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# Hideharu Shintani

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# BIOGRAPHY

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# RESEARCH INTEREST

- Gas plasma sterilization
- HPLC, Ion chromatography
- Analytical Chemistry
- Pharmaceutical Science
- Sterilization Validation

# RECENT PUBLICATIONS

- Sterilization mechanism of nitrogen gas plasma: induction of secondary structural change in protein. Sakudo A, Higa M, Maeda K, Shimizu N, Imanishi Y, **Shintani H**. Microbiol Immunol. 2013 Jul;57(7):536-42. doi: 10.1111/1348-0421.12061.
- Confirmation of the sterilization effect using a high concentration of ozone gas for the bio-clean room. Iwamura T, Nagano K, Nogami T, Matsuki N, Kosaka N, **Shintani H**, Katoh M. Biocontrol Sci. 2013;18(1):9-20.
- Dengue virus presence and surveillance in Okinawa (Review). Sakudo A, Onodera T, **Shintani H**, Ikuta K. Exp Ther Med. 2012 Jan;3(1):15-17. Epub 2011 Oct 21.
- Validation study and routine control monitoring of moist heat sterilization procedures. **Shintani H**. Biocontrol Sci. 2012 Jun;17(2):57-67. Review.

- Validation of sterilization procedures and usage of biological indicators in the manufacture of healthcare products. **Shintani H**. Biocontrol Sci. 2011 Sep;16(3):85-94. Review.
- Methods of rapid microbiological assay and their application to pharmaceutical and medical device fabrication. **Shintani H**, Sakudo A, McDonnel GE. Biocontrol Sci. 2011 Mar;16(1):13-21. Review.
- Fundamentals of prions and their inactivation (review). Sakudo A, Ano Y, Onodera T, Nitta K, **Shintani H**, Ikuta K, Tanaka Y. Int J Mol Med. 2011 Apr;27(4):483-9. doi: 10.3892/ijmm.2011.605. Epub 2011 Jan 25. Review.

# HPLC

## INSTRUMENTATION

# INTRODUCTION

- HPLC stands for "High-performance liquid chromatography"(sometimes referred to as High-pressure liquid chromatography).

- High performance liquid chromatography is a powerful tool in analysis, it yields high performance and high speed compared to traditional columns chromatography because of the forcibly pumped mobile phase.

- HPLC is a chromatographic technique that can separate a mixture of compounds

- It is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of a mixture.

•**Chromatography** : physical method in which separation of components takes place between two phases-a stationary phase and a mobile phase

•**Stationary phase** : The substance on which adsorption of the **analyte** (the substance to be separated during chromatography) takes place . It can be a solid, a gel, or a solid liquid combination

•**Mobile phase** : solvent which carries the analyte (a liquid or a gas)

Chromatographic techniques are divided into different types based on :

The type of chromatographic bed used
i.e. column chromatography (gas chromatography) and planar chromatography(paper and thin layer)

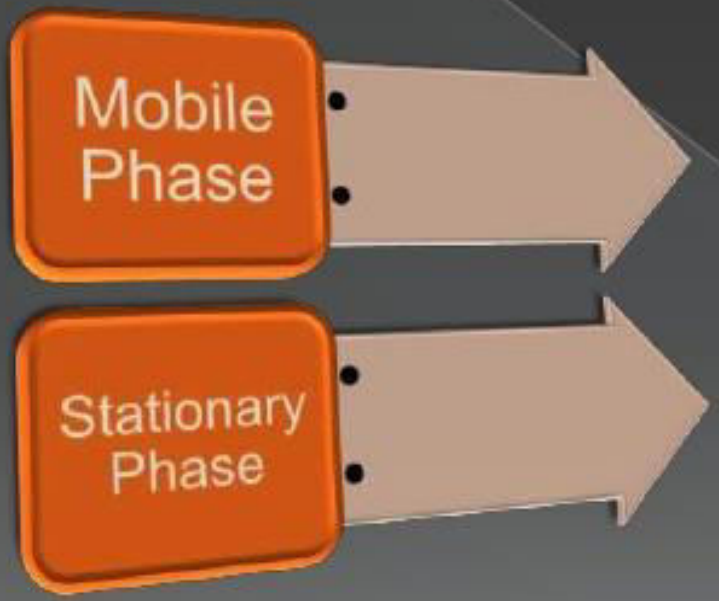
The physical state of mobile phase
i.e. gas chromatography and liquid chromatography

