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LAAN-E-LC 215

Fundamentals of HPLC

2 _ Role of column and Separation Modes in HPLC

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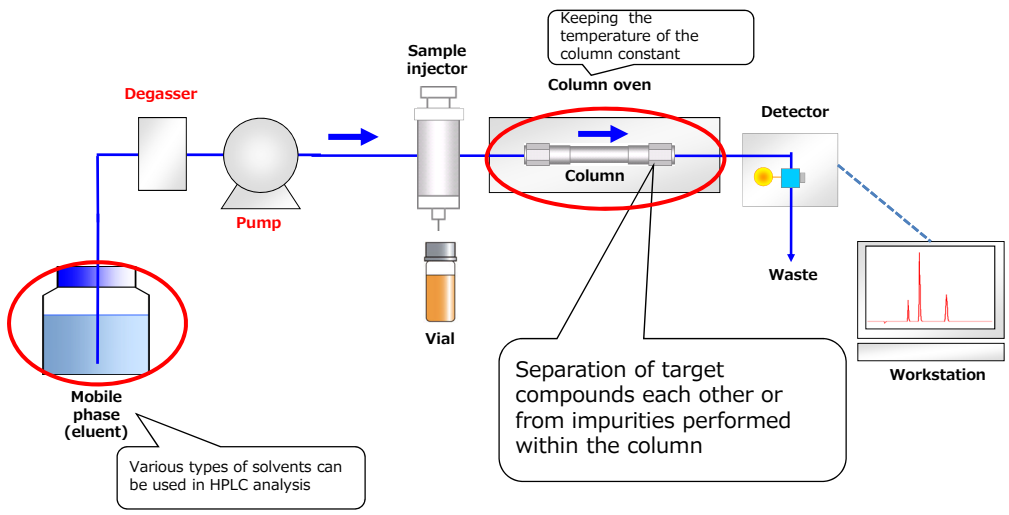
Column and mobile phase in the HPLC configuration



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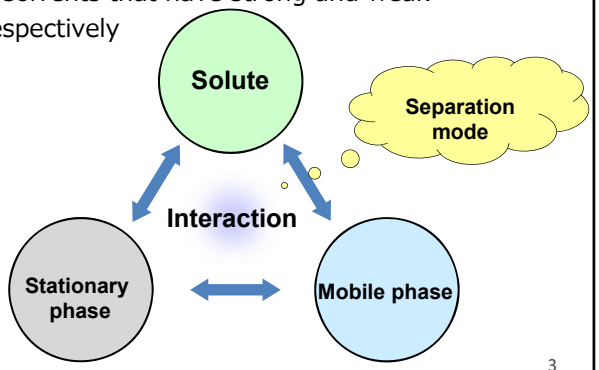
Factors affecting separation in HPLC

- **Separation mode**
- Separation of target compounds from co-existing matrix are accomplished using various interactions provided from the combination of the column and the mobile phase.
- Separation mode mainly depends on the column chemistry
- Mobile phase basically consist of two solvents that have strong and weak elution power to target compounds respectively
  (Except size exclusion mode)



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Typical separation modes

- **Reversed phase chromatography (Reversed phase mode)**
- **Normal phase chromatography (Normal phase mode)**
- **Ion exchange chromatography (Ion exchange mode)**
- **Size exclusion chromatography (Size exclusion mode)**

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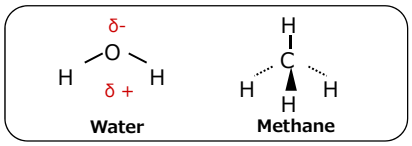
Polarity and hydrophobicity of the compounds affecting separation

What is polarity ?

・・・ **In physical chemistry, it's defined by the dipole moment of compound.**

e.g. Water is a polar compound,
Methane is a non-polar compound.



・・・ **In chromatography, compounds with highly hydrophilic functional groups are called as polar compounds (Hydrophilic).**

Hydroquinone is a polar compound in chromatography even its dipole moment is zero.

e.g. Hydrophilic groups : hydroxyl group, carboxyl group, amino group (High polarity).
Hydrophobic groups : alkyl chains (methyl groups etc.), benzene rings (Low polarity)

In reversed phase analysis, compounds with higher hydrophilic groups elute faster
(Compounds with higher hydrophobic groups elute slower).

