

Automated Fraction Re-Analysis

Does it really make sense?

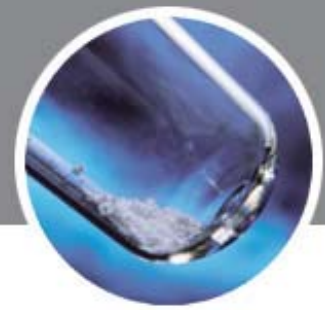


Presented by: **Udo Huber**



- 1995 PHD in organic chemistry from the University Karlsruhe/Germany
- 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





- **Introduction**

- Why purification, why re-analysis?
 - Where is my target compound?
 - Workflow
 - What is purity?

- **Is my compound pure enough?**

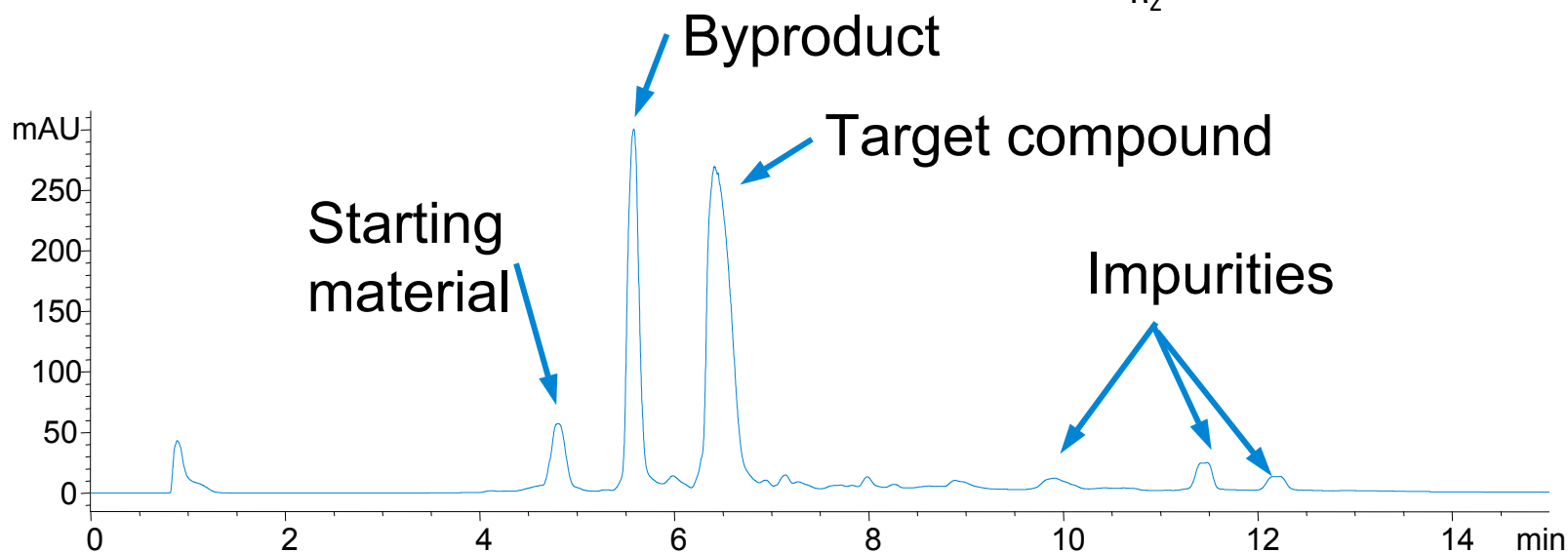
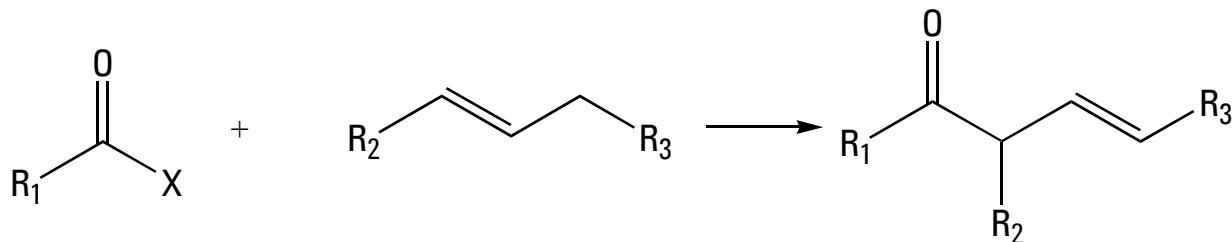
- Possible errors in automated fraction re-analysis
 - The right point in the workflow to check the purity
 - Other aspects

- **A dedicated system for preparative and analytical work**

- Sharing the DAD and MSD



Why purification at all?



- Target compound goes to activity testing.
- Therefore it must be 100 % pure.



Why re-analysis?



1. If more than one fraction was collected for the same sample re-analysis has to be done to identify the fractions containing the target compound.
2. Check purity of the target compound to make sure it is pure enough for activity testing.



More than one fraction



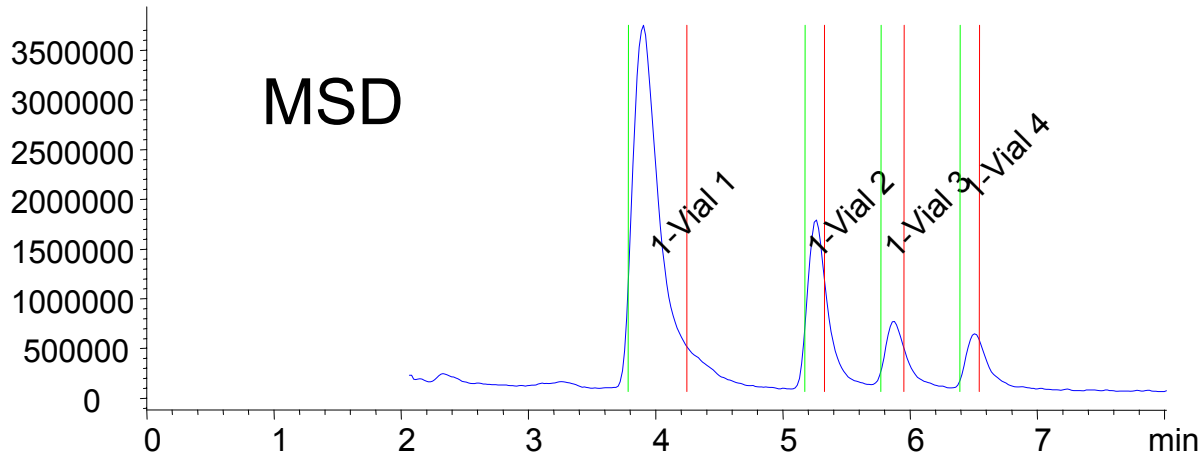
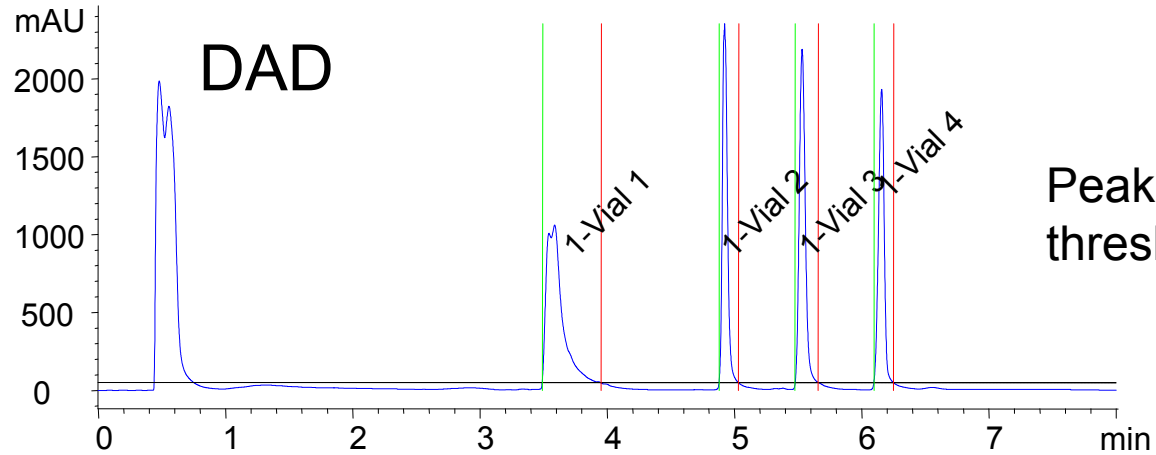
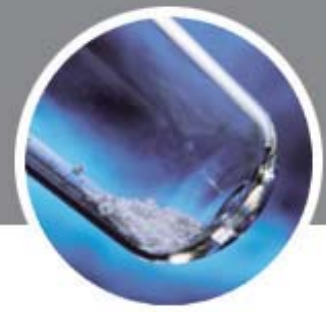
- ▶ Why do I get more than one fraction?
 - ⇒ Time- or peak-based fraction collection