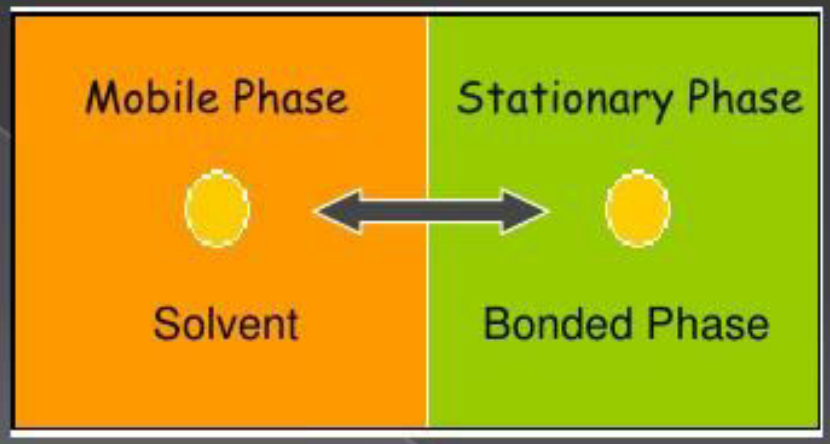
The separation mechanism
i.e. ion-exchange and size exclusion

HPLC is a type of **liquid chromatography** where the sample is forced through a **column** that is packed with a stationary phase composed of irregularly or spherically shaped particles, a porous monolithic layer , or a porous membrane by a liquid (mobile phase) at high pressure.

# TYPES OF PHASES :-

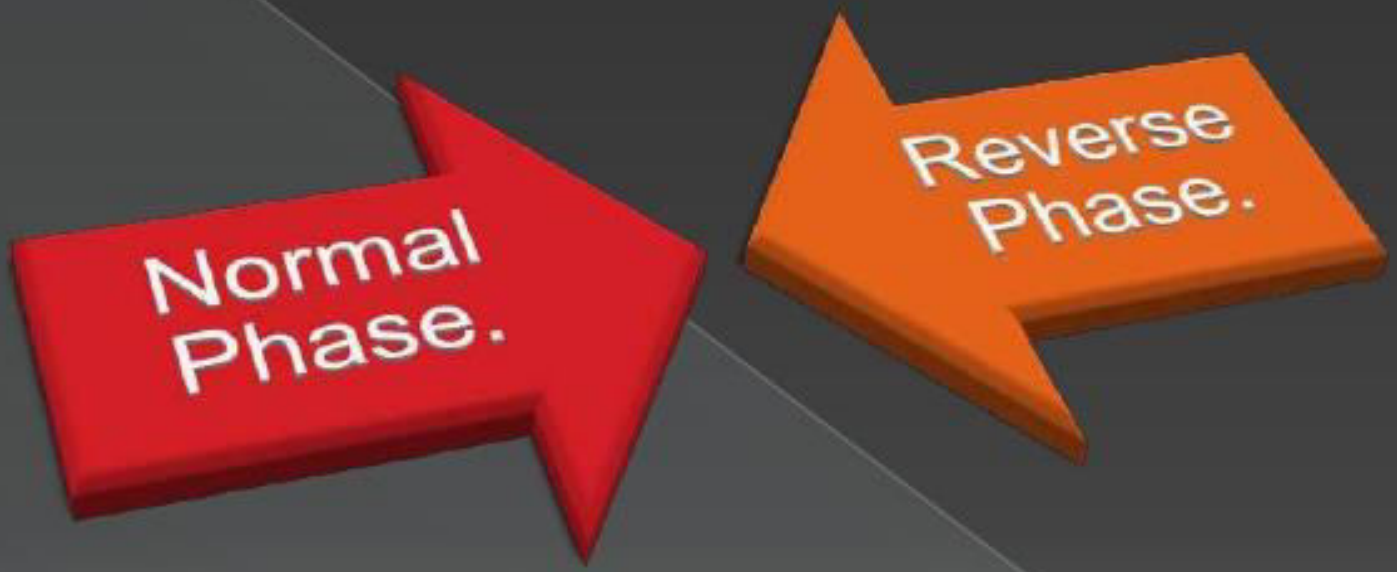
Separation is based on the analyte's relative solubility between two liquid phases




*Partitioning :-*

# HPLC - Modes




## Normal Phase.

- Polar stationary phase and non-polar solvent.

