

# How to Fix Common Problems



# Basic Policies for Fixing Problems

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## ■ Elimination method

- "Once you eliminate the impossible, whatever remains, no matter how improbable, must be the truth" - Sherlock Holmes

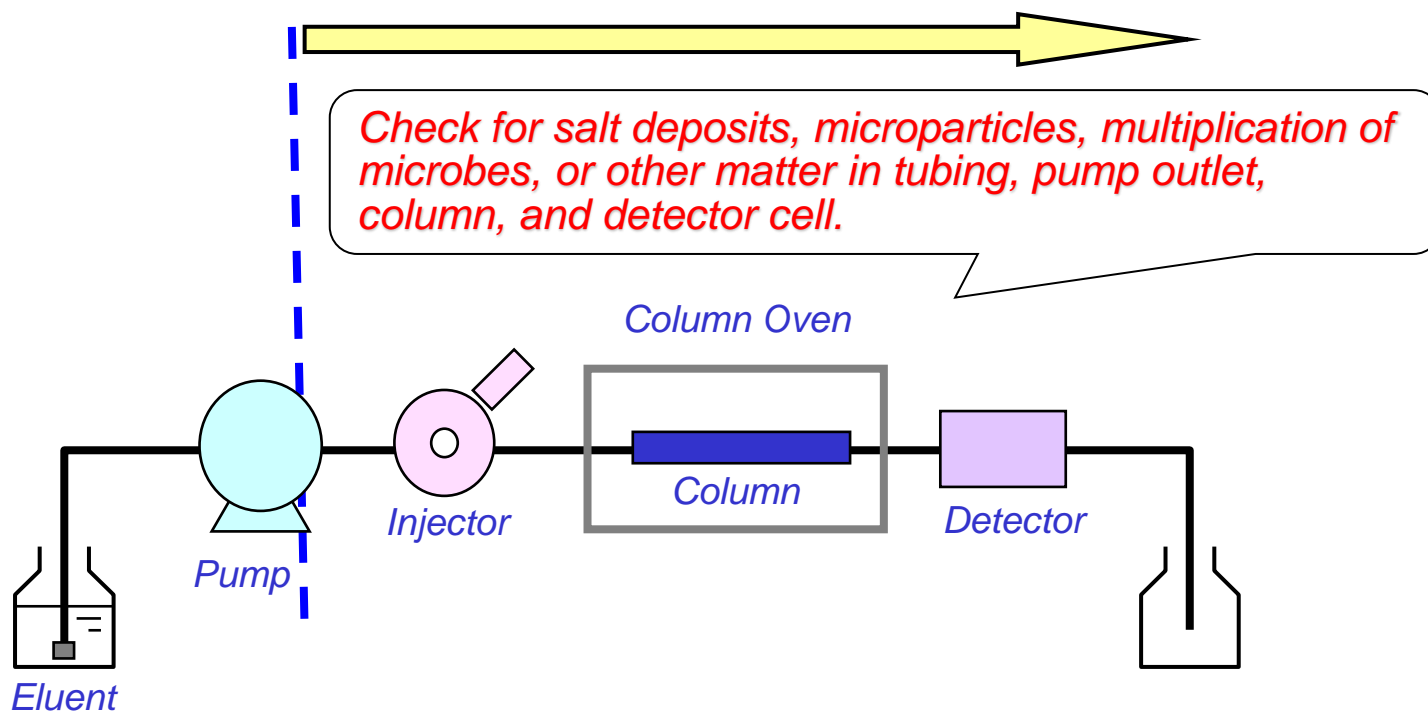
## ■ Order of problem analysis

- Items with a high probability of being a cause
- Items that can be tested easily and cheaply
- Items able to eliminate a large number of possibilities
  - ◆ Such as factors that allow eliminating multiple possibilities by a single verification

# Pressure Increase

# Pressure Increase ... What to Check First

- Check where clogging has occurred
  - Check the change in pressure downstream from the pump head by disconnecting each section, one at a time, starting at the downstream end.



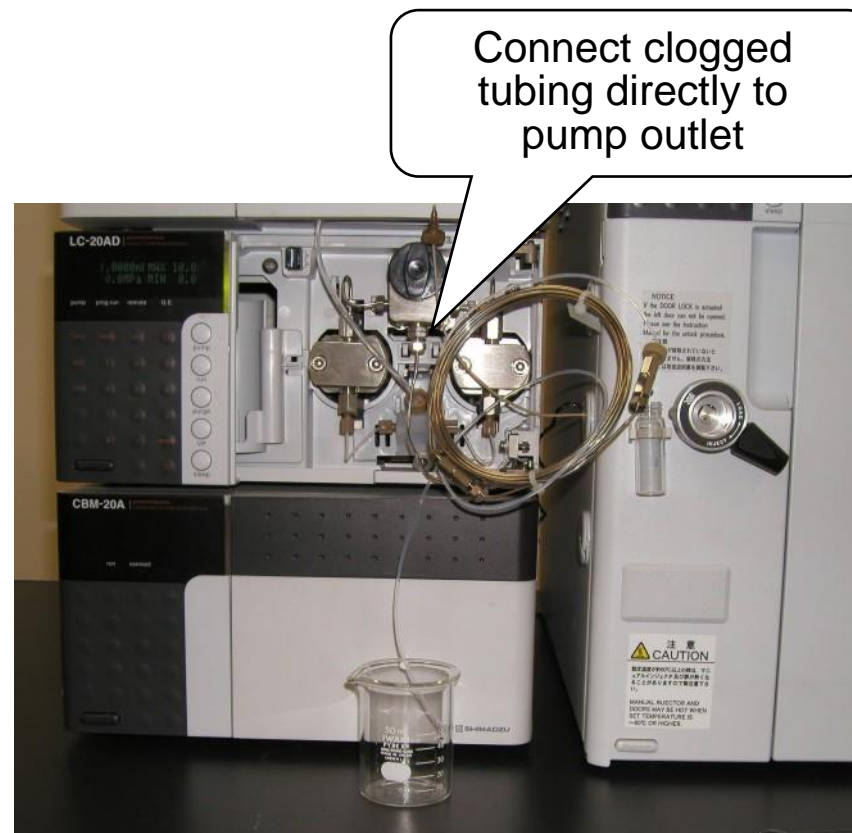
# Clogged Tubing

## ■ Cause

- High probability it is microparticles

## ■ Countermeasure

- Connect opposite end of tubing to pump outlet and flush with water
  - ◆ If necessary, use a high flow rate



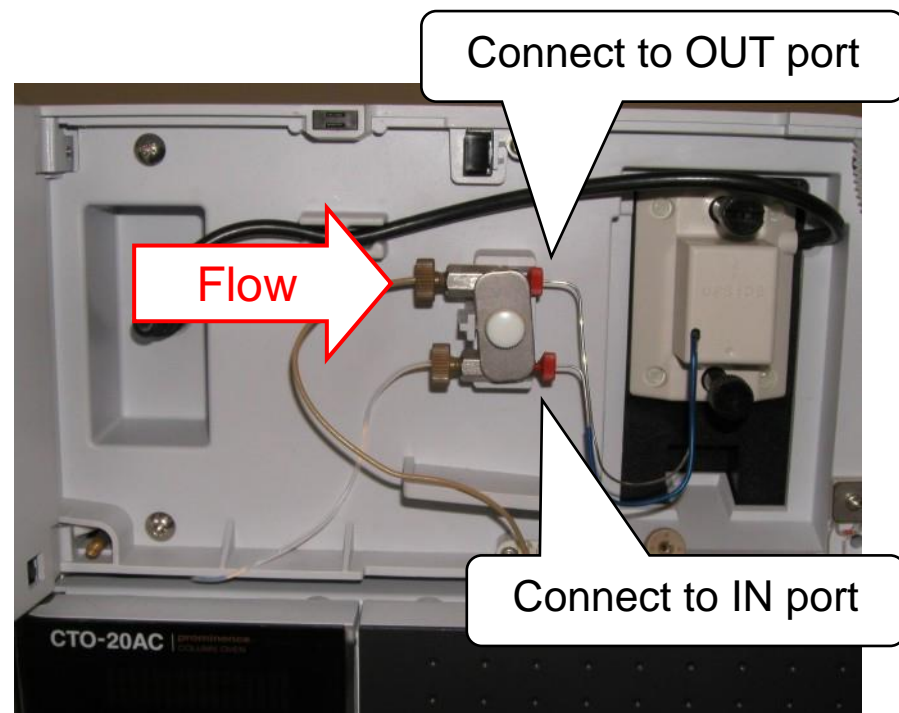
# Clogged Flow Cell

## ■ Cause

- Just like for tubing, high probability of microparticles

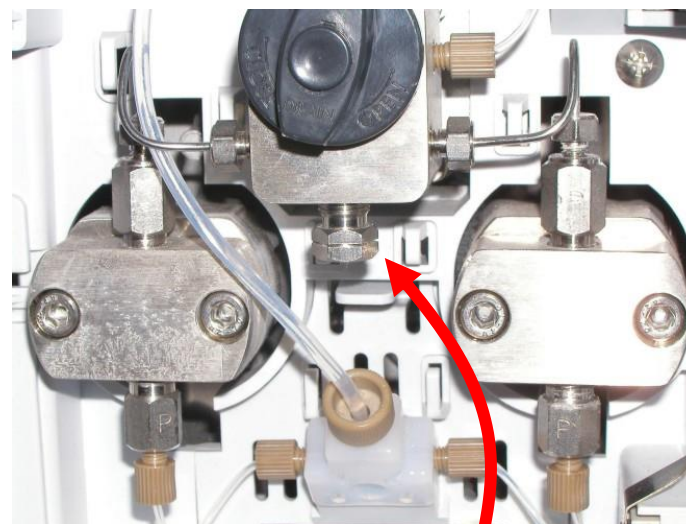
## ■ Countermeasure

- Connect the inlet and outlet in the **opposite direction** and flush with water
  - ◆ Be especially careful of not exceeding the cell pressure capacity



# Clogged In-Line Filter

- Filter (flit) at pump outlet
  - Replace if clogged
- Filters at other locations
  - Filters may be located within the gradient mixer, at the column inlet, at the detector inlet, or other locations.



