High Performance Liquid Chromatography (HPLC) in Environmental Forensics

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Environmental Forensics

- 'Environmental forensics' is a combination of analytical and environmental chemistry, which is useful in the court room context.
- Involves
  1. Field analytical studies
  2. Data interpretation
  3. Modelling
- Connected with the attribution of pollution events to their causes.

Analytical Studies in Environmental Forensics

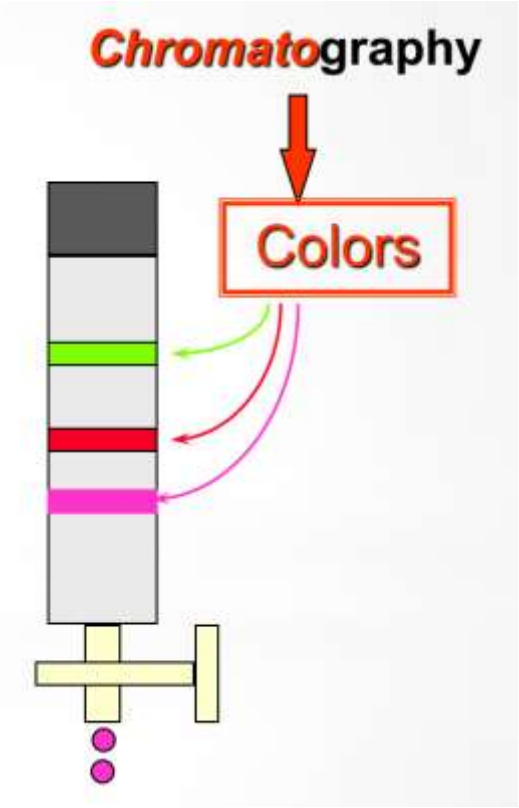
- To get the complex information on environmental quality.

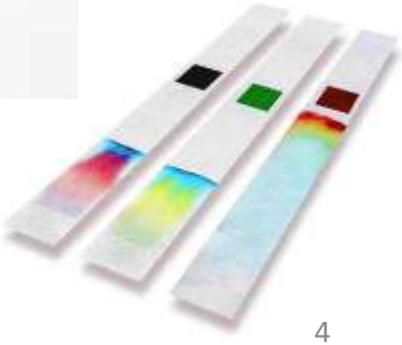
- Methodical requirements:
  - High sensitivity of measurements
  - Producing analytical information continuously in real time or with only negligible delay
  - High resolution of results characterized by short response time of the instruments
  - Long time of autonomous operation

- Gravimetric/titrimetric/spectroscopic/chromatographic etc.

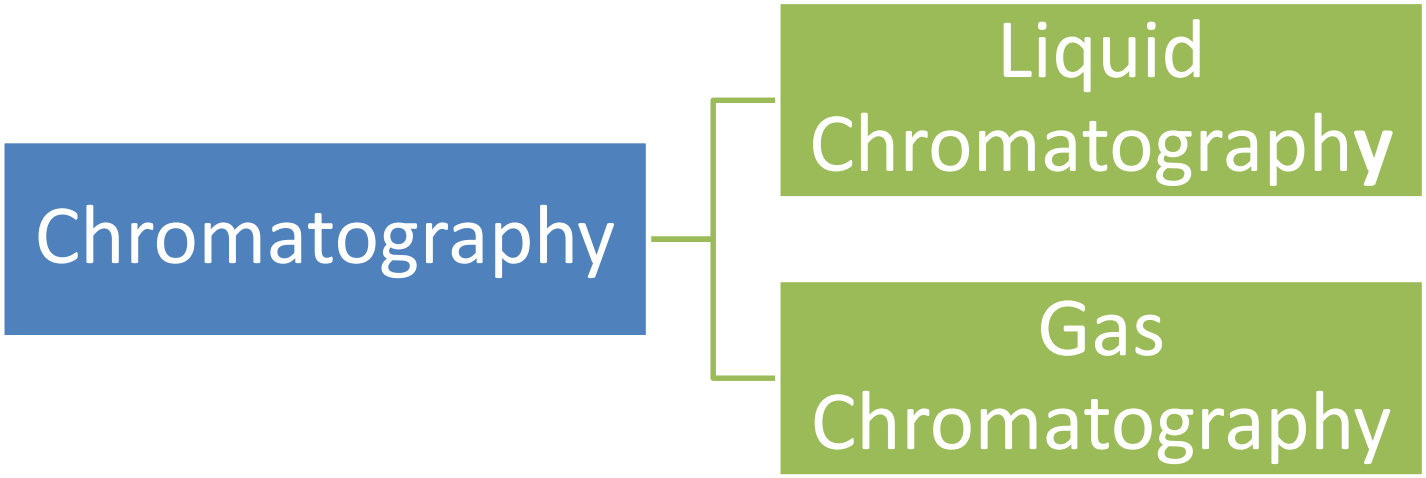
Concept of Chromatography

- Chromatography is an analytical method that the compounds are physically separated prior to measurement
- The main purpose of chromatography is to separate and quantify the target sample in the matrix





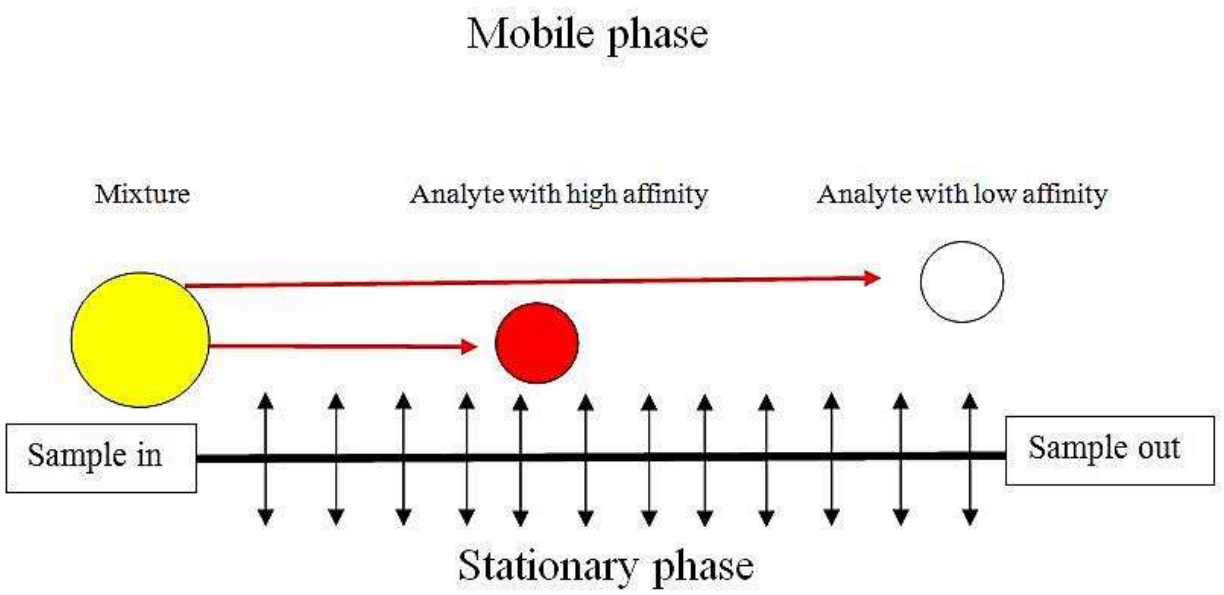
Concept of Chromatography



- **High**
- **Performance**
- **Liquid**
- **Chromatography**

Performs Quantitative & Qualitative Analysis

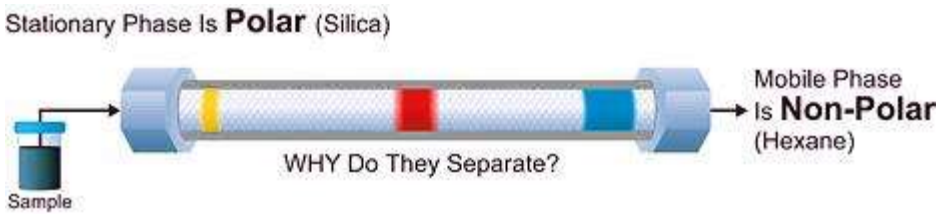
HPLC Column



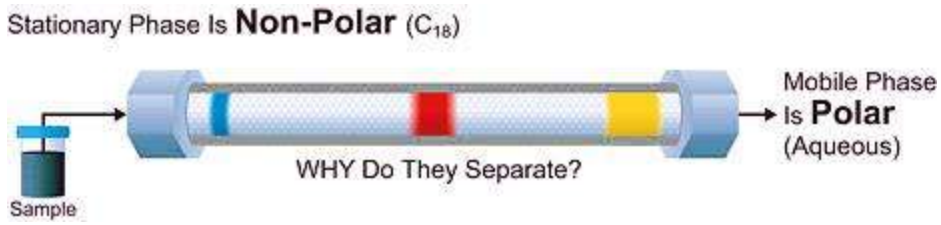
- Mobile Phase – Liquid
- The affinity with the mobile phase and stationary phase varies with the solute which cause separation

Normal Vs Reverse Phase Chromatography

	Stationary Phase	Mobile Phase
Normal Phase	Polar (Hydrophilic)	Non-polar (Hydrophobic)
Reversed Phase	Non-polar (Hydrophobic)	Polar (Hydrophilic)



Normal-Phase Chromatography



Reversed-Phase Chromatography

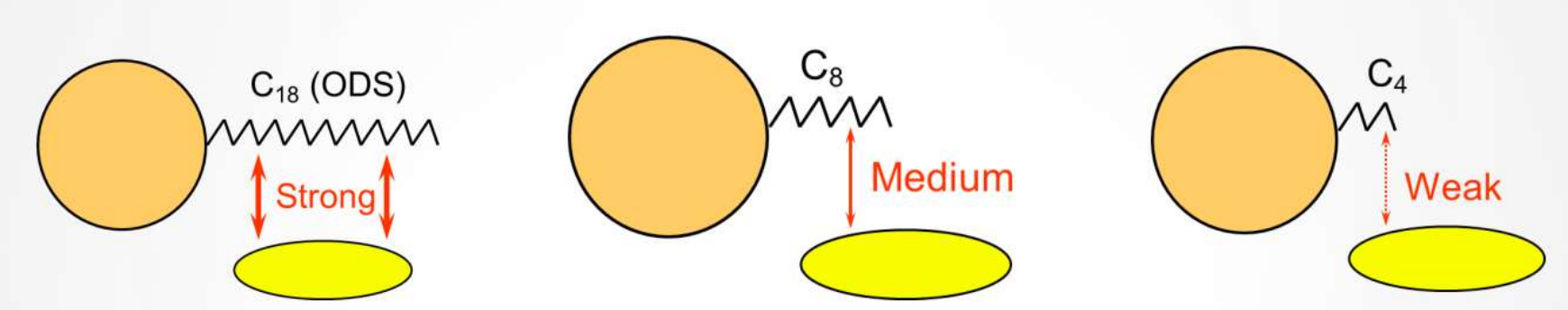
Normal Phase HPLC

- **Stationary Phase (Polar)**
  - Silica gel: -Si-OH
  - Cyano type: -Si-$CH_2CH_2CH_2CN$
  - Amino type: -Si-$CH_2CH_2CH_2NH_2$
  - Diol type: -Si-$CH_2CH_2CH_2OCH(OH)$-CH_2OH

- **Mobile Phase (Non-polar)**
  - Aliphatic hydrocarbons
  - Aromatic hydrocarbons
  - Ethers, etc.

