Welcome to our E-Seminar:

Injection of high-concentration and high-volume samples in preparative HPLC

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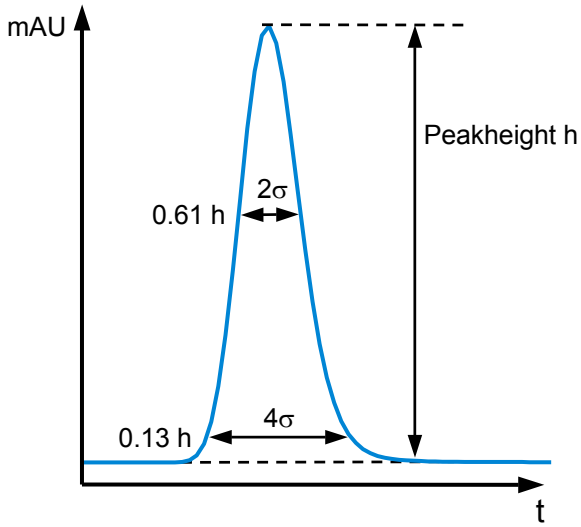
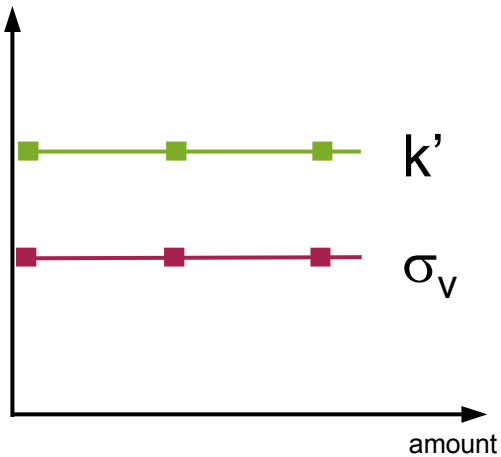
Column loading and overloading





Goal:

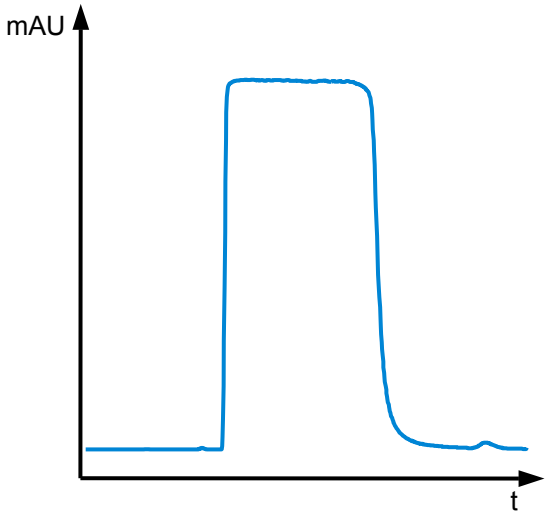
- Quantification of compounds

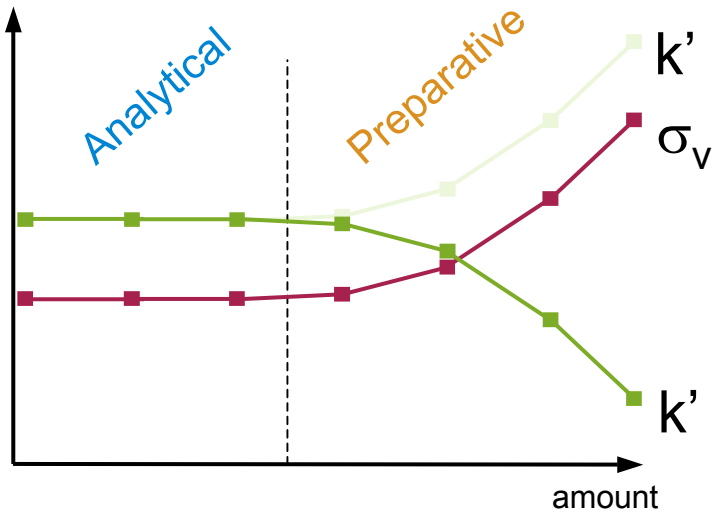
Important parameters:

- Resolution
- Peak symmetry
- Peak width

Capacity factor	$k' = \frac{t}{t_0} - 1$
Peak width	σ_v

Column loading and overloading



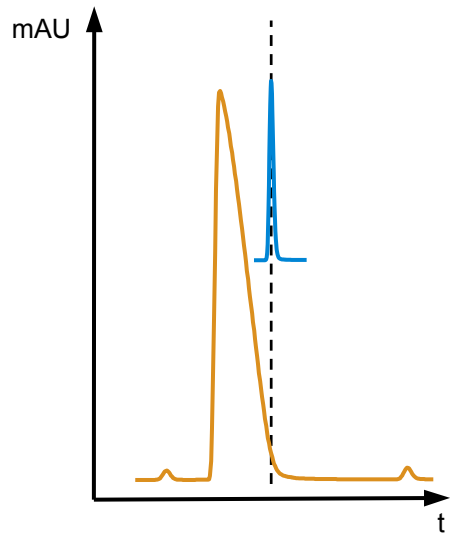


Goal:

- Separation of compounds

Important parameters:

- Throughput
- Yield
- Purity



Column loading and overloading

Concentration Overloading	Volume Overloading
• Determined by solubility of compound in mobile phase • “Preparative” area of adsorption isotherm • Throughput determined by selectivity • Particle size of stationary phase of low influence	• Determined by injection volume • “Analytical” area of adsorption isotherm • Throughput determined by column diameter • Small particle size required
Usually a combination of concentration and volume overloading is done. Concentration overloading is preferred because higher throughput is possible.	