# Clogged Column

## ■ Likely causes

- Insoluble matter in the sample or eluent
- Adsorption of sample components
- Structural changes, dissolution, or crumbling of packing material

## ■ Countermeasures

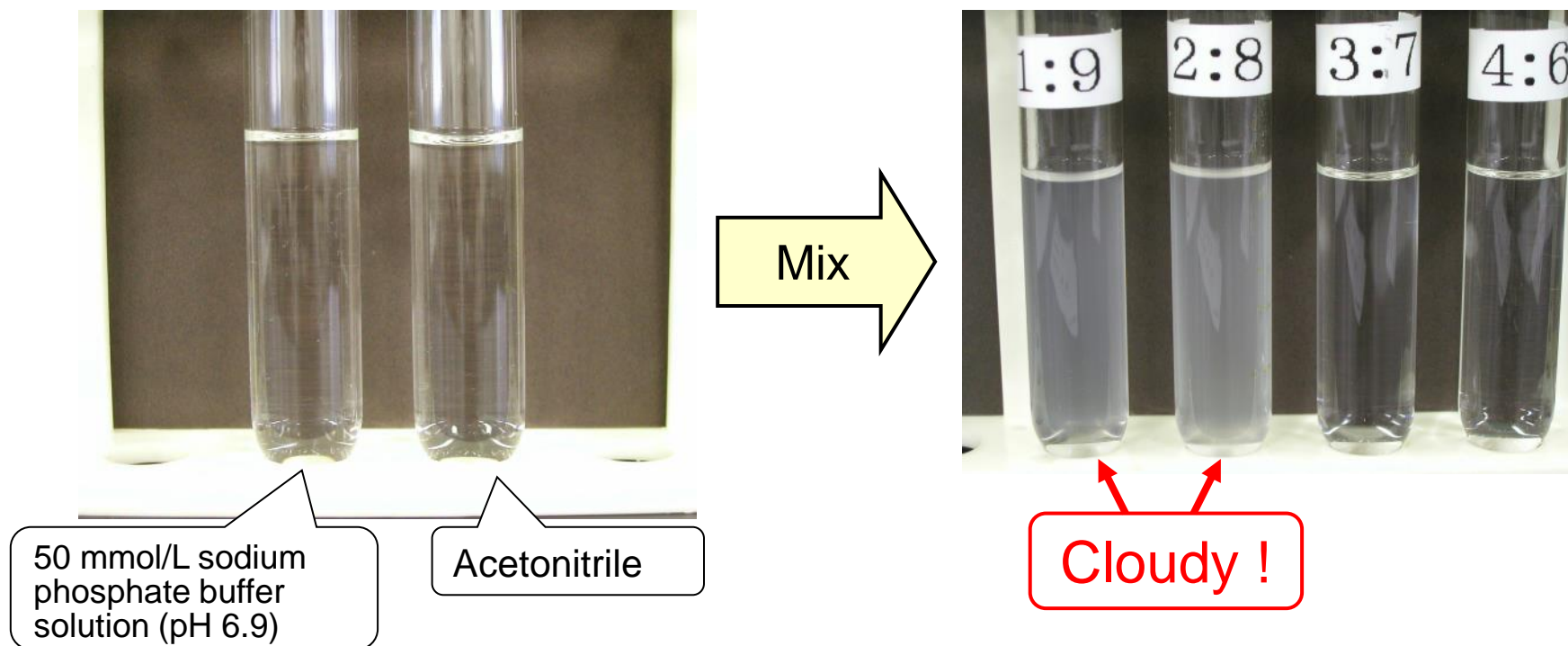
- Flush with rinsing solvent
  - ◆ Connect directly to pump outlet
  - ◆ To select a rinsing solvent, refer to the column instruction manual
- Other methods described in the column instruction manual
  - ◆ Flush in opposite direction as analysis
  - ◆ Replace the column inlet filter, etc.





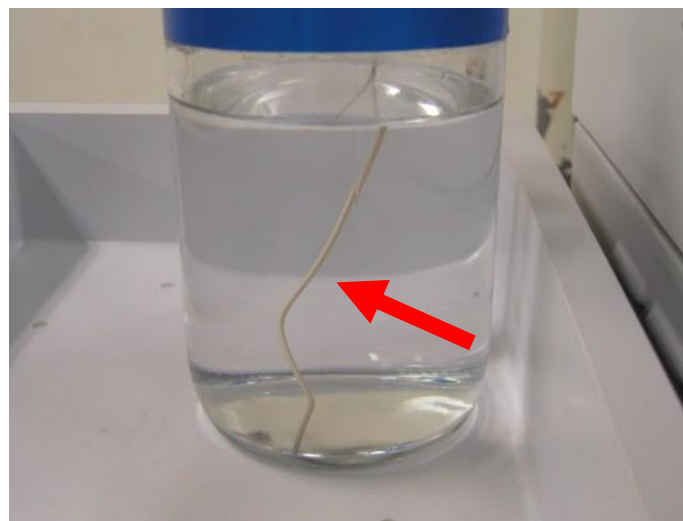
# Preventing Clogging (1)

- Avoid precipitation of salts due to mixing buffer solutions and organic solvents
  - Be especially careful when using gradient analysis
  - Test the mixture in advance to ensure it does not precipitate



## Preventing Clogging (2)

- Avoid precipitation of salts due to evaporation of solvents
  - Cover bottles (upper photo)
  - Insert the end of the drain tube below the surface of the waste liquid (lower photo).
  - If not operating the HPLC system for more than a few days, purge the flow lines with a salt-free solvent.



# Preventing Clogging (3)

- Presence of microparticles
  - Eliminate insoluble substances contained the solvent
    - ◆ Filtration or centrifugal separation
  - Eliminate insoluble substances contained the eluent
    - ◆ Filtration
- Multiplication of microbes
  - Be especially careful using aqueous solutions that do not contain organic solvents.
  - Do not store for long periods. Store refrigerated in airtight containers
    - ◆ Beware of salt precipitation due to cooling
  - Do not simply add more to replenish solvent



# Things to Keep in Mind on a Daily Basis

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## ■ Eluents

- Filtrate if salt concentration is high
- Do not leave salts within flow lines when the instrument is not in use
- Use up perishable eluents in a short time
- Note the purity of solvents used
  - ◆ For water in particular, make sure the water purifying equipment is maintained properly and purified water is controlled properly
- Deliver eluents at a pressure that allows plenty of spare pressure capacity

## ■ Samples

- Always filtrate actual samples
  - ◆ Separation by centrifuge is also possible
  - ◆ If necessary, use cleanup processes as well, such as deproteinization
- Verify compatibility with eluent
- Rinse the injector after injecting samples with high salt concentrations

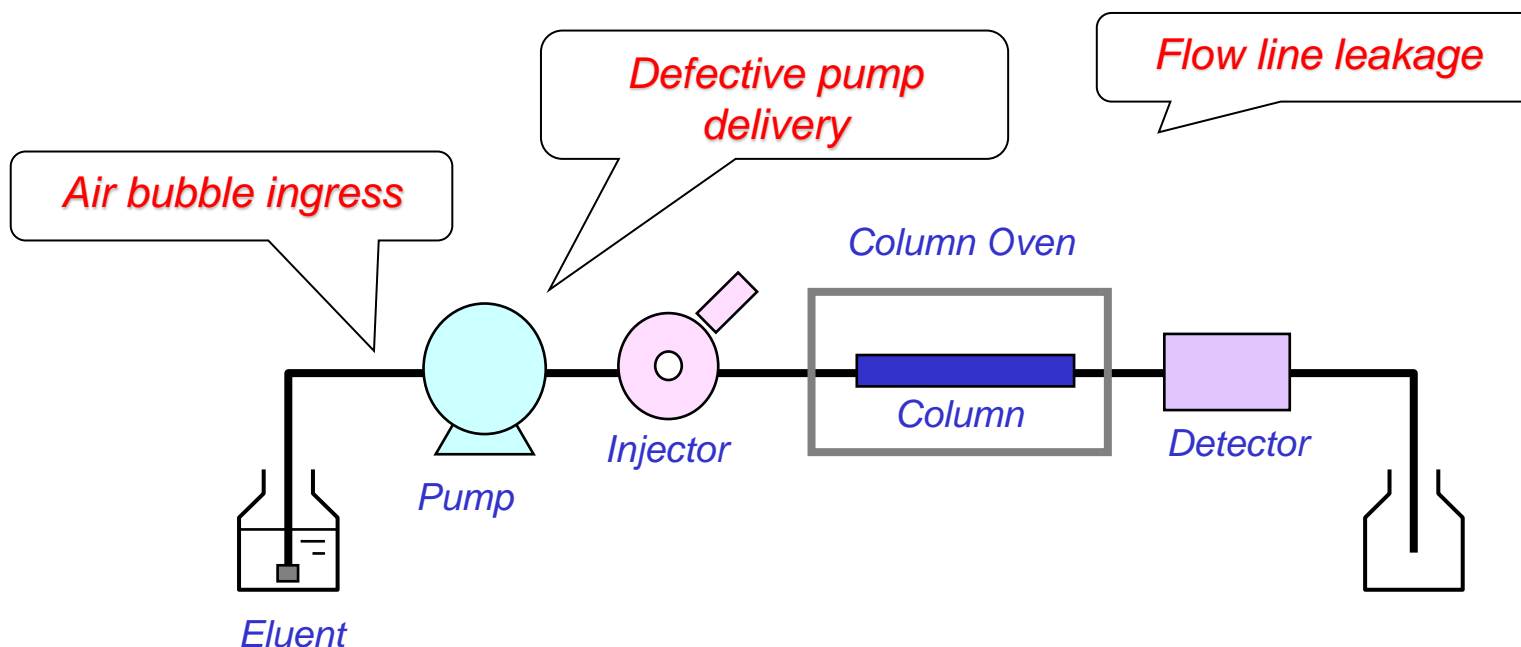
## ■ Instruments

- Always keep replacement filters available
- Keep a record of normal pressures

# Pressure Fluctuation

# Pressure Fluctuation ... What to Check First

- First suspect air bubbles inside the pump
  - Check also for pump delivery problems



# Ingress of Air Bubbles into Pump

- Remove air bubbles
  - Attach a syringe to the drain tube and draw out the air bubbles while purging



# Clogged Suction Filter

## ■ What to check

- Use particular caution if air bubbles are found inside the suction tube
- Temporarily remove the filter and verify that solvent can be delivered normally

## ■ Countermeasure

- Rinse or replace the suction filter
  - ◆ To rinse, immerse in appropriate solvent and clean in an ultrasonic cleaner