Reverse Phase Chromatography

Stationary phase: Non-polar



Mobile phase: Polar

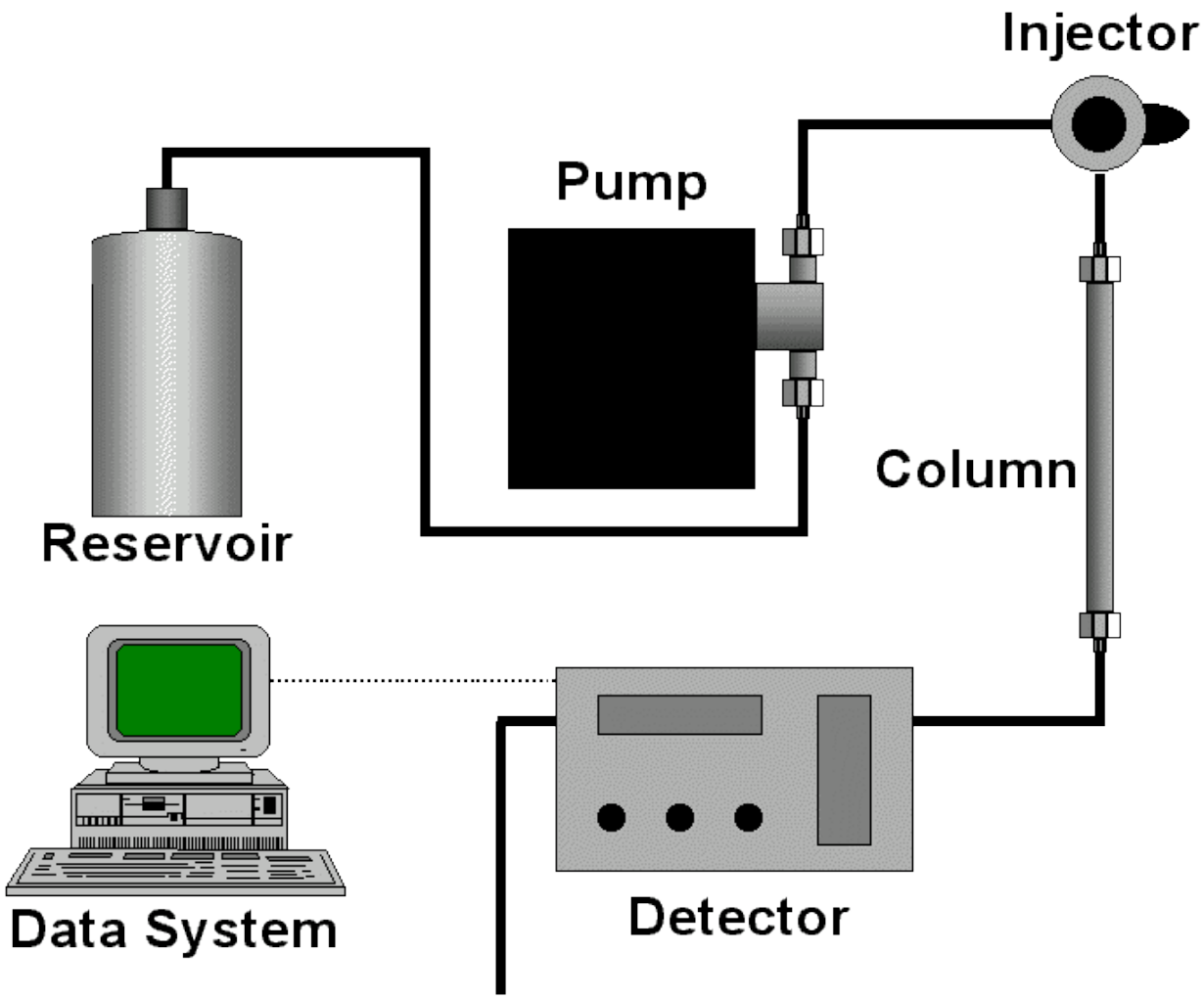
- Water
- Water-soluble organic solvent: Methanol, Acetonitrile, Tetrahydrofuran

The mixing ratio of the water and organic solvent has the greatest influence on separation.

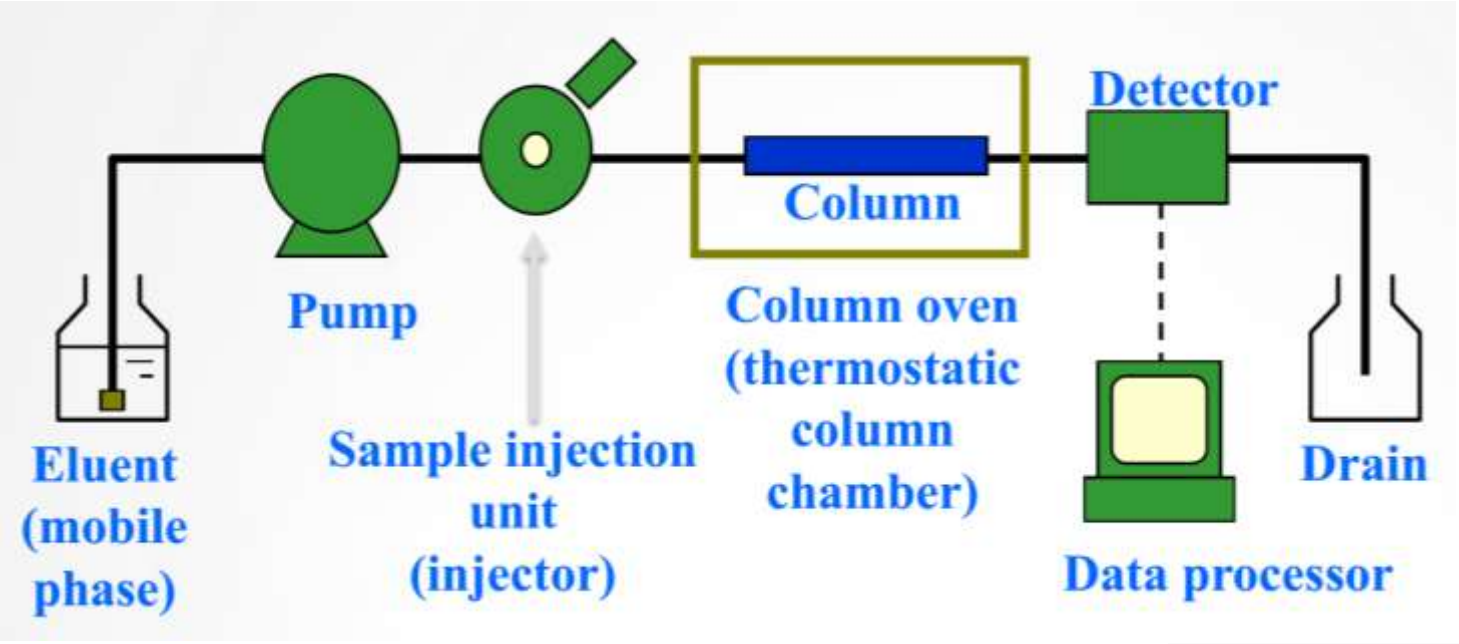
Normal Vs Reverse Phase HPLC

Normal Phase	Reverse Phase
Effective for separation of structural isomers	Wide range of applications like pesticides, antibiotics
Offers separation selectivity not available with reversed phase	Stationary phase has long service life
Stabilizes slowly and is prone to fluctuations in retention time	Stabilizes quickly
Eluents are expensive	Eluents are inexpensive and easy to use

