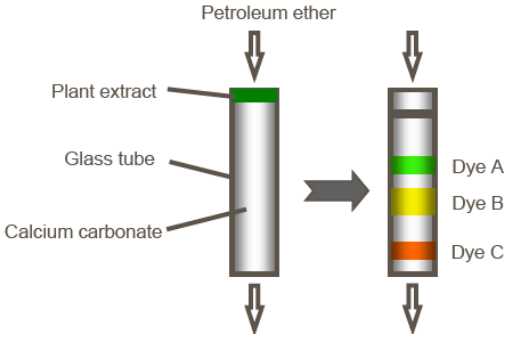
Introduction of HPLC Principle
&
Configuration of an HPLC system

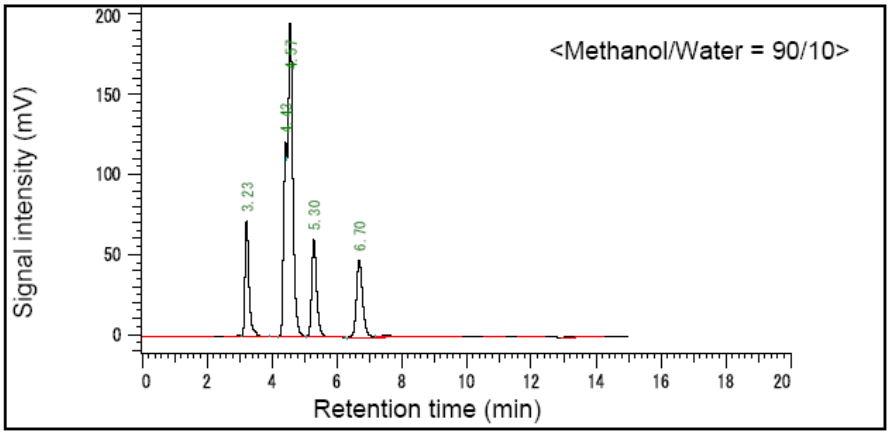
Hitachi High-Technologies Corporation
Analytical Instrument Dept.,
Jimmy Ho
Specialist

1. Principle of chromatography

Chromatography is a technique by which a mixture sample is separated into components. Although originally intended to separate and recover (isolate and purify) the components of a sample, today, complete chromatography systems are often used to both separate and quantify sample components.



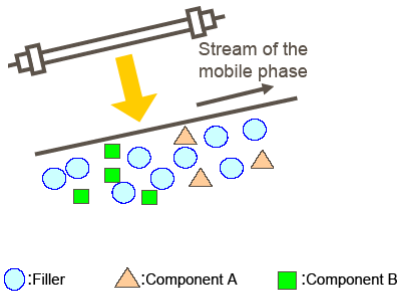
"Chromatography" represents a separation technique; whereas a **"chromatograph"** is a system for performing chromatography. The chart displaying the time-dependent change in signal intensity as a result of the separation is called a **"chromatogram"**.

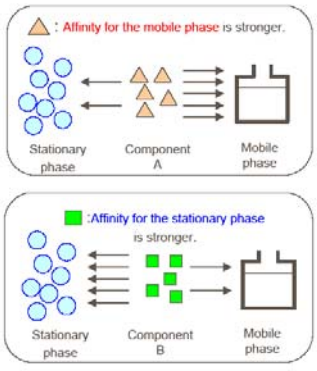


Type of chromatography

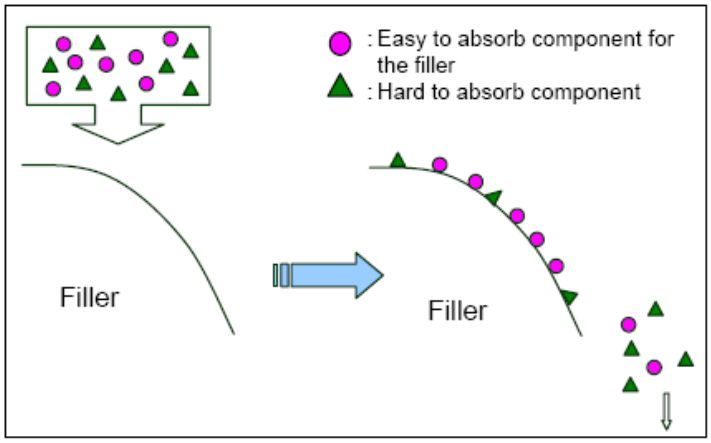
Mobile phase	Stationary phase	Analysis	Sample Types
Gas	Solid/Liquid	Gas chromatography (GC)	Samples that are gaseous at ordinary temperatures and samples that vaporize when heated • Odorous samples such as petrochemicals, perfumes, and thinner are easier to analyze by GC. • High molecular weight compounds are measured after pyrolysis.
Liquid	Solid/Liquid	Liquid chromatography (LC)	Liquid samples and solvent-soluble solid samples • Compared to GC, LC has a wide range of measurement subjects. • High molecular weight compounds can be analyzed, if soluble in solvent.

How is a sample separated into its components in the column? The speed of a migrating sample component depends on whether the component has an affinity for the stationary or mobile phase. This affinity appears via various actions: adsorption, partition, ion exchange, etc.