## Reverse Phase.

- Non-polar stationary phase and a polar solvent.

# FOUR TYPES OF HIGH PERFOMANCE LIQUID CHROMATOGRAPHY :-

1. PARTITION CHROMOTOGRAPHY
2. ION EXCHANGE CHROMATOGRAPHY
3. Size exclusion chromatography
4. AFFINITY CHROMOTAGRAPHY

# 1. PARTITION CHROMOTOGRAPHY :-

Partition chromatography uses a retained solvent, on the surface or within the grains or fibres of an "inert" solid supporting matrix as with paper chromatography; or takes advantage of some additional coulombic and/or hydrogen donor interaction with the solid support. Molecules equilibrate (partition) between a liquid stationary phase and the eluent. Known as Hydrophilic Interaction Chromatography (HILIC) in HPLC, this method separates analytes based on polar differences. HILIC most often uses a bonded polar stationary phase and a non-polar, water miscible, mobile phase. Partition HPLC has been used historically on unbonded silica or alumina supports. Each works effectively for separating analytes by relative polar differences, however, HILIC has the advantage of separating acidic, basic and neutral solutes in a single chromatogram.

# 2. ION EXCHANGE CHROMATOGRAPHY

Ion-exchange chromatography is a process that allows the separation of ions and polar molecules based on their charge. It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids. The solution to be injected is usually called a *sample*, and the individually separated components are called *analytes*. It is often used in protein purification, water analysis, and quality control

Ion exchange chromatography retains analyte molecules on the column based on coulombic (ionic) interactions. The stationary phase surface displays ionic functional groups (R-X) that interact with analyte ions of opposite charge. This type of chromatography is further subdivided into cation exchange chromatography and anion exchange chromatography. The ionic compound consisting of the cationic species $M^+$ and the anionic species $B^-$ can be retained by the stationary phase.

# 3. SIZE EXCLUSION CHROMATOGRAPHY :-

Size exclusion chromatography (SEC), also known as gel permeation chromatography or gel filtration chromatography, separates particles on the basis of size. It is generally a low resolution chromatography and thus it is often reserved for the final, "polishing" step of purification. It is also useful for determining the tertiary structure and quaternary structure of purified proteins. SEC is used primarily for the analysis of large molecules such as proteins or polymers. SEC works by trapping these smaller molecules in the pores of a particle. The larger molecules simply pass by the pores as they are too large to enter the pores. Larger molecules therefore flow through the column quicker than smaller molecules, that is, the smaller the molecule, the longer the retention time.

# 4. AFFINITY CHROMOTAGRAPY :-

This is the most selective type of chromatography employed. It utilizes the specific interaction between one kind of solute molecule and a second molecule that is immobilized on a stationary phase. For example, the immobilized molecule may be an antibody to some specific protein. When solutes containing a mixture of proteins are passed by this molecule, only the specific protein is reacted to this antibody, binding it to the stationary phase. This protein is later extracted by changing the ionic strength or pH.

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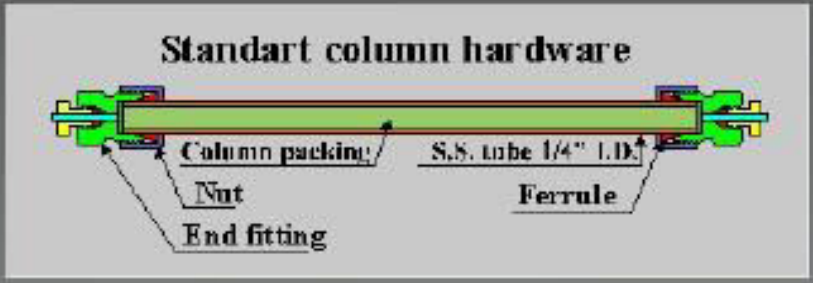
# LIQUID CHROMATOGRAPHIC COLUMN