Suction filter



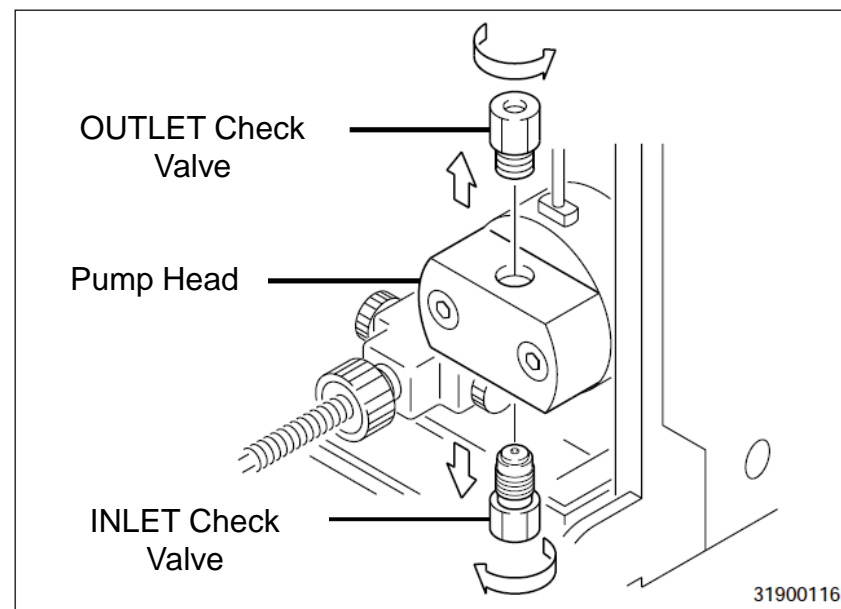
# Defective Pump Delivery (1)

- Countermeasure (1) -  
Rinse inside the pump
  - Rinse by pumping  
2-propanol for a while
    - ◆ Deliver solvent by  
connecting a back  
pressure tube to the pump  
outlet



# Defective Pump Delivery (2)

- Countermeasure (2) - Rinse or replace parts
  - Rinse or replace check valves
  - If necessary, replace the plunger seal

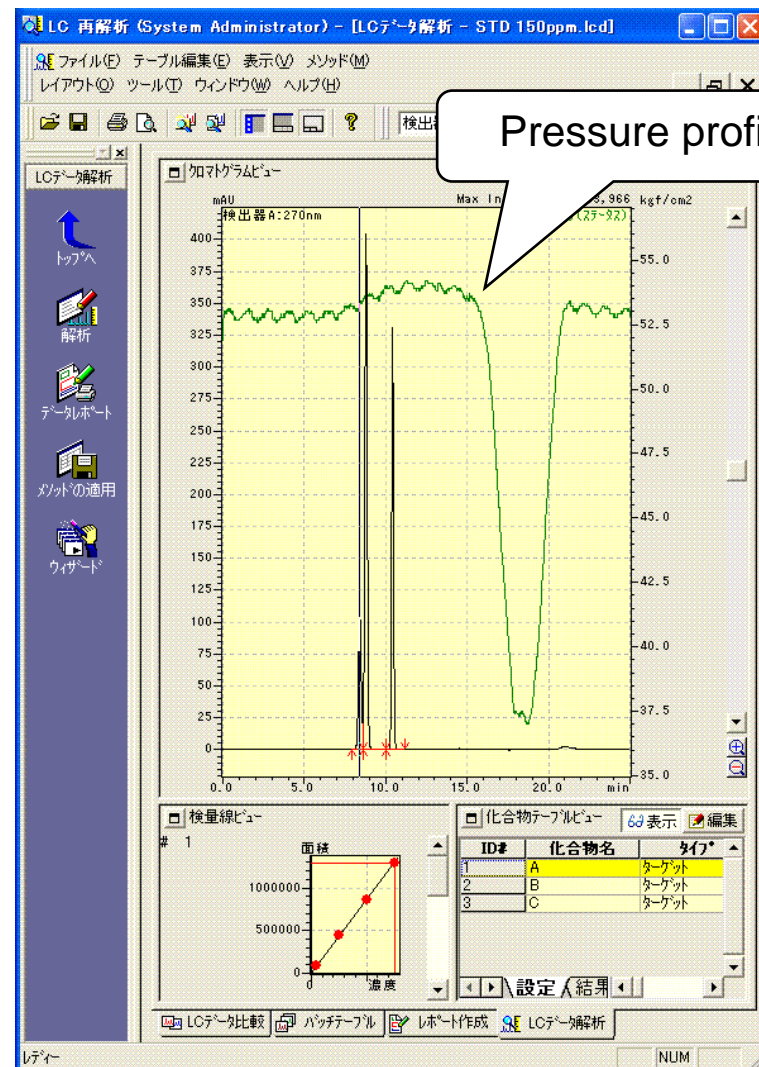
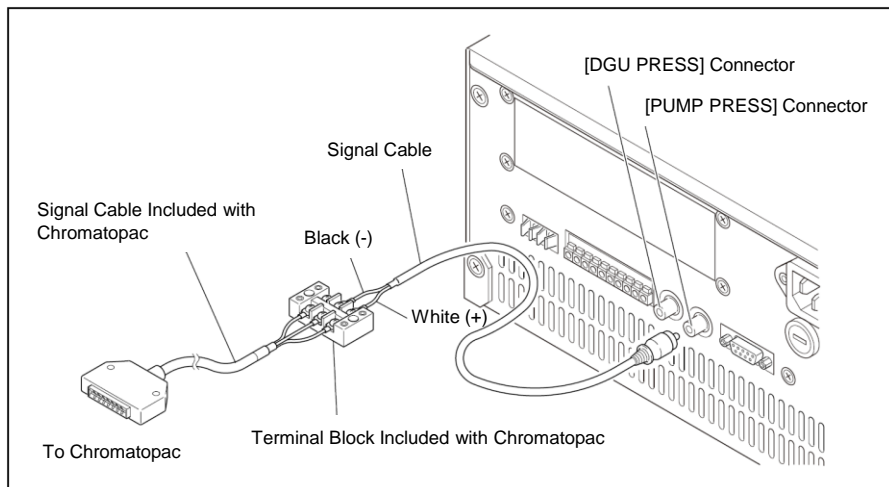


Check valves



# Check the Pressure Profile

- Output a pressure profile along with the chromatogram
  - If using Workstation, output it in the software window (right)
  - From the pump, output as an analog signal (below)



# Things to Keep in Mind on a Daily Basis

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## ■ Air bubbles

- Degas online
- Place the eluent reservoir higher than the pump
- Keep room temperature as constant as possible
  - ◆ Avoid air bubbles generated due to changes in solution temperature

## ■ Pump

- Always keep replacement consumable parts available
  - ◆ Plunger seals
  - ◆ Check valves, etc.
- Keep a record of usage
  - ◆ Particularly column back pressure

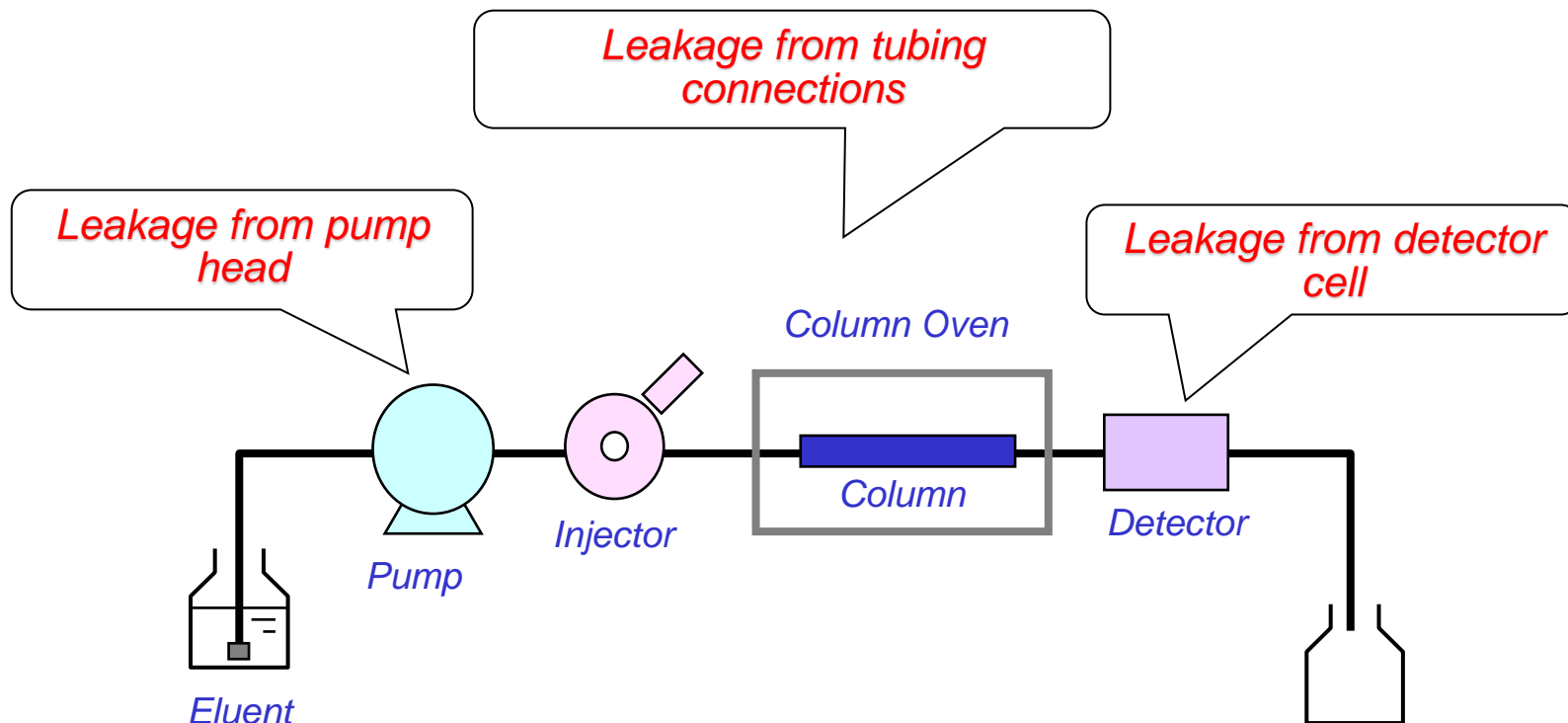
## ■ Eluents

- Displace solvents with miscible solvents
  - ◆ Check valves are especially prone to malfunction when displacing aqueous solutions containing inorganic salts with a 100 % organic solvent

