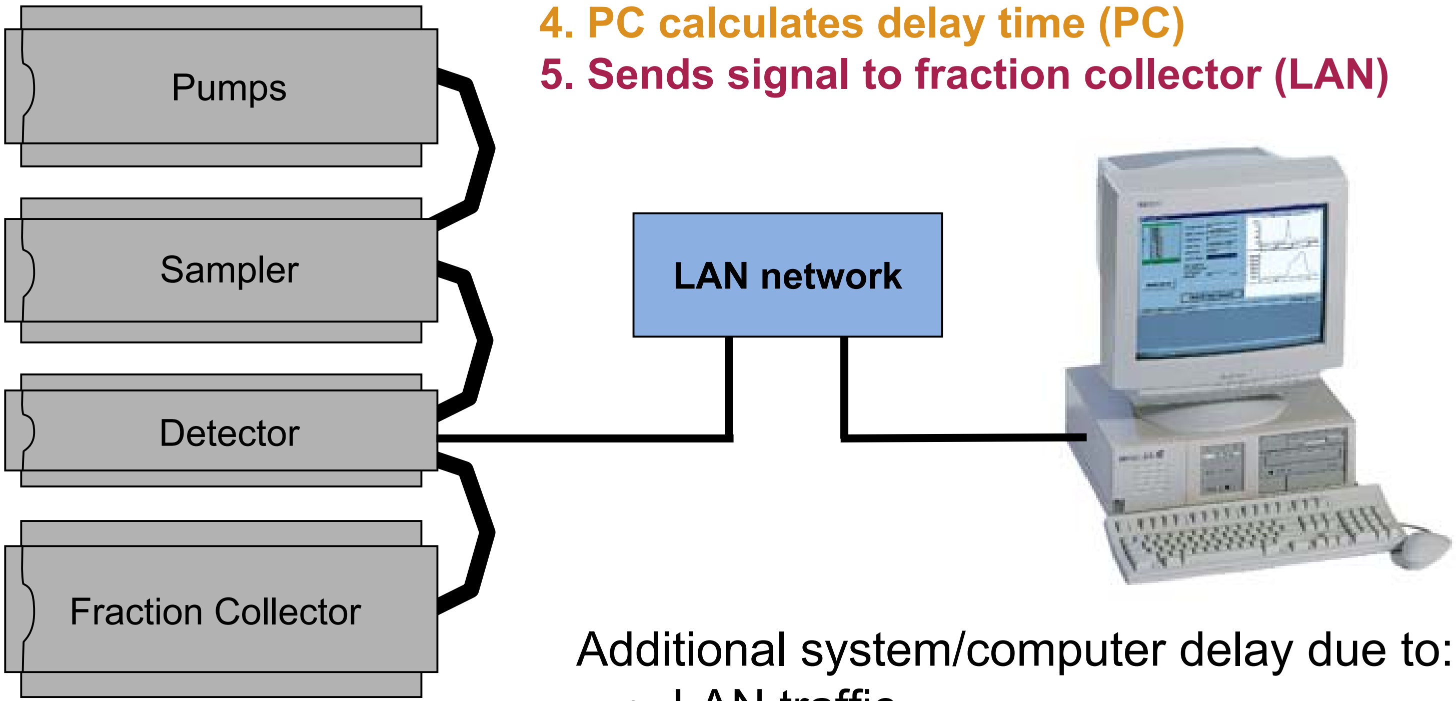
- Additional detector delay time for DAD, MWD and VWD is automatically adjusted by the detector firmware.

- $t_{detector} < t_{D1}$

- Recommendation: Peakwidth should be set to the second-lowest setting for the UV detectors.

What is a system/computer delay?

1. Detector sends signal to PC (LAN)
2. PC processes signal and acknowledges a peak (PC)
3. PC gets flow rate from pump (PC)
4. PC calculates delay time (PC)
5. Sends signal to fraction collector (LAN)