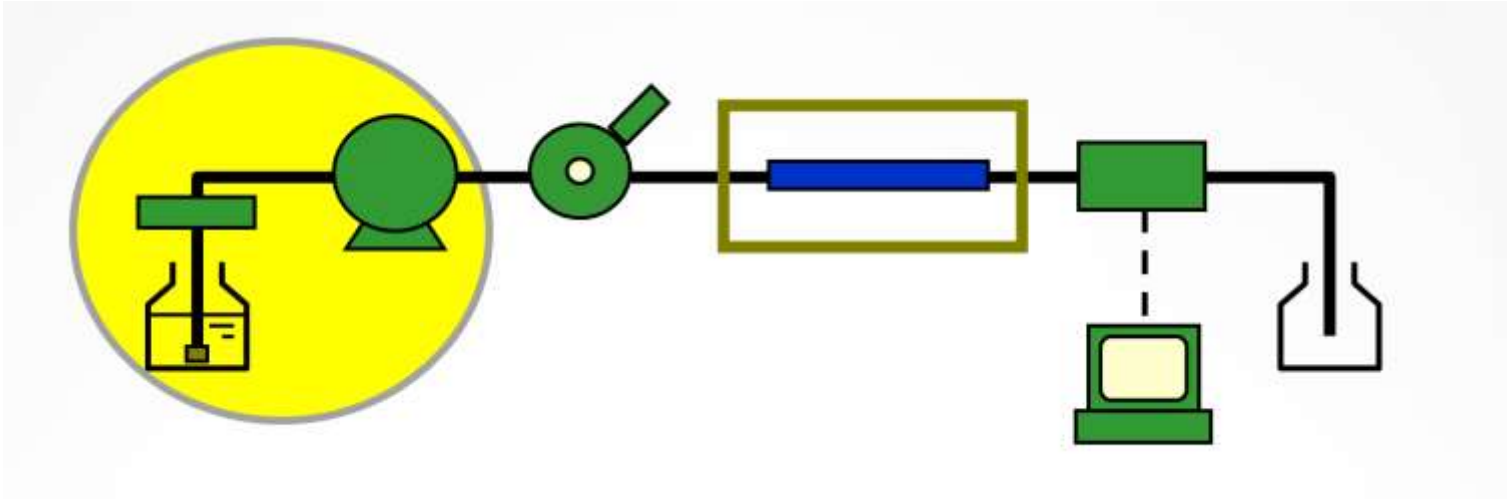
HPLC Basic Components



HPLC Basic Components

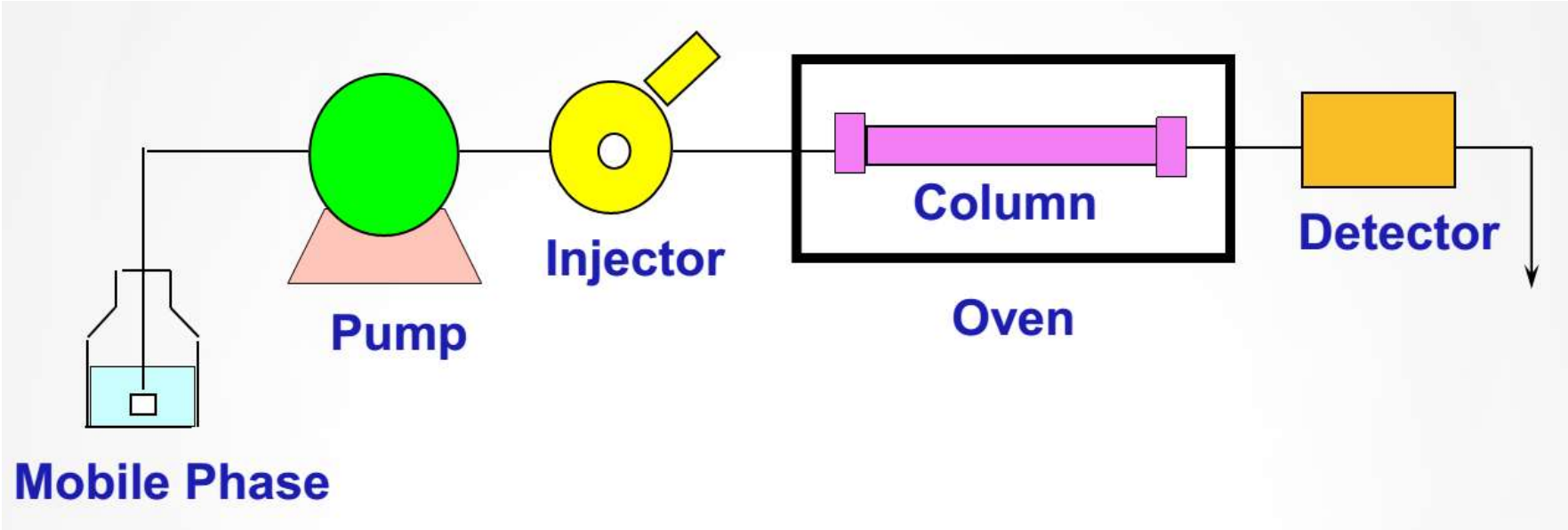


Solvent Delivery in HPLC



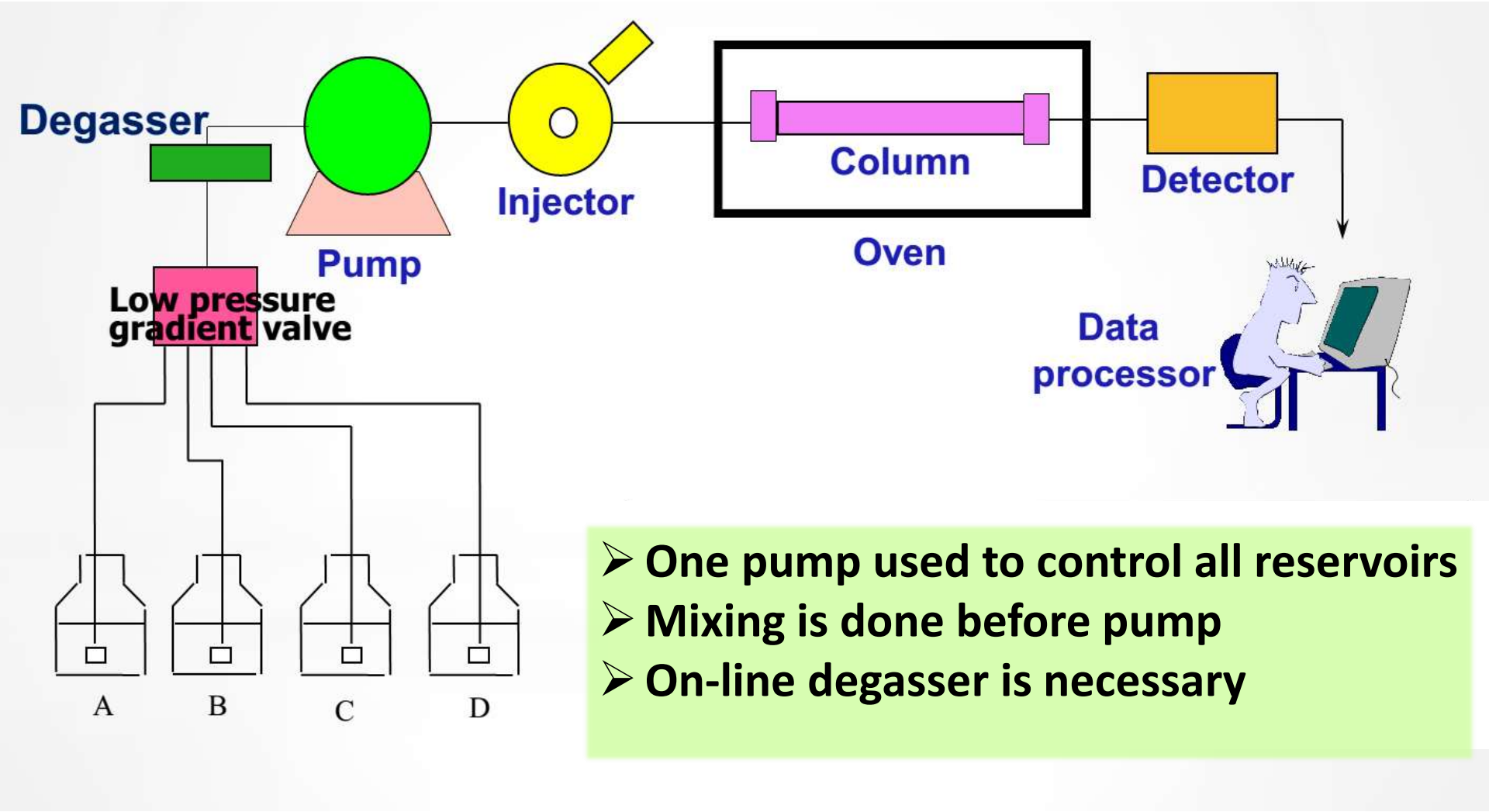
- **Isocratic system**
  - Constant eluent composition
- **Gradient system**
- Varying eluent composition
  - ✓ HPGE (High Pressure Gradient)
  - ✓ LPGE (Low Pressure Gradient)

Isocratic System



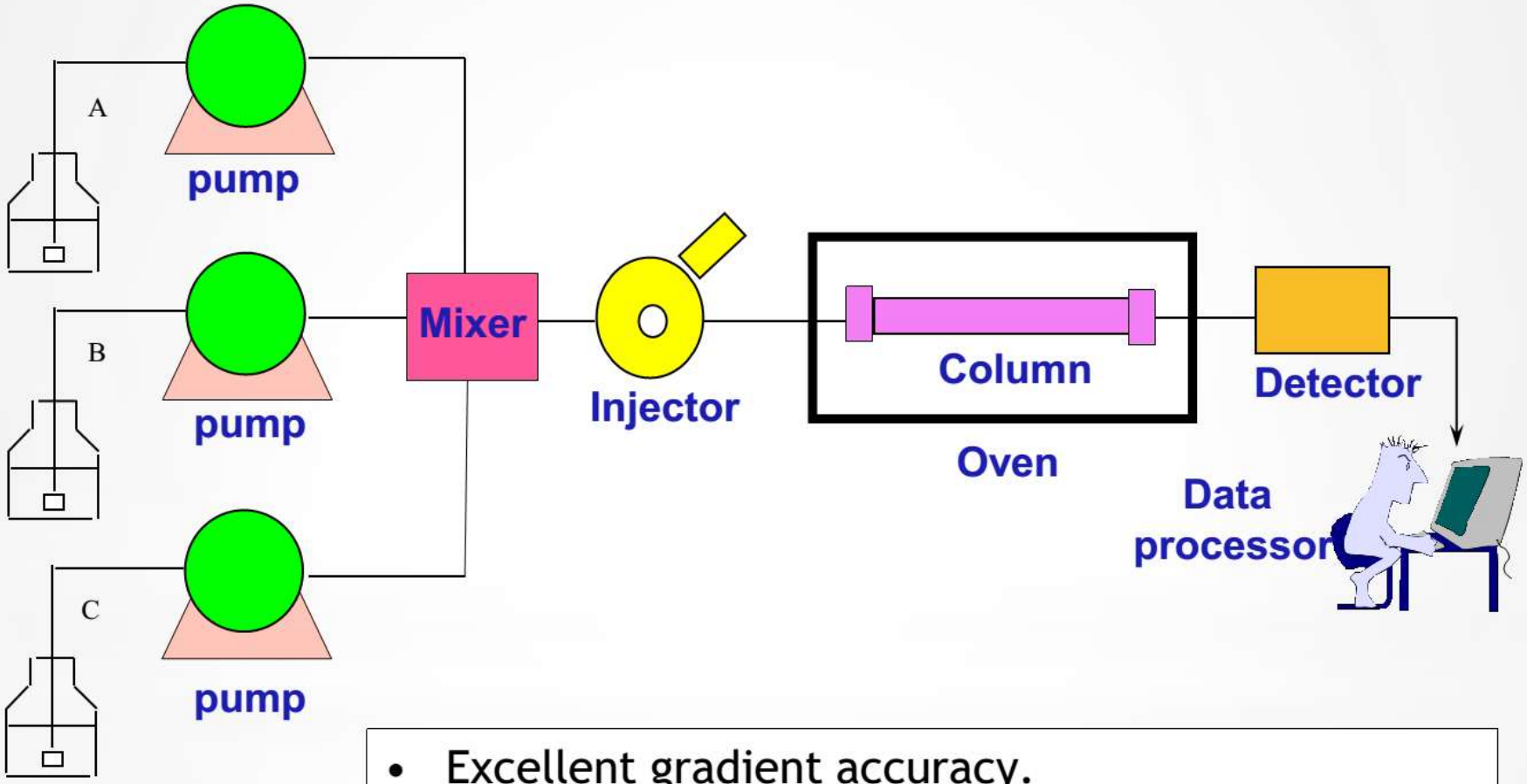
- Simple system with one pump and one solvent reservoir.
- If more than one solvent is used, solvents should be premixed.

Low Pressure Gradient System



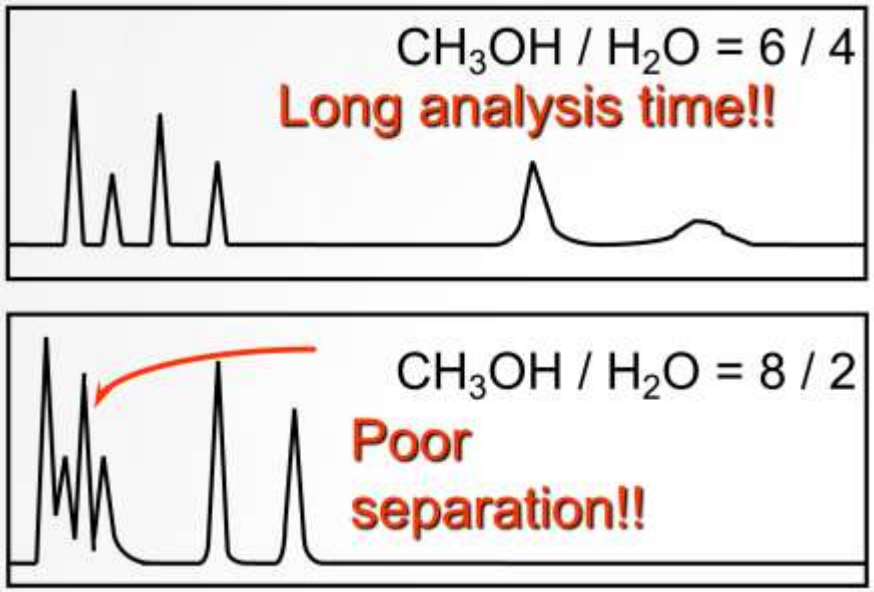
- One pump used to control all reservoirs
- Mixing is done before pump
- On-line degasser is necessary

High Pressure Gradient System

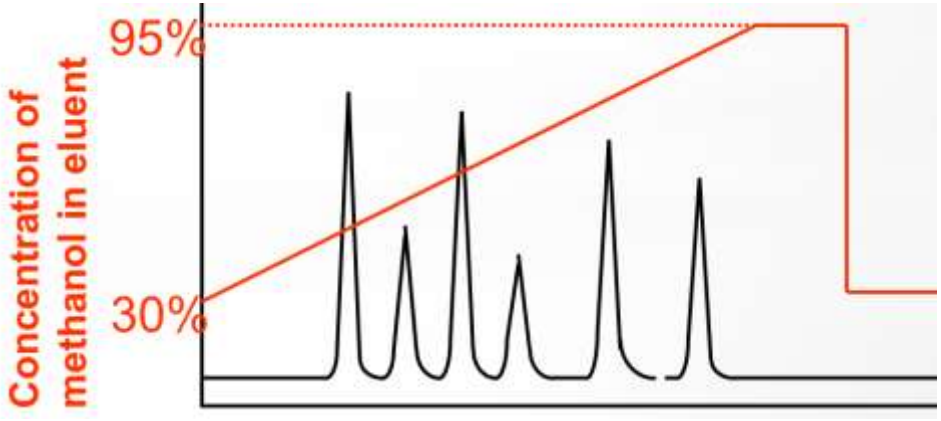


- Excellent gradient accuracy.
- Need one pump per solvent used.
- On-line degassing may not be critical.