- To identify the right fractions I need:
 - either standard for the compound ⇒ analytical system
 - or an MSD

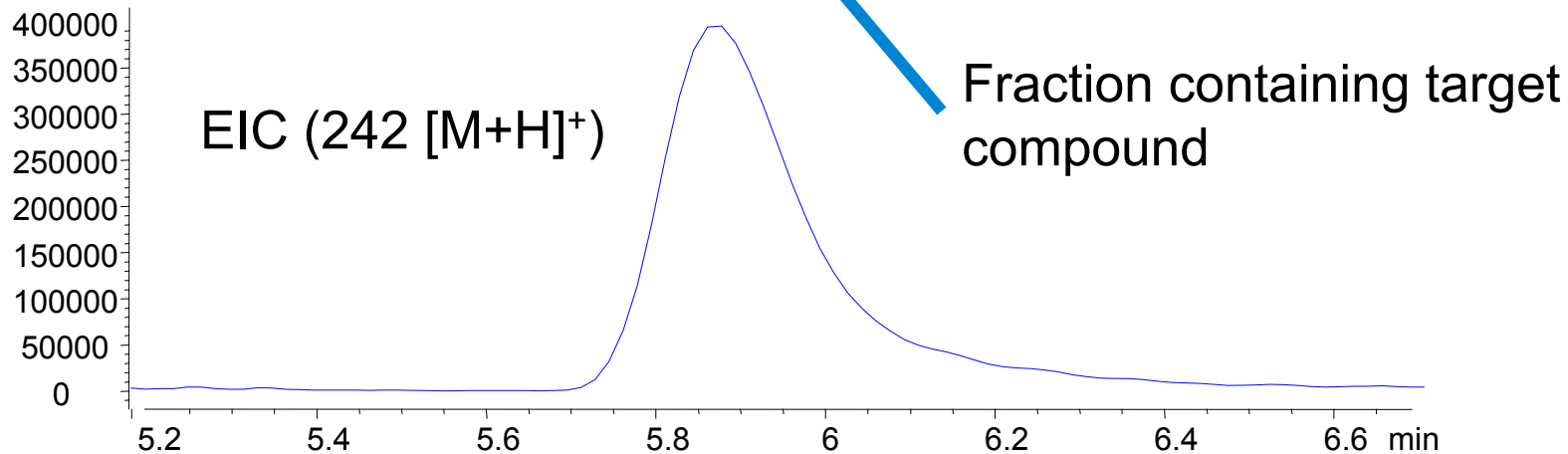
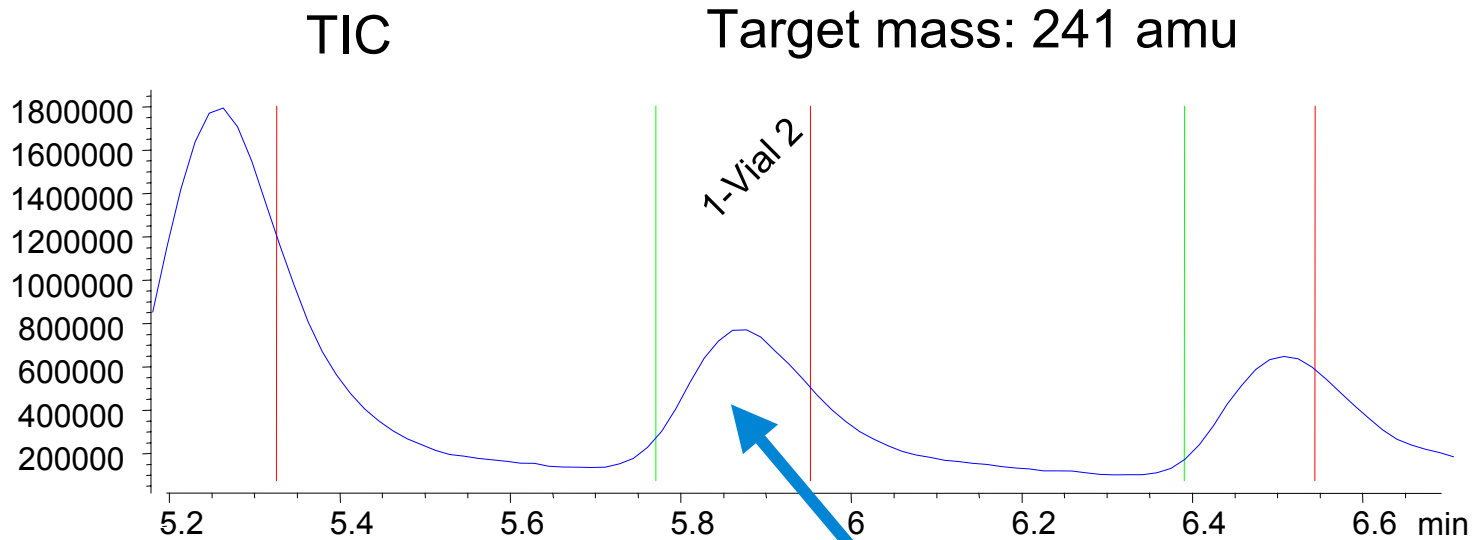
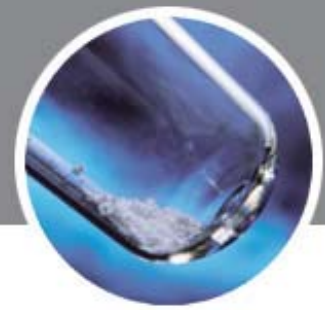
- – Prep system has no MSD
 - if it had an MSD I didn't get more than one fraction
 - ⇒ analytical system



Strategies for purification on a system equipped with MSD



Strategies for purification on a system equipped with MSD



Purity check – Sample workflow



1. Sample submitted by chemist
Solvent often DMSO/DMF



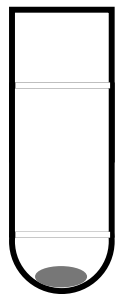
2. Sample purified
Mobile phase water/acetonitrile



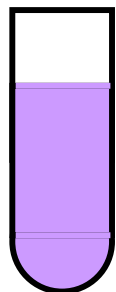
3. Fraction taken
Mobile phase water/acetonitrile



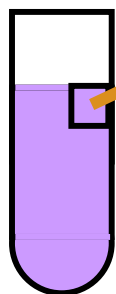
Sample workflow



4. Solvent evaporated
Compound weighed



5. Compound re-dissolved
Certain concentration, solvent often DMSO/DMF



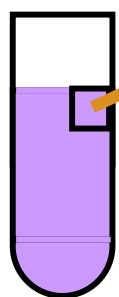
6. Portion taken to activity testing
(either DMSO solution, diluted with aqueous buffers or without DMSO)
Rest of solution stored



Important questions



- Confirm that target compound is going to activity testing, usually done by MS
- Measure purity of compound going to activity testing, must be higher than 90 - 95 %