# Flow Line Leakage

# Flow Line Leakage ... What to Do First

- Verify the leak location
  - Wipe up any leakage and also check for seeping



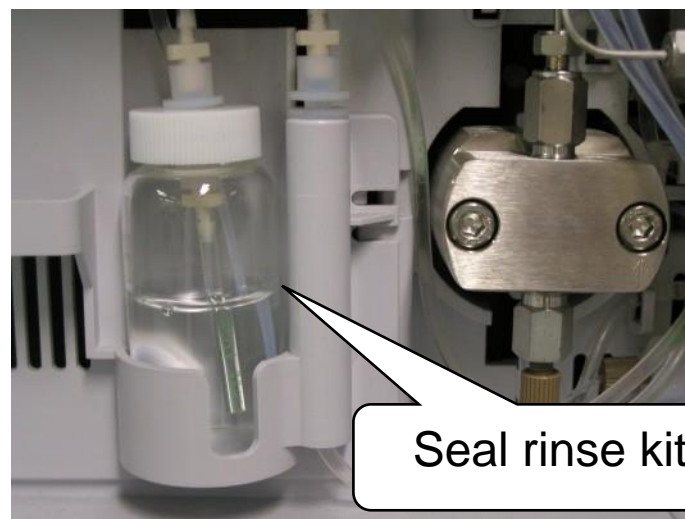
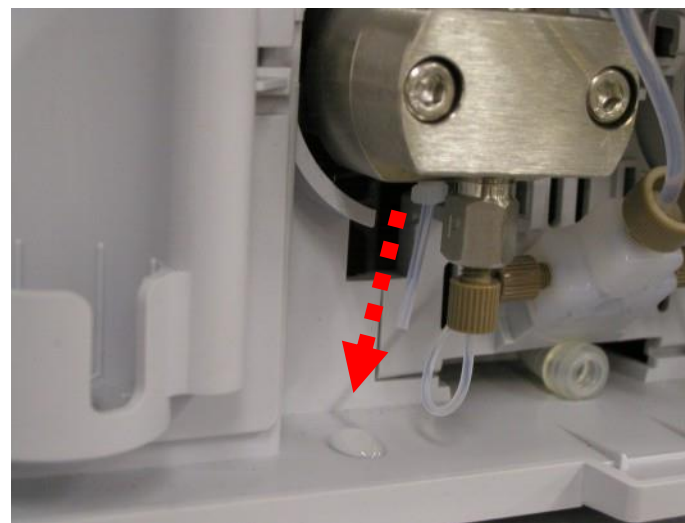
# Leakage from Pump Head (1)

## ■ Phenomena

- Leakage from base area where the head is attached
- Increased water content in seal rinse kit

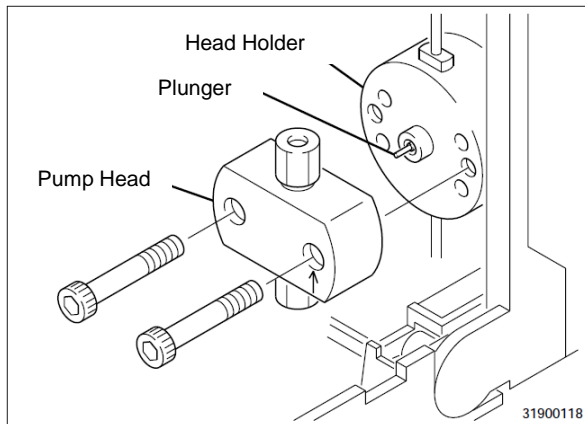
## ■ Cause

- **Deterioration of plunger seal**
  - ◆ In rare cases, the plunger itself can be damaged

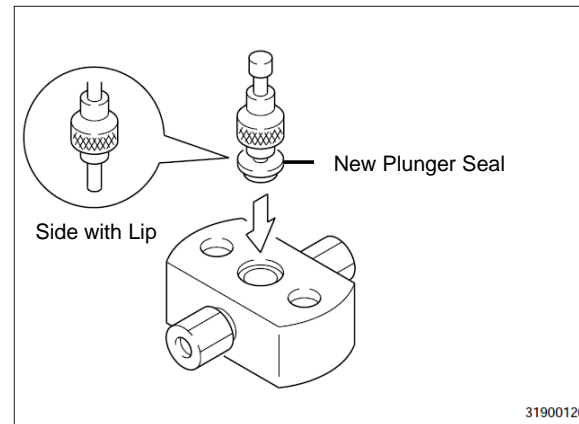


# Leakage from Pump Head (2)

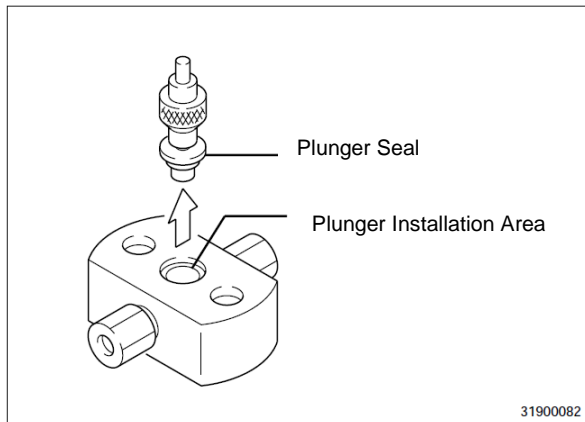
## ■ Replacing the plunger seal



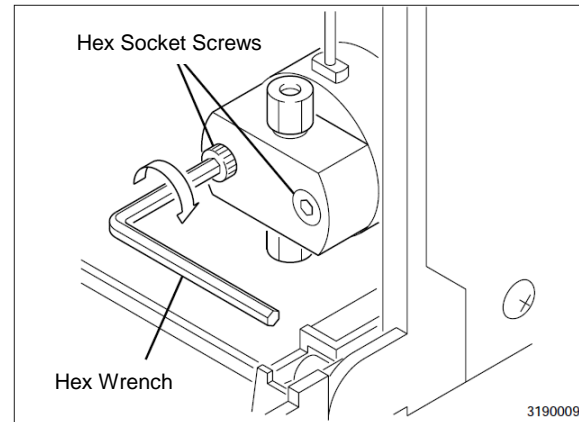
1. Remove the head



3. Install a new seal



2. Remove the old seal

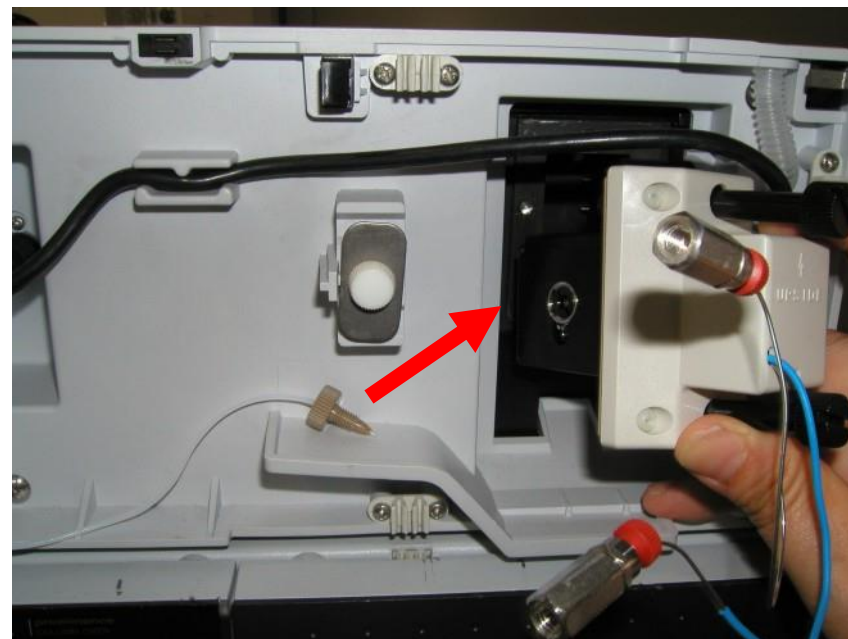


4. Reinstall the head in its original position and reset the delivery volume counter



# Leakage Inside the Detector

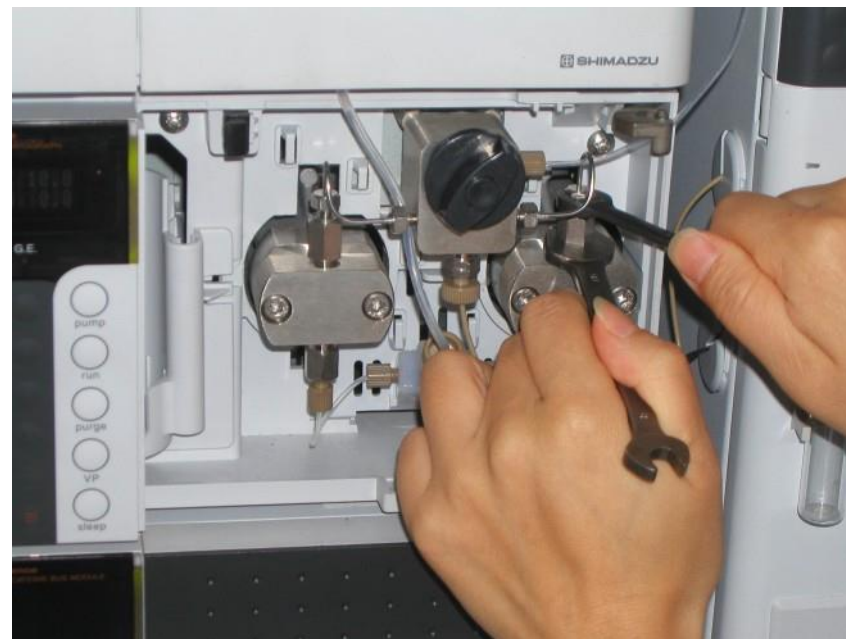
- Absorbance detector and fluorescence detector
  - Remove the flow cell and replace part causing the leak
    - ◆ Be very careful not to break the lens or cell
- Other detectors
  - Follow instructions in the instruction manual



# Leakage from Tubing Connections

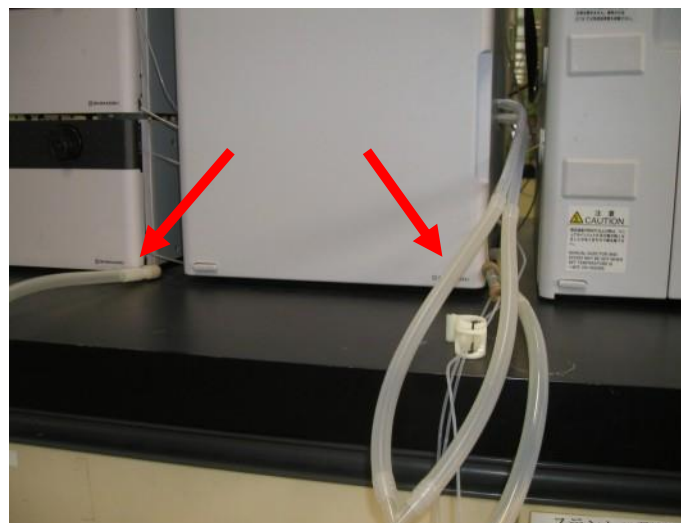
## ■ Countermeasures

- Further tighten connections slightly
- If that fails, replace the joint
  - ◆ Joint parts gradually deform, regardless of the material