Column loading and overloading

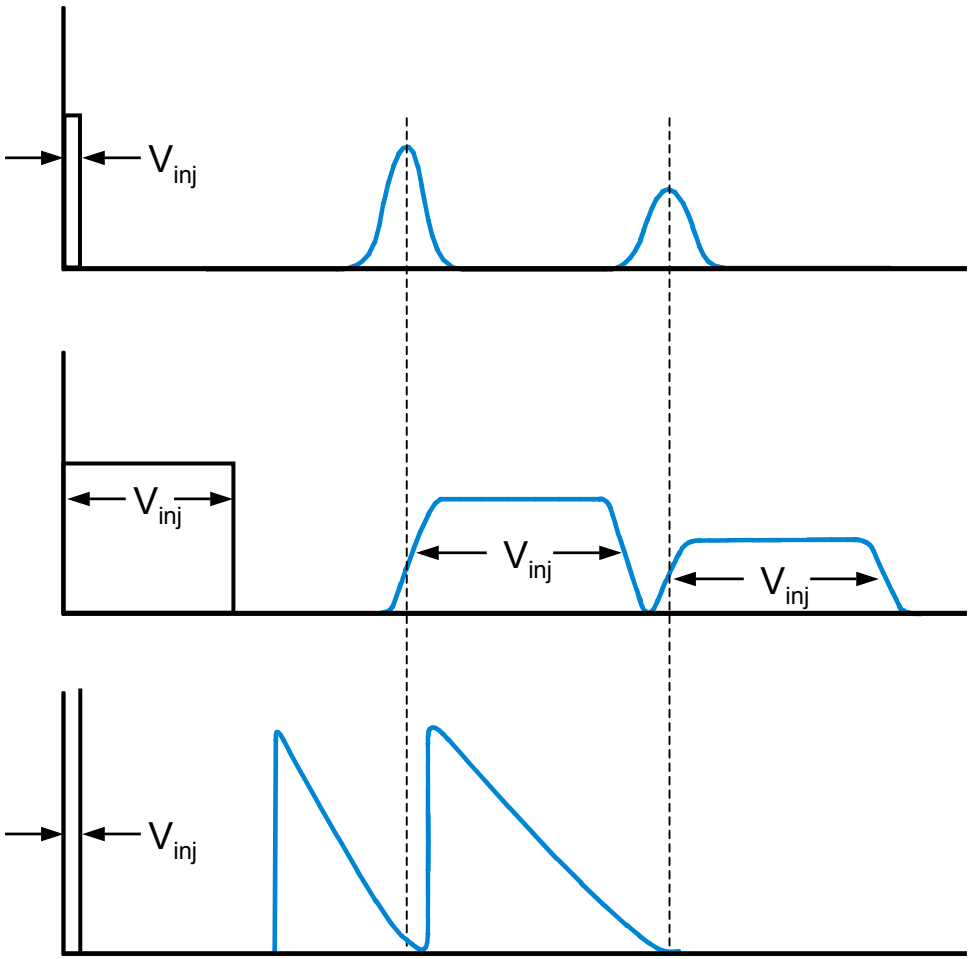
Concentration overloading

- Sample not soluble in mobile phase of gradient starting conditions
- Precipitation, leads to blocking of the flow path

Volume overloading

- Volume limited by max. injection volume of autosampler and sample container

Column loading and overloading



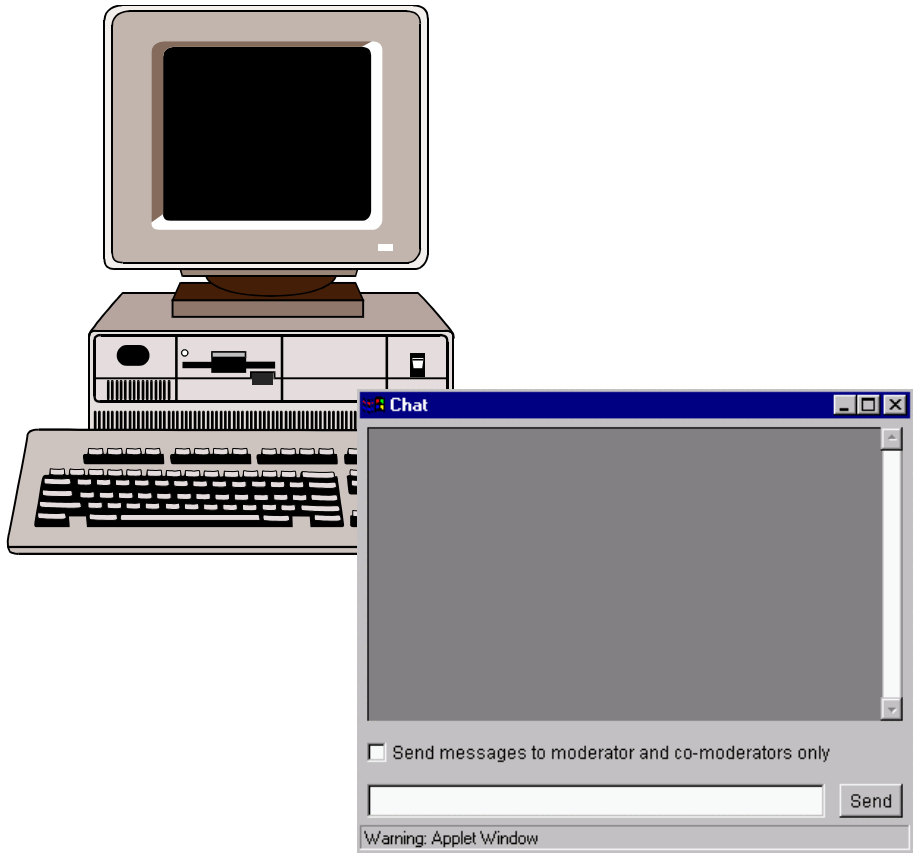
Analytical run

Volume overloading

Concentration overloading

Break Number 1

Please type your question into the Chat Box at any time during the presentation.