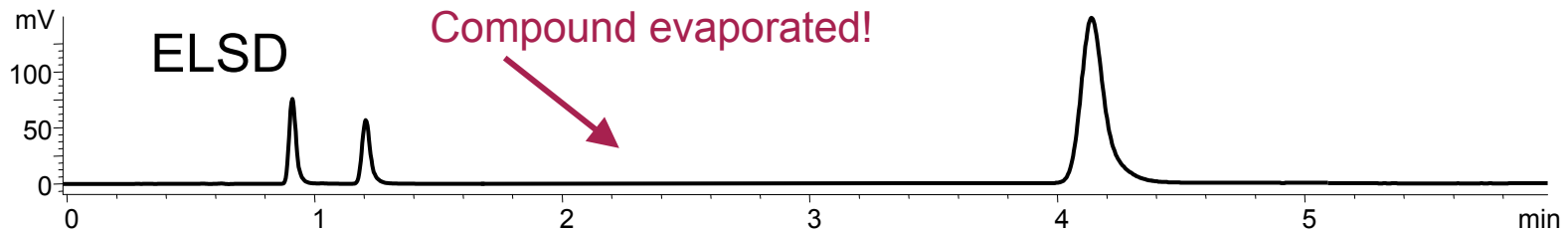
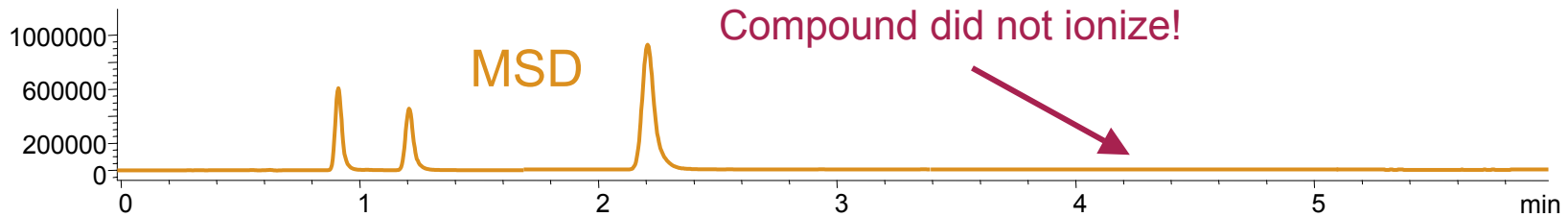
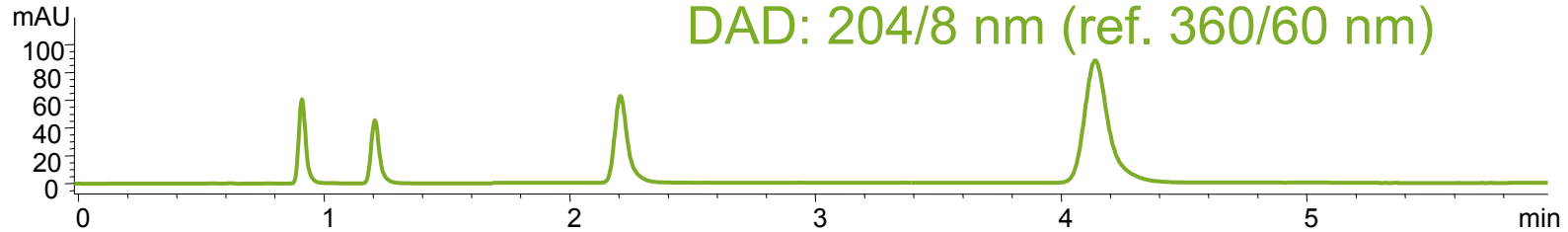
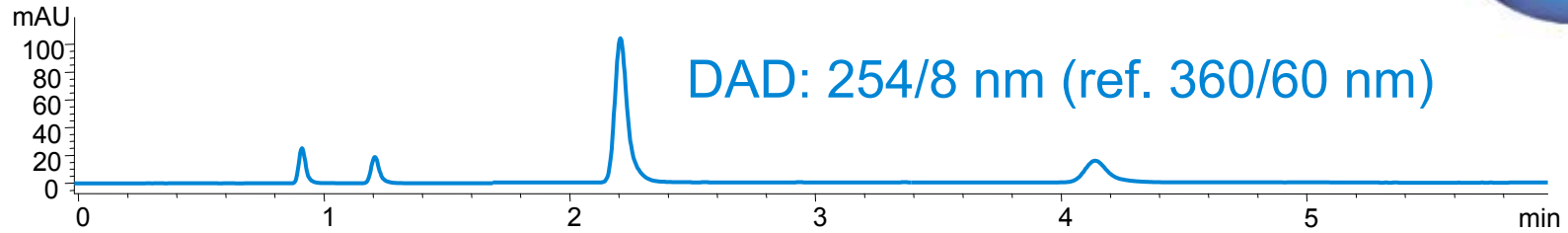


Activity testing

A representative portion of the sample going to activity testing must be re-analyzed to check the purity!



What is purity?





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Question for Presenter:

There are no questions pending.

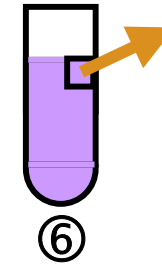
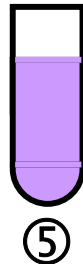
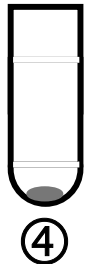
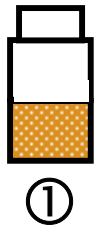
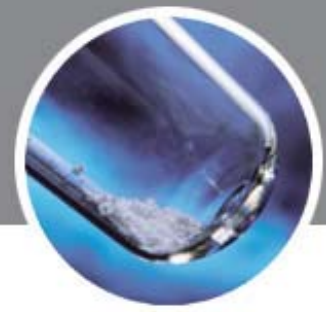
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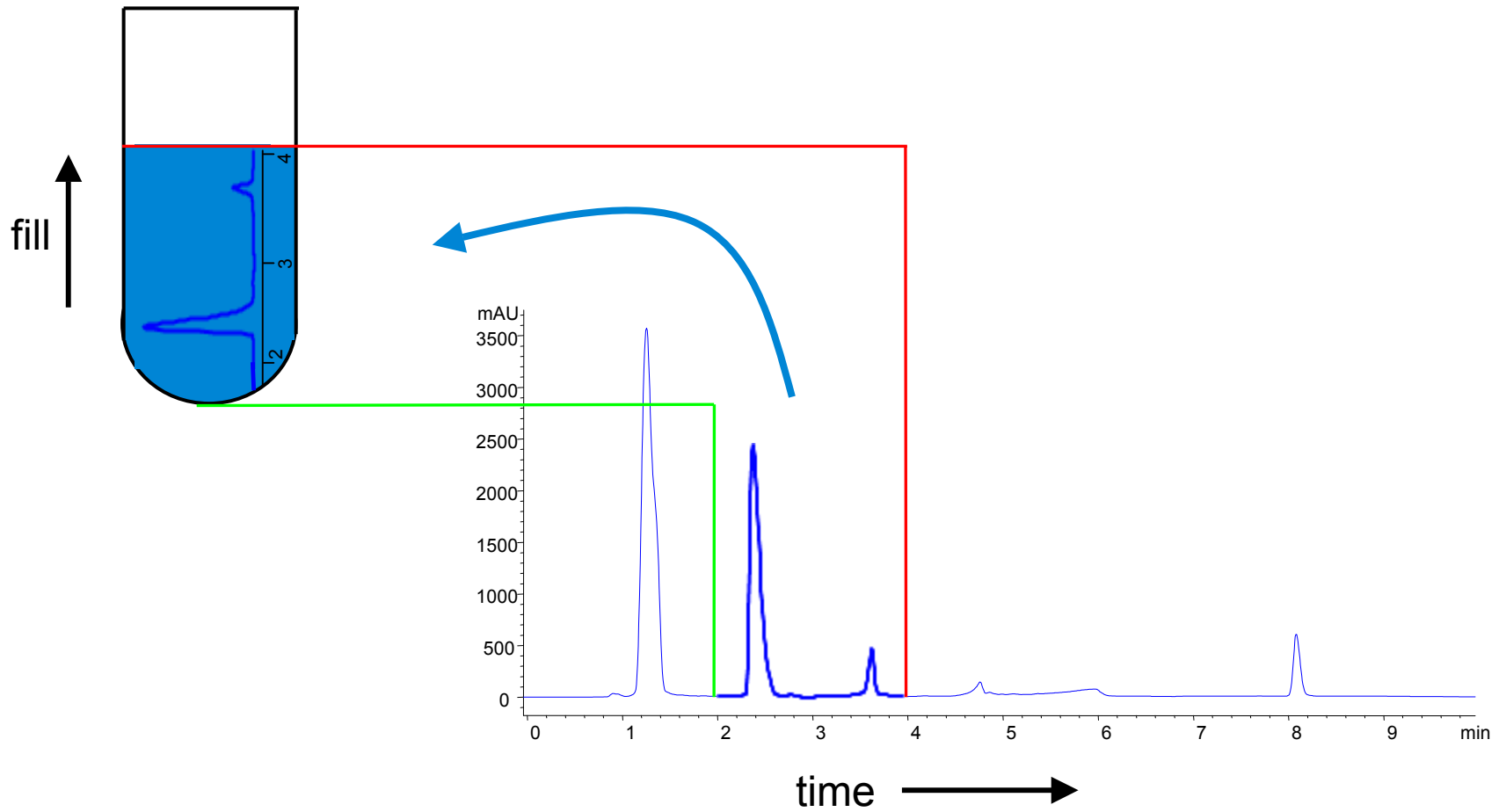
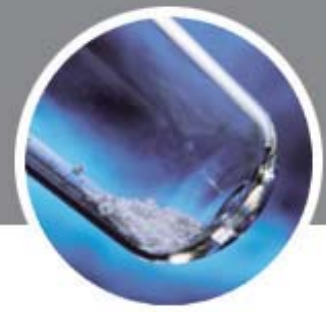
Fraction re-analysis – where in the workflow?