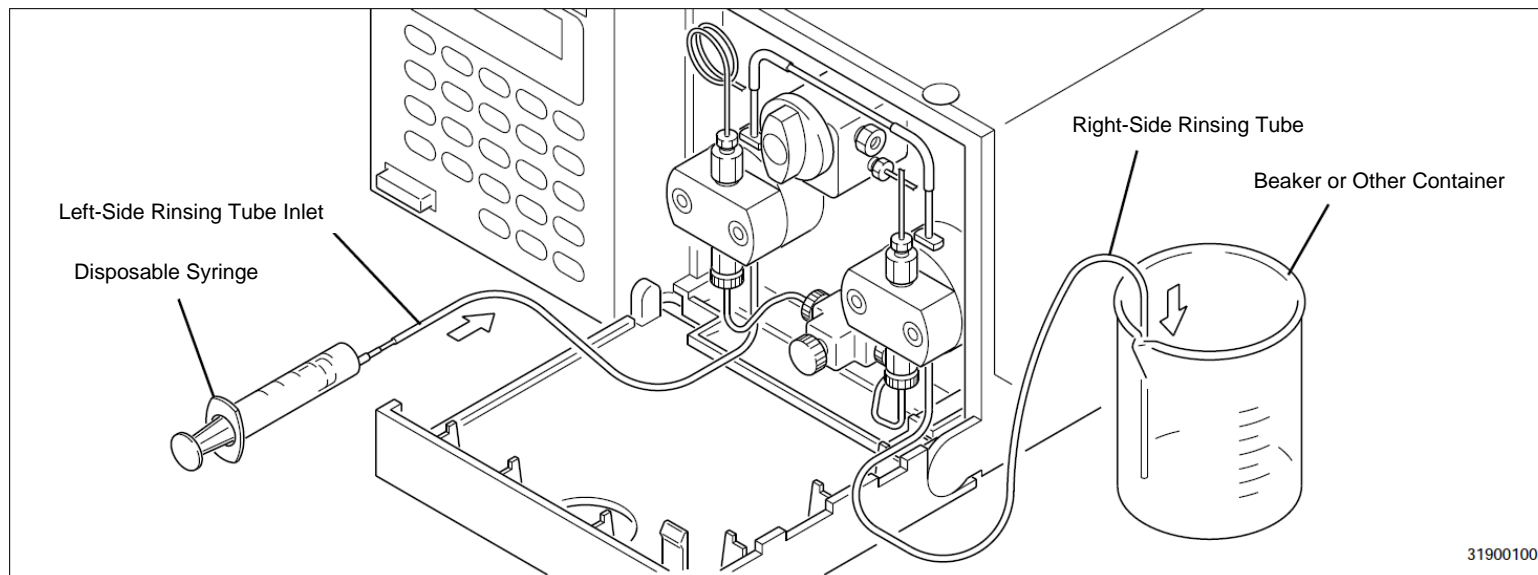
# Things to Keep in Mind on a Daily Basis (1)

- Install a drain tube and tray for leakage
  - Many HPLC systems now include an internal leakage sensor
  
- Always keep replacement parts available, such as plunger seals
  - Some pumps use a delivery volume counter to indicate when to replace parts



# Things to Keep in Mind on a Daily Basis (2)

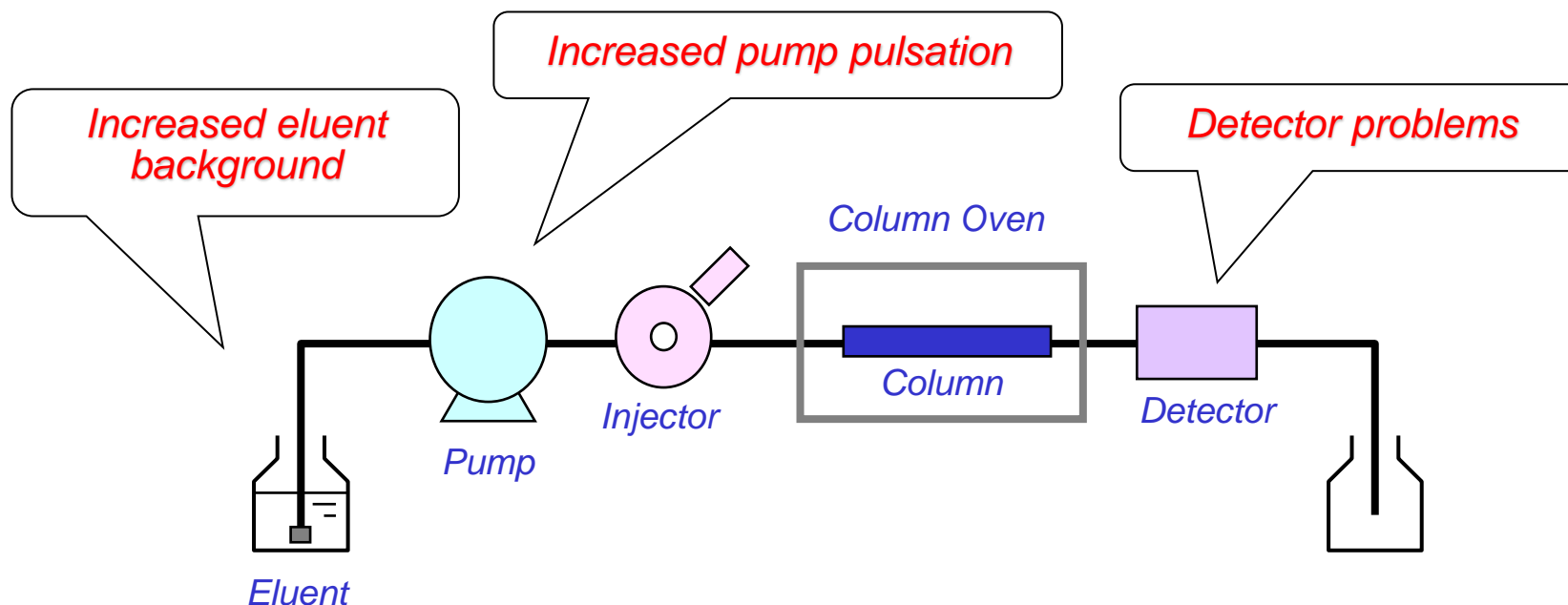
- Rinse plunger seals regularly
  - Automatic rinsing kits are also commercially available



# Noise Increase

# Noise Increase ... What to Do First

- First, verify whether or not the noise cycle matches the pump stroke
  - Also verify how the eluent was prepared and the detector status



# Narrow Down Possible Causes

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- If the noise cycle matches the pump stroke ...
  - Pump pulsation
  - Increased eluent background
- If the noise cycle does not match the pump stroke ...
  - Cause is probably the detector

