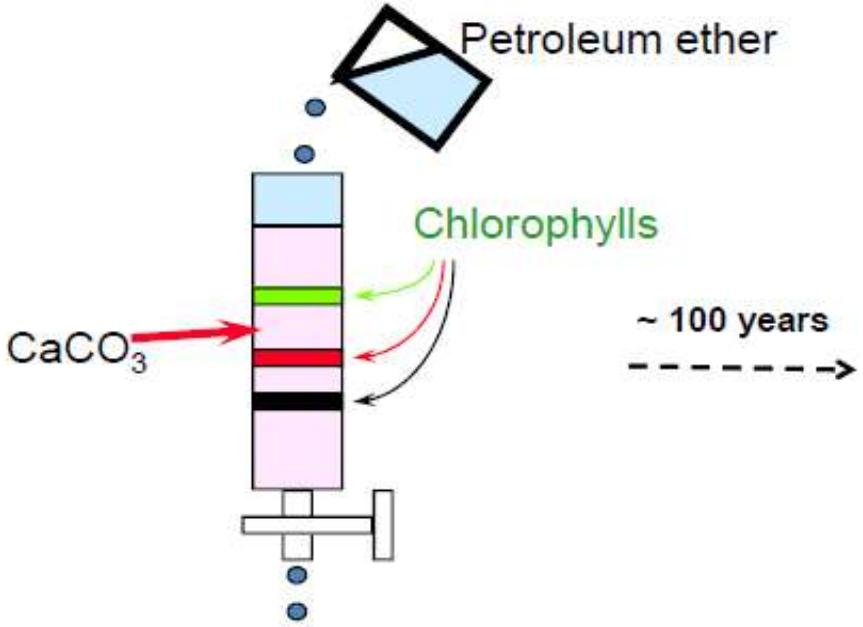
HPLC

(Basic Principles, Operations and Maintenance and Troubleshooting)

HPLC in recent time

M. Tswett first developer of chromatography in 1905



Liquid chromatography was invented to separate pigments from plant by using petroleum ether to wash the components passing through powdered chalk packed in a long tube.

High resolution, high sensitivity and high throughput fully PC controlled system

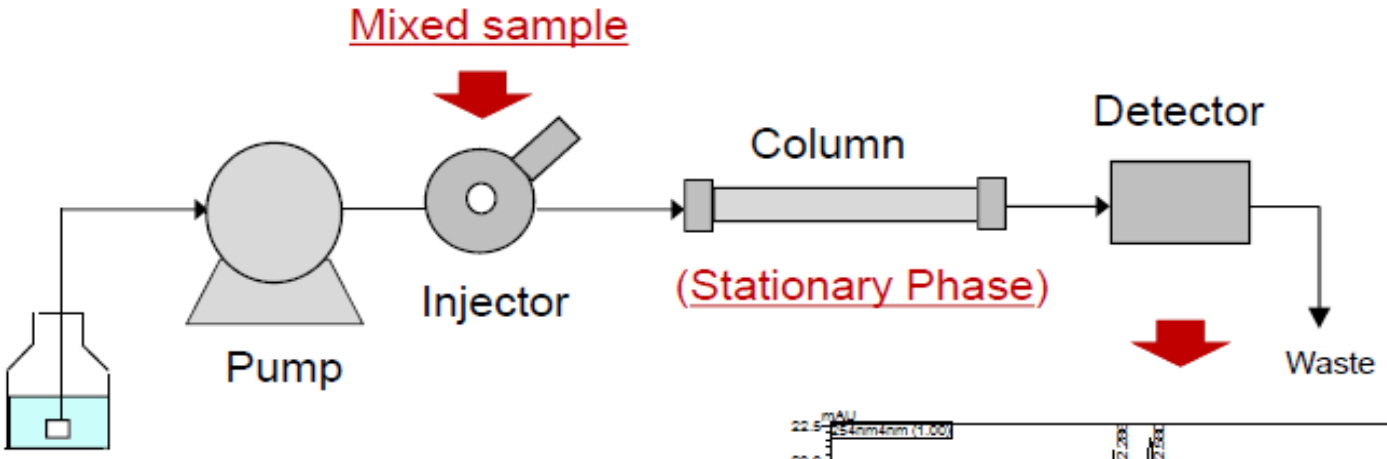
Characterization

- Liquid Chromatography (LC) is an analytical method that the compounds are physically separated prior to measurement using an appropriate detector

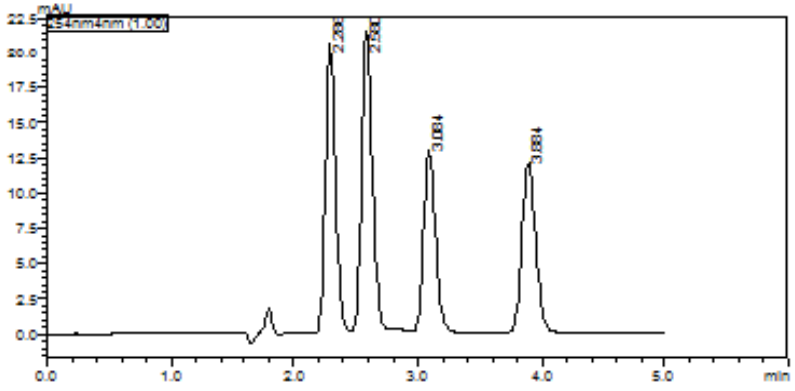
- High Performance (Pressure) Liquid Chromatography (HPLC) is the analytical technique to combine modern separation science and high sensitivity detection technology for analysis of trace level components in mixed samples

- The progress of HPLC in recent years derive to Ultra Fast LC (UFLC) and Ultra High Performance Liquid Chromatography (UHPLC).

Instrumentation

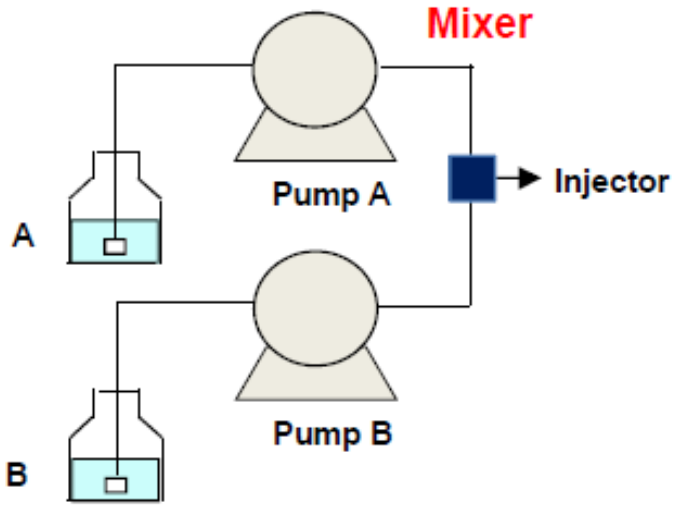


1) Mobile phase delivery pump(s)
2) Injector (manual or auto-sampler)
3) Column (stationary phase)
4) Detector and output system (PC with workstation)



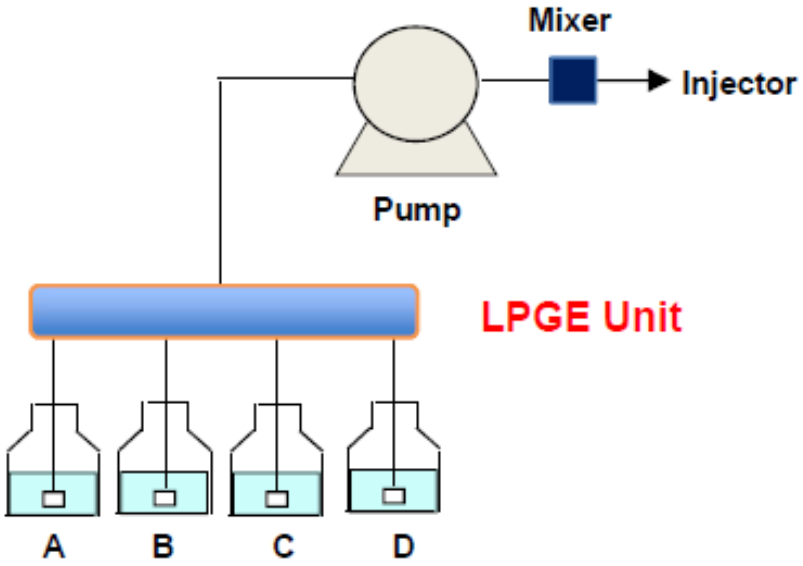
Chromatogram (UV-254 nm) with separated peaks (components)

Basic Configuration of HPLC



High Pressure Binary Gradient

- Mixing after pumps
- **Best gradient accuracy (key advantage)**
- Two mobile phases only



Low Pressure Gradient configuration

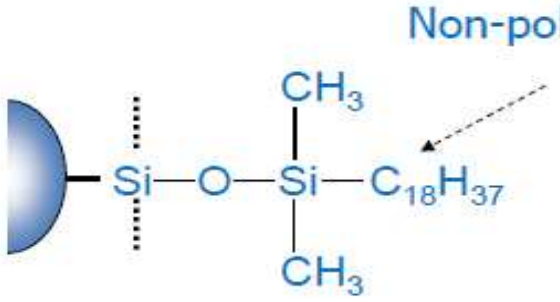
- Mixing before pump using a LPGE unit
- **Four mobile phases (key advantage)**
- Less gradient accuracy