-OH Hydroxy group (Hydrophilic group)
-CH_3 Methyl group (Hydrophobic group)

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Normal phase and Reversed phase

	Stationary phase	Mobile phase
Normal phase	High polarity (hydrophilic)	Low polarity (hydrophobic)
Reverse phase	Low polarity (hydrophobic)	High polarity (hydrophilic)

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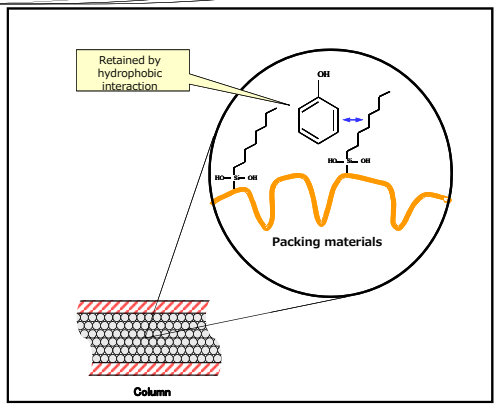
Reversed phase chromatography

- **Mechanism of retention**
  - Separation mode using hydrophobic interaction
- **Features**
  - Wide range of applications
    ->Applicable to most of the compounds
  - High separation efficiency
    ->Easy to get sharp peaks
    ->Analysis time can be short.
  - Neutral hydrophilic compounds are poorly retained
    ->Not suitable for carbohydrates such as sugar



- **Typical mobile phase**
  - Weak elution power: Water or Buffer
    → Adjust the retention of ionic compounds by pH
  - Strong elution power : Organic solvents such as methanol and acetonitrile
    → Adjust the retention by concentration of organic solvent
- **Typical columns**
  - ODS (Octadecyl: C18), C8 (Octyl), TMS (Trimethyl), Phenyl, etc.

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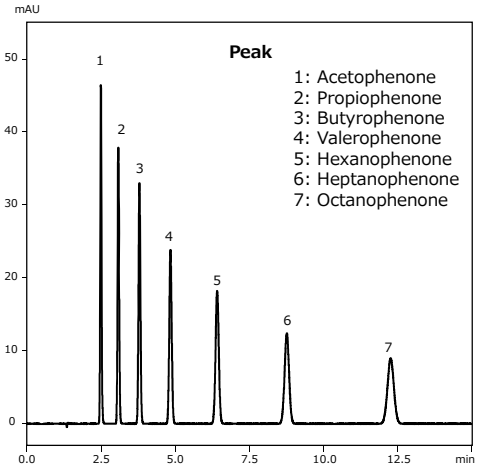
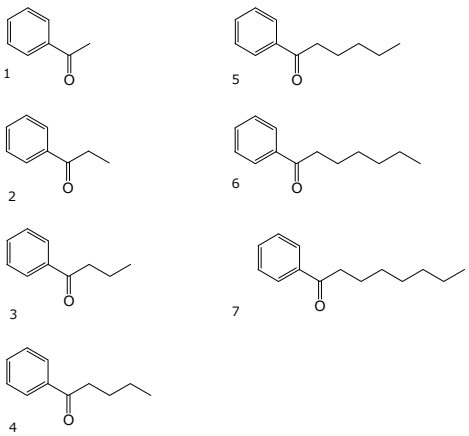
Typical analysis of reversed phase chromatography

- **Separation by difference in hydrophobicity**
  - Lower hydrophobic compounds elute faster





Analytical conditions
Column: Shim-pack VP-ODS (150 mm x 4.6 mm I.D.)
Mobile phase: Water/acetonitrile = 30/70
Flow rate: 1 mL/min
Column temperature: 40 ℃
Detection: UV at 245 nm