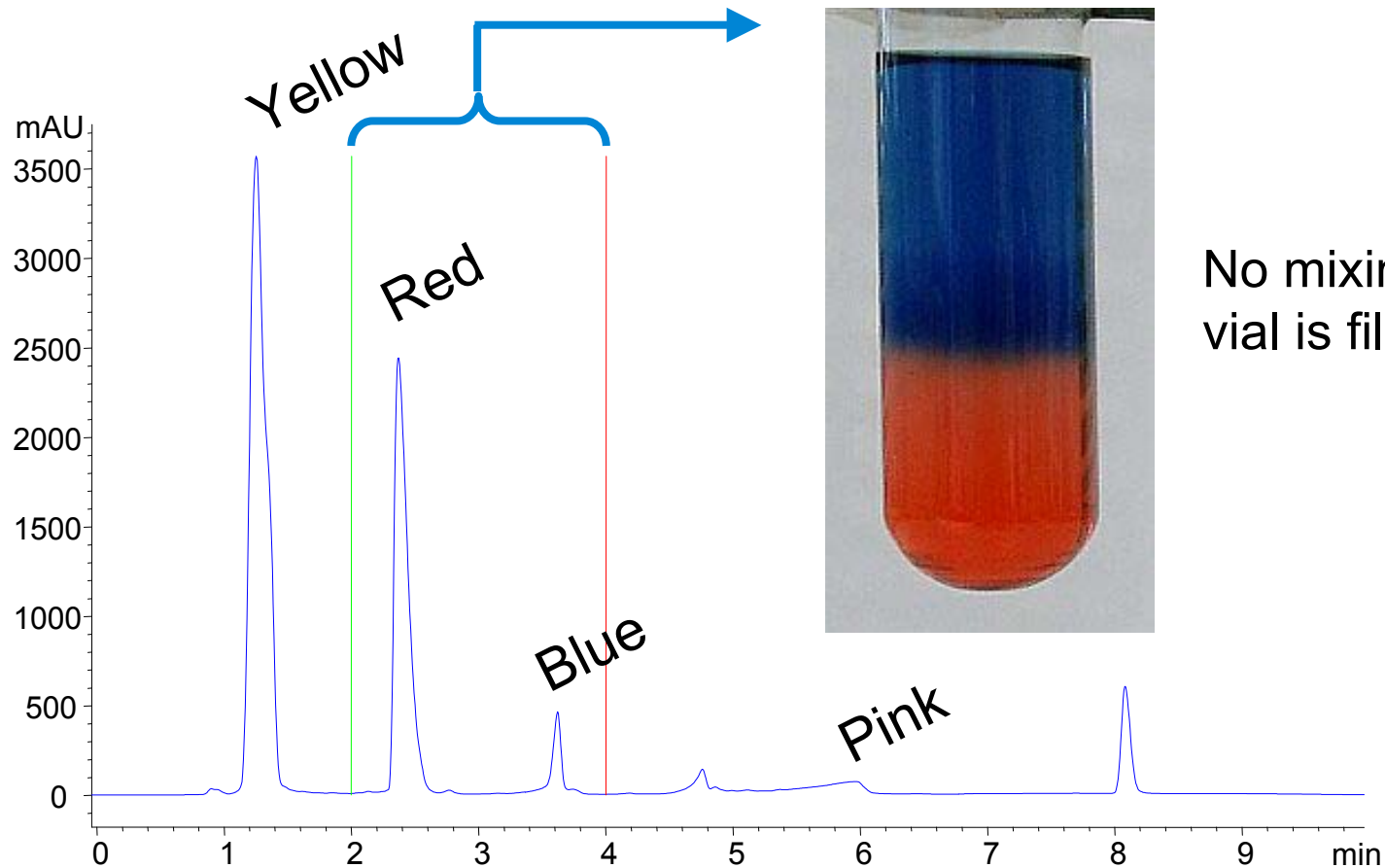
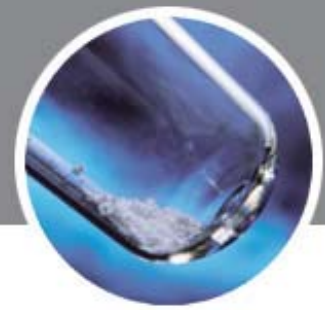
Automated fraction re-analysis:
Directly from fraction container (step 3)

- Advantage: High level of automation
- Disadvantages: ???

Possible errors in when doing automated fraction re-analysis (1)



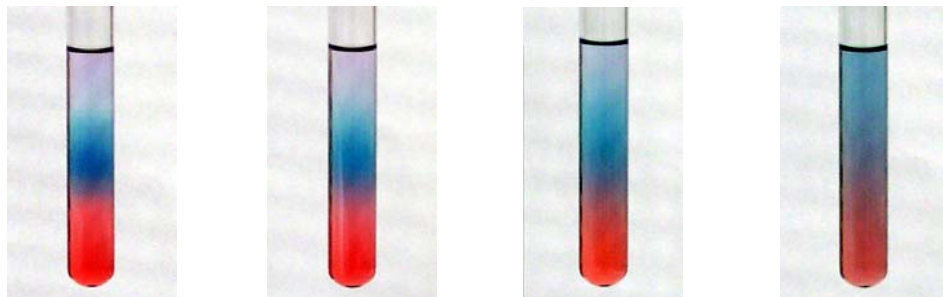
Possible errors in when doing automated fraction re-analysis (1)



Possible errors in when doing automated fraction re-analysis (1)