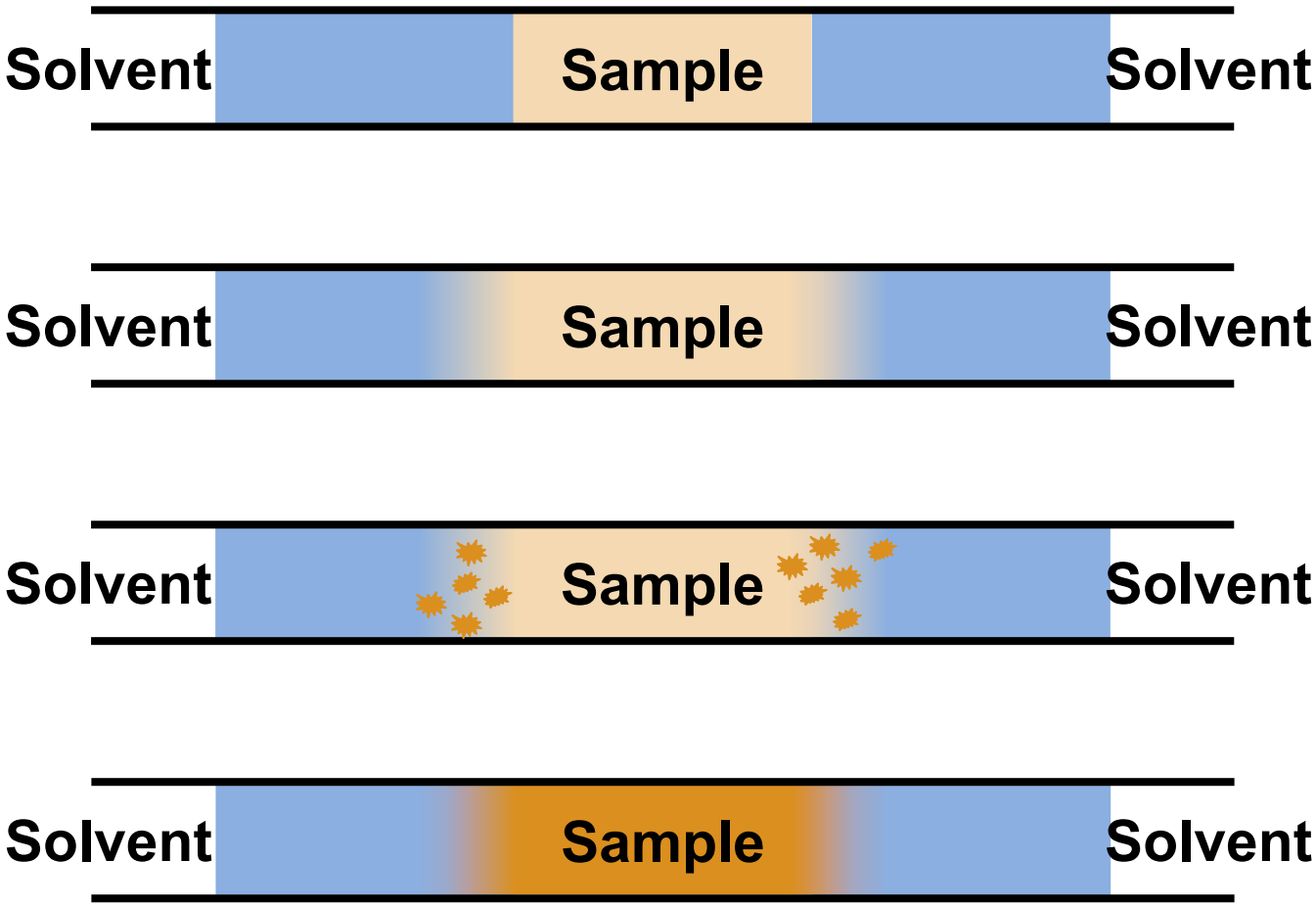
High-concentration samples

When injecting high-concentrated samples, precipitation in the injection valve can be a problem. This blocks the injection valve and it has to be taken apart and cleaned.

Reason:

- Sample is dissolved e.g. in DMSO
- Starting composition of the gradient is e.g. water/acetonitrile 90:10
- As soon as the sample mixes with the mobile phase precipitation occurs

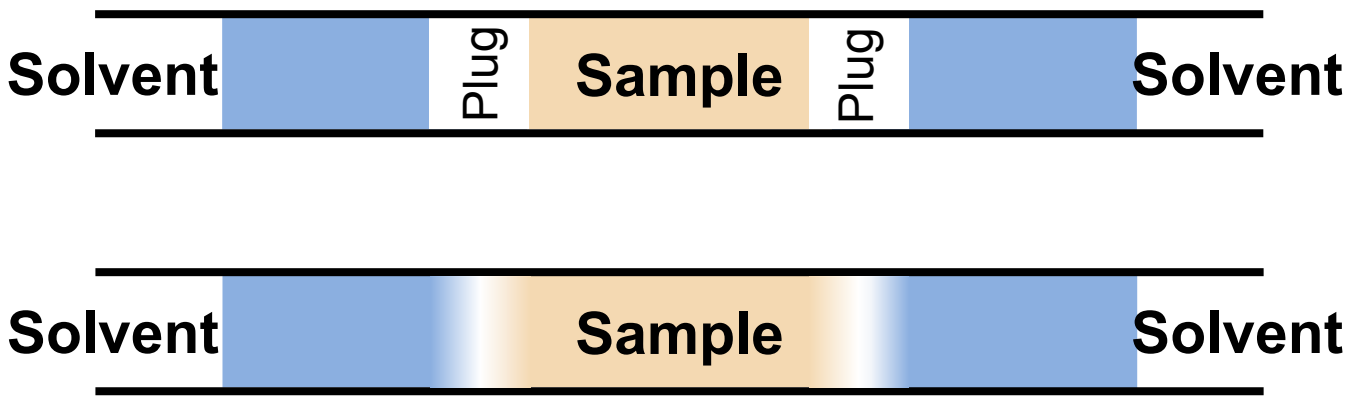
Sample precipitation



Sandwich injection

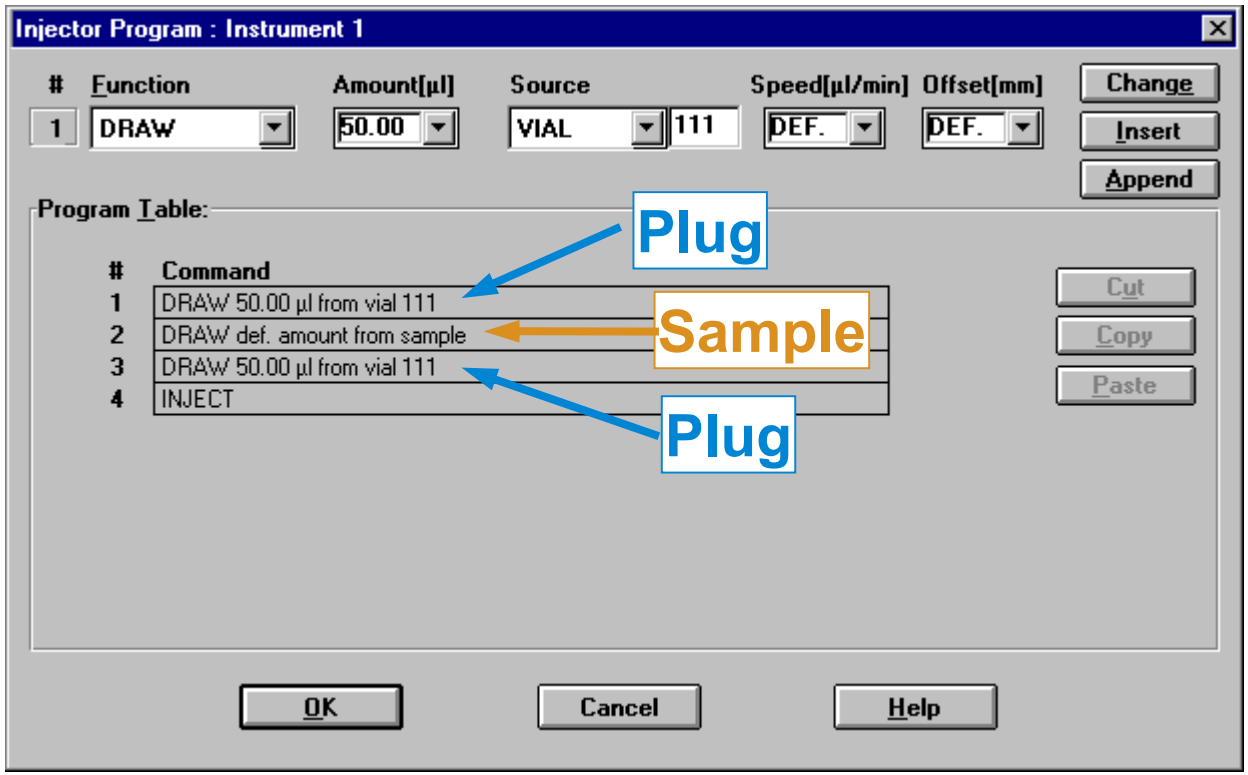
Sandwich Injection:

- Sample is sandwiched between two plugs of the sample solvent.
- Mixing with mobile phase occurs only at the beginning and the end of the plugs, where the sample concentration is zero.

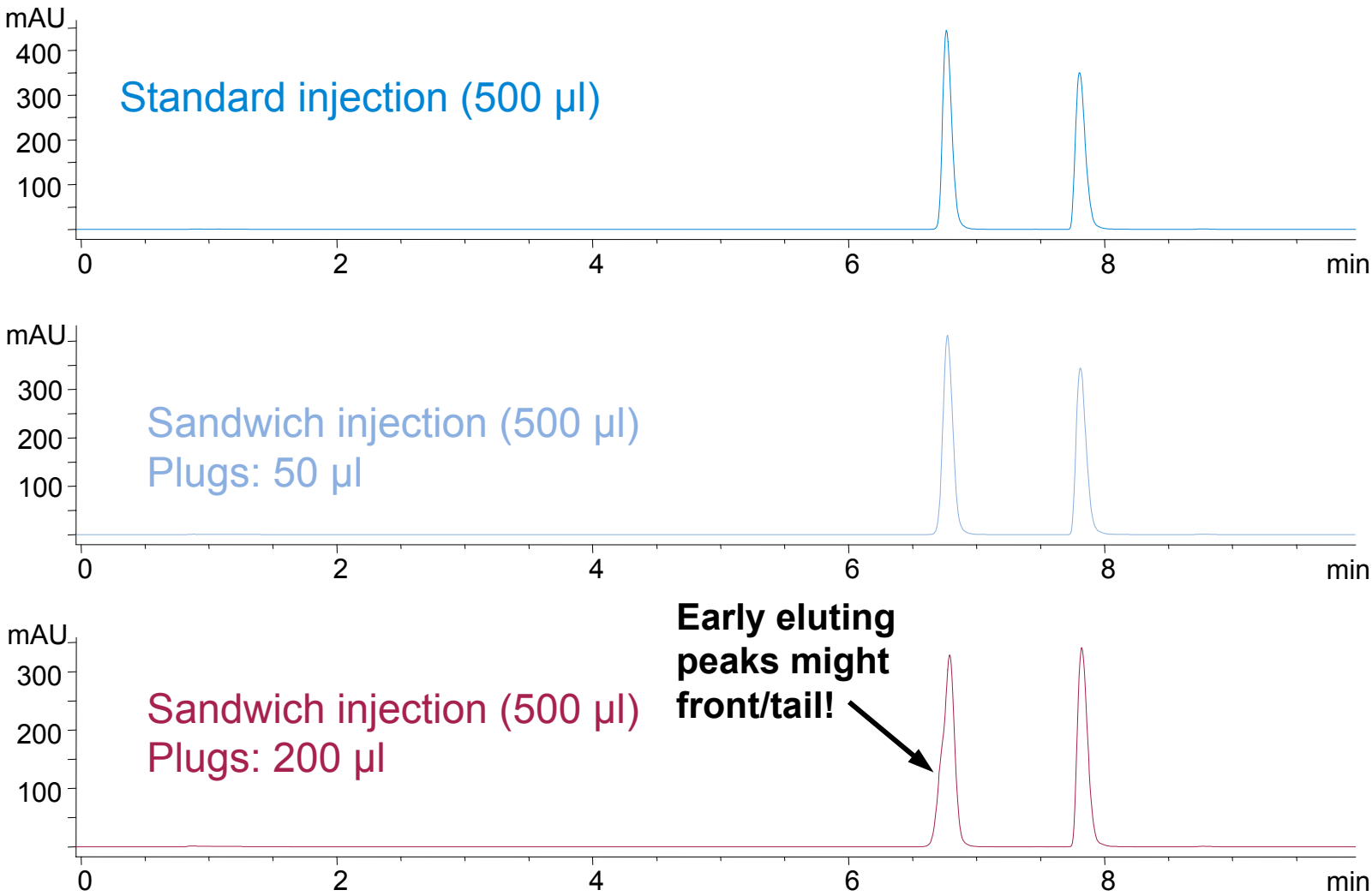


Injector program

- Sandwich is generated using a ChemStation injector program.
- A vial containing the sample solvent must be placed into the injector.



Application example



Advantages of sandwich injection

Advantages of sandwich injection:

- Sample does not come in contact with the mobile phase until it reaches the column. Therefore no precipitation in the critical part of the flow path can occur.

- No hardware re-configuration required. Sandwich injection can be done on a standard system using an injector program.

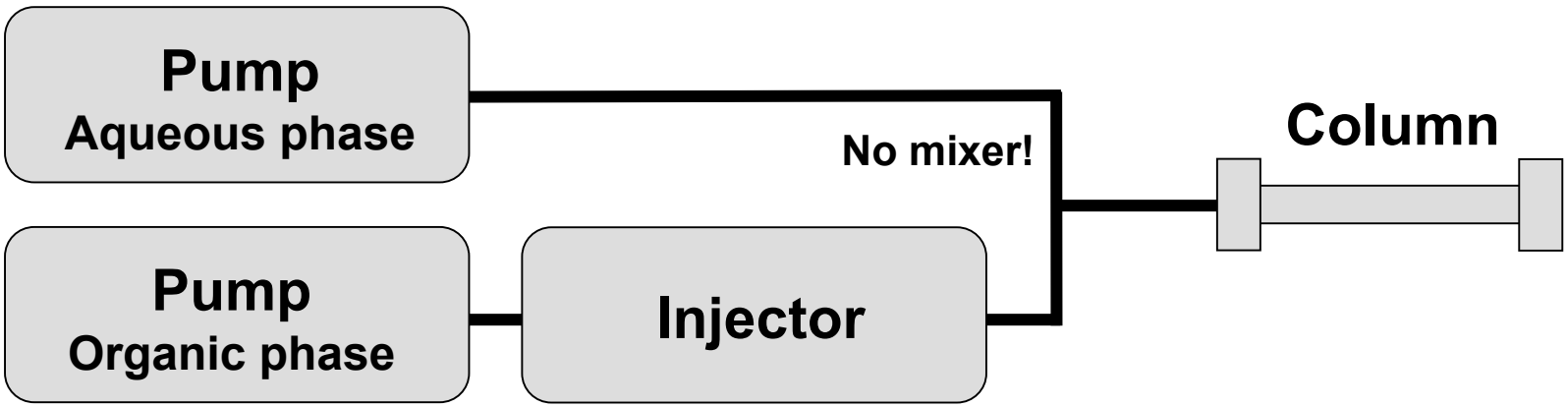
Disadvantages of sandwich injection

Disadvantages of sandwich injection:

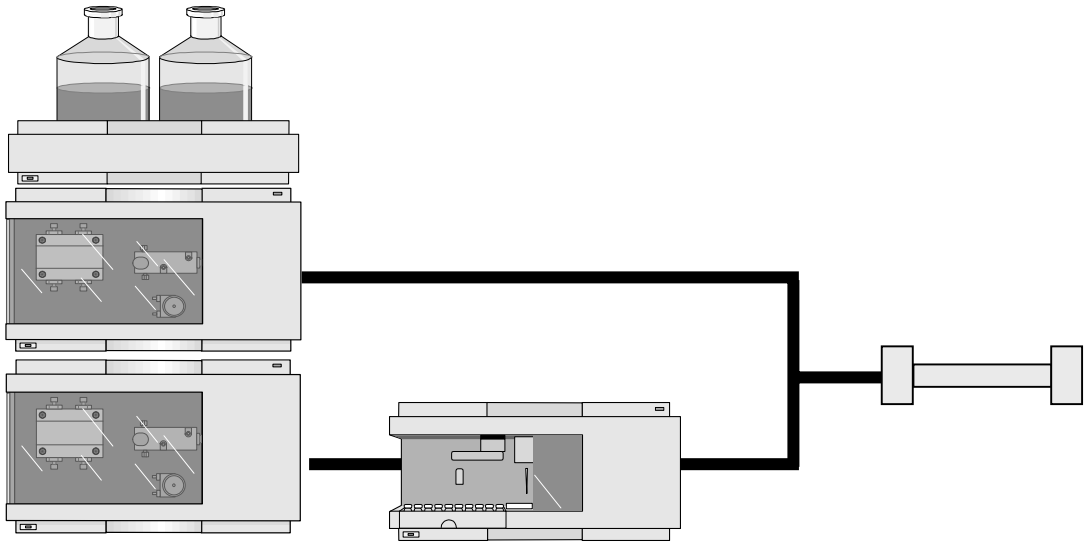
- The sample must be retained on the column in a rather narrow band. The strong sample and plug solvent leads to a significant band broadening of the sample on the column head.
- If the ratio of plug and sample volume compared to the column volume is too large some of the compound can elute with the solvent front as column breakthrough.
- The plug and sample solvent might disturb the equilibrium of the stationary phase.

Organic phase injection

- Sample is injected onto the column using only the organic phase.
- Mixing with aqueous phase occurs on the column (in the T-piece shortly before the column).