Aim of Gradient System



Isocratic system



Gradient system

High/Low Pressure Gradient System

➢ **High-pressure gradient system**

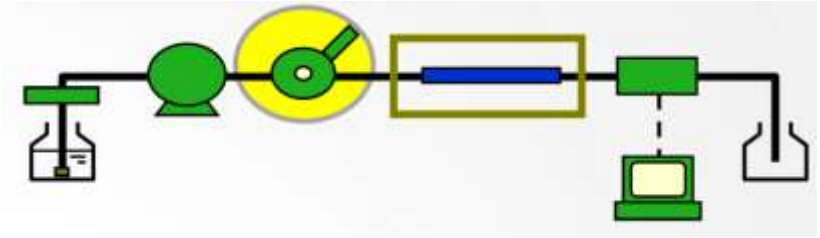
- High gradient accuracy
- Complex system configuration (multiple pumps required)

Ultrasonic Bath

➢ **Low-pressure gradient system**

- Simple system configuration
- Problems caused by dissolved air in the eluent
  - Unstable delivery by pump
  - More noise and large baseline drift in detector cell
  - In order to avoid these problems, the eluent must be degassed by Degasser

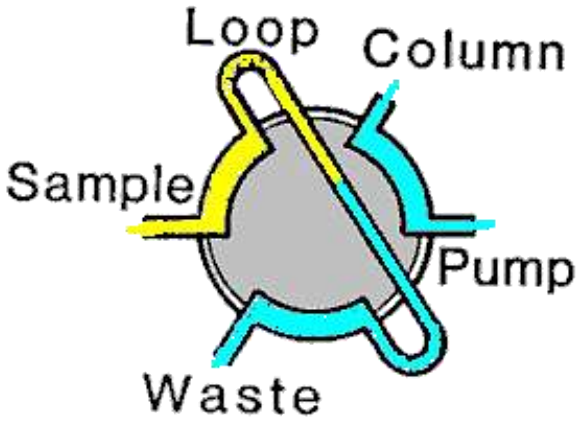
Sample Injection Unit (Injector)



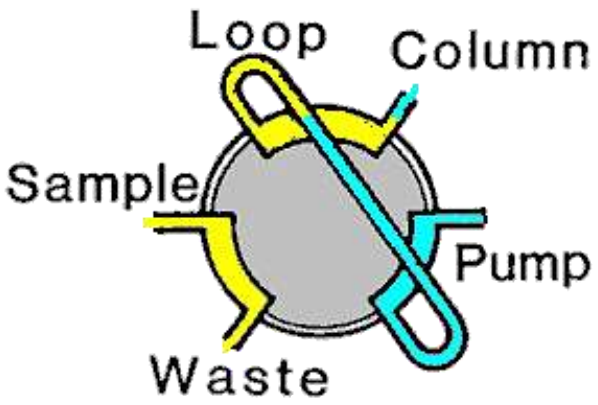
Manual injector & Auto sampler

"LOAD" position → the sample can be injected into the sample loop, which is separated from the flow path

“INJECT" position → The eluent travels through the loop from the pump then delivers the sample to the column

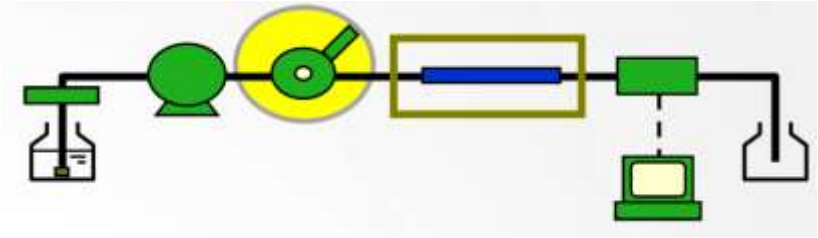


LOAD



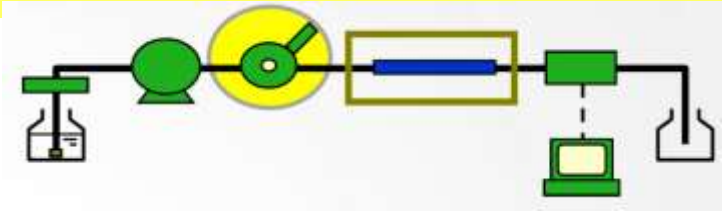
INJECT

Sample Pre-treatment



- To improve the accuracy of quantitative values
- To improve sensitivity and selectivity
- To protect and prevent the deterioration of columns and analytical instruments
- To simplify measurement operations and procedures
- To stabilize target substances

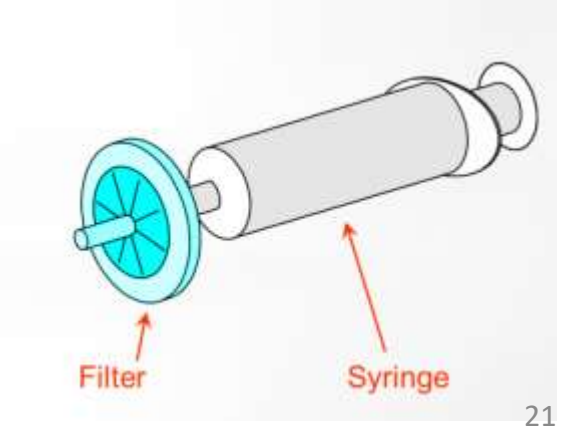
Sample Pre-treatment



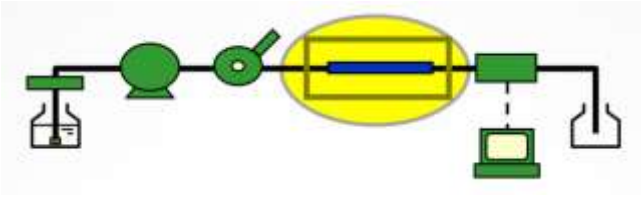
Not to be injected:

- Insoluble substances (e.g., microscopic particles and precipitates)
- Substances that are precipitated in the eluent
- Substances that irreversibly adsorb to the packing material
- Substances that dissolve, or chemically react, with the packing material

✓ In general, filter every sample before injection.
✓ It is convenient to use a disposable filter with a pore diameter of approx. 0.45 µm.
✓ Centrifugal separation is applicable for samples that are difficult to filter.



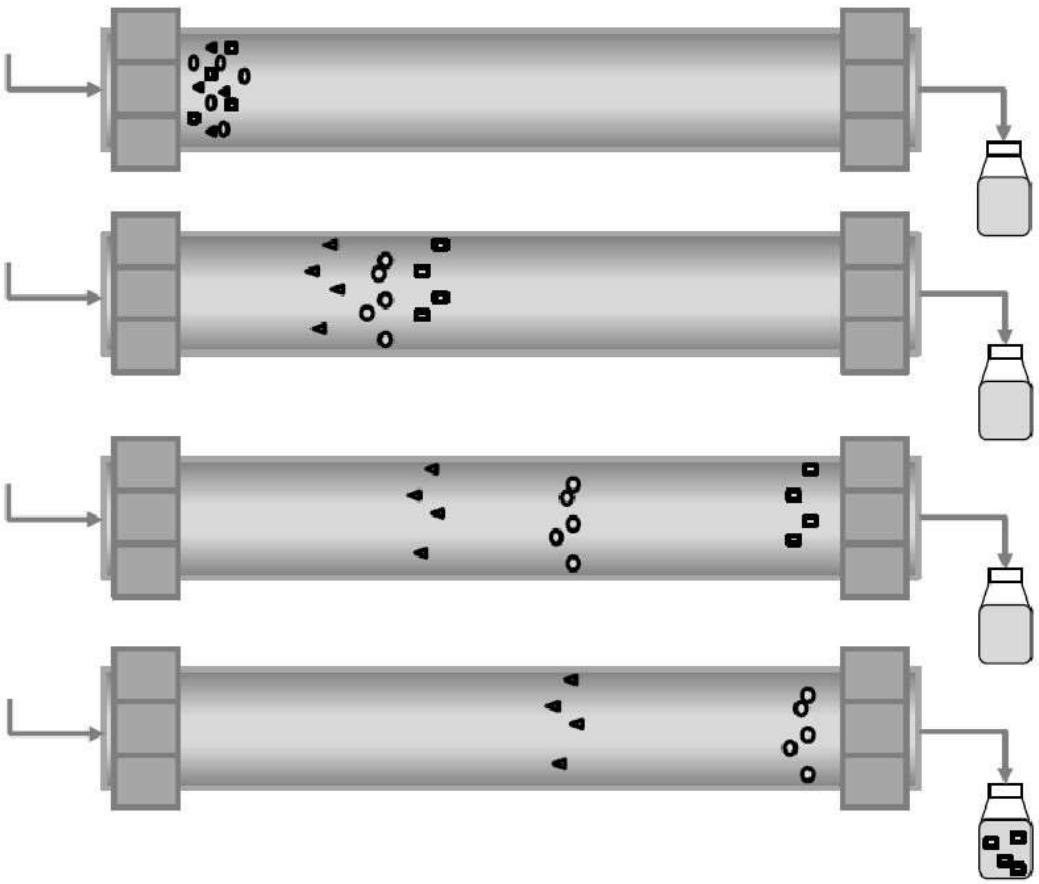
Fate of Sample in HPLC Column

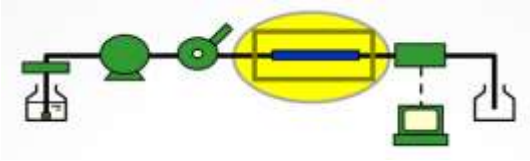


- The column oven is used to maintain a constant column temperature.
- If the column temperature were allowed to vary during qualitative or quantitative analysis, the elution time of the components would change, so that an accurate analysis could not be performed.
- An analysis temperature between 25 and 50^{o}C is often selected.