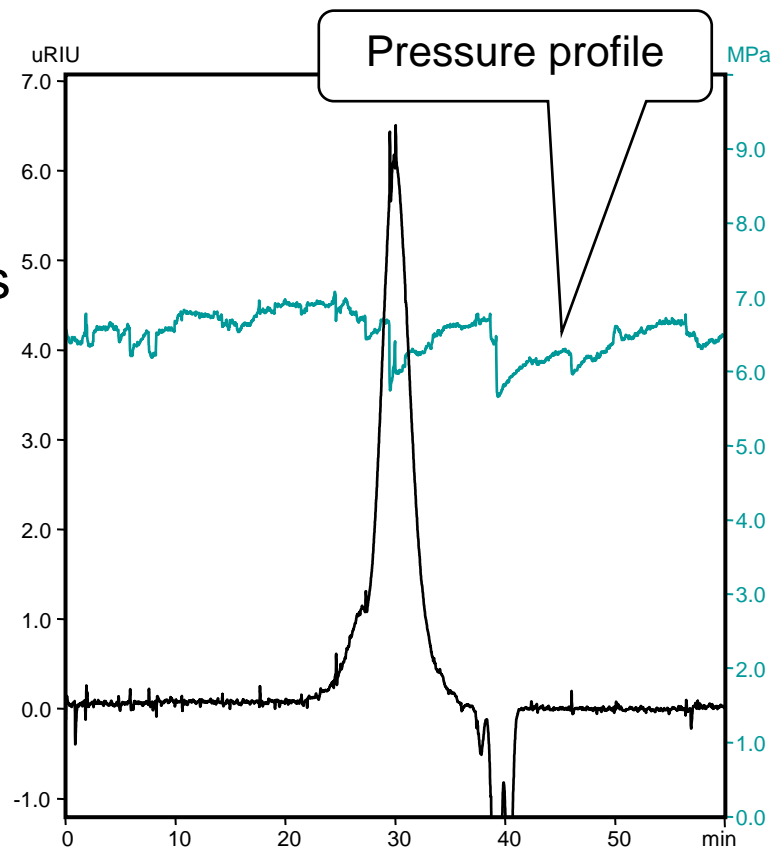
# Pump Pulsation

## ■ Phenomena

- Generally corresponds with fluctuations in pressure

## ■ Countermeasure

- Implement same measures as described in 2. Pressure Fluctuation





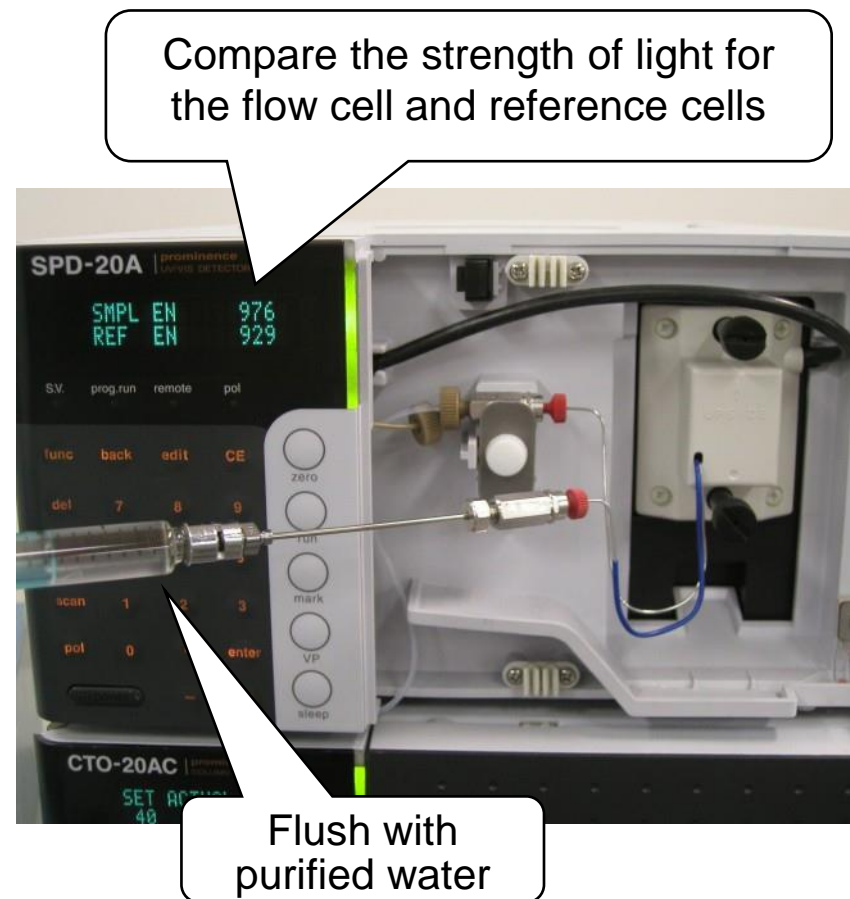
# Increased Eluent Background

## ■ What to check

- For absorbance detectors or electroconductivity detectors, check the background level while bypassing the column

## ■ Countermeasures

- Prepare new eluent and replace reagents
- Rinse flow lines and eluent reservoir



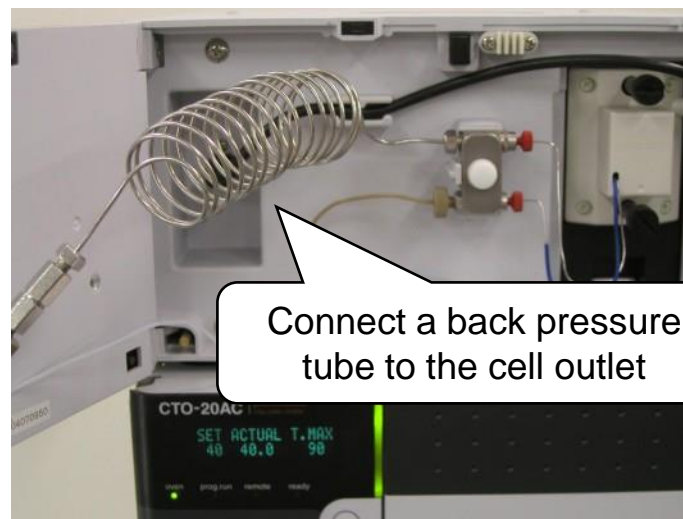
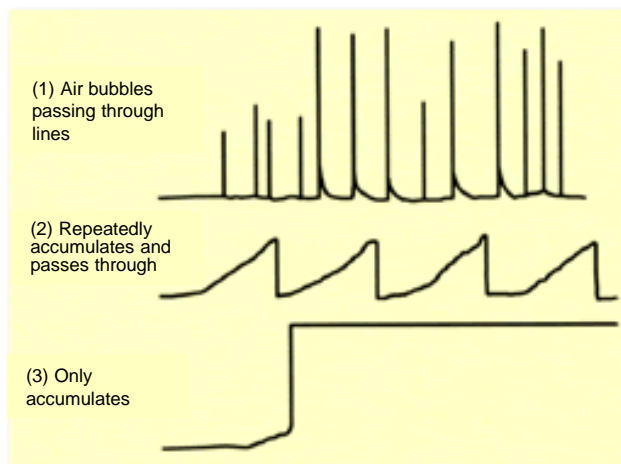
# Ingress of Air Bubbles into the Detector Cell

## ■ What to check

- If a baseline like the one to the right is obtained, suspect air bubbles inside the flow cell

## ■ Countermeasures

- Rinse the cell with organic solvent (such as 2-propanol)
- Connect a tube to the cell outlet and apply back pressure
  - ◆ Use caution, as the cell pressure capacity differs depending on the detector



# Noise Increase from the Detector Itself

## ■ What to check

- If the noise cycle does not match the pump stroke, suspect the detector

## ■ Countermeasures

- Rinse the flow cell using the prescribed method (upper photo)
  - ◆ For evaporative light scattering detectors, rinse the nebulizer
- For photometric detectors (such as absorbance, fluorescence, or evaporative light scattering detectors), replace the light source lamp (lower photo)



# Things to Keep in Mind on a Daily Basis

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## ■ Eluents

- Establish methods for controlling and storing reagents
- Keep water purifier systems maintained
- Always have extra reagent available
- Keep a record of normal background value

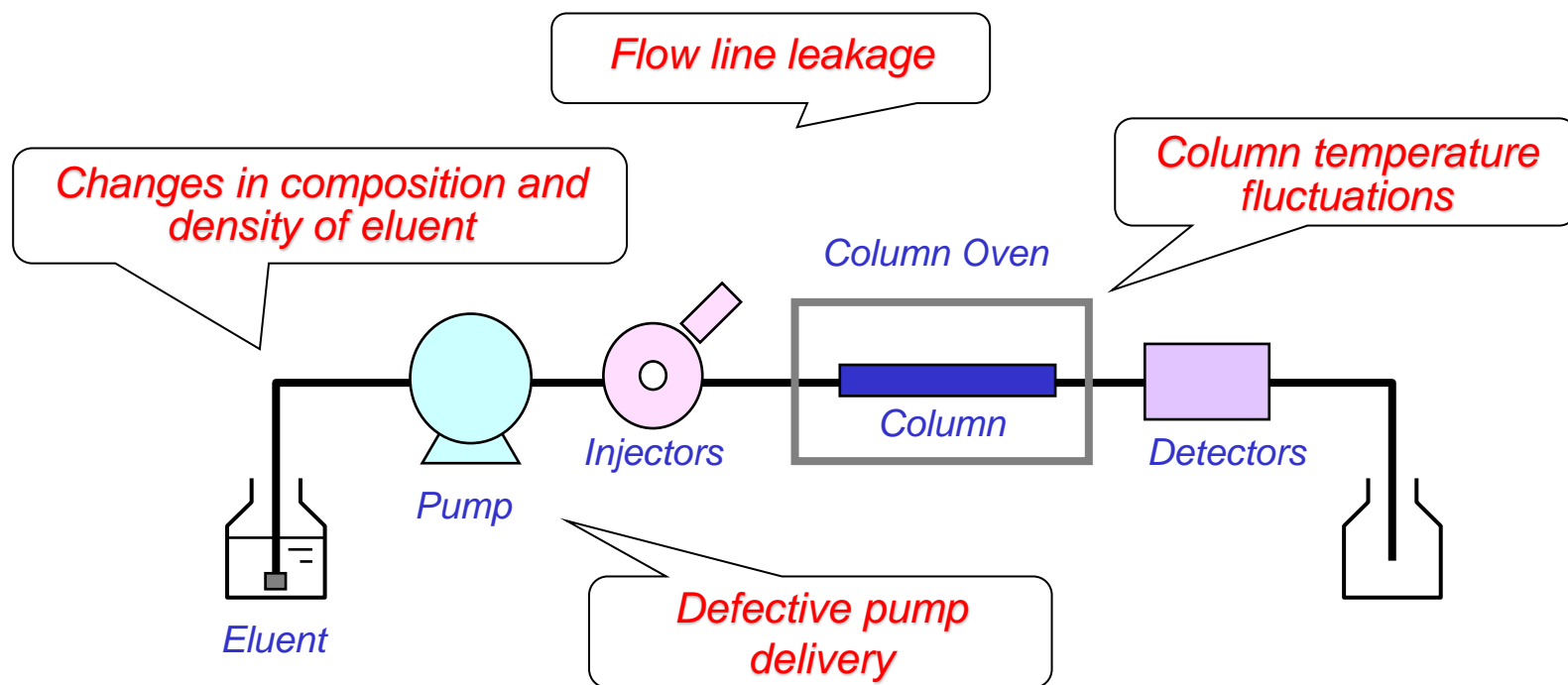
## ■ Detectors

- Keep a record of usage status for consumables and keep an extra supply available
  - ◆ For optical detectors, be aware of the cumulative usage hours for light source lamps
- Keep a record of normal background values and baselines
  - ◆ For absorbance detectors, verify the light strength for sample cells and reference cells

# Retention Time Fluctuation

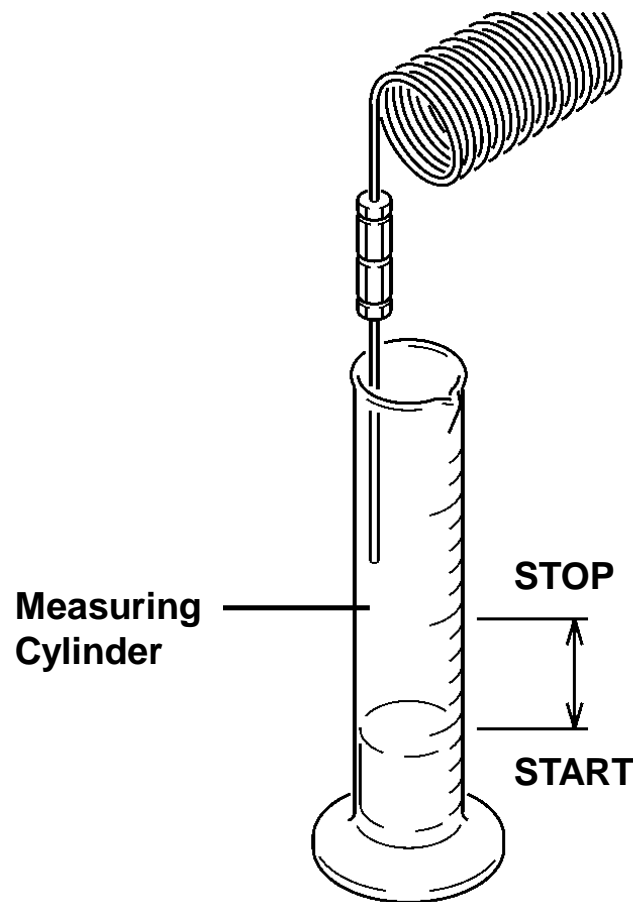
# Retention Time Fluctuation ... What to Do First