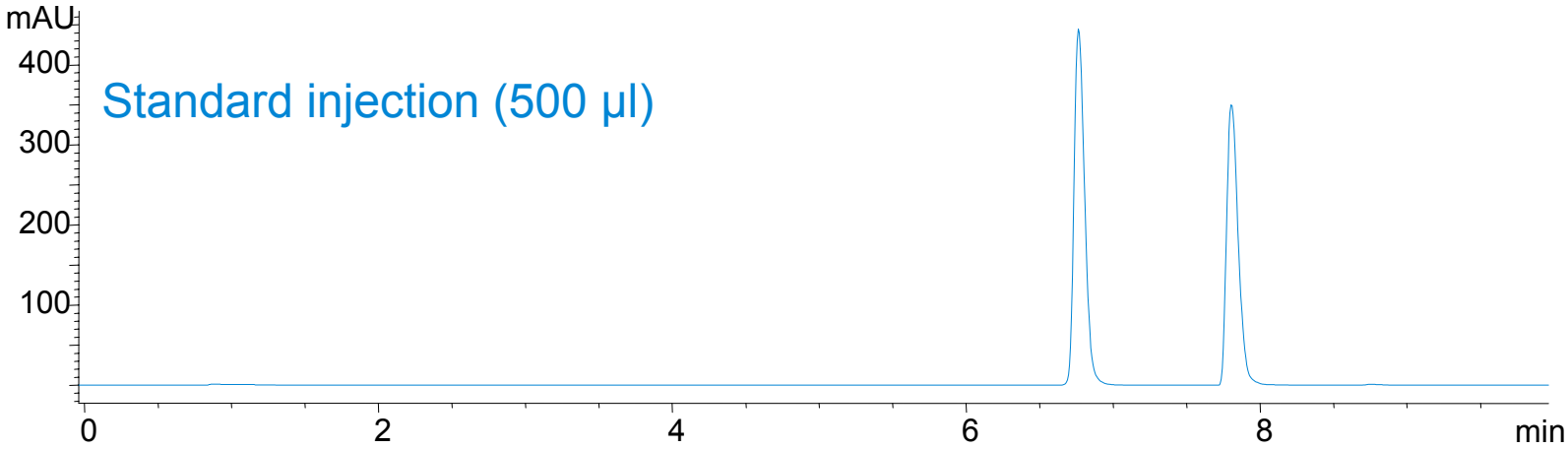


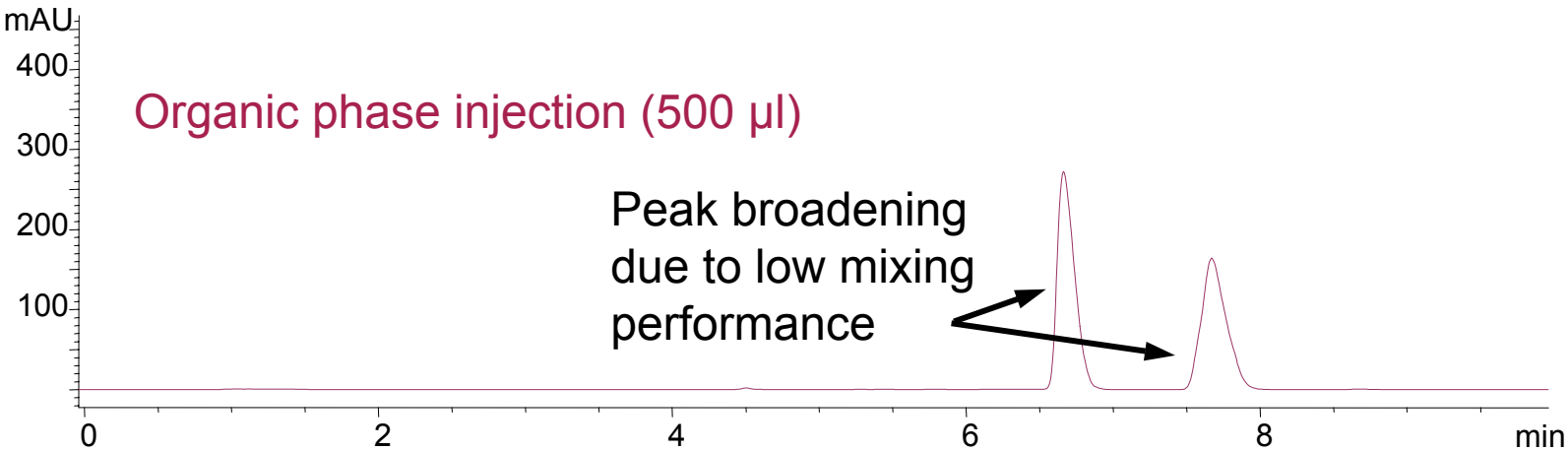
Organic phase injection



- Due to the design of our preparative pump, two isocratic pumps form a gradient pump, the system setup is very simple.
- Since the two physical pumps are treated as one binary pump in the software gradient programming is very easy (no flow-gradient programming)!

Application example





Advantages of organic phase injection

Advantages of organic-phase injection:

- No strong sample solvent is required, which could lead to additional peak broadening or column breakthrough.

- Since the Agilent 1100 Series preparative pump in the gradient version consists of two physical pumps anyway the gradient programming is very easy. It can be set up in the same way as for the standard configuration, no flow gradient programming is necessary.

Disadvantages of organic phase injection

Disadvantages of organic-phase injection:

- Sample precipitation can still occur in the mixing tee or on the head of the pre-column. While precipitation on the column head usually leads only to an increased pressure clogging in the mixing tee is critical.

- Setting up the purification system for organic-phase injection requires re-plumbing of the system. That means it is not possible to perform organic-phase and standard injection on the same system without hardware changes.

Which technique should be used?

Sandwich injection:

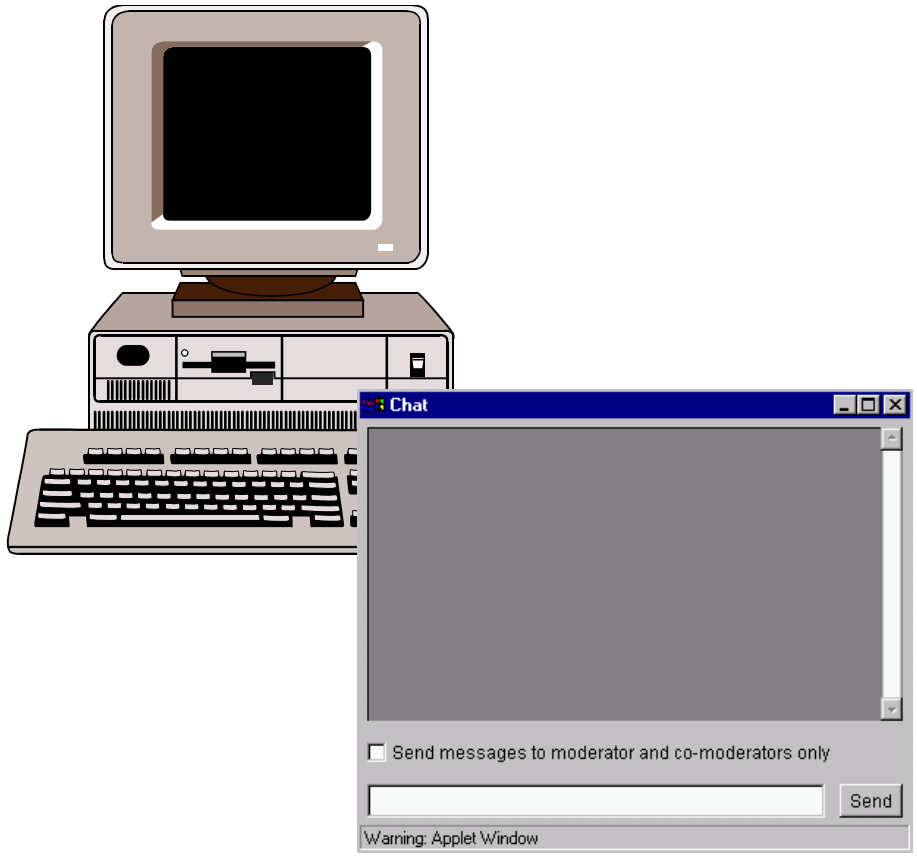
- For highly-concentrated samples where chromatographic performance is not critical.

Organic phase injection:

- For medium-concentrated samples where good chromatographic performance is required.