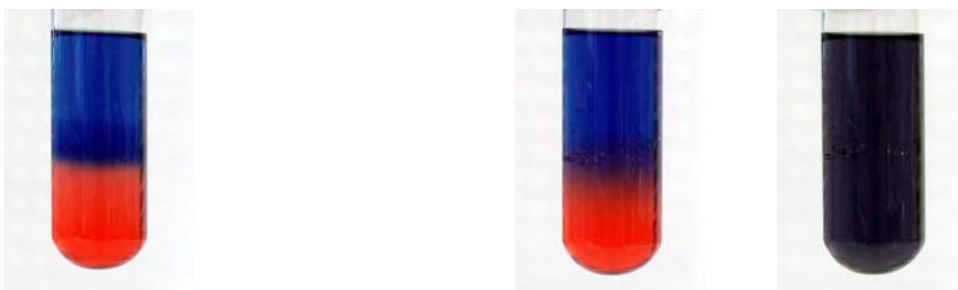
T = 0 h T = 6 h T = 24 h T = 96 h



Flow = 1 mL/min



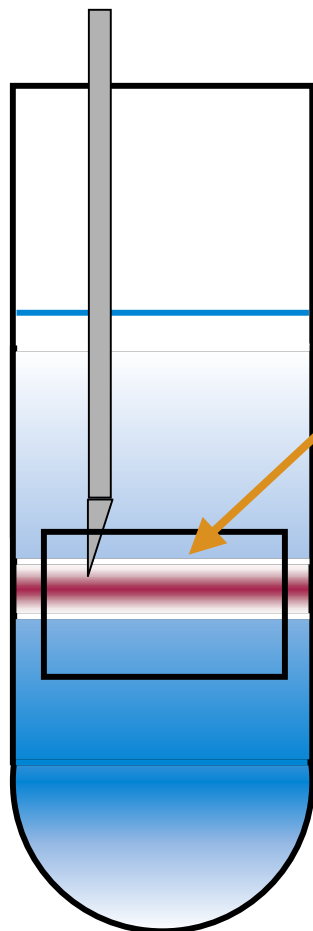
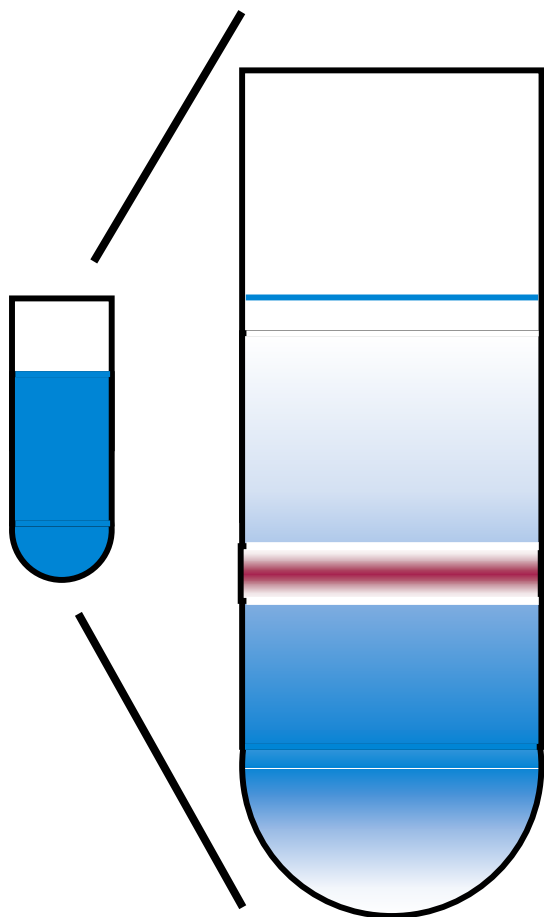
Flow = 4.5 mL/min



Flow = 20 mL/min

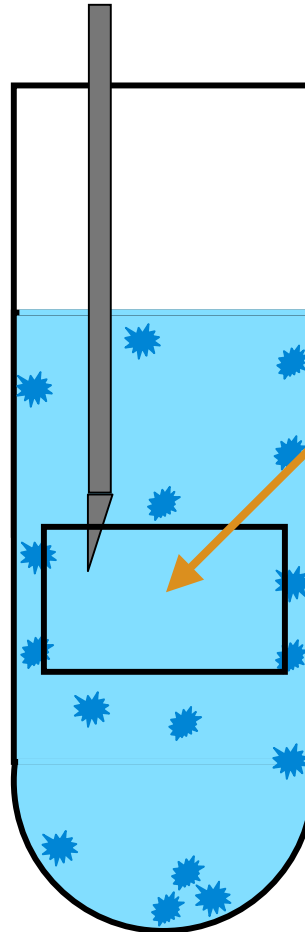
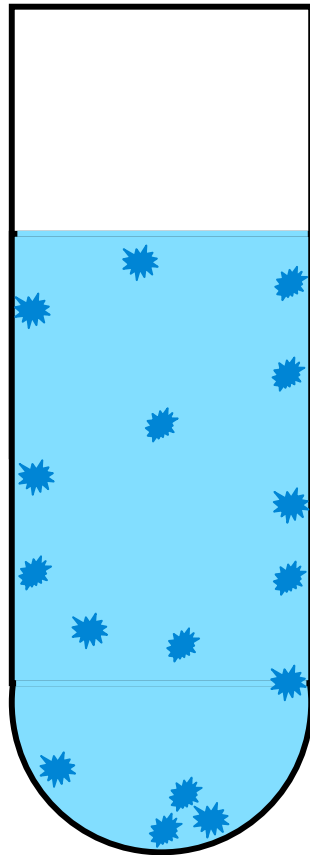
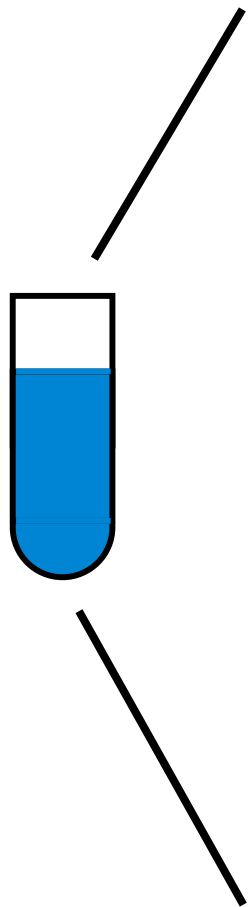
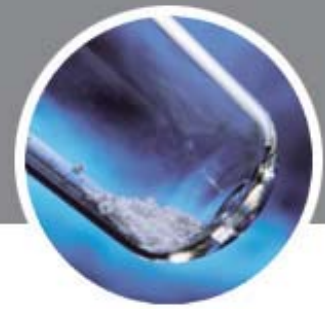


Possible errors in when doing automated fraction re-analysis (1)



Portion drawn for re-analysis. **Is this a representative sample?**

Possible errors in when doing automated fraction re-analysis (2)

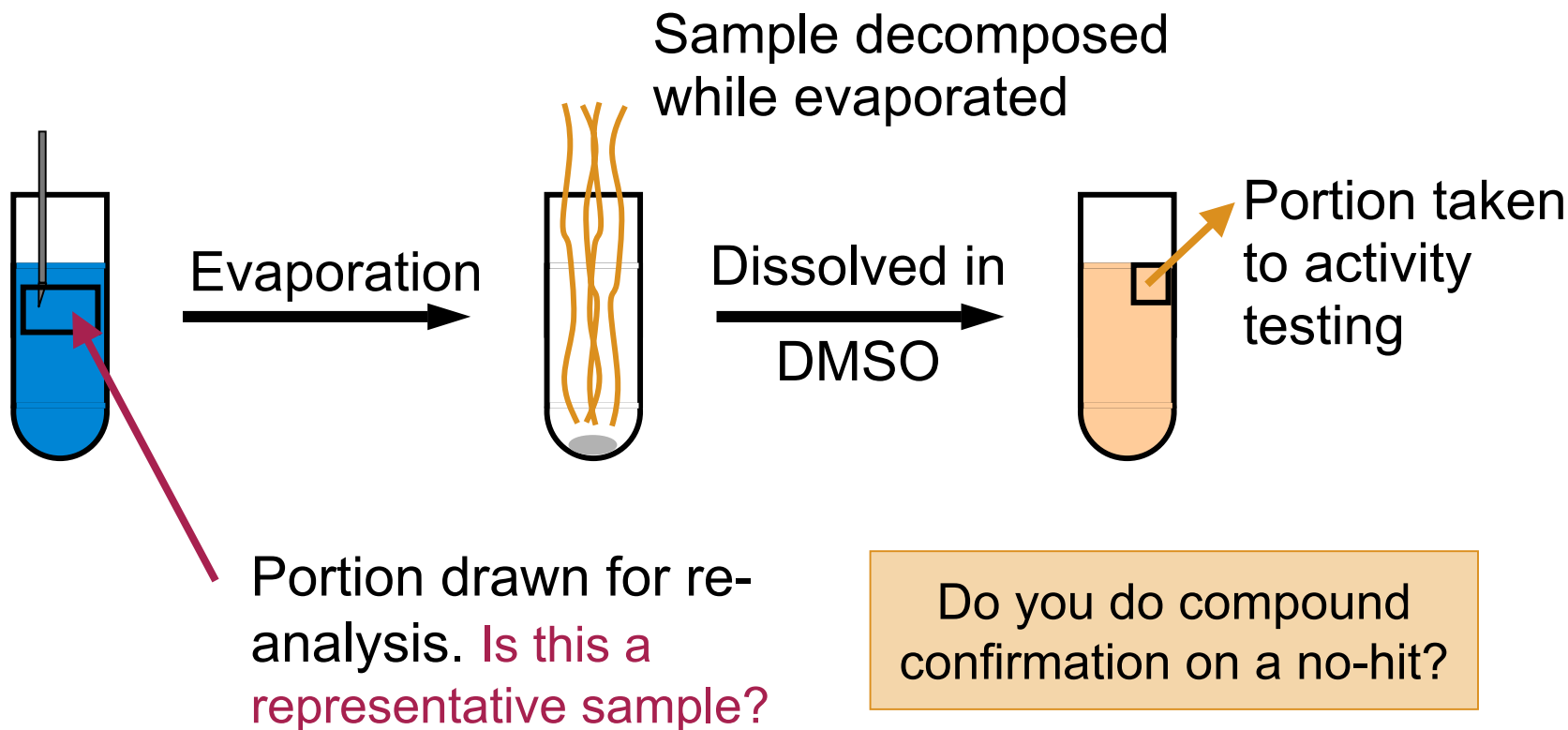


Portion drawn for re-analysis. **Is this a representative sample?**

Sample was submitted in DMSO

Mobile phase is water/ACN

Possible errors in when doing automated fraction re-analysis (3)



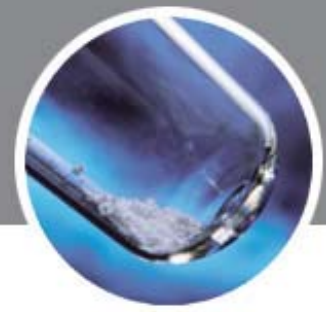
What is the solution?