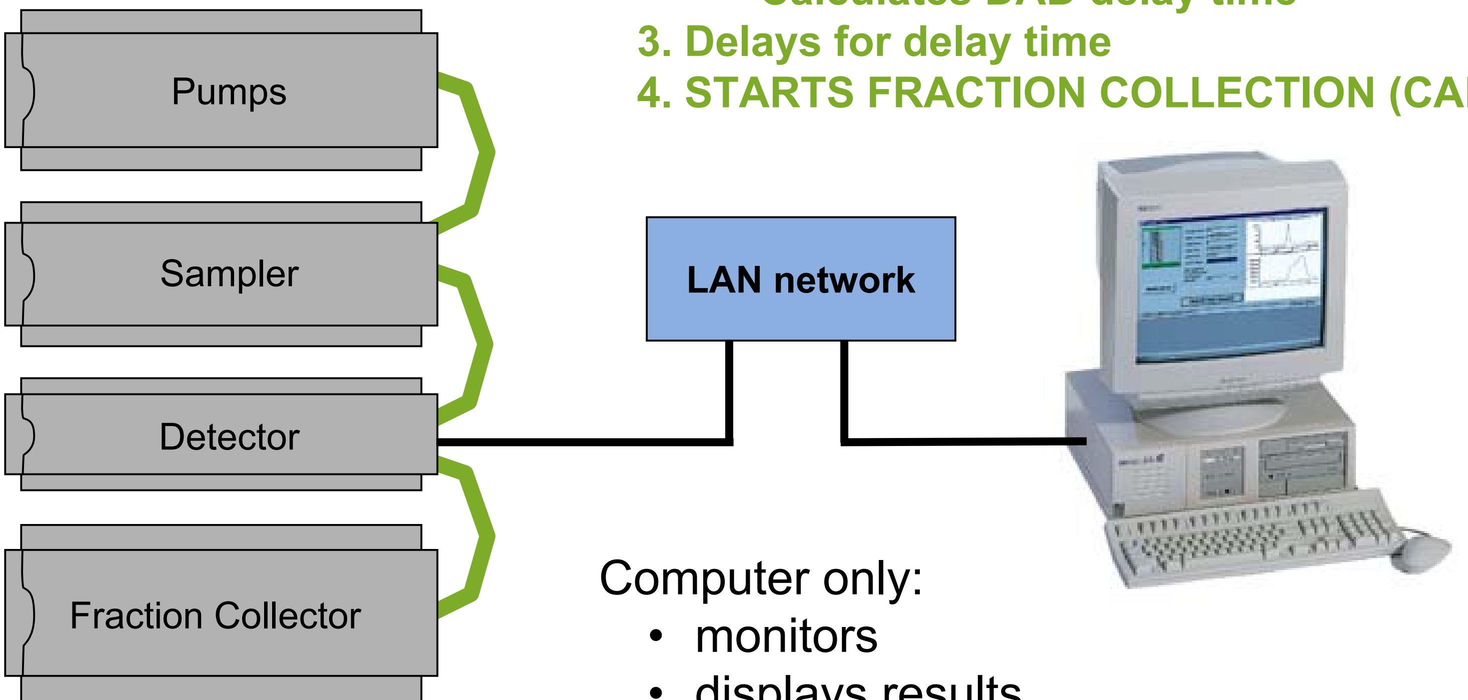


Additional system/computer delay due to:

- LAN traffic
- CPU usage

The CAN network: Integrated intelligence

1. Detector detects peak
2. FC acknowledges DAD peak (CAN)
   - Looks up DAD delay volume (CAN)
   - Gets current flow rate from pump (CAN)
   - Calculates DAD delay time
3. Delays for delay time
4. STARTS FRACTION COLLECTION (CAN)



Computer only:

- monitors
- displays results

Break Number 2

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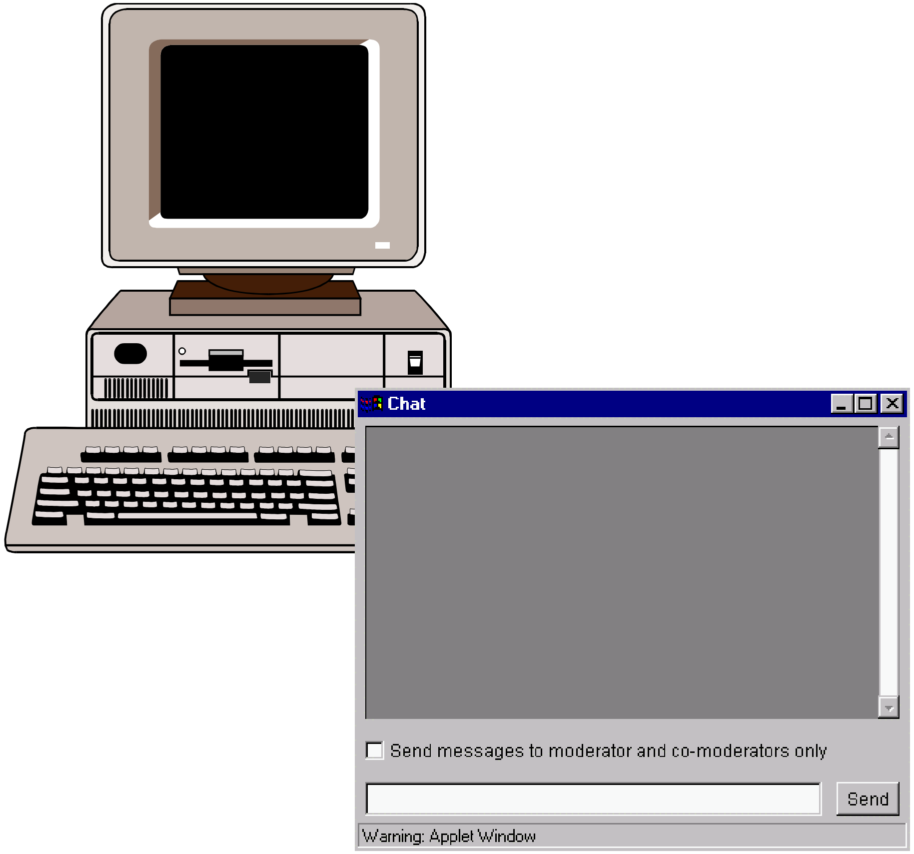
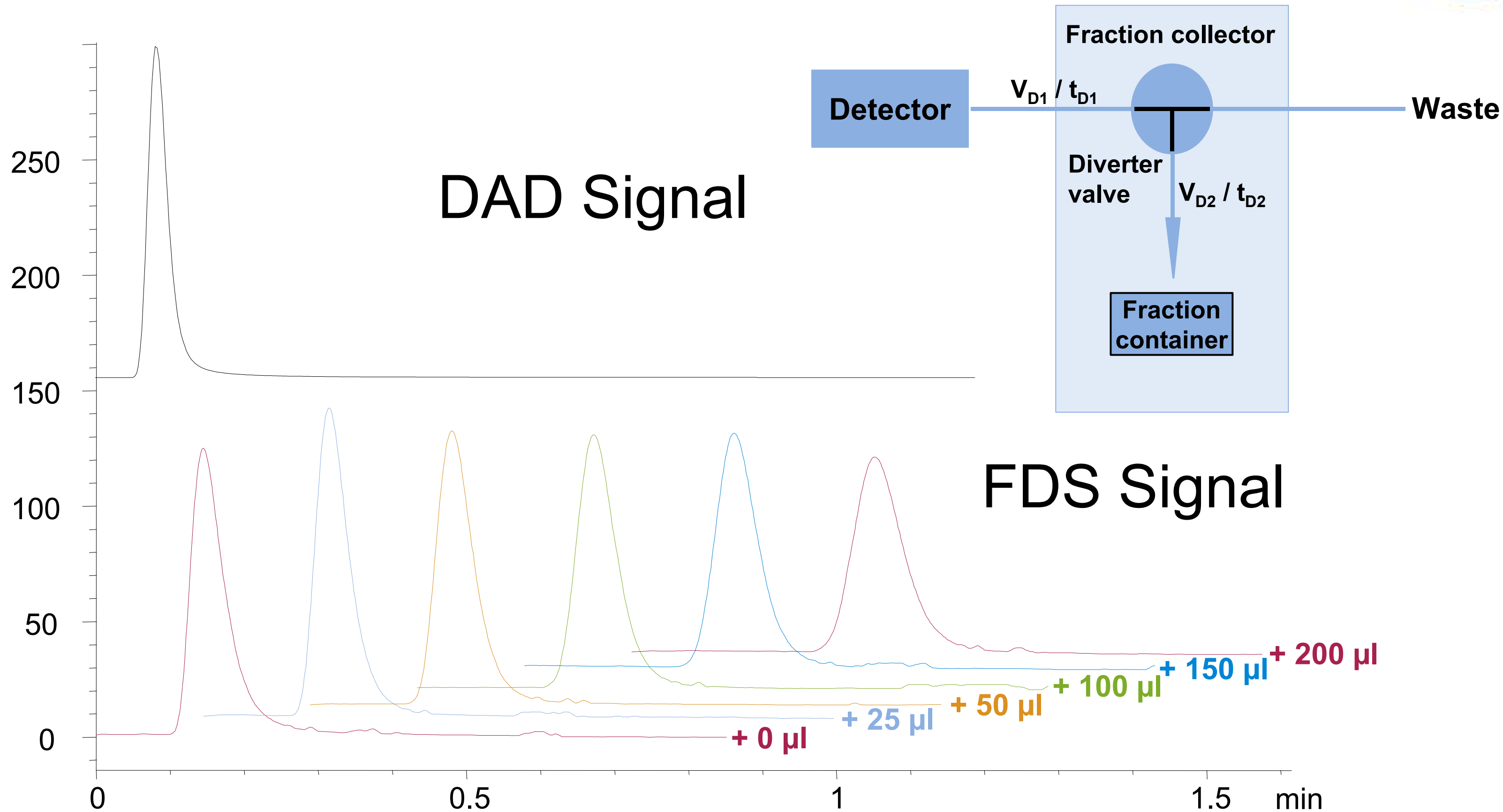
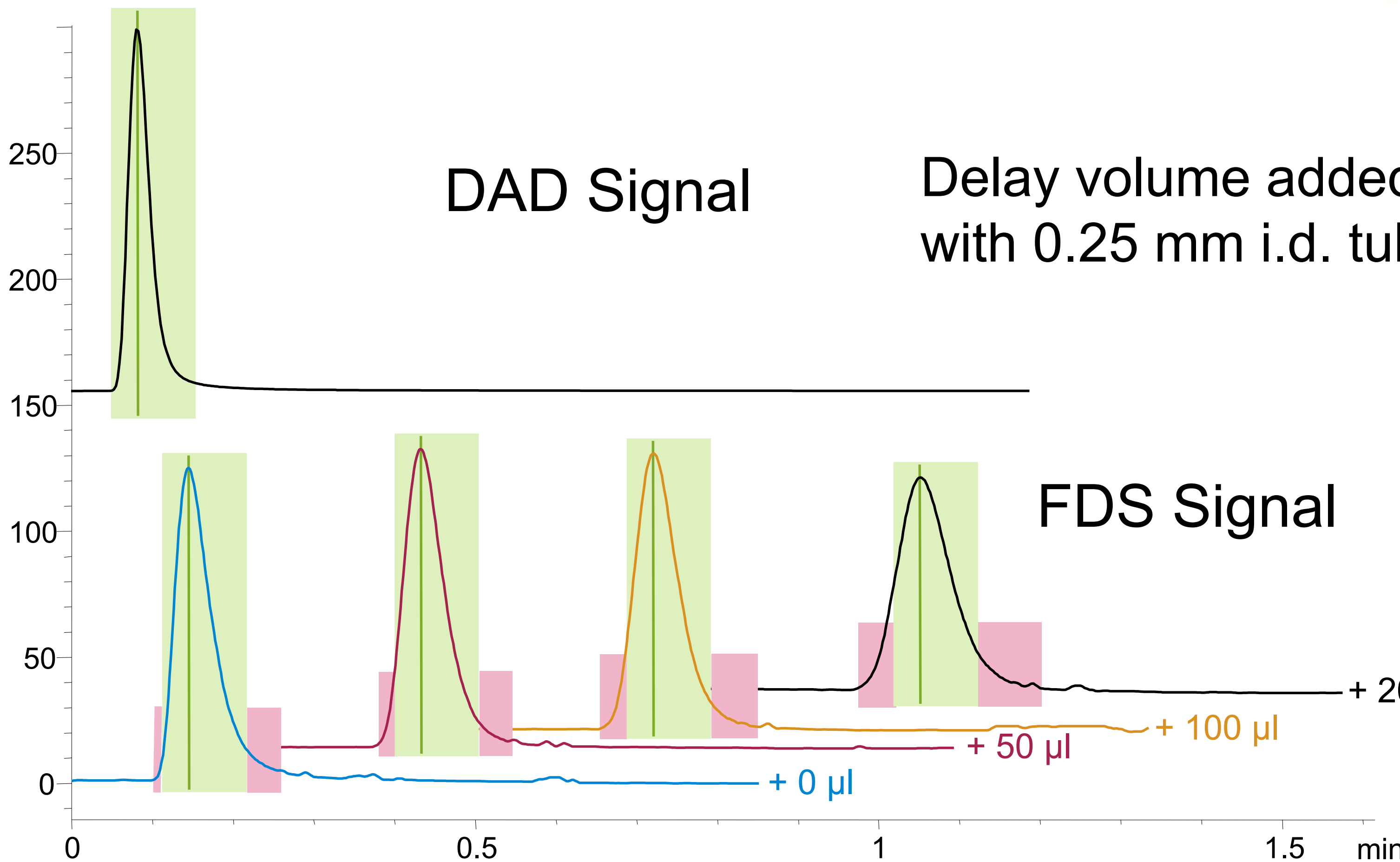