Break Number 2

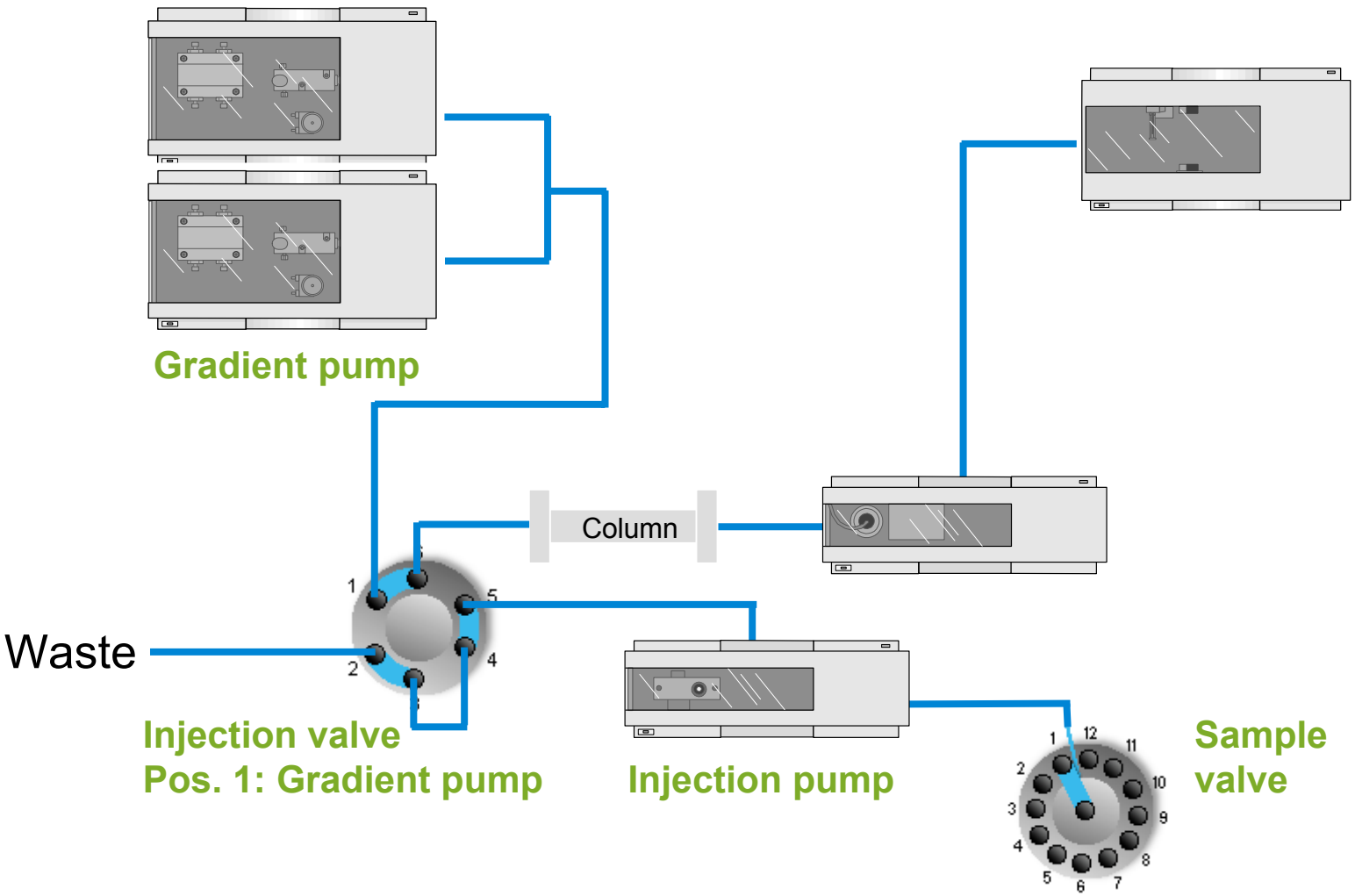
Please type your question into the Chat Box at any time during the presentation.



High-volume samples

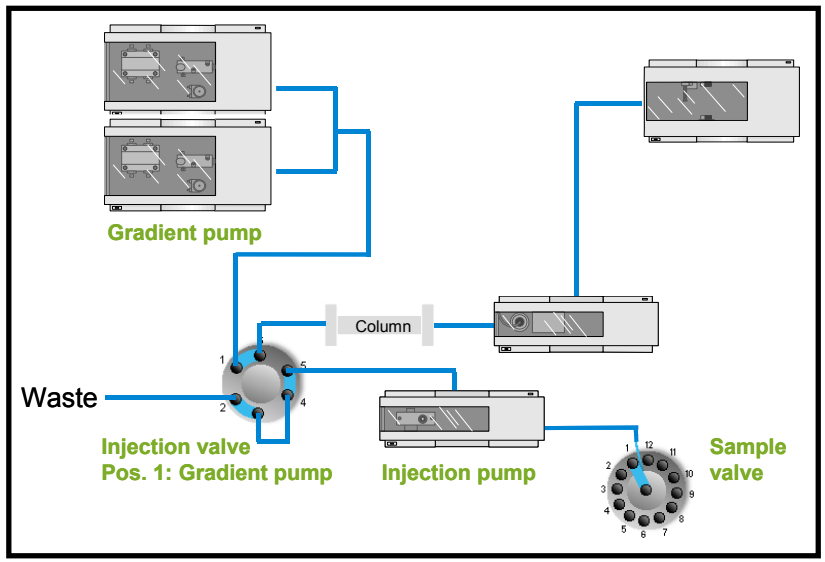
- The usage of our autosampler for sample injection is limited to 5 ml. This is OK for most preparative work on 1 inch columns and flow rates of 20 - 35 ml/min.
- Especially for larger columns (but also for 1 inch columns) higher sample volumes have to be injected. Therefore usually an injection pump is used.
- In our system an isocratic (preparative) pump and a 2-position/6-port valve can be used as injection pump.
- Basically two approaches: Pump fills an injection loop, which is then switched into the flow or direct pumping of the sample onto the column. Second approach is favored.

System configuration



Operation

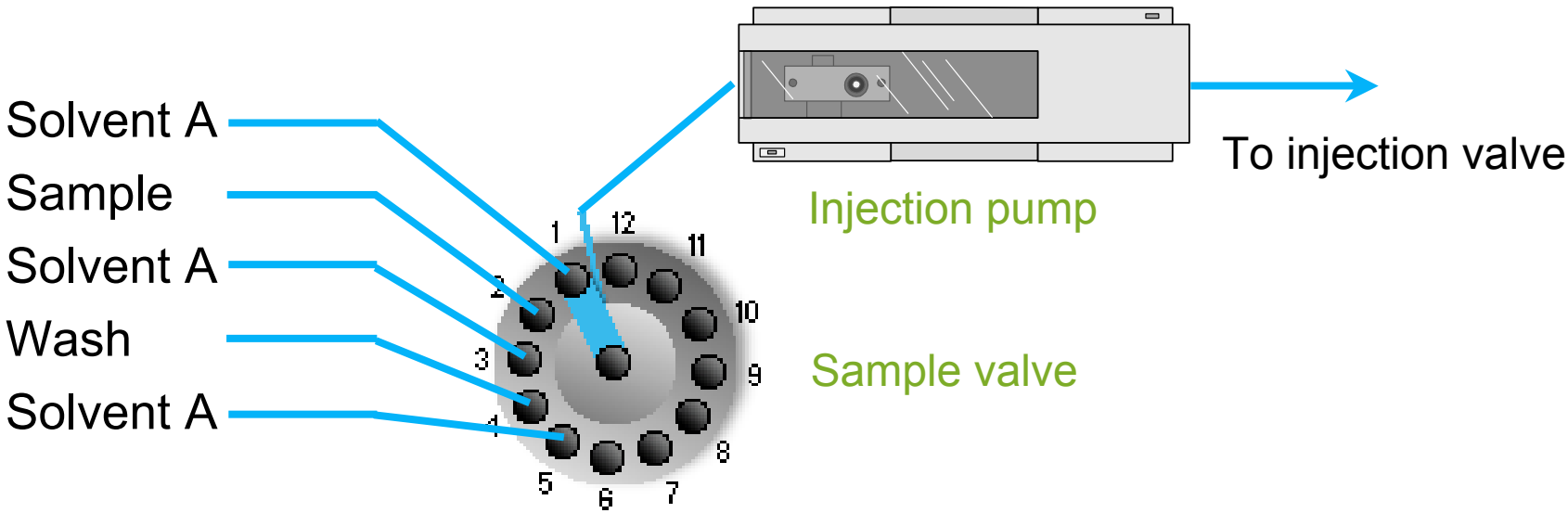
1. At the start of the run the injection valve is in position 1, in which the flow from the gradient pump goes to the column and the flow of the injection pump goes to waste.
2. When the run is started the injection valve switches to position 2 where the flow from the injection pump goes to the column. The injection pump transfers the sample onto the column.