Column Oven

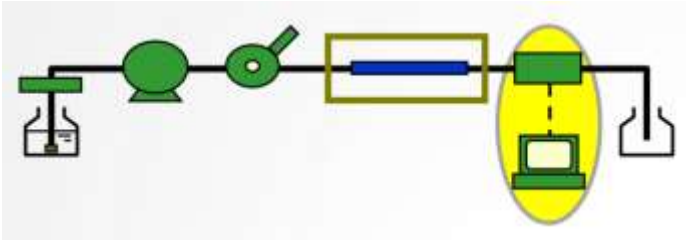
Fate of Sample in HPLC Column

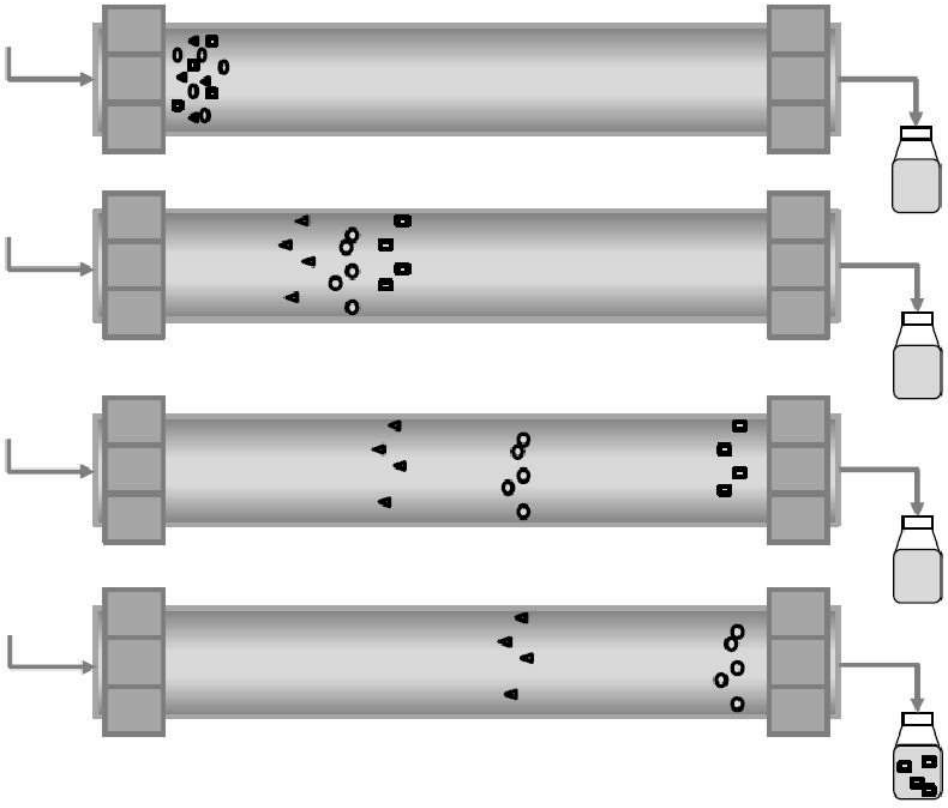


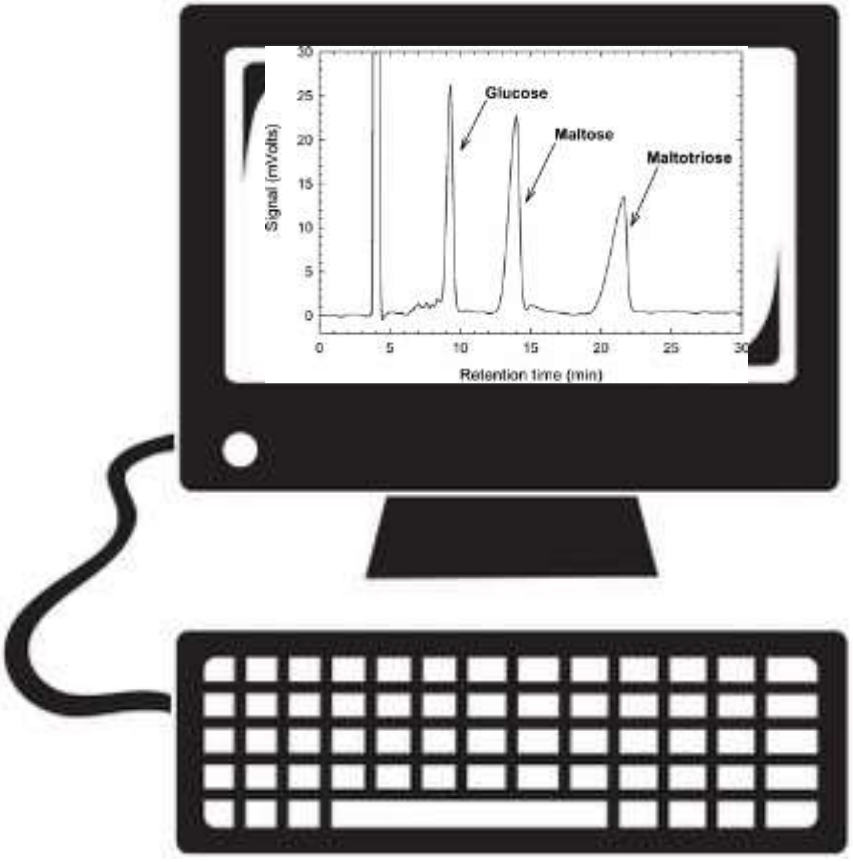


Components of the mixture being eluted off the chromatography column has to be detected……

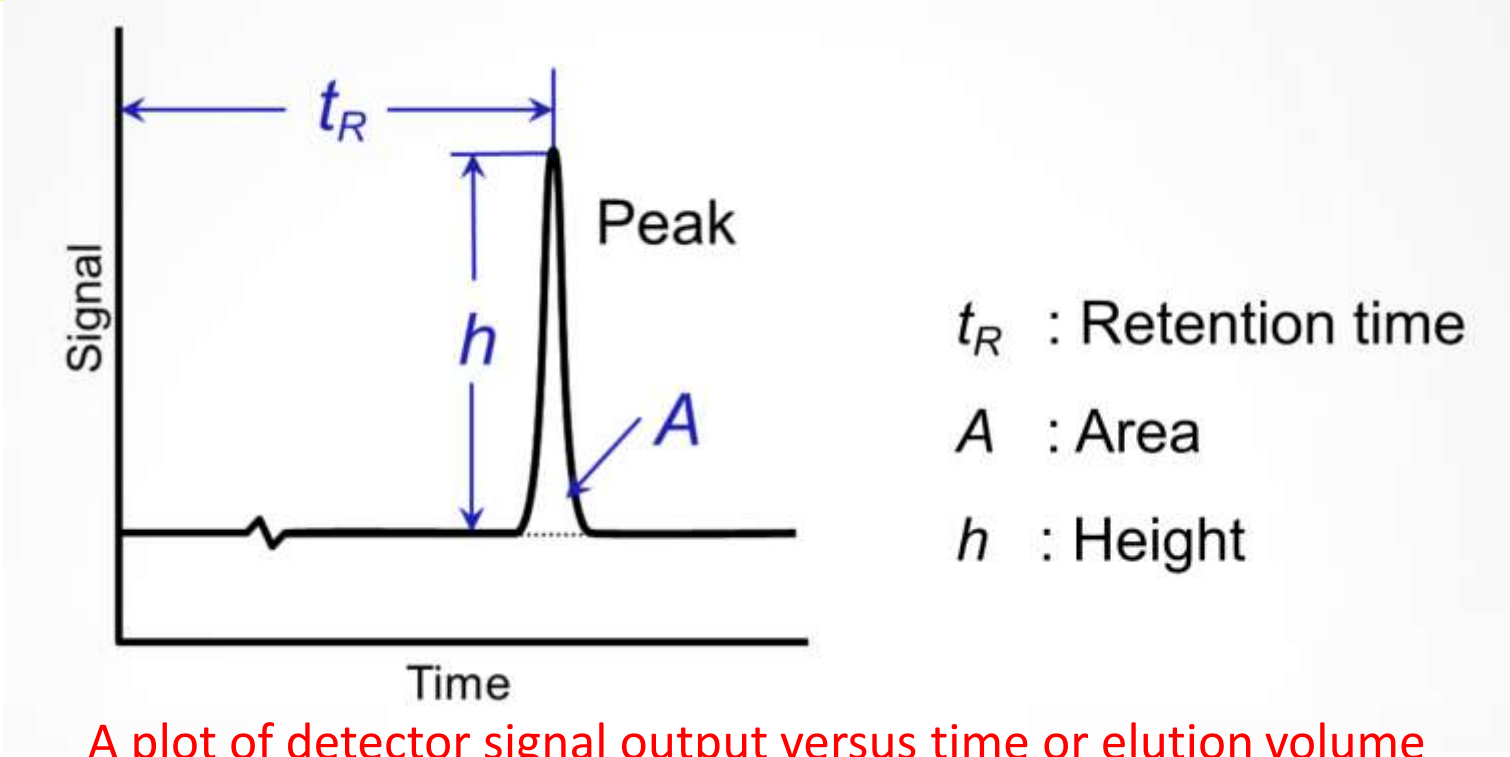
Detectors for HPLC







Chromatogram



A plot of detector signal output versus time or elution volume

Retention time: The time taken by the analyte peak to reach the detector after sample injection.

Peak: The visual representation on the chromatogram based on the detector's electrical response due to the presence of a sample component inside the flow cell.

Detectors for HPLC

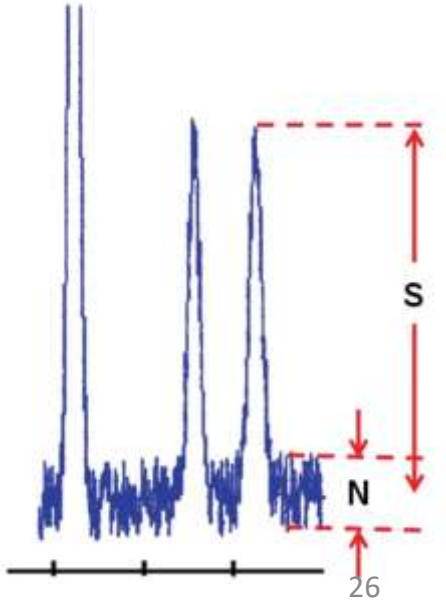
- **Sensitivity**

  Sensitivity towards solute over mobile phase.

- **Selectivity**

  The detector must be able to detect the target substance without, if possible, detecting other substances.