- First, check for pump pressure fluctuations and flow rate
  - Also check for room temperature fluctuations and eluent temperature changes



# Flow Rate Is Not Correct

- If pump is not delivering solvent properly
  - To verify flow rate, use a measuring cylinder and stopwatch to measure flow rate
  - For countermeasures, see 2. Pressure Fluctuation
- If flow lines are leaking
  - For countermeasures, see 3. Flow Line Leakage



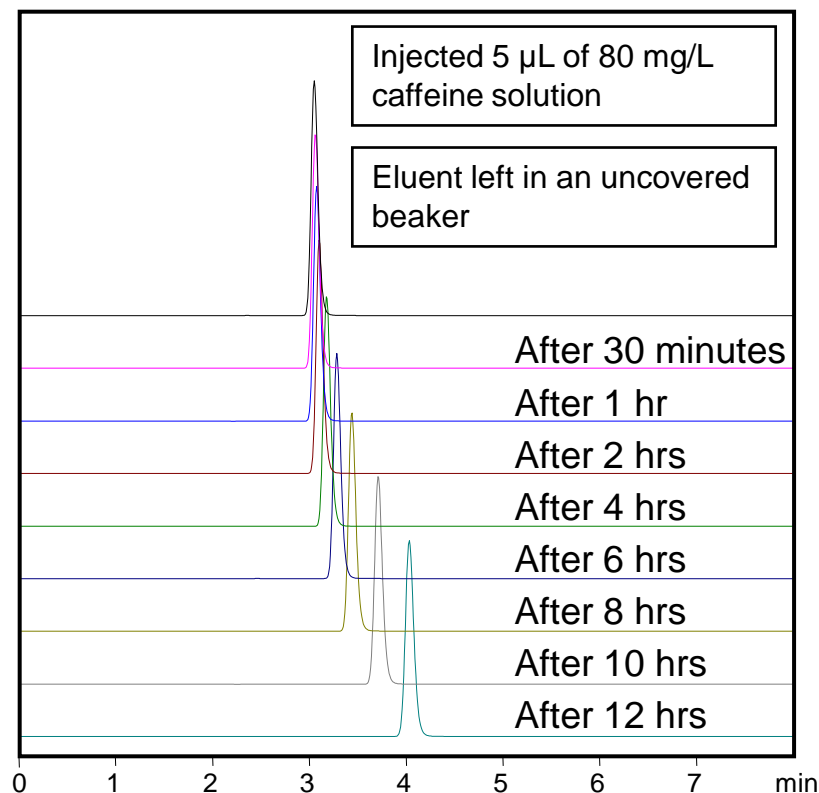
# Changes in Eluent Composition or Density

## ■ Phenomena

- Retention time gradually changes due to change in composition by solvent evaporation (right)
- Retention time changes could also be due to changes in eluent problem resulting from room temperature changes

## ■ Countermeasures

- See 5. Baseline Fluctuation – Eluent Problem
  - ◆ Prepare new eluent
  - ◆ Cover eluent reservoirs
  - ◆ Maintain a constant room temperature



Column: Shim-pack VP-ODS  
(150 mm x 4.6 mm I.D.)  
Eluent: Water / acetonitrile = 8/2 (v/v)  
1.0 mL/min  
Detection: UV 275 nm



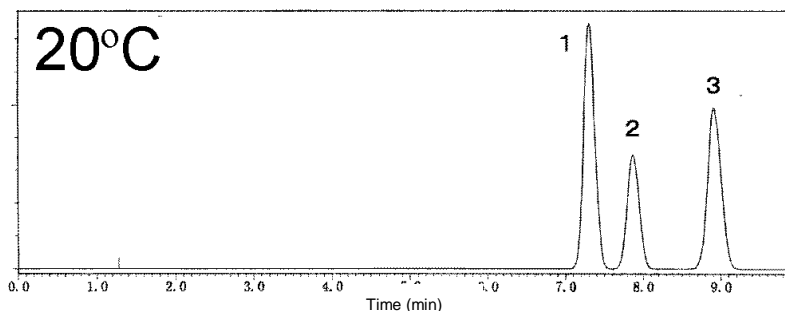
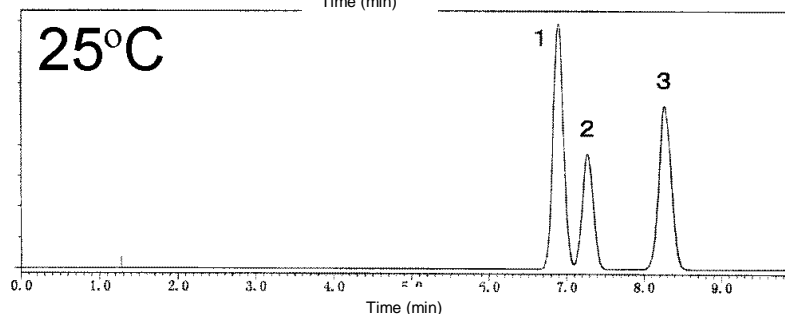
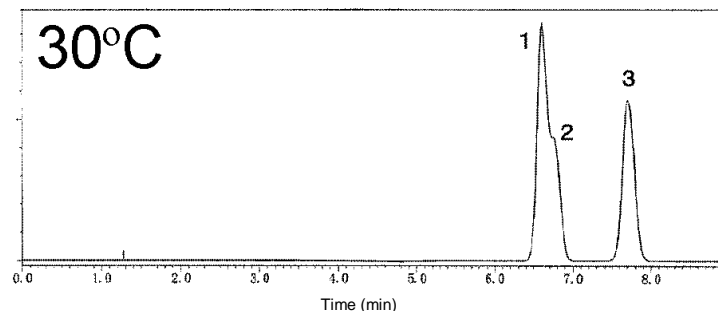
# Effect of Column Temperature

## ■ Phenomena

- Retention and separation vary due to changes in column temperature

## ■ Countermeasures

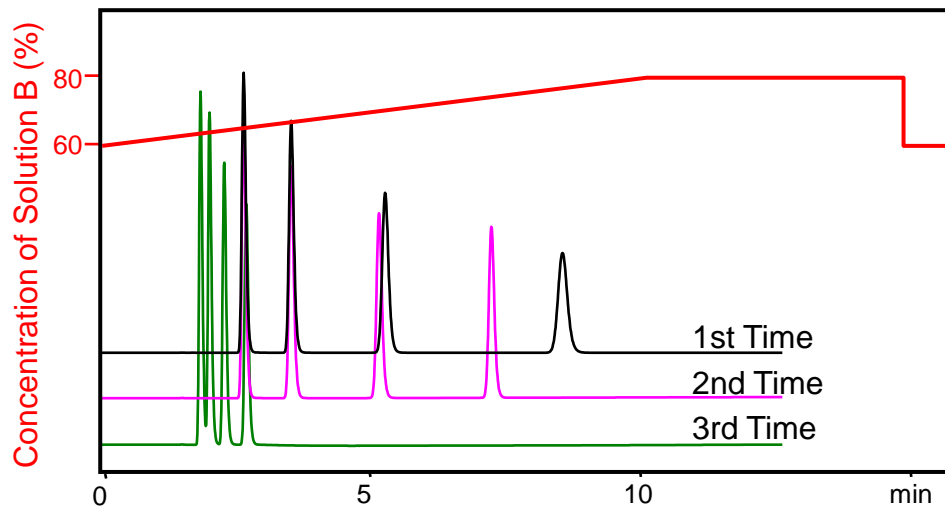
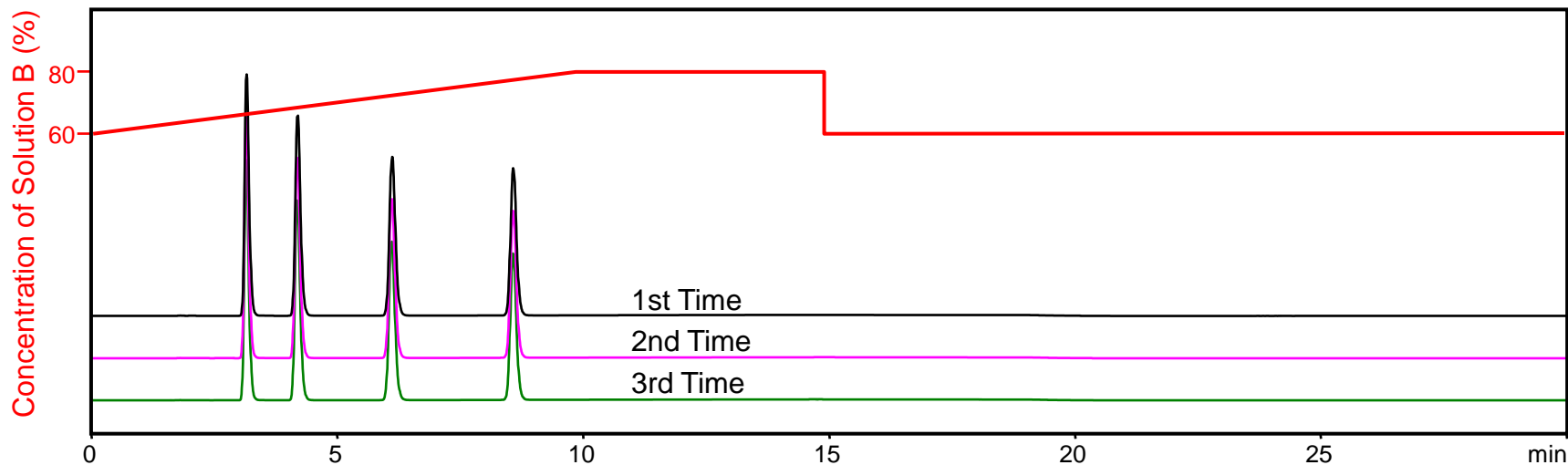
- Use a column oven
- Change the temperature setting
- Connect a preheating tube to the column inlet port
  - ◆ If column temperature and room temperature are different
  - ◆ If eluent flow rate is large



Effects of Column Temperature in Reverse Phase Mode

1: Sorbic acid, 2: Benzoic acid, 3: Methylparaben

# Inadequate Reequilibration in Gradient



Column: Shim-pack VP-ODS  
(150 mm × 4.6 mm I.D.)  
Eluents: A: water, B: methanol  
Flow Rate: 1.0 mL/min  
Detection: UV 260 nm

Injected 5  $\mu$ L each of 50 mg/L  
methylparaben, ethylparaben,  
n-propylparaben, and n-butylparaben