Smooth-bore stainless steel or heavy-walled glass tubing

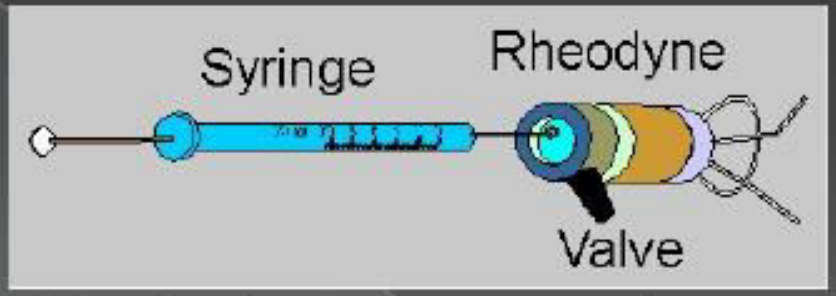
Hundreds of packed columns differing in size and packing are available from manufacturers ($200-$500)

Add columns together to increase length



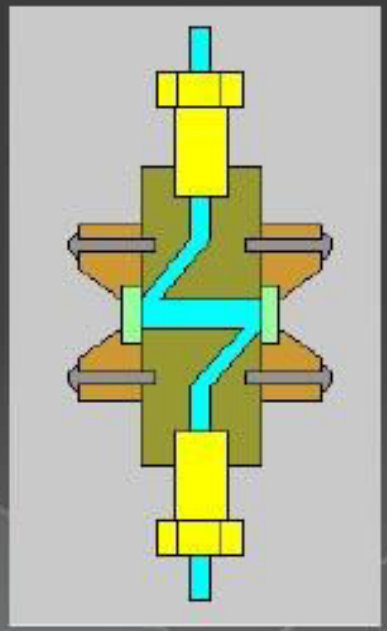
# Sample Injection Systems

- For injecting the solvent through the column
- Minimize possible flow disturbances
- Limiting factor in precision of liquid chromatographic measurement
- Volumes must be small
- .1-500 μL
- Sampling loops
  - interchangeable loops (5-500 μL at pressures up to 7000 psi)



# DETECTOR

- Mostly optical
- Equipped with a flow cell
- Focus light beam at the center for maximum energy transmission
- Cell ensures that the separated bands do not widen



# HPLC detection and characteristics with sensitivity

| Detection | Application | Characteristics |
|---|---|---|
| **RI** | all | Low sensitivity, not applicable to gradient elution |
| **UV** | Almost all except alkane, saturated alkyl | Most popular, 10-100 times more sensitive than RI |
| **Fluorescence** | Compound with fluorescence in nature or derivatization to compounds with fluorescence | Selective and sensitive, 10-50 time more sensitive than UV |
| **ECD** | Compounds with relatively lower oxidation-reduction potential | Selective and sensitive, 10-50 time more sensitive than UV. Sensitivity is almost identical to Fluorescence. |
| **Ms** | all | Most sensitive. Expensive. Handling is complicated. |

# Pretreatment method for the analysis of compounds in complicated matrix

| Procedure | Reagent | Characteristics |
|---|---|---|
| **Liquid-liquid extraction** | Resemble polar solvent to identical compounds of interest | Much solvent use. Evaporation required. Recovery loss is possible. Troublesome. |
| **Manual type solid phase extraction** | Column and solvent selected as identical to the interest of compounds of analysis | Due to handle treatment, pressure variation causes. Pressure variation leads to recovery variation |
| **Automated type solid phase extraction** | Identical to Manual type solid phase extraction | Due to computer handling, no pressure variation, so reproducibility is quite high |
| **Dialysis** | Dialysis reagent | Membrane equilibrium between dialysis inner and outer. Commercially available but not so popular |

# Utility of HPLC

## USES OF HPLC :-

1.This technique is used for chemistry and biochemistry research analyzing complex mixtures, purifying chemical compounds, developing processes for synthesizing chemical compounds, isolating natural products, or predicting physical properties. It is also used in quality control to ensure the purity of raw materials, to control and improve process yields, to quantify assays of final products, or to evaluate product stability and monitor degradation.

2. In addition, it is used for analyzing air and water pollutants, for monitoring materials that may jeopardize occupational safety or health, and for monitoring pesticide levels in the environment. Federal and state regulatory agencies use HPLC to survey food and drug products, for identifying confiscated narcotics or to check for adherence to label claims.

# Pharmaceutica Analytica Acta Related Journals

- Pharmaceutical Regulatory Affairs
- Pharmacovigilance

# Pharmaceutica Analytica Acta Related Conferences

- International Conference and Exhibition on Pharmacovigilance & Clinical Trials
- International Conference and Exhibition on Pharmaceutical Regulatory Affairs
- International Conference and Exhibition on Analytical & Bioanalytical Techniques

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