- **Adaptability to separation conditions**
- **Operability**
- **Low detector noise**
- **Low detection limits**
- **Large linear dynamic range**



Detectors for HPLC

UV-VIS	Ultraviolet / Visible detector
PDA	Photodiode Array detector
RF	Fluorescence detector
CDD	Conductivity detector
RID	Refractive Index detector
ECD	Electrochemical detector
ELSD	Evaporative light scattering detector
MS	Mass spectrometer

Detectors for HPLC

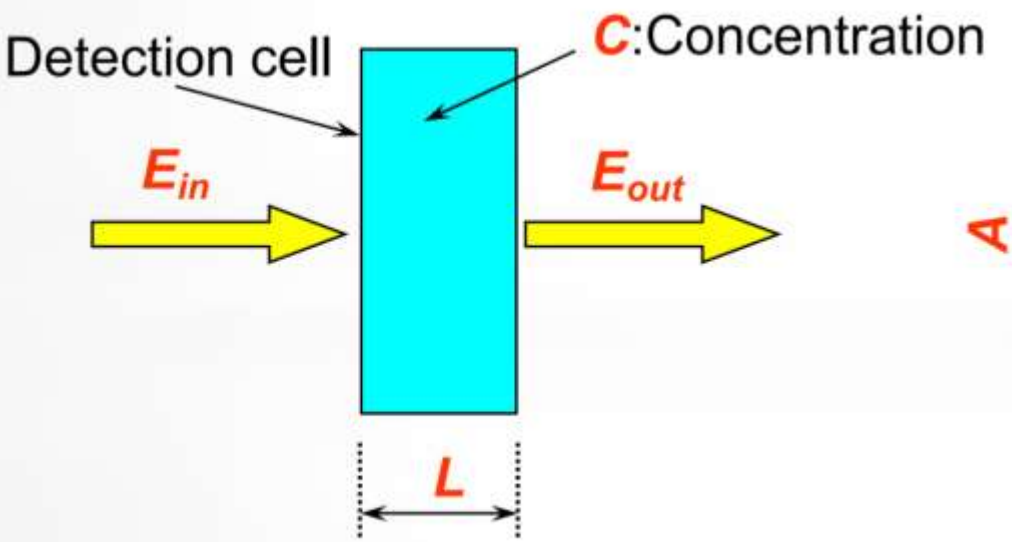
Detectors	Type of compounds can be detected
UV-Vis & PDA	Compounds with aromatic rings or multiple alternating double bonds.
RF F	Fluorescent compounds
CDD	Charged compounds, such as inorganic ions and organic acid.
ECD	For easily oxidized compounds like quinones or amines.
RID & ELSD	For compounds that do not show characteristics usable by the other detectors, eg. polymers, saccharides.

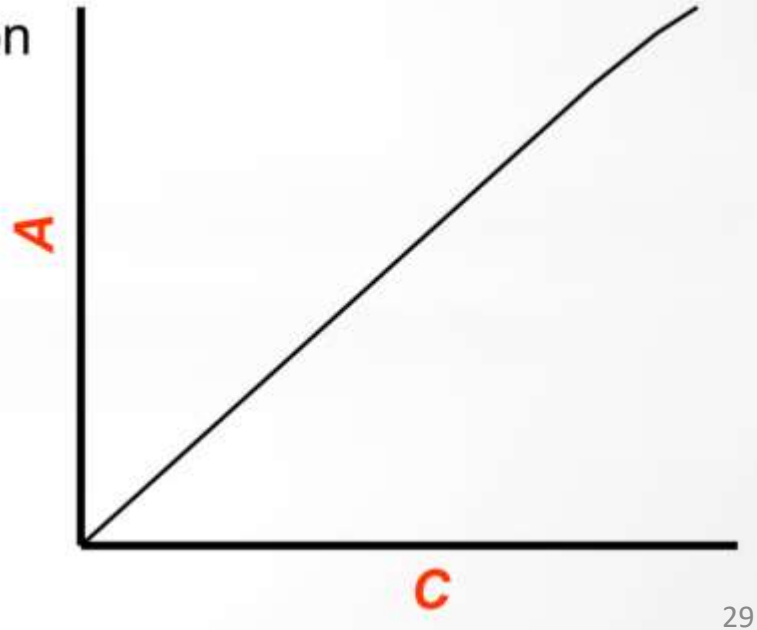
UV-Vis Detector

Beer Lambert's Law

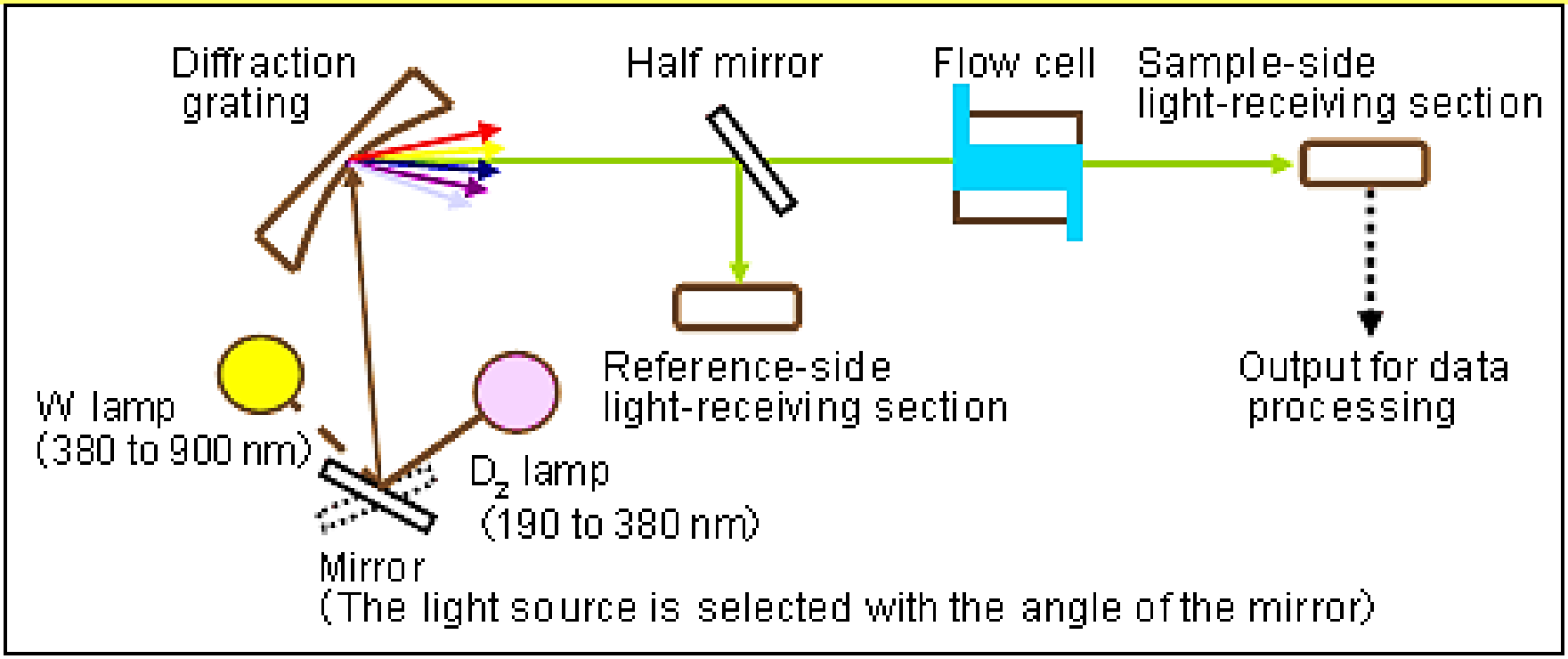
$$A = \varepsilon C L = -\log (E_{out} / E_{in})$$

A : absorbance
ε : molar absorptivity
C : analyte concentration
L : path length of the flow cell
E : energy





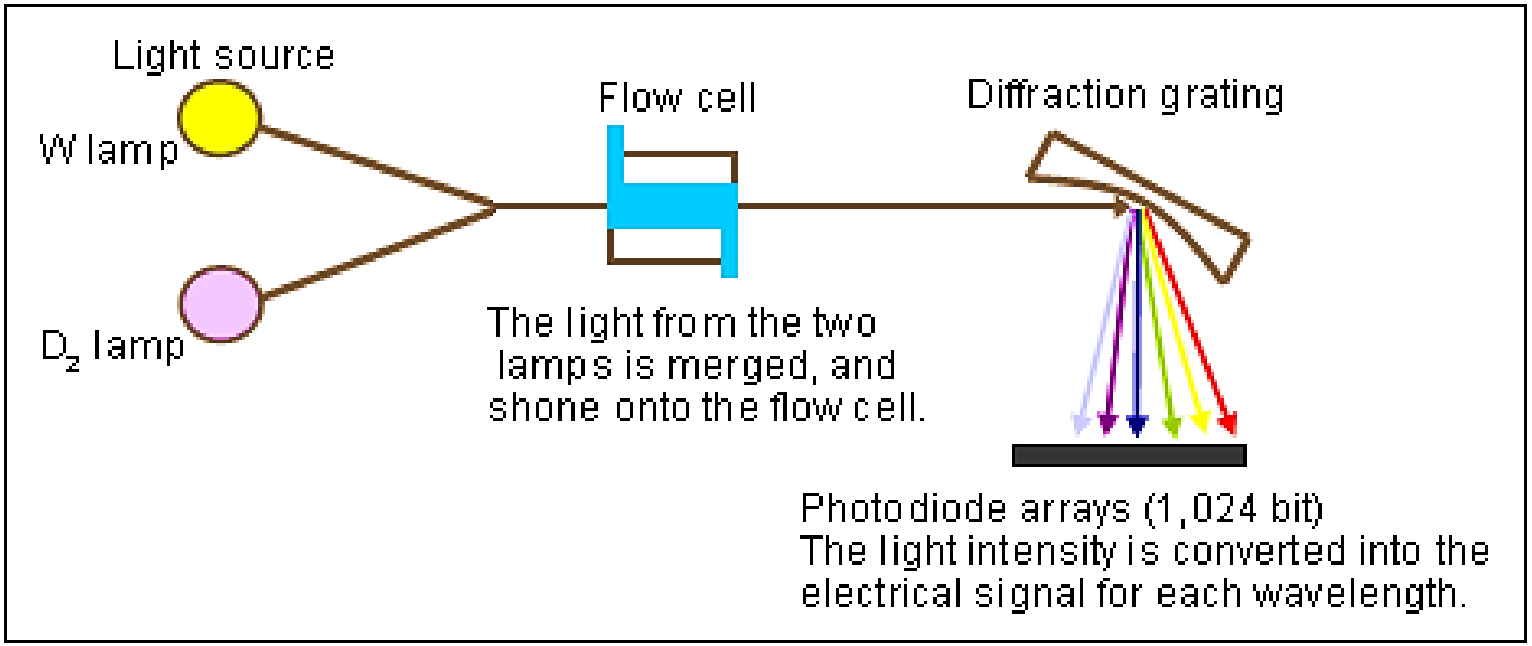
UV-Vis Detector



- Components with a large molar extinction coefficient (ε) can show a large peak even in small amounts.
- Thus, the concentration cannot be determined from peak size.

Photodiode Array Detector (PDA)

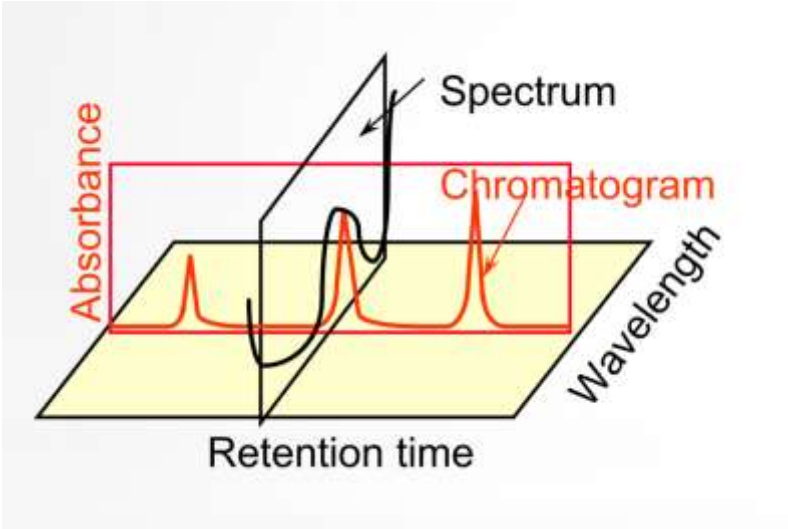
- Uses photodiode array in the detection unit.
  - Information over a wide range of wavelengths at one time
- Spectra are measured at intervals of 1 second or less during separation by HPLC with continuous eluate delivery.