Separation Modes

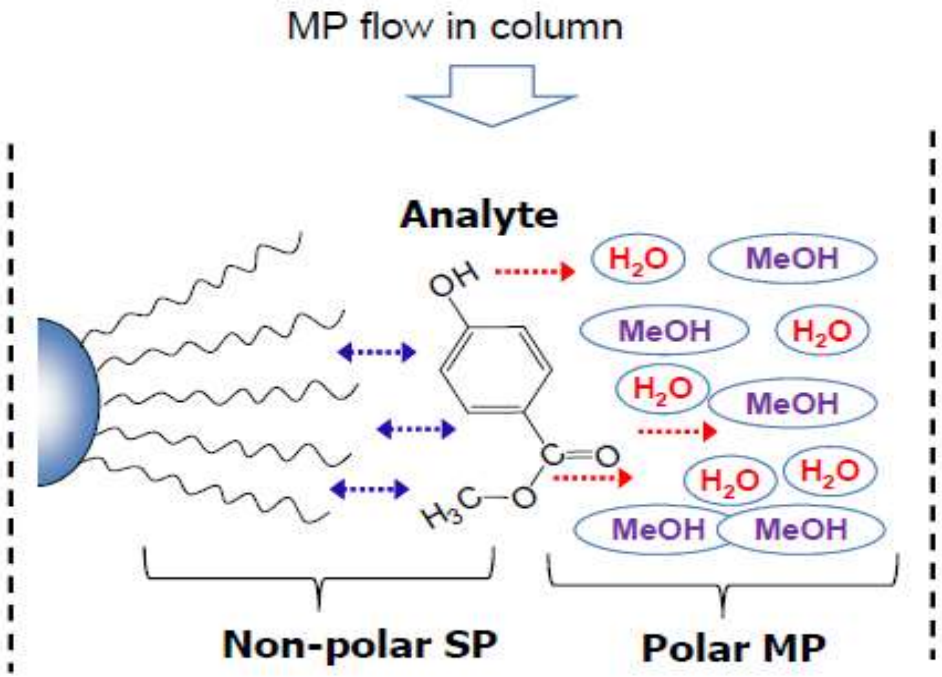
Separation mode	Stationary Phase	Mobile Phase	Type of analyte
Reversed phase	C18, C8, C4, TMS and Phenyl (hydrophobic surface)	Polar solvent: Water and MeOH or MeCN	Organic compounds, less to mid polar
Normal phase	SIL, CN, NH2 (hydrophilic surface)	Non-polar solvent: Hexane, THF etc	More polar organic compounds like sugars, steroid hormones, phospholipids etc
HILIC	SIL, CN, NH2 (hydrophilic surface)	Polar solvent: Water and MeOH or MeCN	More polar organic compounds
Ion Exchange	Quaternary ammonium, sulfone or carboxylic groups	Aqueous	Anions and cations
SEC/GPC	Resin	THF	High polymers, rubbers

Reversed Phase HPLC System

- Hydrophobic surface and hydrophobic interaction



1) The surface of stationary phase (SP) is **hydrophobic** (non-polar) in nature, bonding on the SiO2 support (porous particles)
2) The non-polar parts of analyte tend to interact with non-polar surface. This nonpolar-nonpolar interaction, or namely hydrophobic interaction play main role in retaining the molecule in **reversed phase** (RP) separation.



Reversed Phase HPLC System

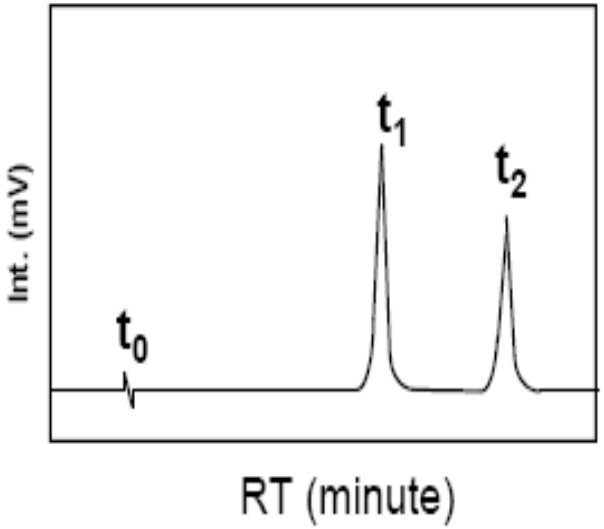
- Non-polar stationary phase (SP) & polar mobile phase (MP)

Type of SP:	Type of MP
• C18 (ODS) type: strong hydrophobicity • C8 (octyl) type: middle hydrophobicity • C4 (butyl) type: weak hydrophobicity • Phenyl type • TMS type • Cyano type	•Water-MeOH (v/v) •Water-MeCN (v/v) •Water-MeCN-with Sodium phosphate (10~100 mM, pH 2.6~6) •Water-MeCN-additives (such as ion-pair reagent)

- Retention (time) of a compound in RP separation depends on:
  - ✓ Strength of retention of stationary phase (column type and length)
  - ✓ Strength of mobile phase (% of organic component)

Peak Parameters

The retention and separation of a group compounds on a column are described by capacity factor (*k'*), Separation factor (α) and resolution (*Rs*).



$$k' = \frac{t_R - t_0}{t_0}$$

k' is a measure of retention of a compound under the separation conditions and column used

$$\alpha = \frac{k'_2}{k'_1}$$

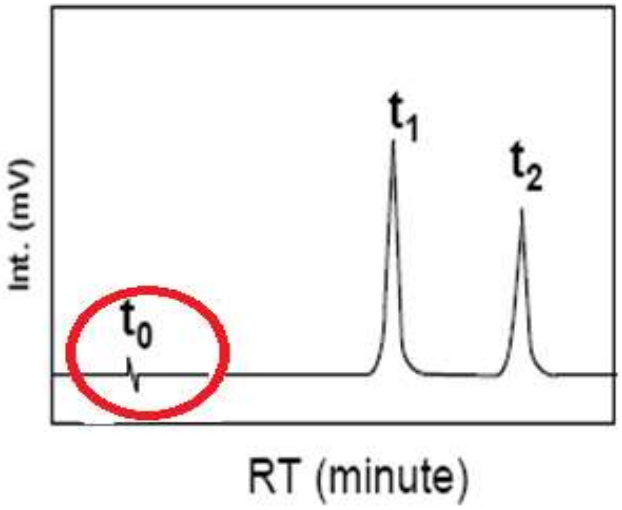
α indicates retention difference of a compound from the closest neighboring peak expressed in capacity rat

$$Rs = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\sqrt{N}\right)\left(\frac{k'}{k'+1}\right)$$

R indicates how good a peak is separated from a neighboring peak.

Factors!!!

The retention and separation of a group compounds on a column are described by capacity factor (*k'*), Separation factor (α) and resolution (*Rs*).



$$k' = \frac{t_R - t_0}{t_0}$$

k' is a measure of retention of a compound under the separation conditions and column used

$$\alpha = \frac{k'_2}{k'_1}$$

α indicates retention difference of a compound from the closest neighboring peak expressed in capacity ratio

$$Rs = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\sqrt{N}\right)\left(\frac{k'}{k'+1}\right)$$

Column Dead Volume

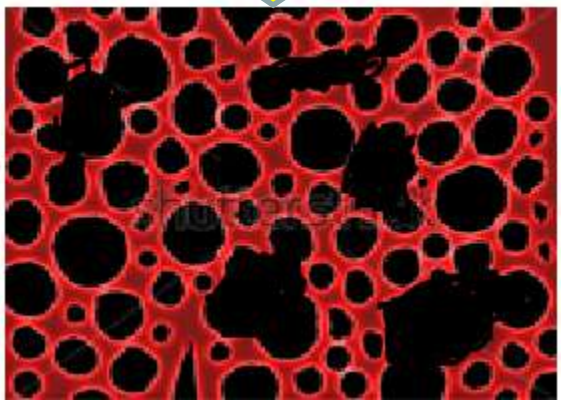
Volume of cylinder = $\pi r^2 L$

Volume of cylinder = $\pi (0.23)^2 (15) = 2.49 \text{ cm}^3 = 2.5 \text{ mL}$

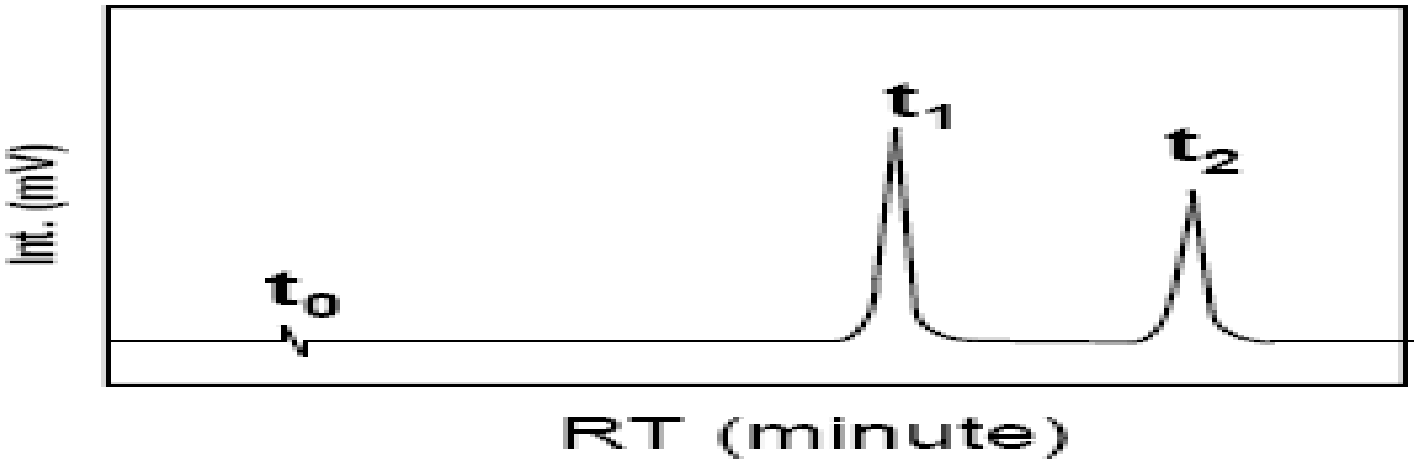
2.5 mL X ~65% (The volume in the column not taken up by packing)= 1.5 mL= 1 column volume