Dispersion for different delay volumes

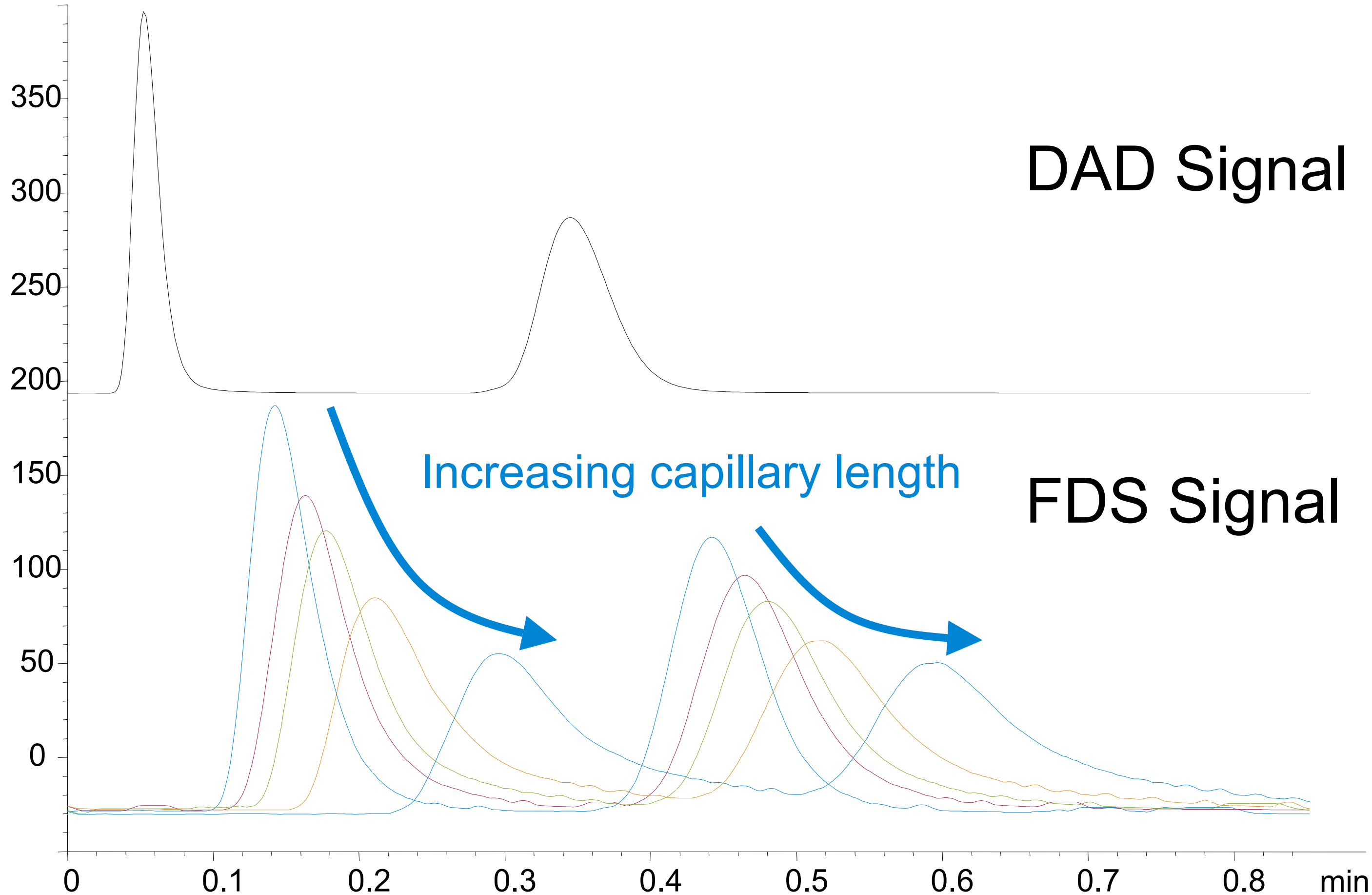


Delay volume added with 0.25 mm I.d. tubing

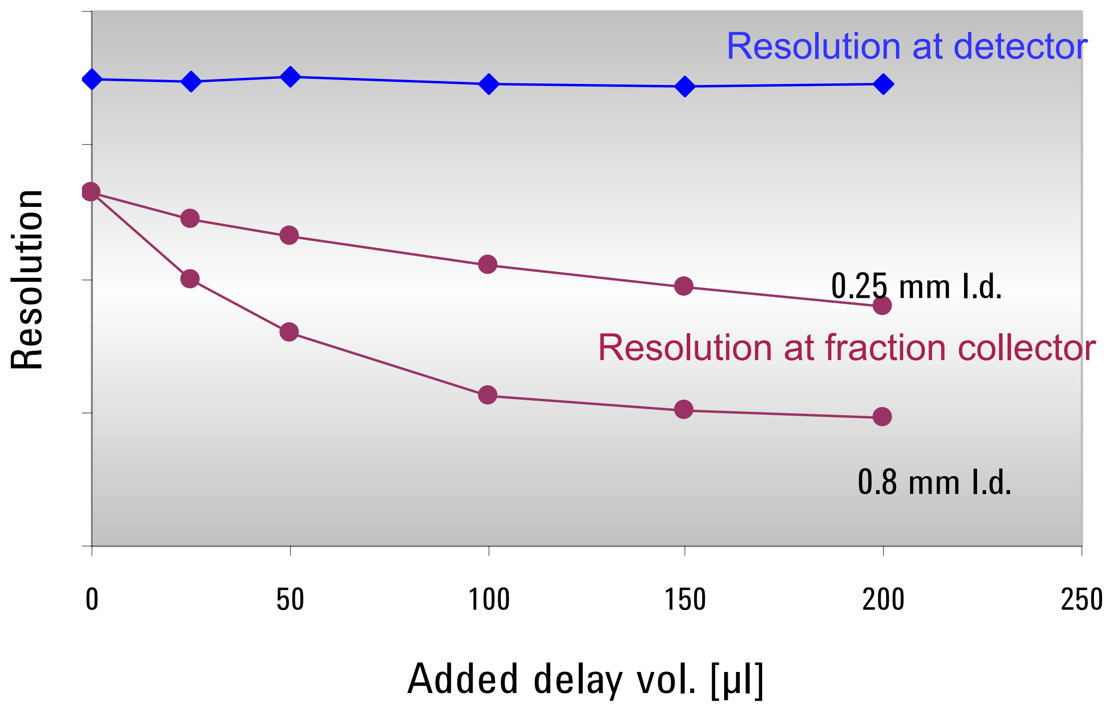
Peak loss due to dispersion



Resolution and peak width



Resolution and peak width



- Standard fraction collector AS with 0.25 mm i.d. capillary from DAD to AFC gives point at 0 delay volume added
- Delay volume was enhanced using a 0.25 mm i.d. capillary or a 0.8 mm i.d. capillary, respectively.
- Dispersion with 0.8 mm i.d. capillary higher for same delay volume due to squared influence of capillary radius on dispersion.

Does it really matter?

"If they [the chemists] want to purify a sample they'll know which one is best! The choice is a system that can actually separate and purify or one that sends e-mails with data the chemists can manipulate."

“[My customer] is achieving recoveries right in line with […]’s presentation [..] (not quite 110%). His quote: ”You guys don't know what you have here" was based …”

“Using a combined inject/collector seriously compromises the pluming of the system and leads to additional broadening and therefore loss of recovery. At […] we see 90% recovery with your system and 60% with others.”

Optimizing your purification system

- **Correct delay volume calibration**
  Fraction delay sensor vs. stop-watch method
- **No timing error due to detector delay**
  Firmware adjustment
- **No timing error due to PC/LAN delay**
  Direct LAN connection, intelligent CAN network
- **Minimizing dispersion with optimized delay volume**
  Delay volume control with optimized design/setup