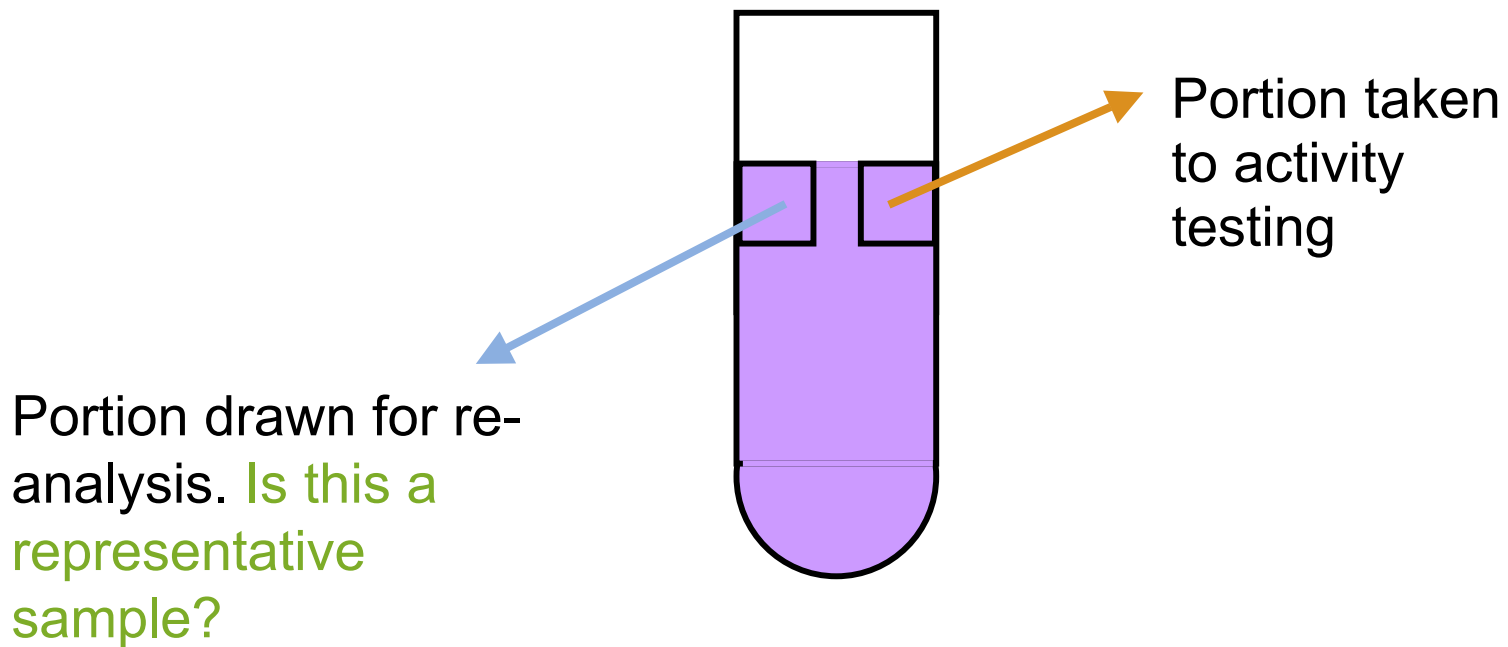
What can I
do???



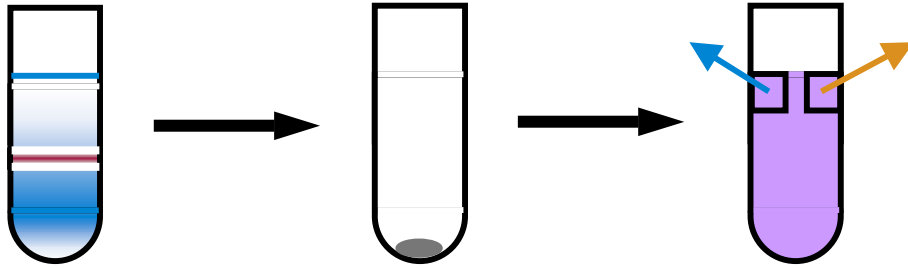
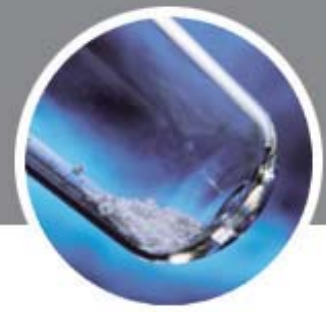
Take a second sample from the solution that goes to activity testing!



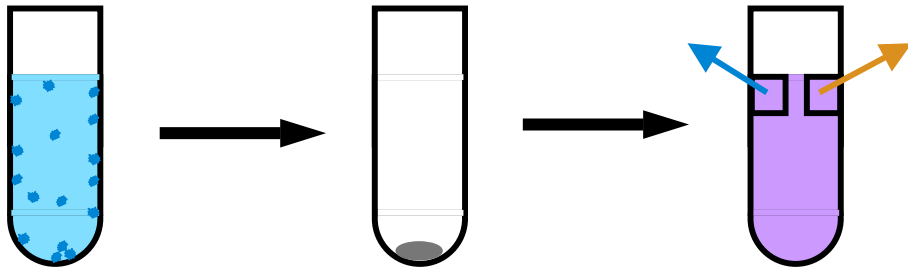
- Let the purification system do what it was designed for!
- Use an analytical system to confirm compound and determine purity.



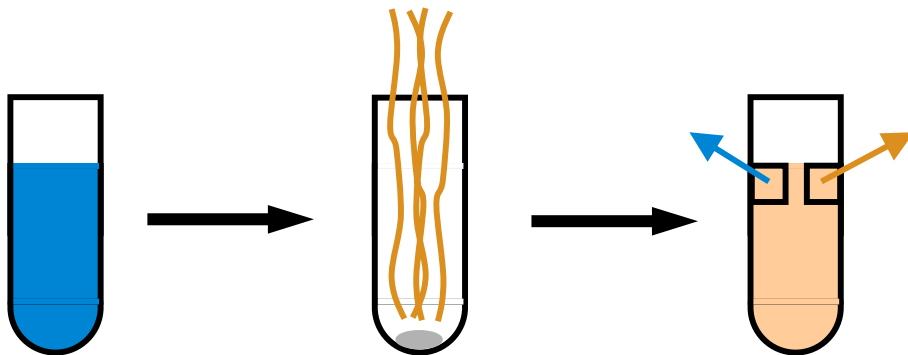
Does this approach solve the problems?



Fraction mixed before re-analysis

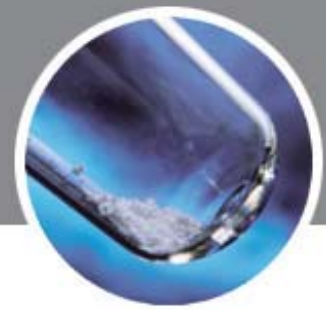


Compounds dissolved before re-analysis



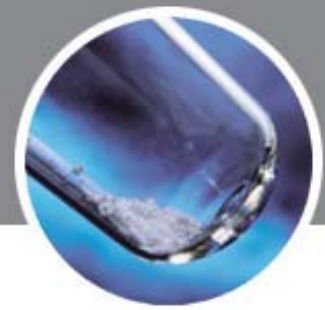
Decomposition discovered during re-analysis





- Using a **combined inject/collector** seriously **compromises the plumbing of the system** and leads to additional broadening and therefore **loss of recovery**. At [...] they see 90% recovery with our system and 60% with others
- You make a very good, in fact the best, **analytical system** already which is **optimised for that specific job**. All the labs interested have [Agilent] 1100 [Series] systems available to them for such use.
- A separate analytical system means our expensive **Prep system is not tied up doing routine analysis** as the real prep runs start to build up in a queue - Not cost effective.
- Performing re-analysis off line means you can specifically **select which fractions you would like to re-analyse** rather than all collected fractions being re-analysed. This **eliminates unneeded analytical runs** which are both time consuming and wasteful.