# Problem with Analytical Conditions Themselves

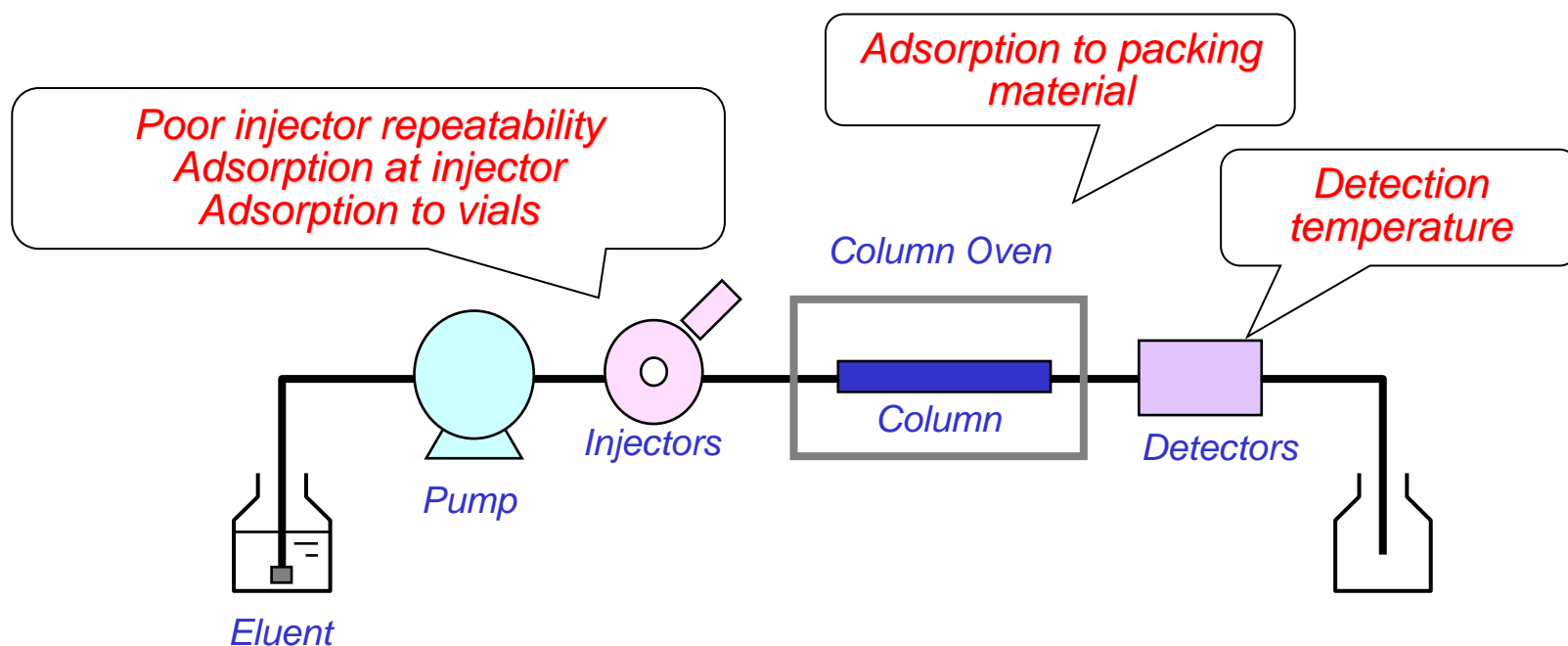
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- Parameters prone to variation in retention strength of packing material
  - Reverse phase eluent is 100 % water
  - Extremely acidic or alkaline eluents in silica columns
- Parameter that require extra time for equilibration
  - Normal phase
  - Reverse phase ion pair, etc.
- Countermeasures
  - Change analytical conditions, beginning with eluent composition
    - ◆ Evaluate robustness as part of parameter evaluation process
  - Use a longer equilibration time
  - Rinse or replace column

# Peak Area Fluctuation

# Peak Area Fluctuation ... What to Do First

- Check that samples are injected with good repeatability
  - Focus especially on injectors



# Variability of Injection Amount

- What to check
  - Assess by repeated injections under identical conditions (right)
    - ◆ Compare to specifications in autosampler catalog
- Countermeasures
  - Rinse needle
  - Remove bubbles from or replace the rinsing solvent
  - Check settings for suction speed, needle depth, etc.
  - Inspect and maintain injector

	Without Rinsing	With Rinsing
1st	43031	48228
2nd	42935	48293
3rd	43637	48434
4th	43864	48060
5th	43679	47951
Average	43429	48193
%RSD	0.96	0.40

Example of Area Repeatability Test of Thiamin Hydrochloride

Sample Solvent: Water  
 Rinsing Solvent: 100 mmol/L aqueous perchlorate solution  
 Sample Concentration: 10 µmol/L  
 Injection Volume: 10 µL

# Adsorption at Injector

## ■ What to check

- Add an adsorption resistant internal standard and compare repeatability with target compounds (right)
  - ◆ For reverse phase, choose a difficult to ionize substance as the internal standard

## ■ Countermeasure

- See 6. Sensitivity Reduction

	Caffeine	Thiamin
1st	50327	48228
2nd	50372	48293
3rd	50479	48434
4th	50259	48060
5th	50224	47951
Average	50332	48193
%RSD	0.20	0.40

Example of Area Repeatability Test of Caffeine and Thiamine Hydrochloride

Sample Solvent: Water  
 Rinsing Solvent: 100 mmol/L aqueous perchlorate solution  
 Sample Concentration: 10 µmol/L each  
 Injection Volume: 10 µL

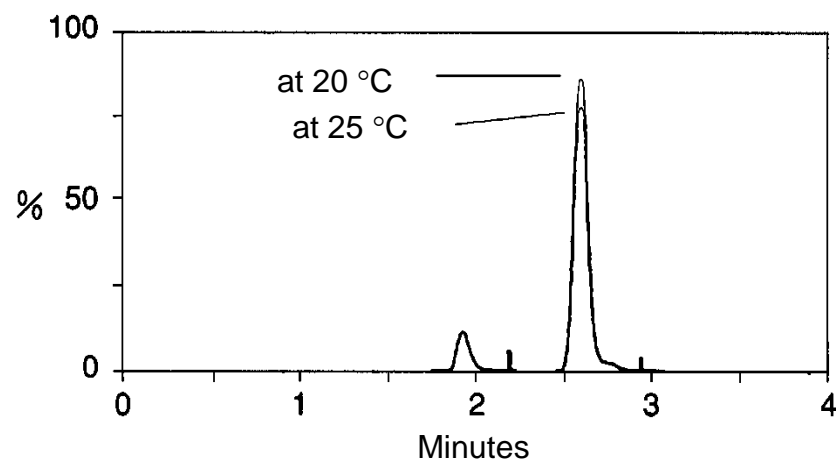
# Detector Temperature Fluctuations

## ■ What to check

- If detector includes temperature control function, monitor cell temperature
  - ◆ Refractive index detectors and electroconductivity detectors, etc.
  - ◆ Even absorbance and fluorescence detectors are commercially available with a temperature control capability

## ■ Countermeasure

- Keep room temperature constant
  - ◆ Especially for fluorescence detectors, the higher the temperature, in general, the lower the sensitivity



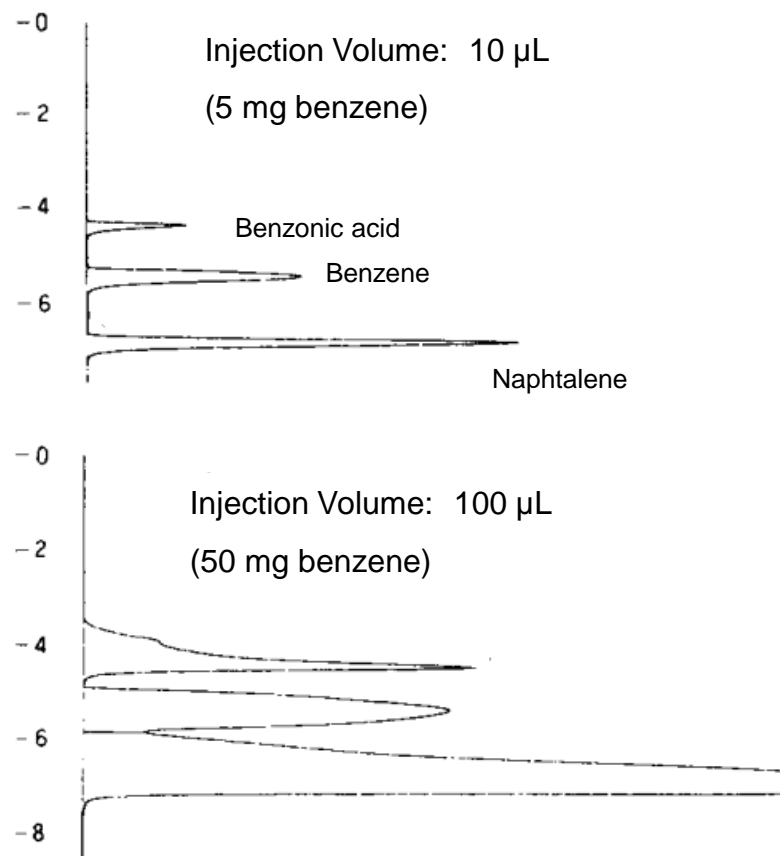
Effect of Cell Temperature on Fluorescence Detection of Acridine



# Poor Peak Shape and Separation

## ■ Countermeasures

- Reduce the injection volume (right)
  - ◆ Reduce the effects of the sample solvent by reducing the injection volume
  - ◆ Reduce the load level on the stationary phase by reducing the sample quantity
- Rinse or replace column
- Optimize the peak processing
  - ◆ Check whether different peak processing is used for each peak



# Things to Keep in Mind on a Daily Basis

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## ■ General

- Perform system suitability tests regularly
  - ◆ Analyze a standard solution 5 or 6 times
  - ◆ Record the retention times, area values, number of theoretical plates, resolution, etc.

## ■ HPLC system and column

- Keep a record of usage
  - ◆ Keep an instrument record of users, usage time, replacement of consumables, etc.
  - ◆ For columns, back pressure must be recorded as well
- Inspect regularly

## ■ Laboratory environment

- Provide an environment with minimal room temperature variations
  - ◆ Make sure air conditioners or heaters do not blow directly on the HPLC system

# Summary

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- Key considerations for routine analysis
  - Existence of pitfalls that cannot be escaped without know-how
  - Practical training of basic know-how and techniques is important
- Examples of common problems and how to fix them
  - For each phenomenon, check the possible sources of the problem
  - Identify likely causes
  - Implement countermeasures for each cause and verify results

# THANK YOU!

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