Photodiode Array Detector (PDA)

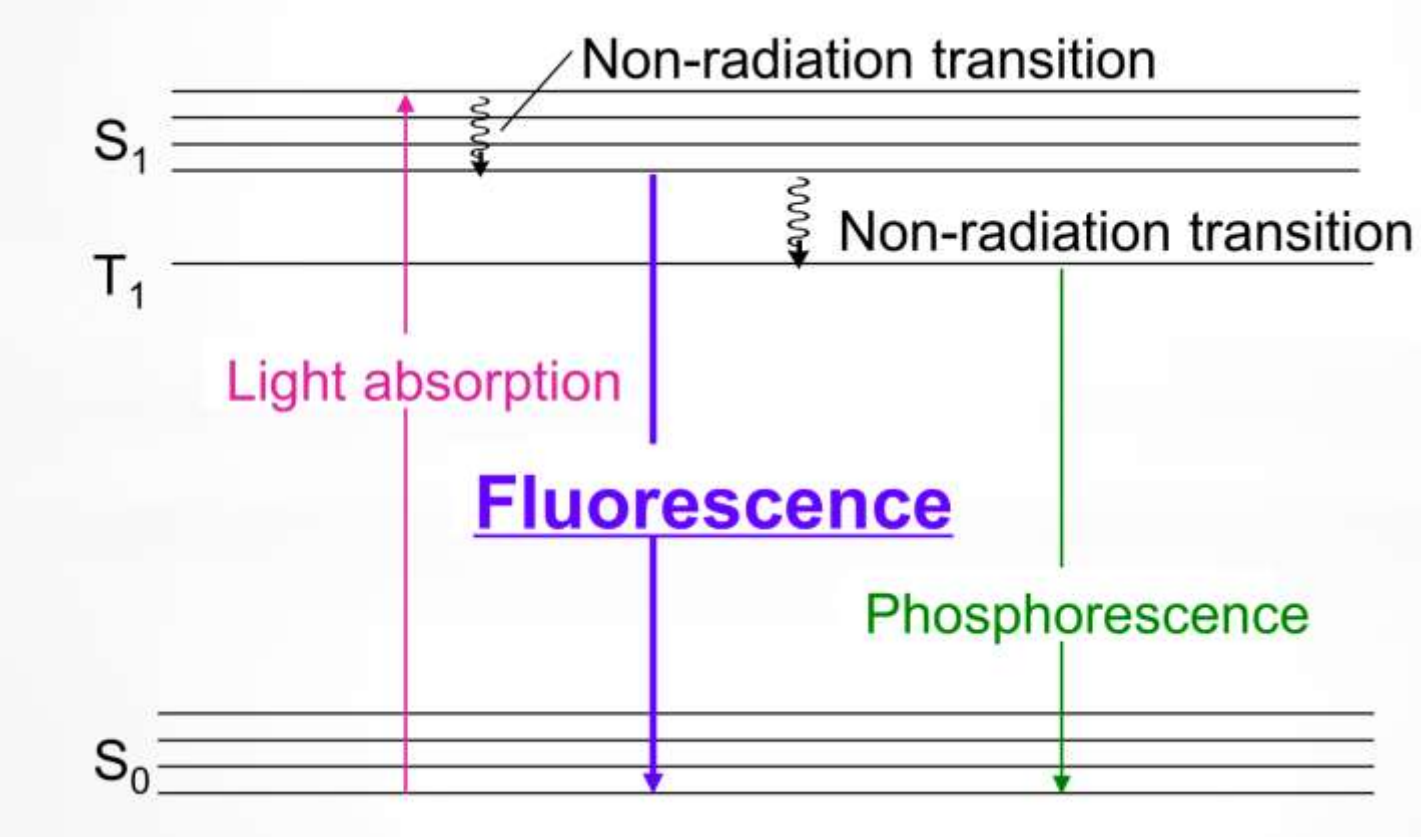
Advantages:

- A Detector could analyze a sample simultaneously at many different wavelengths.
- Useful for compound identification, checking peak purity, as well as finding the optimum absorbance for the compounds.
- UV Visible spectra of many compounds could be stored in the spectrum libraries, which are useful for compound identification.
- Relatively robust to temperature and flow rate fluctuations
- Compatible with gradient elution.



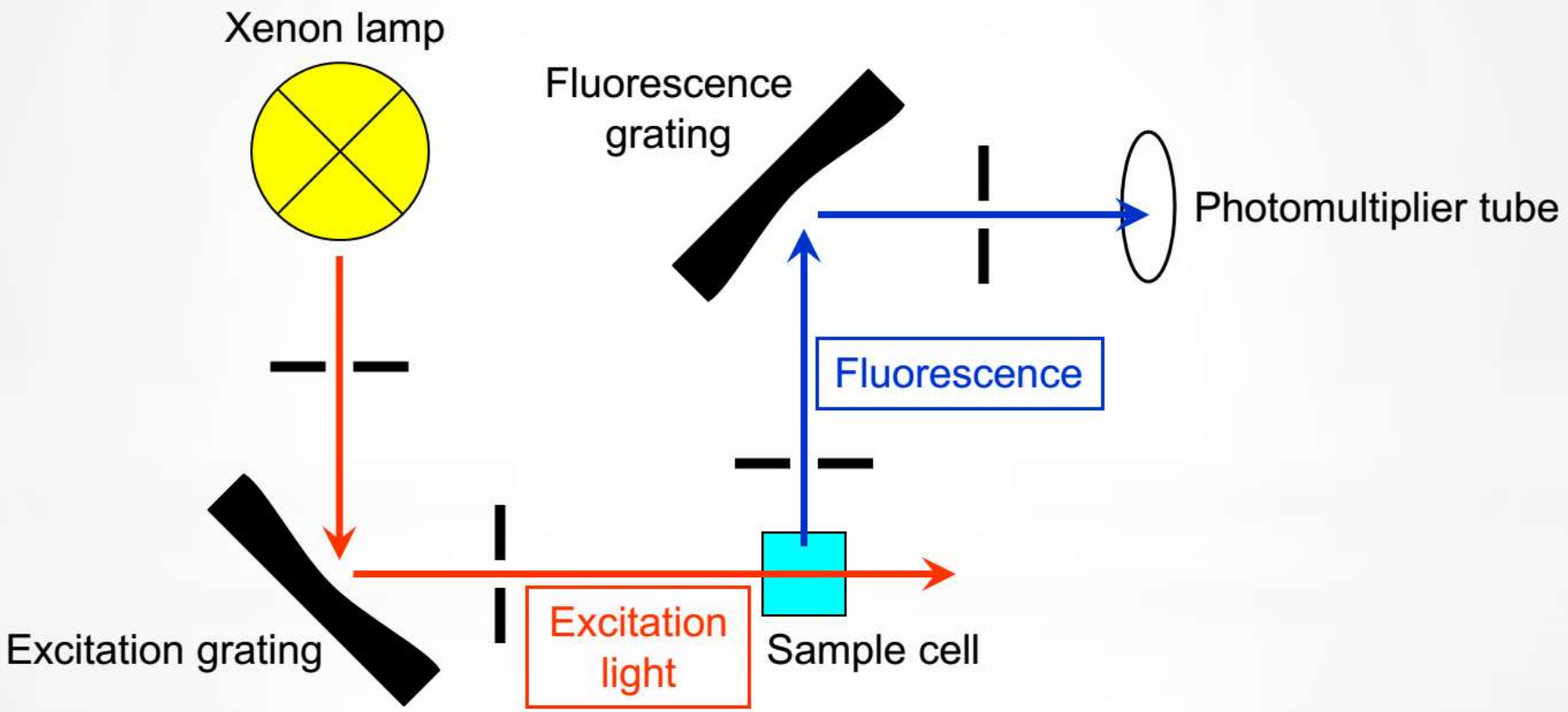
Fluorescence Detector

Greater sensitivity than a UV-VIS detector (10 -1000 times higher)



Fluorescence is a type of luminescence in which the light energy is released in the form of a photon in nanoseconds to microseconds.

Fluorescence Detector



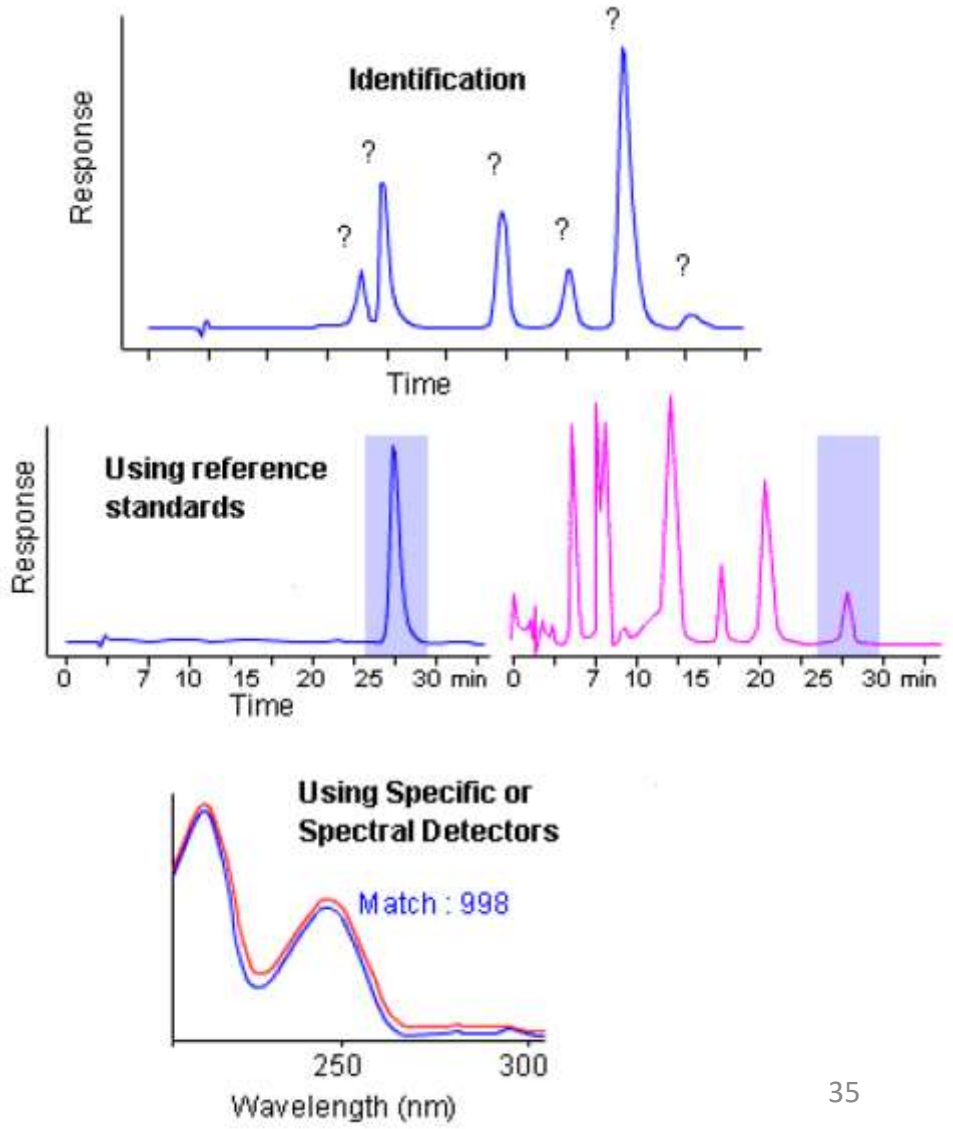
It is possible to detect even a presence of a single analyte molecule in the flow cell.