2 mL

Worn

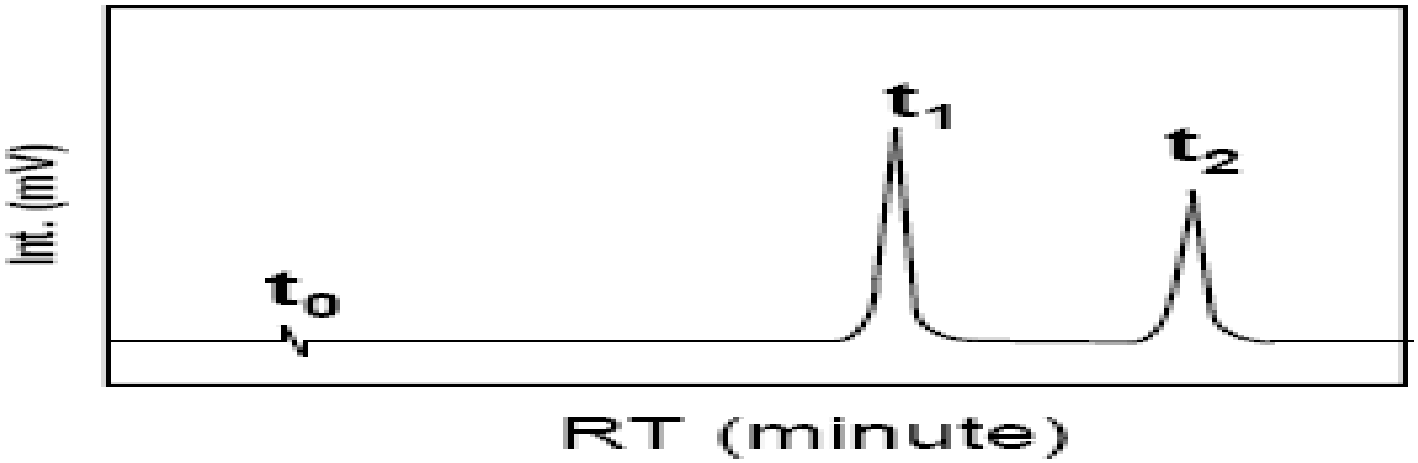


Exercise!!



Peak	t_0 (min)	t_R (min)	k	a	R
p1	0.501	1.871	2.73		
p2	0.501	2.562			

Answer!!!



Peak	t_0 (min)	t_R (min)	k	a	R
p1	0.501	1.871	2.73		
p2	0.501	2.562	4.11	1.50	> 1.5

Normal Phase HPLC System

Polar stationary phase (SP) & Non-polar mobile phase (MP)

Type of SP:

- SIL silica type ≡Si-OH)
- Amino NH2 type: ≡Si-NH2
- Cyano CN type: ≡Si-CN
- Diol type

Type of MP

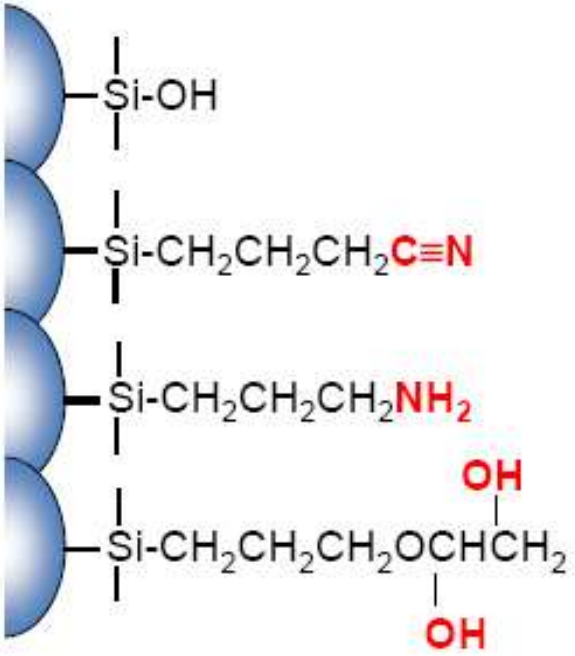
- Ethanol or 2-propanol (IPA) with n-hexane, chloroform etc
- Methylene chloride
- Ethyl ether
- Tetrahydrofuran (THF)
- Acetonitrile (A CN) with water

- About normal phase HPLC:
  - ✓ Need for those compounds are either too hydrophobic or too hydrophilic that are difficult for RP HPLC
  - ✓ Key applications: separation of isomers; sugars

Normal Phase HPLC System

Hydrophilic surface

Stationary phase: polar



Silica gel type:	For general use
Cyano type:	For general use
Amino type:	For sugar analysis
Diol type:	For protein analysis

Converting Normal Phase to Reverse Phase and Vice versa

To convert a normal phase system/column to a reversed phase system/column, flush with a solvent that is miscible with both the current normal phase solvents and ideally

Normal Phase :	Hexane/Ethyl Acetate
Flush :	IPA then Methanol
Finally :	50:50 Methanol/Water
Reversed Phase :	Buffered Aqueous Methanol

To convert a reversed phase system/column to a normal phase system/column, follow a similar path to the one listed previously, but in reverse, for example,

Reversed Phase :	Buffered Aqueous Methanol
Flush :	50:50 Methanol/Water
	Methanol then IPA
Normal Phase :	Hexane/Ethyl Acetate

HILIC System

- HILIC: Hydrophilic Interaction Chromatography
- HILIC is basically a *variation of normal-phase* chromatography (the term of HILIC started from 1990)
- HILIC is mainly used for mid-high polarity organic compounds like melamine (C3H3N6) etc

Type of SP:

•Stationary phase is a POLAR material such as silica, cyano, amino, diol

•Same as normal phase

Type of MP

•aqueous-organic mobile phases: highly organic (> 80%) with a small amount of aqueous/polar solvent

•Same as reversed phase

Various HPLC Detectors

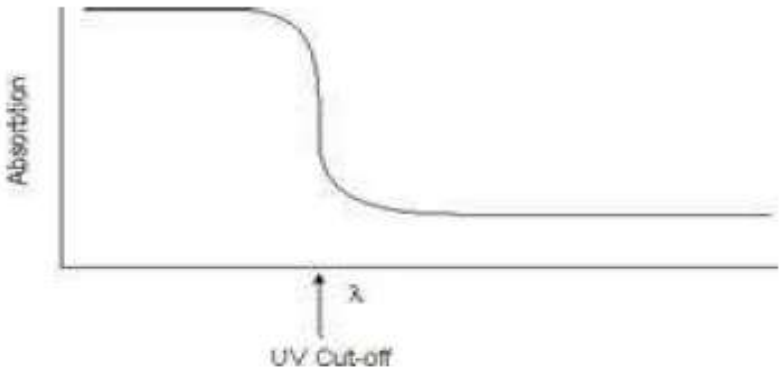
Key features required as HPLC detector:

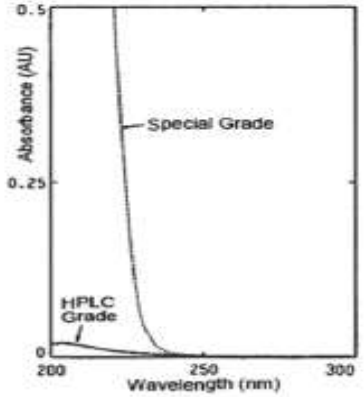
- ✓ Sensitivity: strong response to analyte molecules (but low response to MP)
- ✓ Coverage: applicable to more types of analytes

Abv	Type of detector	Compound	Sensitivity
UV-VIS	Ultraviolet -Visible	With absorption 190~800 nm	High (ppm~ppb)
PDA	Photodiode Array	With absorption 190~800 nm	High (ppm~ppb)
RF	Fluorescence		V High (ppb~ppt)
RID	Refractive Index	any (universal)	Low
ELSD	Evaporative light scattering	Any (universal)	Low-high
CDD	Conductivity	Cations and anions	High
MS	Mass spectrometer	Ionizable compounds	V High (ppb~ppt)