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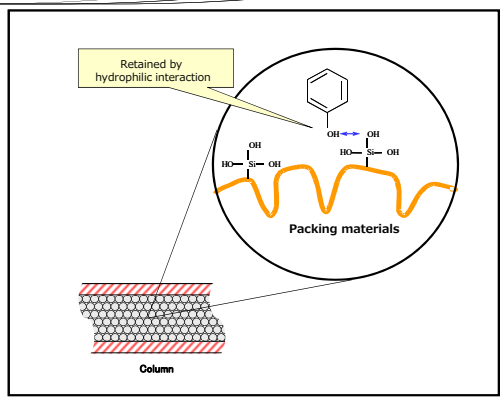
Normal phase chromatography

- **Mechanism of retention**
  - Separation mode using hydrophilic interaction
- **Features**
  - Normally water is not used as mobile phase
    ->Suitable for hydrolyzable compounds
    ->Convenient for preparative purification
  - Saturated hydrocarbon compounds can not be retained
  - Basic compounds adsorbed strongly
  - High structural recognition ability
    ->Suitable for the separation of geometrical isomers



- **Typical mobile phase**
  - Solvents with weak elution power: Low Polarity solvents : hexane
  - Solvents with strong elution power: Highly polarity solvents : ethanol and 2-propanol
    ->Adjust retention by concentration of polarity organic solvent
    ->Adjust retention of ionic compounds by adding acid/base
  - Mixture of acetonitrile and water may be used in particular cases (hydrophilic interaction chromatography, HILIC).
- **Typical columns**
  - SIL (silica gel), CN (Cyano), NH2 (Amino)

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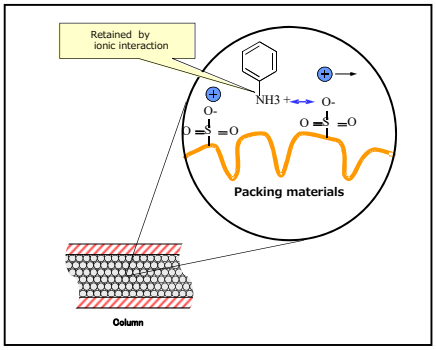
Ion exchange chromatography

- **Mechanism of retention**
  - Separation mode using ion interaction based on ionic bond
- **Features**
  - Suitable for all of the ionic compounds
    ->Suitable for both organic and inorganic compounds
  - Theoretically neutral compounds can not be retained
  - Cations and anions can not be separated in one analysis (Columns can retain one but can not retain the other)



- **Typical mobile phase**
  - Aqueous solution (Mixing with acetonitrile, etc. if necessary) containing salts such as a buffer solution
    ->Higher concentration (ionic strength) buffer results in faster elution
    ->In cation exchange mode, increase the pH to elute faster
    In anion exchange mode, decrease the pH to elute faster.
- **Typical columns**
  - Strong cation exchange (SCX), weak cation exchange (WCX)
  - Strong anion exchange (SAX), weak anion exchange (WAX)

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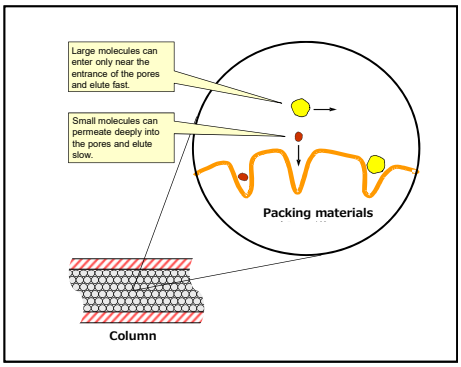
Size exclusion chromatography (SEC)

- **Mechanism of retention**
  - Separate molecules by their solvated size (molecular weight)
- **Features**
  - Separation is only depends on the size of molecules
    ->Larger molecules elute faster
  - Theoretically, all molecules should elute between the exclusion limit and penetration limit of column
    -> Allowable molecular weight range is determined by the characteristics of column
- **Typical eluent**

  <Non-aqueous mode, GPC>
  - Tetrahydrofuran, chloroform, dimethylformamide, etc.

  < Aqueous mode, GFC>
  - Water, buffer
- **Typical columns**
  - Elution time can be controlled by changing columns that have particular molecular weight range respectively
  - Non-aqueous type (organic solvents) and aqueous type (water or aqueous buffer solution)



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Thank you for your attention.