Qualitative Analysis using HPLC

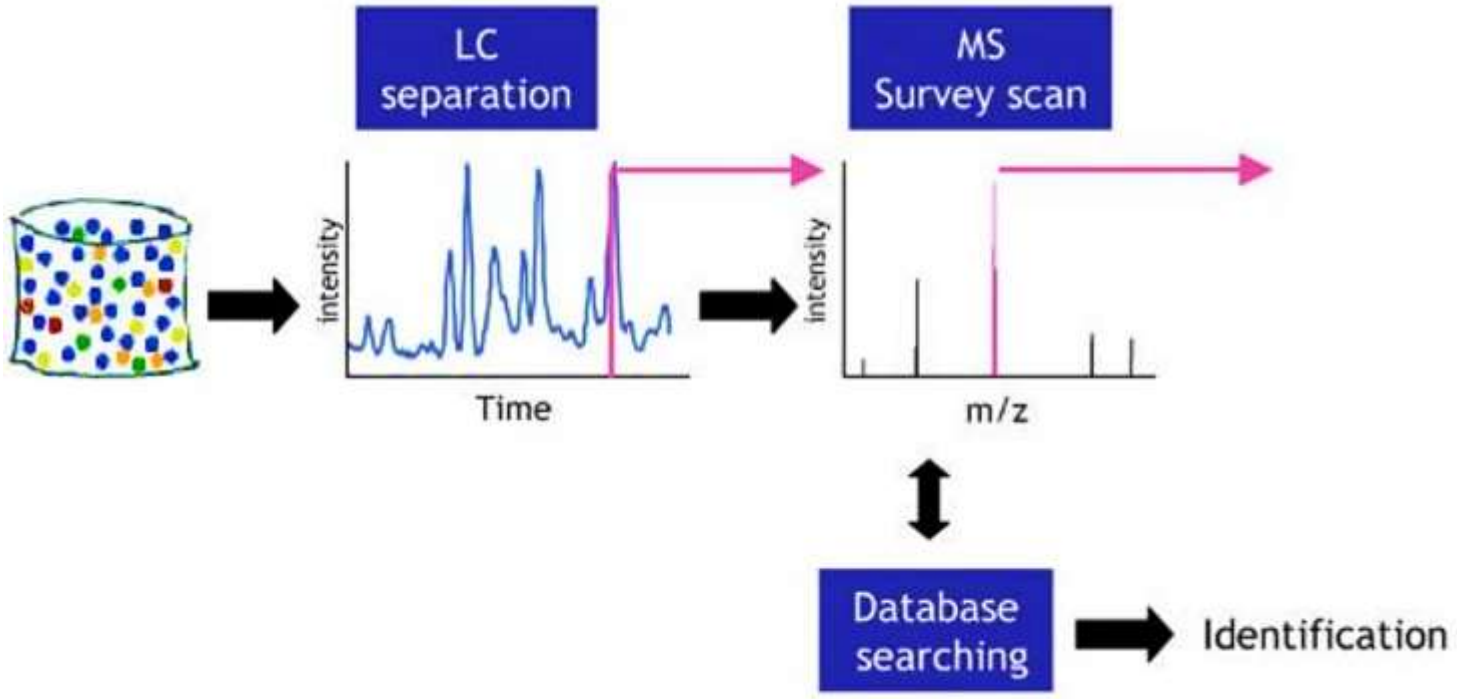
'What' is in the sample?

(1) The sample components are known and peaks within the chromatogram need to be assigned to the known components

(2) A completely unknown sample



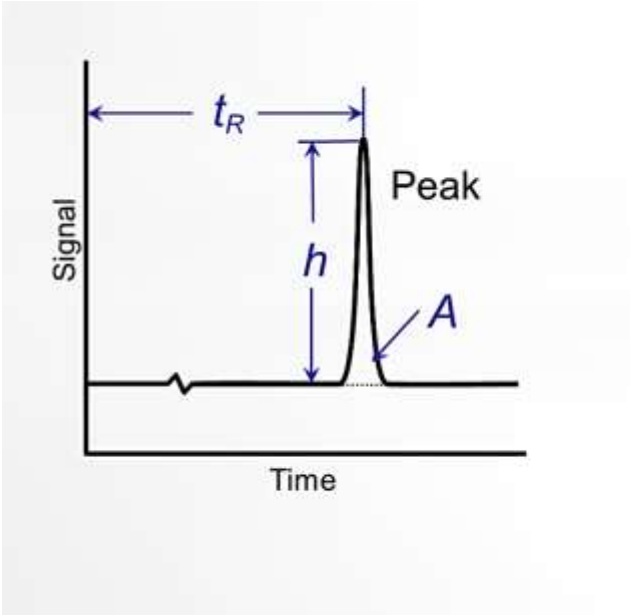
Qualitative Analysis - Mass Spectrometer

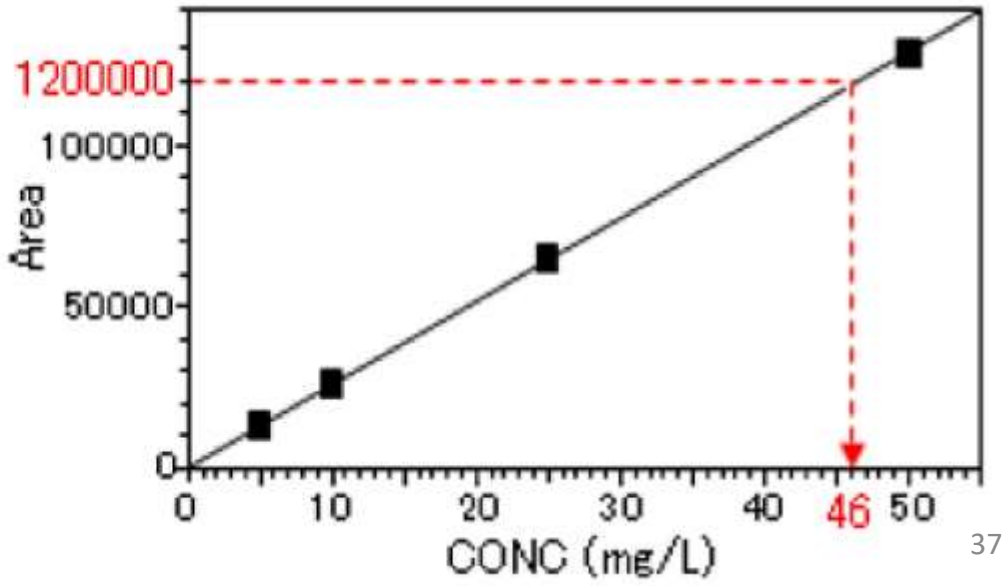


Quantitative Analysis using HPLC

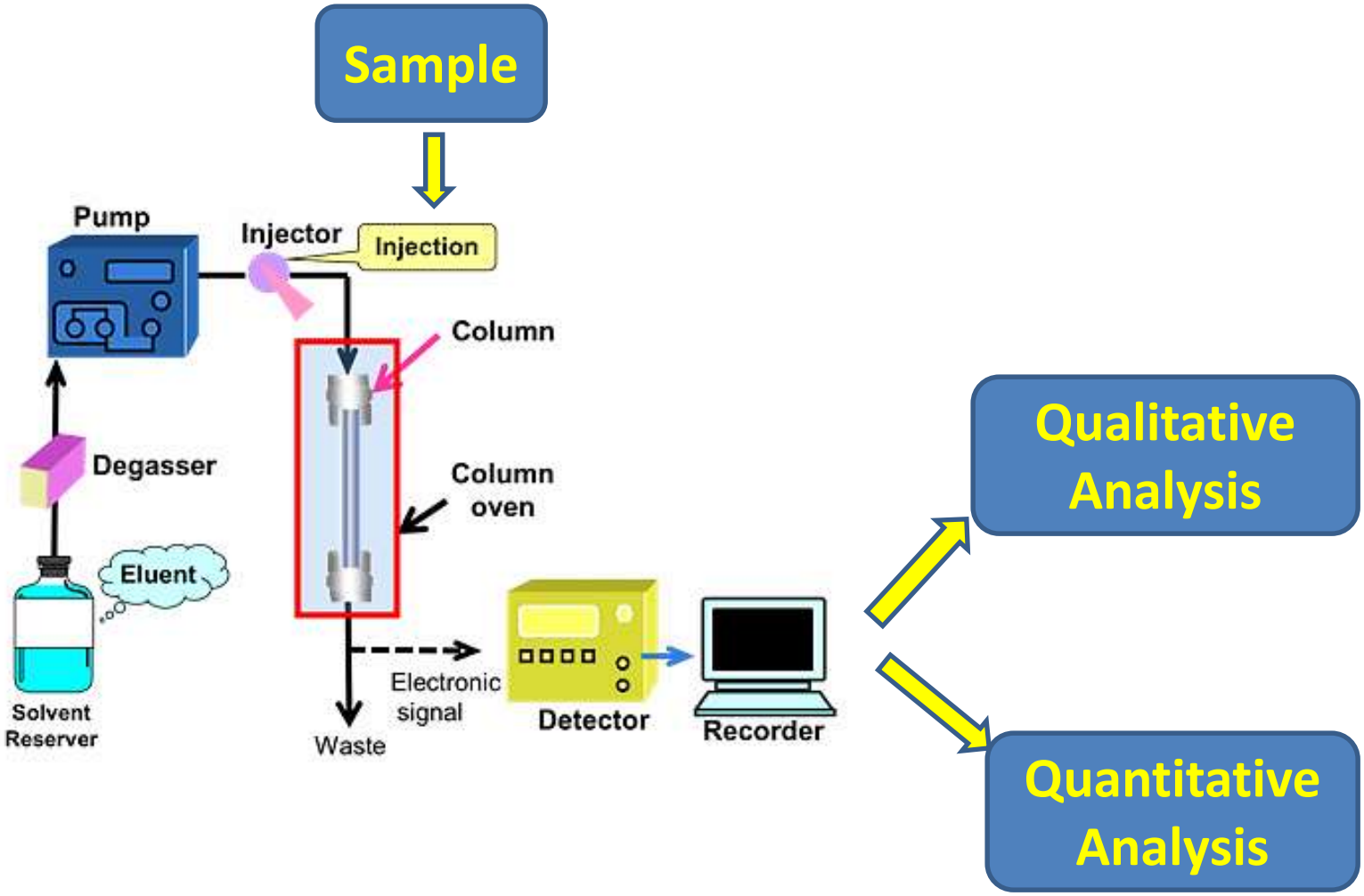
How much????

- Quantitation performed with peak area or height.
- Calibration curve created beforehand using a standard.
- Determine concentration of sample from calibration curve based on its peak area.





Summary


Sample
Pump
Injector
Injection
Column
Degasser
Column oven
Eluent
Solvent Reserver
Waste
Electronic signal
Detector
Recorder
Qualitative Analysis
Quantitative Analysis

Thank You…