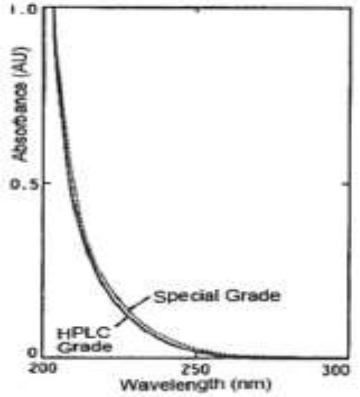
UV-Vis Detector

- Wavelength cut-off: Most solvents are relatively transparent to UV light down to a certain wavelength and below that they absorbs UV strongly.
- Reverse phase mobile phase like MeOH-water or A CN-water have low UV cut-off
- HPLC grade solvent: impurities in mobile phase can affect UV cut-off greatly. Therefore, HPLC grade solvents (Millipore water) are required to use in HPLC analysis





Acetonitrile



Methanol

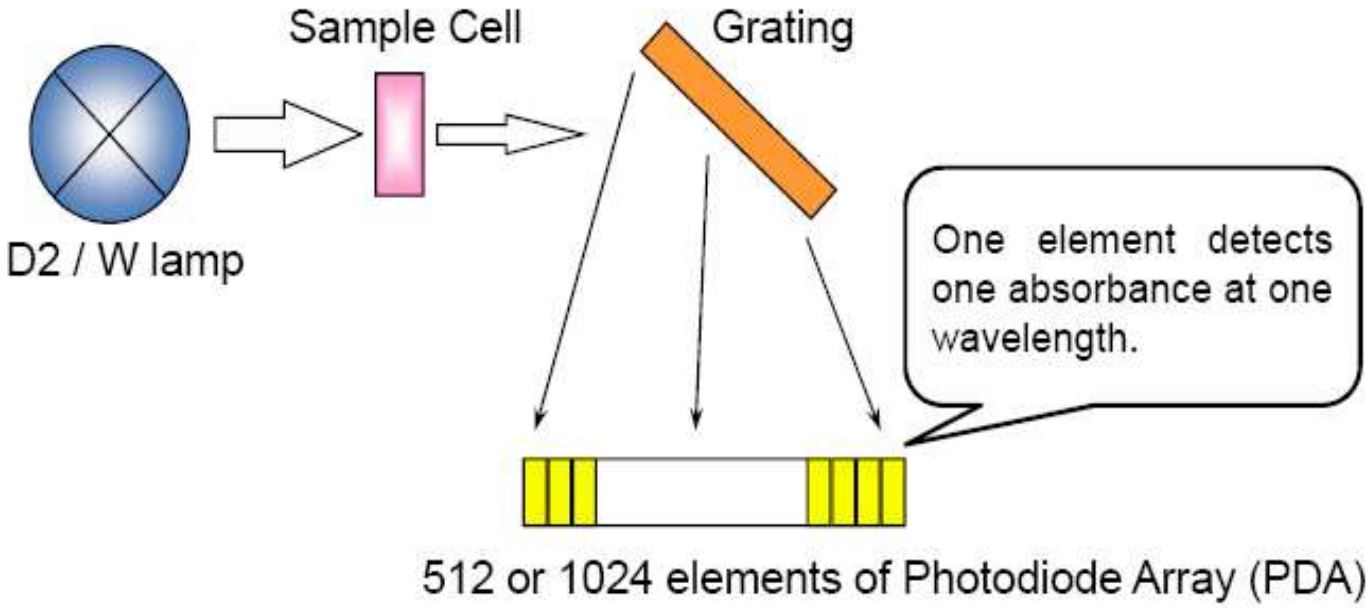
Solvent cut off-value

Acetonitrile	190 nm
Methanol	205 nm
Water	200 nm
Chloroform	245 nm
THF	215 nm
Ethyl acetate	254 nm
Toluene	284 nm
Acetone	330 nm
Pyridine	330 nm

- *Notice: wavelength cut-off of solvent will change if any additive like organic acid, ion-pair reagent is present.*

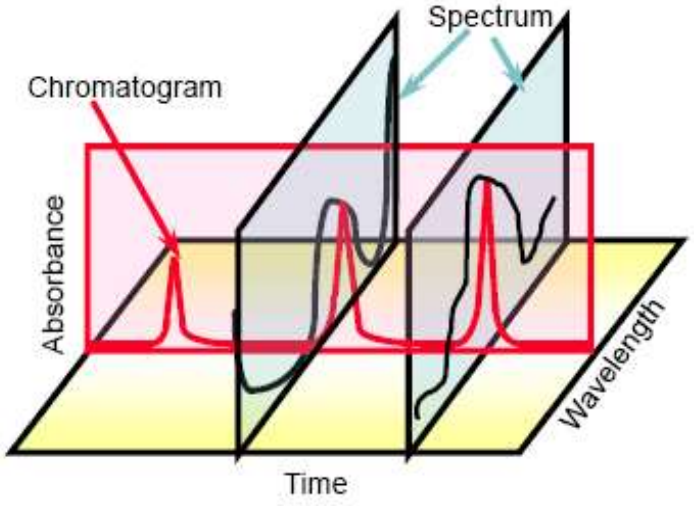
PDA Detector

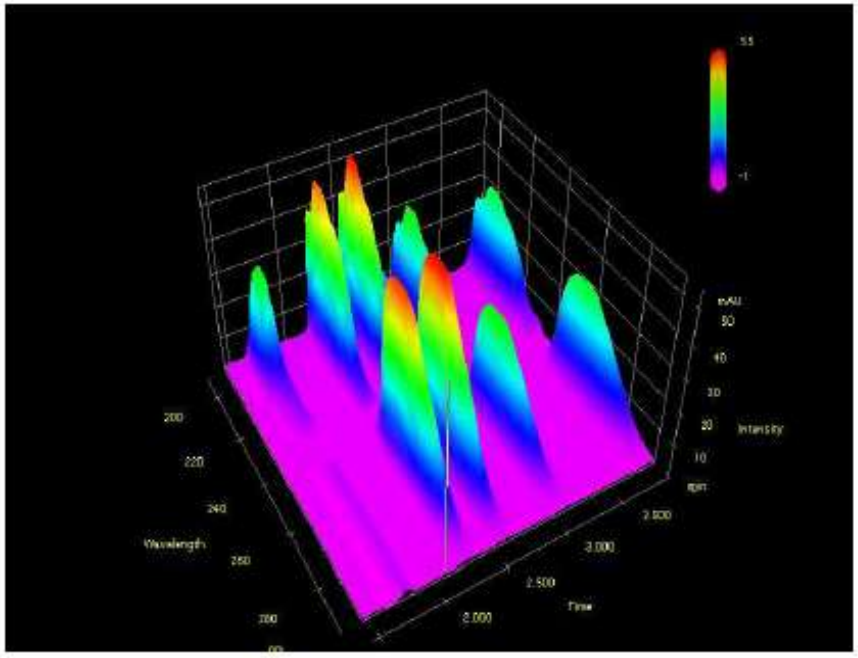
- Key features:
  - ✓ Coverage: organic compounds with UV-VIS absorption (190~900 nm)
  - ✓ Sensitivity: high sensitivity (ppm~ppb), mobile phase low absorption
  - ✓ 3-D detector: UV-VIS spectrum for qualitative analysis



PDA Detector

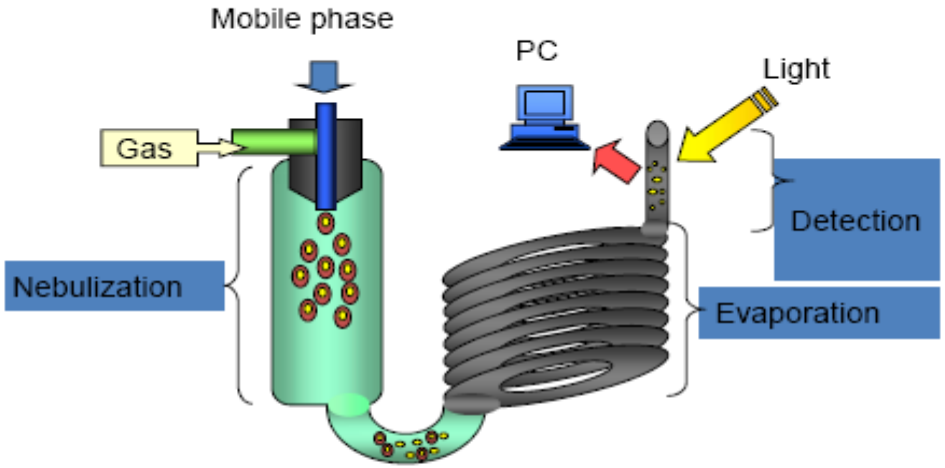
- 3-D detector: RT, Intensity and spectrum





Evaporating Light Scattering Detector (ELSD)

- Detection Principle
  - ✓ Nebulization (N2 or Air gas)
  - ✓ Evaporation of solvent
  - ✓ Detection
- Mobile phase: RP suitable
  - ✓ Aqueous-MeOH, ACN, THF
  - ✓ Evaporation of solvent