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Question for Presenter:

There are no questions pending.

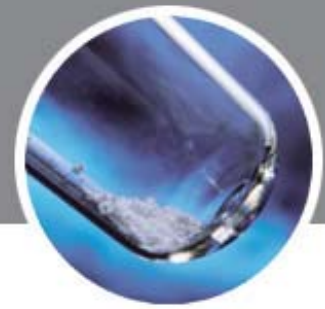
Question & Answer Session

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A single system for purification and analytical work



- Two separated systems
 - absolutely no compromises regarding flow paths
 - preparative AND analytical work in parallel
- Two systems sharing a single MSD and DAD
 - can be operated with ChemStation only (rev. A.10.01), purification software and with Easy Access Plus
 - not possible to do analytical and preparative work in parallel
 - preparative autosampler and pumps used for analytical work (delay volume)

Recommended for:

Preparative flow rates up to 40 mL/min (1 inch columns)

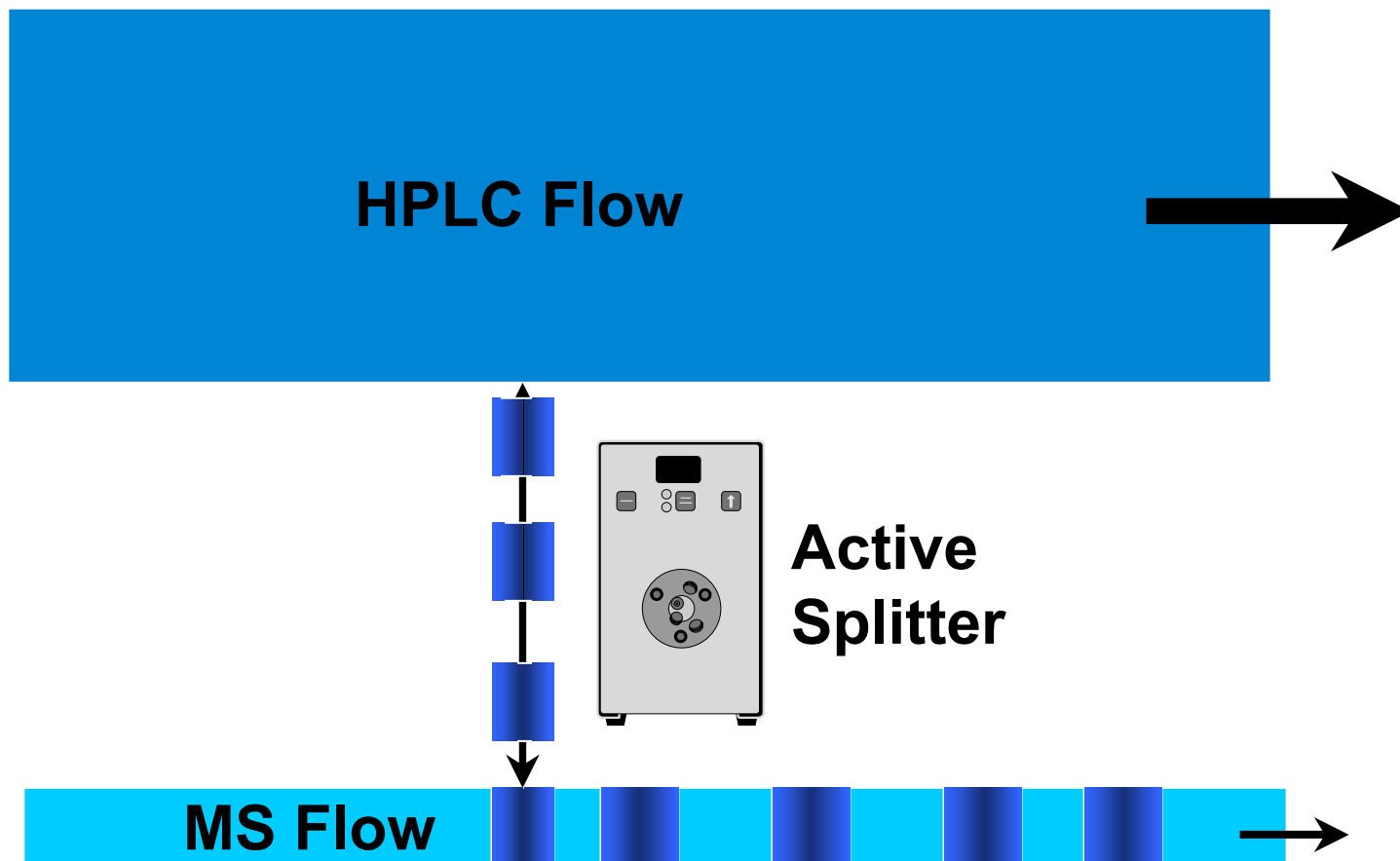
Injection volumes < 900 μ L

Analytical flow rates above 0.8 - 1 mL/min (4.6 mm columns)