3. When the sample is applied to the column the injection valve switches back to position 1 and the gradient is started. During the run the injection pump and the injection valve are washed with an appropriate solvent and are then re-equlibrated.

Sample injection system

- Pos. 1: Starting position, Solvent A gradient start composition
- Pos. 2: Sample
- Pos. 3: Solvent A to flush sample completely onto column
- Pos. 4: Wash solvent to rinse injection valve and injection pump
- Pos. 5: Solvent A to remove wash solvent from inj. pump and inj. valve



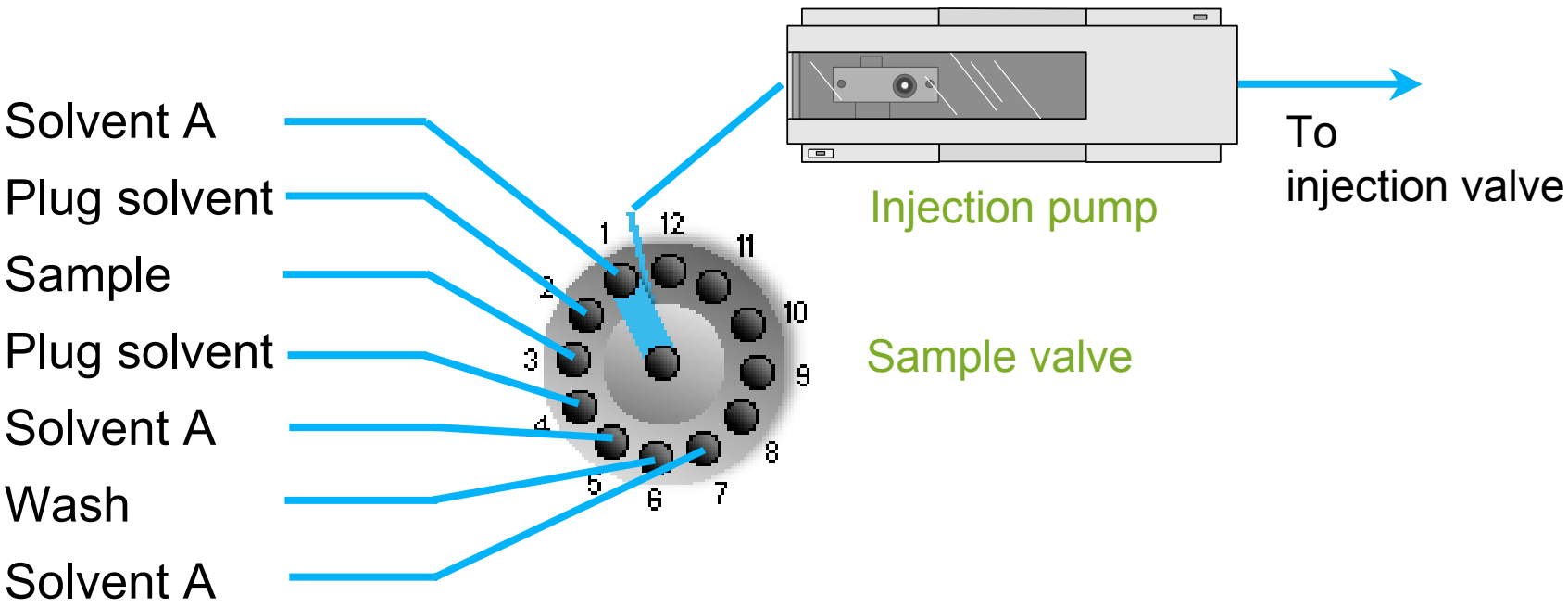
Pumps and valves timetables

Time	Gradient pump		Injection	Injection pump	Sample	Comment
	(% B)	Flow (mL/min)	valve pos.	Flow (mL/min)	valve pos.	
0.0	5	20	1	0	1	Starting conditions
0.1	|	20	2	|	|	Injection valve to injection pump pos.
0.2	|	1	|	0	2	Sample valve to sample pos., lower gradient pump flow
0.3	|	|	|	10	|	Start injection pump
* 1.3	|	1	|	|	3	Sample valve to solvent A pos., transfer sample onto column
** 1.8	5	20	1	|	|	Injection valve to gradient pump pos., increase gradient pump flow
1.9	|	|	|	|	4	Sample valve to wash solvent pos.
** 2.4	|	|	|	|	5	Sample valve to solvent A pos., transfer sample onto column
** 2.9	|	|	|	10	|	
3.0	|	|	|	0	|	Decrease flow of injection pump
11	95	|	|	|	|	End of gradient

* timing depends on sample volume to be injected

** timing depends on injection system volume (capillaries, inj. pump, valve)

Sandwich injection with the injection pump system



Scope and limitations

- ChemStation only (A.10.01), system cannot be used with Purify.
- Time-, peak- and mass-based fraction collection
- Injection pump cycle controlled by injection pump and valve time-table.
- Delay volume calibration procedure not available without autosampler.

Scope and limitations

- A blank run must be set up in the ChemStation since there is no autosampler in the system to give a start pulse.
- The run starts not with the injection (switching of the injection valve back to the gradient pump position) but with the start of the injection cycle. Advantage: If compound of interest brakes through the column it is still collected!
- For connecting solvent A, sample and wash solvent to the valve capillaries with rather large diameter (1.5 mm i.d., e.g. from solvent selection capillary kit G1160-68706) must be used. The capillary from the sample to the sample valve should be as short as possible.

Injection of high-concentration and high-volume samples

- **Column loading and overloading**
  - Concentration and volume overloading
  - Limiting factors of column overloading
  - Characteristic peak shapes
- **High-concentration samples**
  - Problems with high-concentration samples
  - Sandwich injection
  - Organic phase injection
  - Advantages and disadvantages of both methods
- **High-volume samples**
  - Configuration of a system with injection pump
  - Sample injection system
  - Sandwich injection with the injection pump