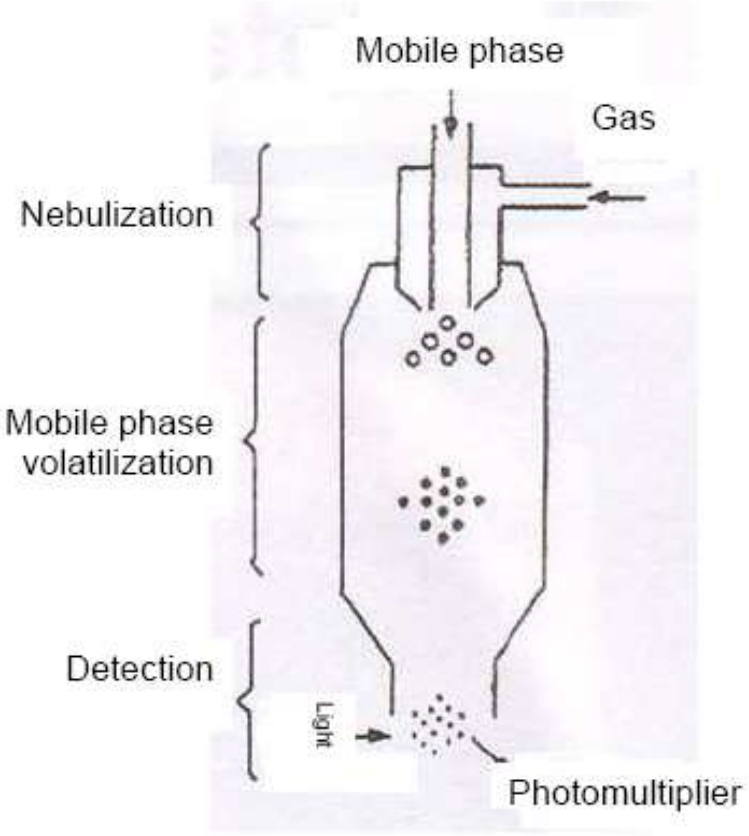
- Advantages in applications
  - Non and weakly-chromophoric compounds (e.g. carbohydrates, sugar alcohols, alcohols, terpenoids, surfactants, macromolecules and polymers).
  - Gradient method (e.g. lipids, phospholipids, triglycerides, fatty acids, amino acids, natural macromolecules, synthetic polymers).

ELSD Mechanism

1) **Nebulization**
Effluent and nebulizer gas form aerosol (very small droplets), proceeding to the next stage. Larger droplets impinging on the wall and are expelled as waste.

(2) Mobile phase evaporation
The solvent evaporates as aerosols passing through a temperature-controlled "drift tube"

(3) Detection
A strong light beam directed at the exit of the drift tube is scattered due to the particulates enters the angle-offset photomultiplier for detection.



Chromatography._Animation_(IQOG-CSIC).mp4

True_Universal_Detection ELSD.mp4

Maintenance

HPLC System Maintenance Strategy

- Purpose of maintenance:
  - Ensure the system work properly
  - Reduce machine downtime
  - Ensure analysis results reliable
  - Reduce the repairing cost

- Strategies of maintenance
  - Preventative maintenance periodically: conscientious preventative maintenance can minimize the number of problems that can occur
  - Keep a log book of instrument use history
  - Replacement of consumable parts regularly
  - Keep tool kits from the supplier

1. **Logbook for system record**
   - System usage logbook – users, samples and date info
   - Maintenance and troubleshooting logbook

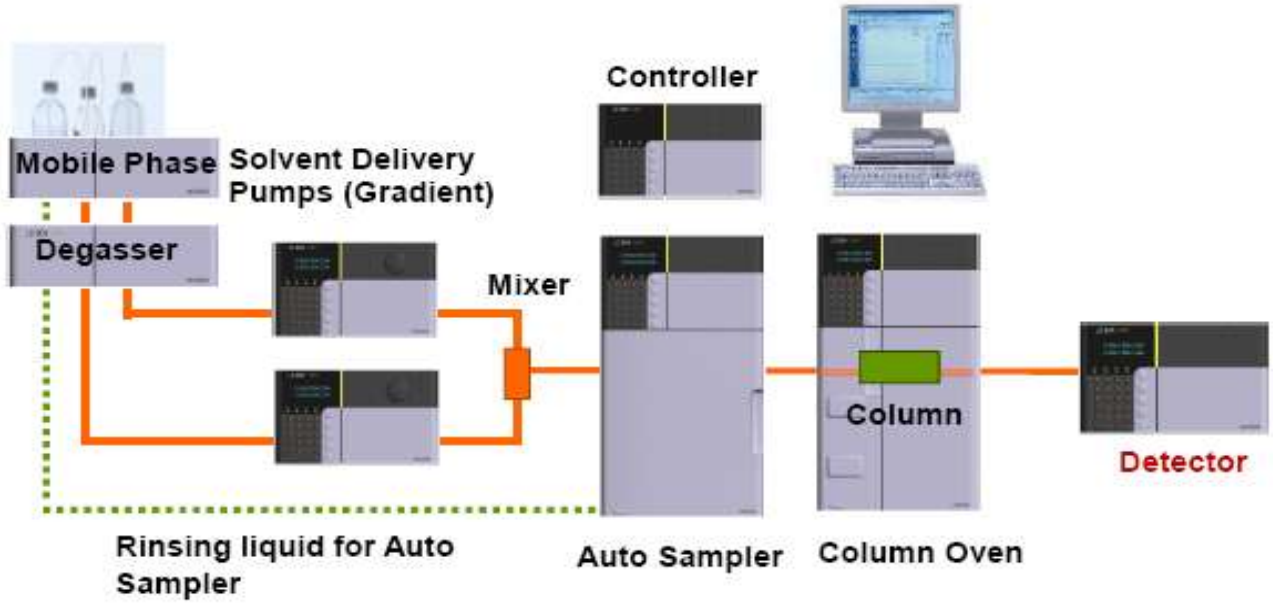
2. **Periodic preventative maintenance**
   - Solvent delivery system (degasser, pump etc)
   - Autosampler
   - Detector

3. **Replacement of consumable parts**
   - Regular replacement of consumable parts like UV lamp
   - Important consumable parts should be replaced periodically before failure

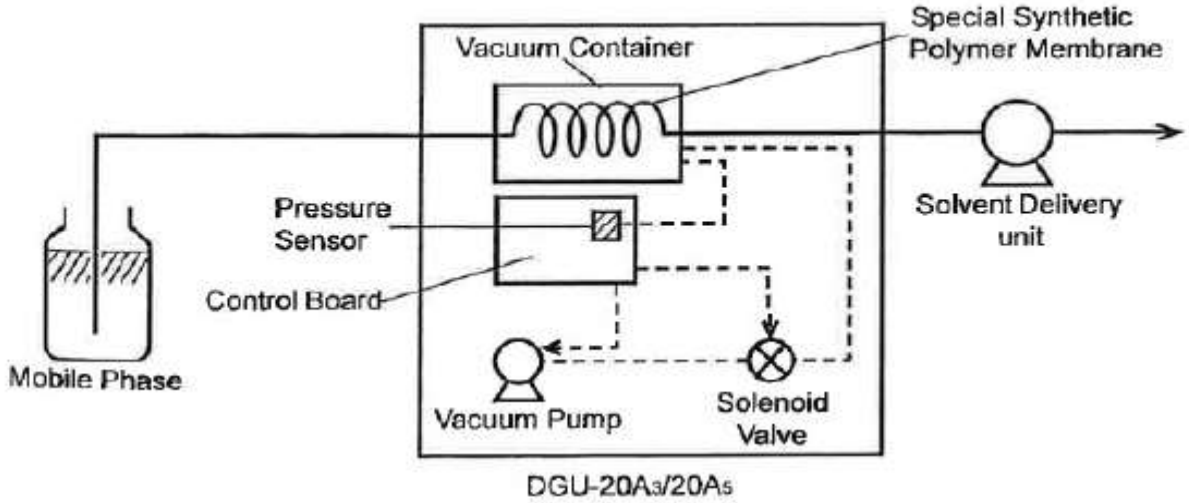
4. **Spare parts supply**
   - Consumable part list (recommended by supplier)
   - General spare parts are not suggested to keep

5. **Tool kit**
   - Kits provided by supplier should be kept well (special tools)

Individual HPLC Module Preventive Maintenance

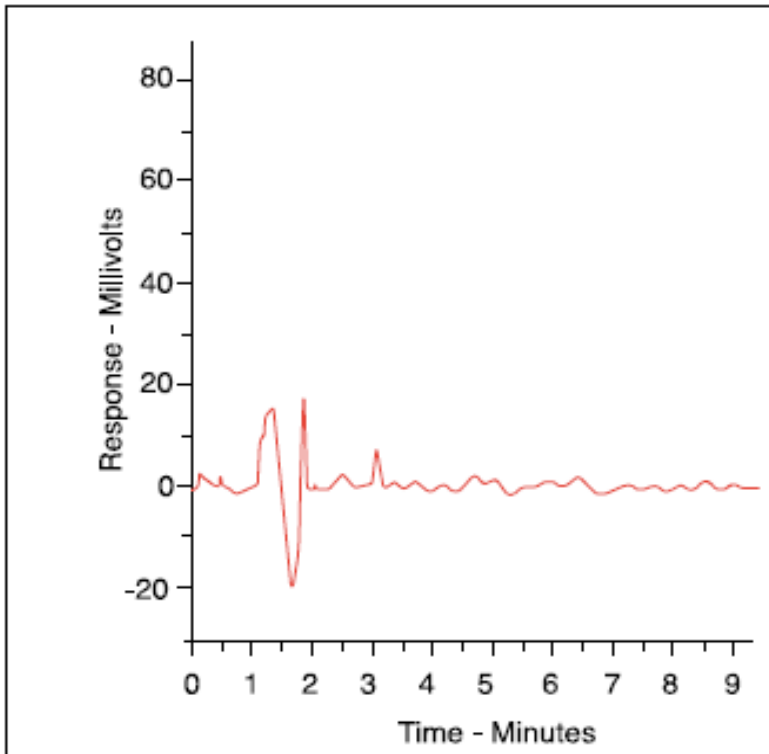


- Units need preventative maintenance
  - Solvent delivery system (degasser, pump)
  - Autosampler
  - Detectors (UV-Vis, PDA, RF, RID etc.)