Further details will be described in an Application Note



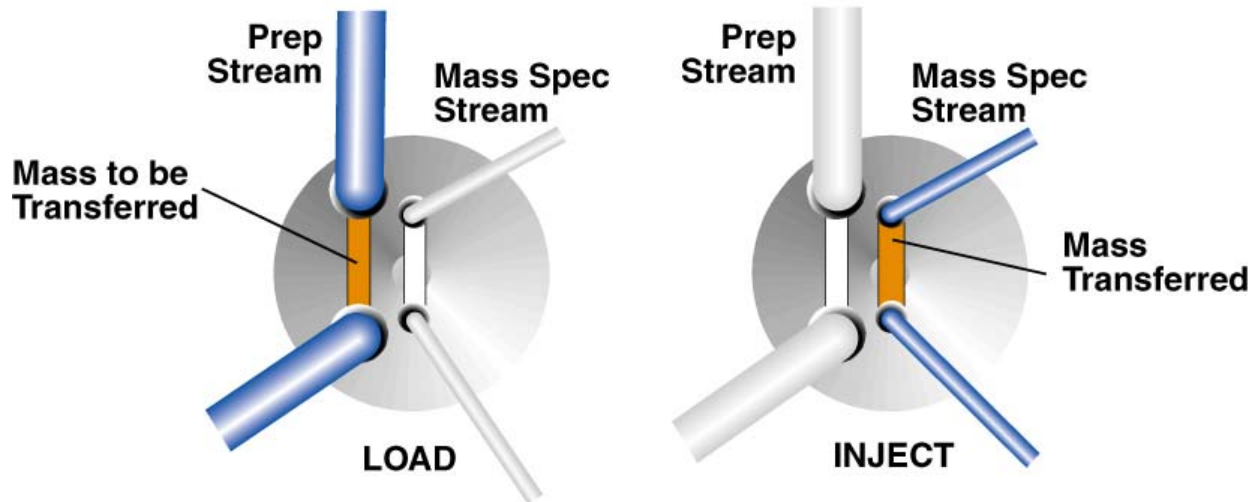
The Agilent active splitter



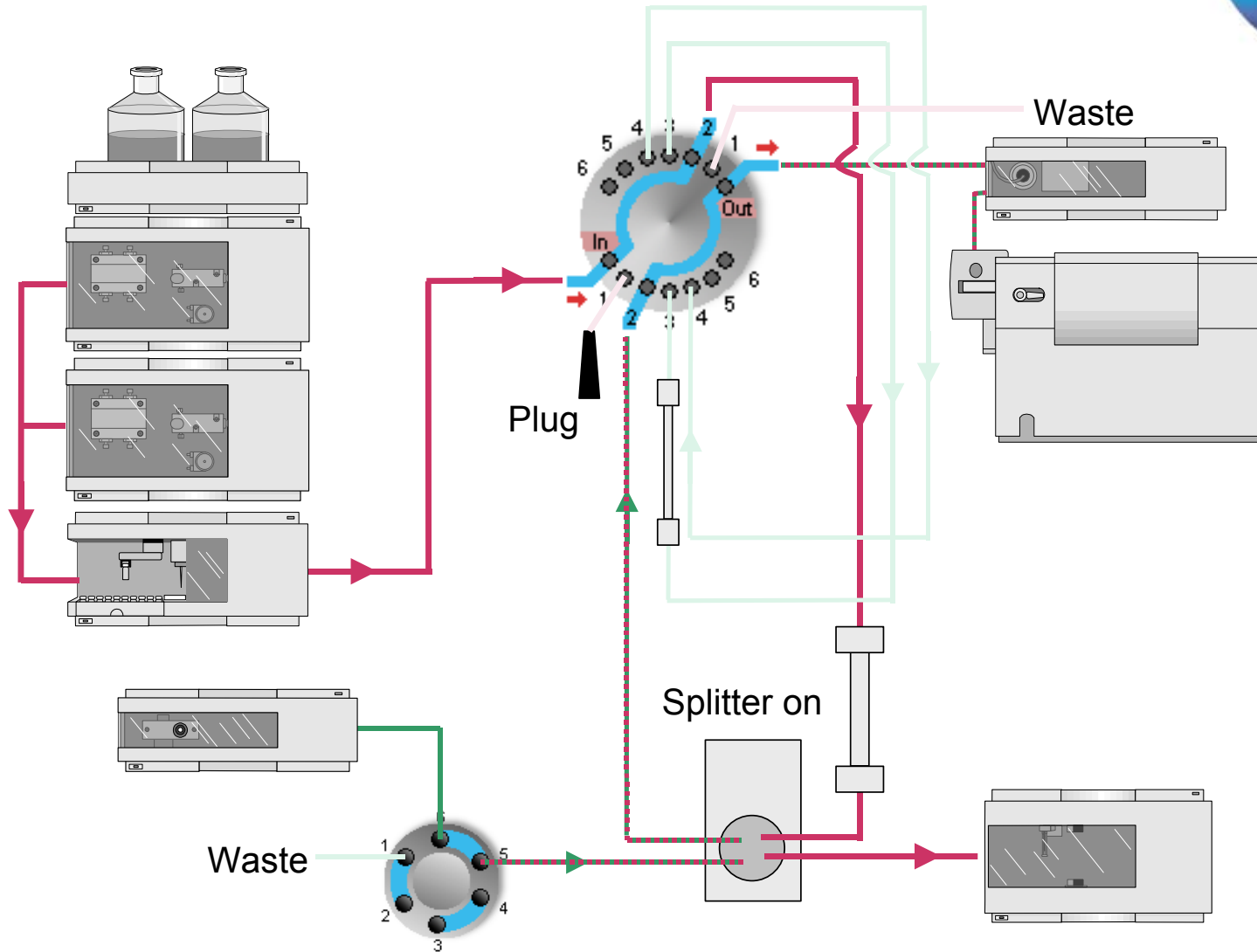
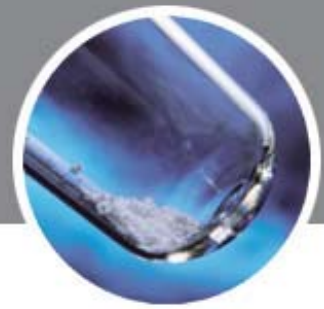
The Agilent active splitter



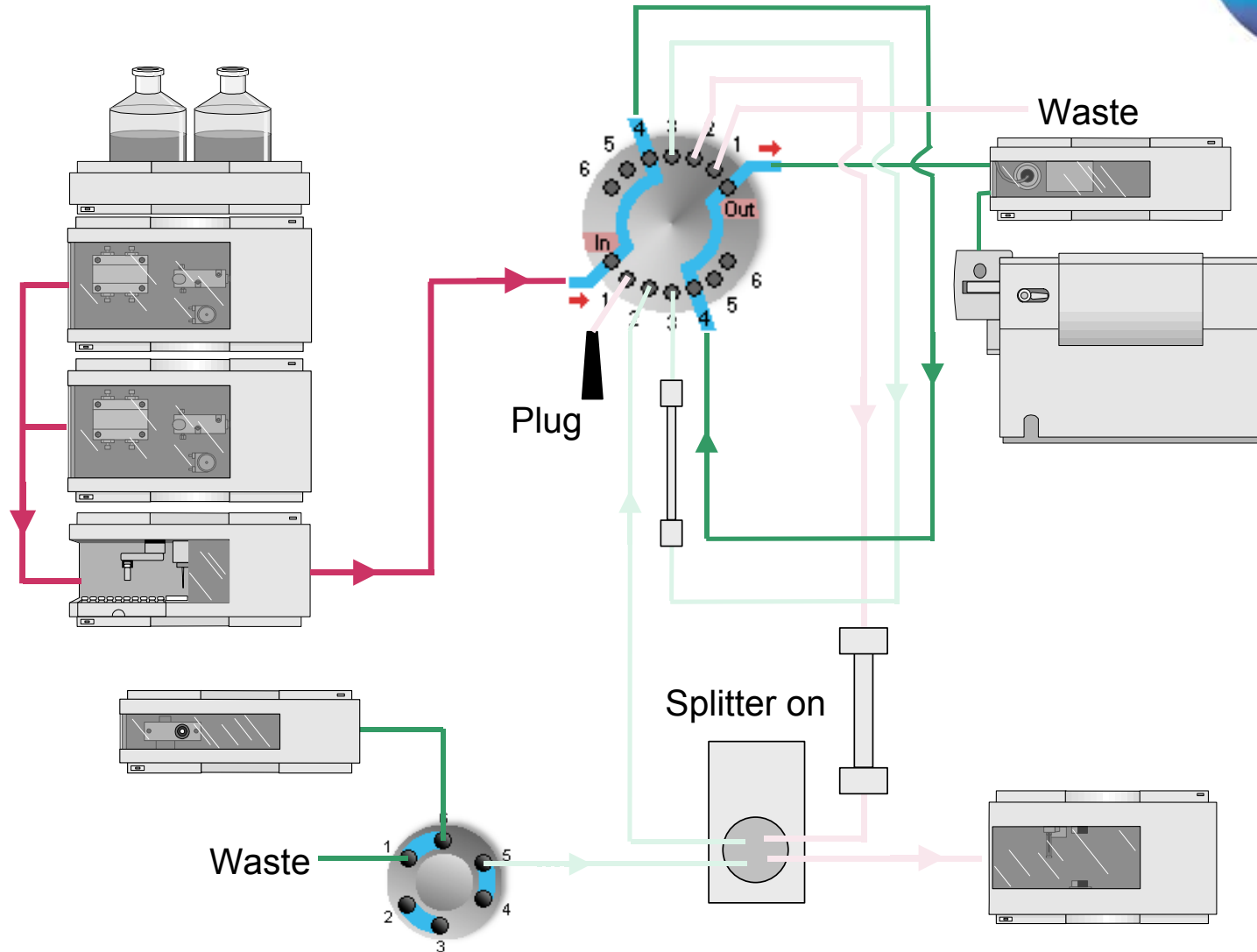
- Agilent active splitter provides control of the transfer of material from one flow stream to another.
- Rotor seal includes three selectable aliquot volumes (22 nL, 100 nL, or 300 nL).
- Gating frequency (0.25-2.0 Hz) and aliquot volume determines the rate of mass transfer from source stream to destination stream.



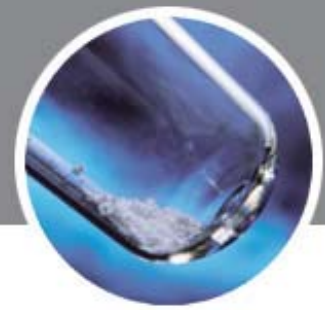
Two systems sharing a single MSD and DAD – preparative work



Two systems sharing a single MSD and DAD – direct injection



Automated fraction re-analysis – does it really make sense?



- **Introduction**

 - Why re-analysis, where is my target compound and what is purity?

- **Possible errors in automated fraction re-analysis**

 - Concentration gradient

 - Crystallization

 - Decomposition

 - How to avoid those problems?

- **A dedicated system for preparative and analytical work**

 - Sharing the DAD and MSD