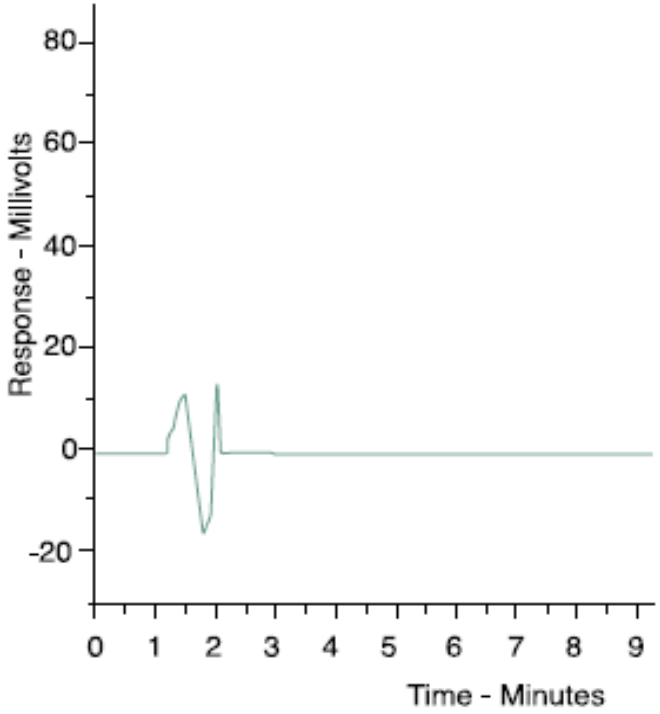


DGU-20A3/20A5

- Degassing is a very important step in HPLC analysis.
- Poor degassing may cause
  - bubbles in flow line and affect elution
  - the detector baseline drift
- Bubbles will form when mobile phase becomes supersaturated with air



Mobile Phase – Not Degassed



Mobile Phase – Degassed

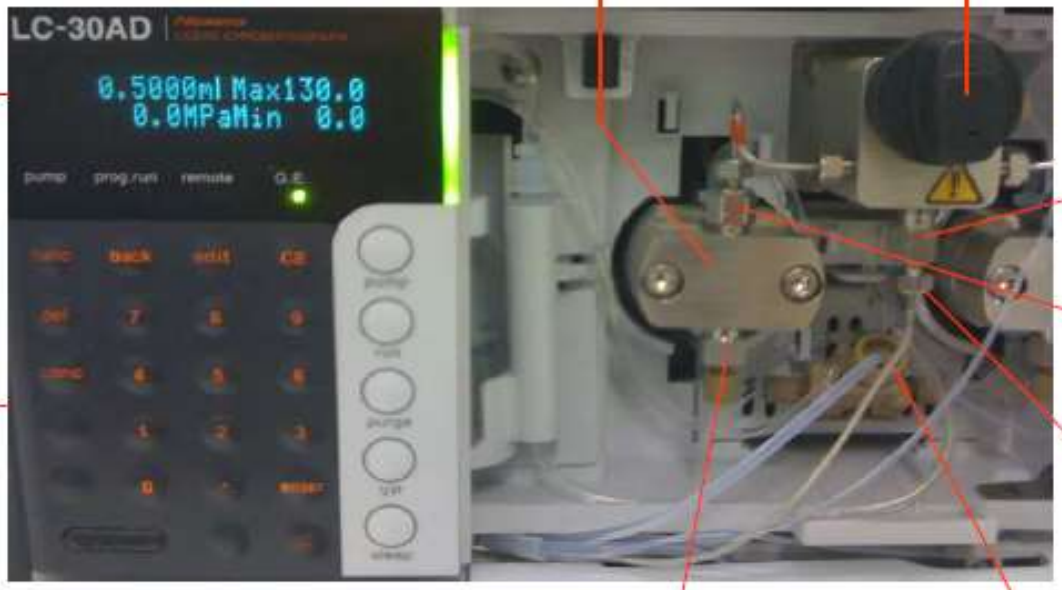
Possible problems from the Pumps

- Low pressure:
  - Leakages or Loosen connection
  - Air bubbles are trapped in solvent line
- Pressure fluctuation:
  - Check valve problems (contaminated)
  - Mobile phase insufficient degassing
  - Plunger is spoilt (surface scratched)
- Too high pressure
  - Flow lines (between pump and column) are clogged. It is more often for UHPLC, because the ID of tubing is very small (0.1 mm).
  - Line filter (installed at pump outlet) is clogged

Display panel

Pump head

Drain valve knob



Line filter

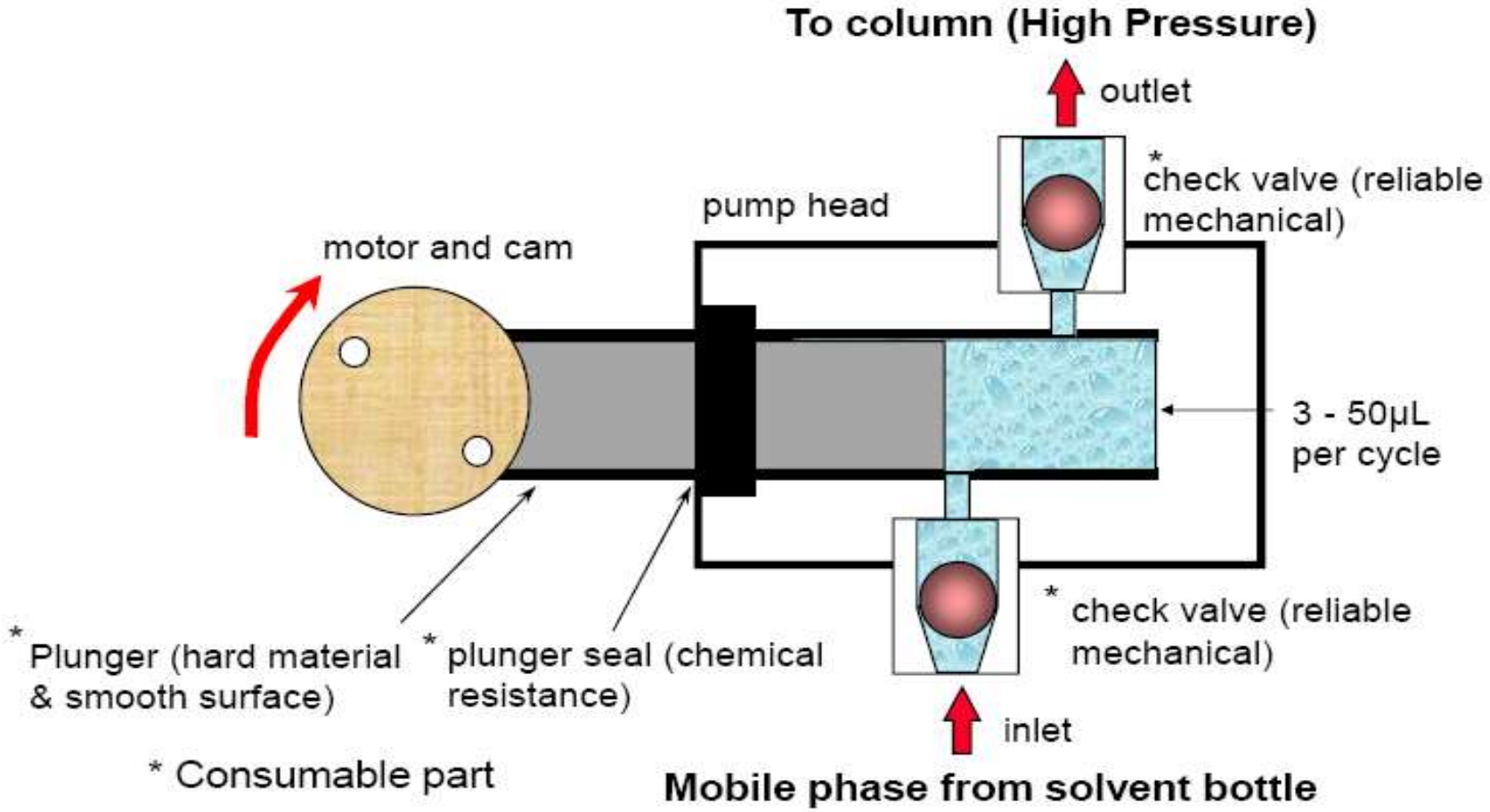
Outlet check valve

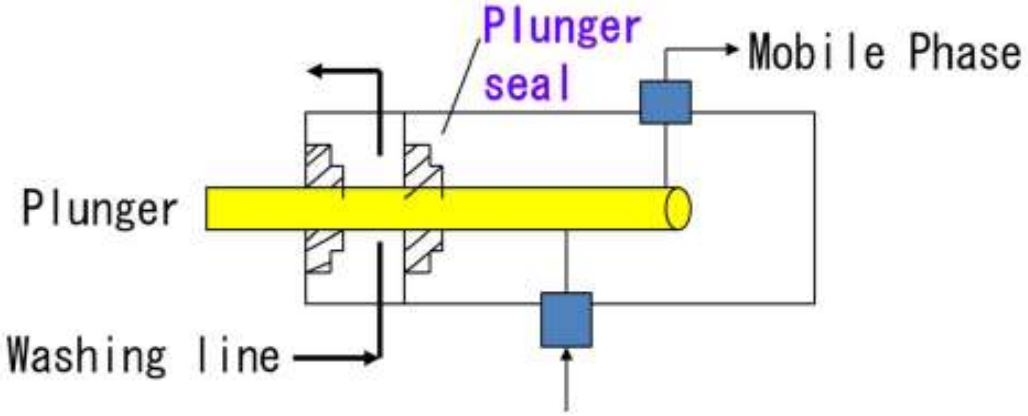
Pump outlet

Keypad

Inlet check valve

Pump inlet

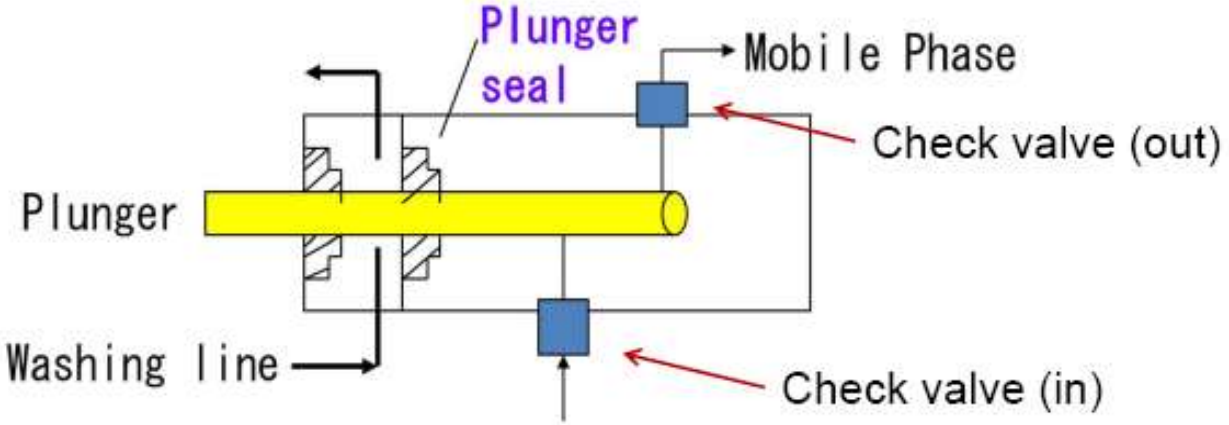

To column (High Pressure)
outlet
* check valve (reliable mechanical)
pump head
motor and cam
3 - 50µL per cycle
* check valve (reliable mechanical)
* Plunger (hard material & smooth surface)
* plunger seal (chemical resistance)
inlet
* Consumable part
Mobile phase from solvent bottle



Problem : Plunger seal is worn. Main cause for pump leakage

Symptom : Pressure is not stable or mobile phase is leaking

Solution : Change plunger seal



Problem : Pressure fluctuation

Symptom : No flow or pressure is fluctuating strongly after purging

Solution : Take off and clean the check valve by sonicating in IPA and water

- To keep particles from solvent bottles out of the delivery system
- Types of suction filter
  - Stainless:
    - Not suitable for mobile phase added with TFA
    - Difficult for changing
  - Improved stainless: improved one for stainless suction filter
  - Ceramic: for ion chromatography system

Stainless filter

Improved stainless filter

Ceramic filter

Replacement and cleaning of suction filter

General operation steps:

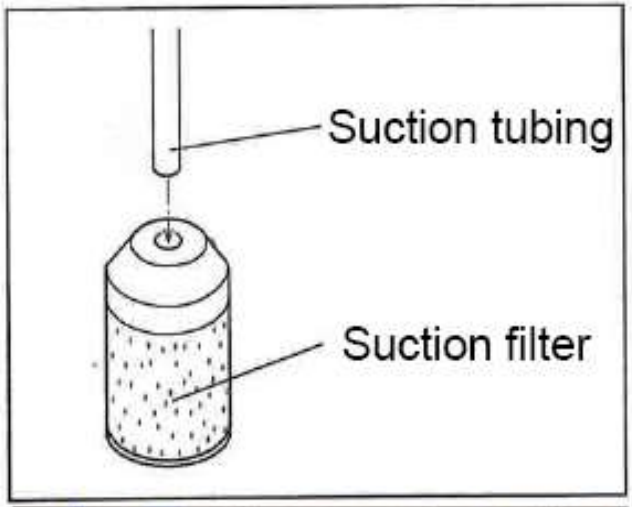
Step 1: Pull the suction filter out of the tubing

Step 2: Clean the suction filter in 2-propanol, in an ultrasonic cleaning device for 5 minutes

Step 3: Insert the suction tubing into the filter

Step 4: Turn the power switch on and pump water at 1 mL/min for 10 minutes

Step 5: Check that air bubbles do not accumulate inside the solvent tubing. If they do, replace the tubing



Air bubbles accumulate on the surface of the filter, indicate the solution is dirty already

Auto sampler common problems

- Clogging issue:
  - Dirty samples or high concentration samples may cause clogging in piping, sample loop and needle
  - Lead to high pressure and leakage
- Leaking:
  - High pressure valve may leak due to wearing off of rotor and rotor stator
  - Connector in high pressure line may leak due to over high pressure caused by clogging
- Needle
  - For any reason (like misalignment of sample rack or hit the bottom of the sample vial), needle bent may happen
- Sample carryover:
  - Peaks appear in blank samples after real sample running
  - Affect the analysis results

UV-VIS and PDA detector common Problem

- D2/W lamp low energy issue
  - Life time of lamp is limited. Lamp energy will decrease after the life time and cause reduced sensitivity and higher baseline noise
  - Recommended life time of D2 and W are normally 2000 hours

- Flow cell
  - Flow cell may leak if there is back pressure (like MS detector after UV detector can give some back pressure)
  - Flow cell may trap bubble. If there are bubbles, the UV adsorption will be affected.

When to Change the Lamp

- Check D2 lamp energy:
  - Set wavelength to 220nm, and check the energy of reference cell. Change D2 lamp if the energy is lower than 400.

- Check W lamp energy:
  - Set wavelength to 540nm, and check the energy of reference cell. Change W lamp if the energy is lower than 500.

Good Laboratory Practice for HLPC

1. Preparation of Solvents :

Correct solvent preparation is very important. It can save vast amounts of time spent troubleshooting spurious peaks, base-line noise etc.

2. Quality :

All reagents and solvents should be of the highest quality. HPLC grade reagents may cost slightly more than lower grade reagents, but the difference in purity is marked. HPLC grade reagents contain no impurities to produce spurious peaks in a chromatogram baseline whereas AR grade reagents do contain trace levels of impurity, which may produce spurious baseline peaks.

Important: *Do not store HPLC grade water in plastic containers. Additives in the plastic may leach into the water and contaminate it. Always store HPLC grade water in glass containers*

Good Laboratory Practice for HLPC

3. Buffers :

All buffers should be prepared freshly on the day required. This practice ensures that the buffer pH is unaffected by prolonged storage and that there is no microbial growth present. Changes in pH and microbial growth will affect chromatography.

If buffer solutions are stored, be aware that they have a finite lifetime. Refer to pharmacopoeia monographs or similar for further guidance on buffer shelf life.


250 mL Reservoir
Clamp
Funnel Base
1000 mL Flask
Cap

Filtration Apparatus

Good Laboratory Practice for HLPC

4. Filtration :

Ideally, all HPLC solvents should be filtered through a 0.45 µm filter before use. This removes any particulate matter that may cause blockages. After filtration, the solvents should be stored in a covered reservoir to prevent contamination with dust etc. Filtering HPLC solvents will benefit both your chromatography and the wear and tear of the HPLC system. Pump plungers, seals and check valves will perform better and lifetimes will be maximized.

Good Laboratory Practice for HLPC

Pump

Injector

Wash

Column

Flow Cell

Hundred Problems Thousand Solutions

Troubleshooting Process

To execute the strategy a systematic approach, which will work for any problem, is required. The systematic approach should follow a logical sequence, so that the exact cause of the problem can be found.

1. **Gather the facts – not theories.**
2. **Check the simplest things first – it's easier.**
3. **Compare the performance obtained to the expected performance.**
4. **List possible causes.**
5. **Work through the possible causes in a step-by-step manner checking the outcome from any changes made.**
6. **As a last resort – get help from elsewhere, for example your instrument supplier help desk or your local technical support department.**

SYMPTOM:	Possible cause:	Corrective action:
No flow or pressure	**a** System leak	**a** Locate and repair
	b Injection valve improperly positioned	**b** Check valve for proper rotation
	c Air in the pump	**c** Disconnect system at pump outlet and pump at maximum flow rate with MeOH until no more bubbles appear; replace mobile phase
	d Faulty pressure gauge	**d** Check for proper flow at the system outlet and insert a different gauge
	e No mobile phase	**e** Check solvent reservoir and refill
	f Solvent delivery system not pumping	**f** Check that the pump is plugged in and turned on; check the pressure limit switch
	g Pump starvation	**g** Check the boiling point of the solvent for potential pump cavitation

SYMPTOM:	Possible cause:	Corrective action:
Pump pressure up but no flow through system	**a** Particulate matter clogging inlet system or head of column	**a1** Filter mobile phase and sample **a2** Check syringe for a barb(s) breaking septa pieces off into the system
	b Leak in system	**b1** Check all system fittings and repair **b2** Check detector cell for leaks
	c Plugged detector line	**c** Turn pump off immediately and carefully clean lines and cell
	d Injection valve improperly positioned	**d** Check valve for proper rotation
	e Column inlet clogged with dirt accumulation	**e** Clean inlet and/or replace column

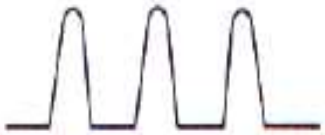
SYMPTOM:	Possible cause:	Corrective action:
Baseline stepping and peaks are flat-topped; Baseline does not zero	**a** Recorder gain and damping improperly adjusted	**a** Properly adjust gain and damp
	b Improper grounding	**b** Check system ground
	c Saturated electronics	**c** Reduce sample size

SYMPTOM:	Possible cause:	Corrective action:
Baseline spiking	**a** Air bubbles passing through detector	**a1** Degas mobile phase **a2** Locate and repair all leaks **a3** Flush air out of pump and check valves **a4** Check boiling point of mobile phase
	**b** Improper system ground	**b** Check for proper grounding
	c Electronic interference from other lab equipment turning on and off	**c** Check for other equipment turning on and off on same circuit and remove (e.g., constant temperature bath)
	d Loose electronic connections	**d1** Check all connections and plugs **d2** Check for vibrations **d3** Check for loose fitting source lamp
	e *Rf* feedback	**e** Properly grounded equipment

SYMPTOM:	Possible cause:	Corrective action:
Baseline drifting	**a** Dirt in detector sample or reference cells	**a** Flush detector cells with solvent or carefully clean cell
	b Temperature gradient over the system	**b1** Check for drafts **b2** Insulate column and column inlet/outlet lines or use a constant temperature jacket **b3** Move instrument away from direct sunlight
	c Contamination bleed in system	**c1** Check for septum bleed and replace with proper septum **c2** Check for column bleed: i. Previous sample(s) buildup — wash the column ii. Column-mobile phase incompatibility — replace column or mobile phase **c3** Stationary phase bleed (particularly at elevated temperatures); check stationary phase solubility in mobile phase; change mobile phase and column, or add stationary phase to mobile phase, or add a heavily loaded precolumn to the system **c4** Uneluted peaks; wash column
	d System leak	**d** Locate and repair
	e Bubble trapped in detector sample or reference cell	**e1** Flush out cell **e2** Degas mobile phase **e3** Locate and repair any system leaks **e4** Add suitable back pressure to detector outlet
	f Solvent immiscibility or immiscible pools (previous solvent not thoroughly flushed out)	**f** Flush system with compatible solvents until only the desired mobile phase is present
	g Mobile phase not in equilibrium with column	**g** Continue to flush system until equilibrium is established
	h Mobile phase/sample vaporizing	**h** At elevated temperatures check boiling point of mobile phase
	i Contamination in mobile phase	**i** Change mobile phase
	j Failing detector source	**j** Replace with new source
	k Recorder problems	**k** Short out detector; if drift continues, check

SYMPTOM:	Possible cause:	Corrective action:
Noisy baseline	a Air bubbles passing through detector	a1 Degas mobile phase a2 Flush pump check valves clear of air a3 Check all fittings for air leaking into mobile phase stream; look for salt-like deposits and stains near fittings; tighten appropriately
	b Column packing passing through detector	b Check column outlet for proper column plug and screen
	c Leak in system	c Locate and repair
	d Pulses from pump	d Add a pulse dampener and/or restrictor
	e Pulse dampener(s) and/or restrictors not properly flushed	e Disconnect solvent delivery system from injector and purge with suitable solvent(s)
	f Bubbles in detector sample or reference cell	f Check for bubbles entering detector and flush out air
	g Dirt in detector	g1 Disconnect detector from system and back flush with suitable solvents g2 Clean detector cell
	h Detector source failing	h Check and replace source
	i Temperature effects on detector cell input tubing	i1 Insulate inlet tubing i2 Move instrument away from drafts and/or direct sunlight
	j Recorder improperly grounded	j Check recorder and properly ground
	k Noisy electronics	k1 Check appropriate detector and recorder circuits; consult instrument manual k2 At high detector attenuation check source lamp k3 Check for dirty or loose electronic contacts; also check for instrument vibration

SYMPTOM:	Possible cause:	Corrective action:
Negative peaks	**a** Polarity reversed on detector or recorder	**a1** Switch polarity to other position **a2** Reverse detector leads
	b Negative peaks in UV trace	**b** Check for non-UV absorbers in system or sample impurities
	c Negative peak at V_O	**c1** Result of pressure surge due to sample introduction; don't quantitate peaks at V_O **c2** Air sampled, improve sample introduction technique

SYMPTOM:	Possible cause:	Corrective action:
Flat bottom peaks	**a** Bubbles in detector	**a** Degas mobile phase or add suitable back pressure to detector cell
	**b** Dirt buildup on detector cell windows	**b** Clean detector cell
	c Optics out of adjustment	**c** Check alignment or call service representative
	d Light output in reference beam lower than on sample side	**d** Clean cell and check with operation manual or service representative

SYMPTOM:	Possible cause:	Corrective action:
Very rounded peaks	**a** Operating beyond linear dynamic range of detector	**a** Reduce sample size
	**b** Recorder gain is too low	**b** Adjust recorder gain
	c Column-sample interaction (e.g., absorption)	**c1** Check sample chemistry and change column **c2** Increase temperature **c3** Change ionic strength or pH
	d Column dried out at ends	**d** Replace column
	e Column overload	**e** Reduce sample size
	f Contamination on detector cell windows	**f** Clean detector cell

SYMPTOM:	Possible cause:	Corrective action:
Loss of resolution	a Column overload	a Reduce sample size
	b Loss of column efficiency	b Adjust mobile phase or replace/regenerate column
	c Loss of column liquid phase	c Replace column
	d Dirty column	d Wash column with suitable solvents or replace
	e Distorted column bed (cracked, compressed)	e Repack or replace column
	f Used wrong column or mobile phase	f Change system

SYMPTOM:	Possible cause:	Corrective action:
Increased retention volume	a System flow rate decreased	a Check and increase flow rate; if flow rate decreasing, check and repair any leaks
	b Column temperature too low	b Insulate or jacket column
	c Column activity changing	c Solvent stripping H_2O or stationary phase off of column; add H_2O or liquid phase to mobile phase

SYMPTOM:	Possible cause:	Corrective action:
Decreased retention time	a System flow rate increased	a Check pump for proper setting
	b Column activity changing	b Change column
	c Wrong mobile phase	c1 Change mobile phase
		c2 Check for changes in mobile phase ratio

SYMPTOM:	Possible cause:	Corrective action:
Low sensitivity	a Inadequate flow rate	a Adjust flow rate
	b Sample not compatible with detector	b Check sample chemistry and adjust detector or change it
	c Insufficient sample	c Increase sample size
	d Sample not eluting from column	d Check sample chemistry; change mobile phase and/or column
	e Dirty detector cell windows	e Clean cell
	f Gas bubble(s) in detector cell	f1 Degas mobile phase
		f2 Apply suitable back pressure to detector output
	g Detector attenuation too high	g Adjust attenuation
	h Detector and/or recorder out of calibration	h Check detector and recorder calibration; recalibrate if necessary
	i Failing/faulty detector source	i Change detector source
	j Recorder in wrong millivolt range	j Check setting and adjust

SYMPTOM:	Possible cause:	Corrective action:
No peaks; no response	a Detector/recorder not on	a Check and turn on
	b Detector/recorder not plugged in	b Check and plug in
	c No sample injected	c Check injection/injector for complete sample introduction; clean or change syringe or valve
	d Failure in the electronics	d1 Check and replace fuse
		d2 Check service manual

Please DO!!!

- **Good Laboratory habits**
  - Log book for system records
  - Periodic Preventative maintenance
  - Read manual/instruction for additional information
- **If any problem happens**
  - Observe the instrument carefully
  - Check the simple things first
  - Remember the last thing that happen before the problem started
  - Isolate the source of the problem
  - Normally, the hardware would not be damaged easily

References

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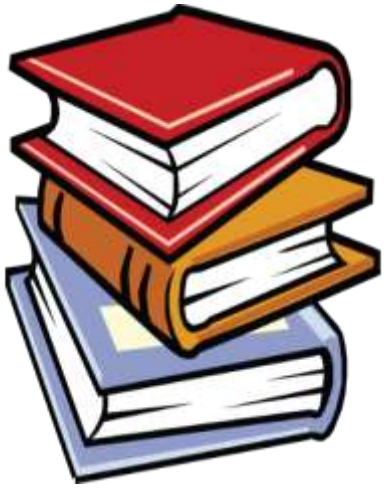

Thank You