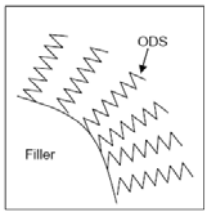


Type of separation mode

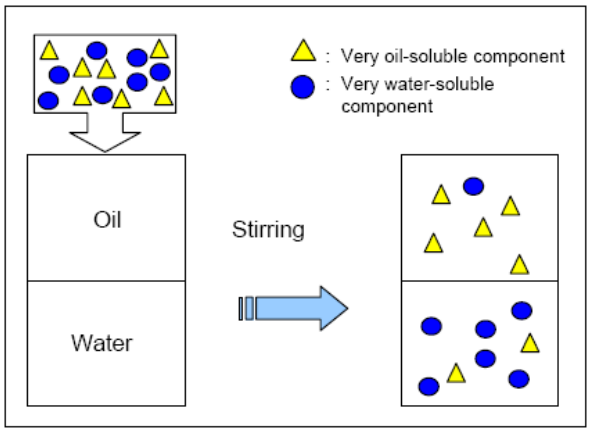


Pattern diagram illustrating adsorption mode

Type of separation mode

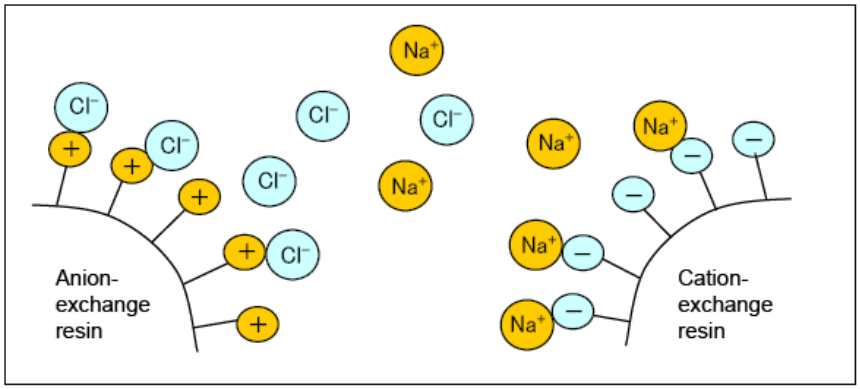


Surface of an ODS filler



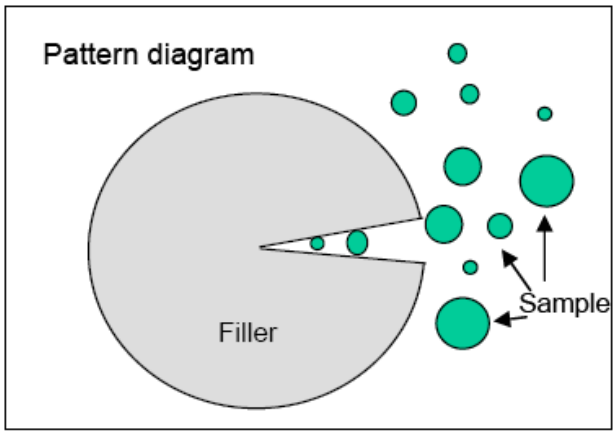
Pattern Diagram illustrating partition mode

Type of separation mode



Pattern diagram illustrating ion exchange mode

Type of separation mode

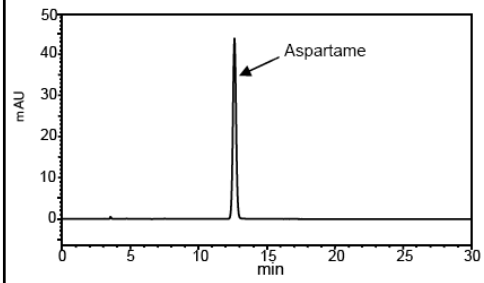


Pattern diagram illustrating size exclusion mode

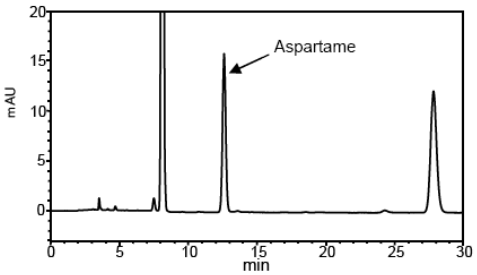
Chromatography is based on the principal that **under the same conditions, the time between the under the same conditions, the time between the injection of a component into the column and the elution of that injection of a component into the column and the elution of that component is constant component is constant**. This characteristic is used to perform qualitative or quantitative analysis.

<Qualitative analysis>

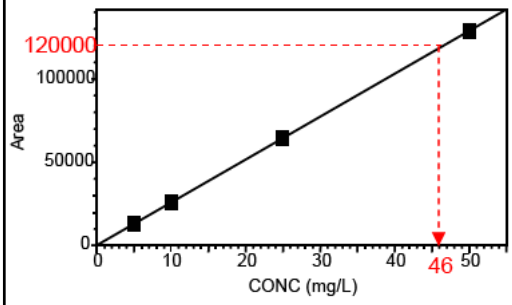
[Chromatogram of the standard sample]



[Chromatogram of the beverage]

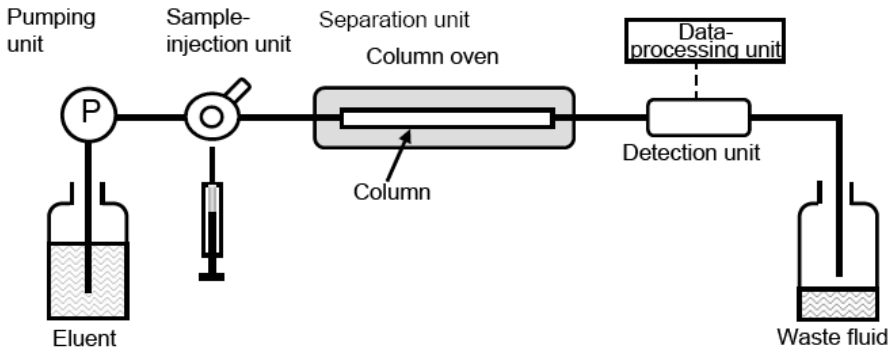


<Quantitative analysis>

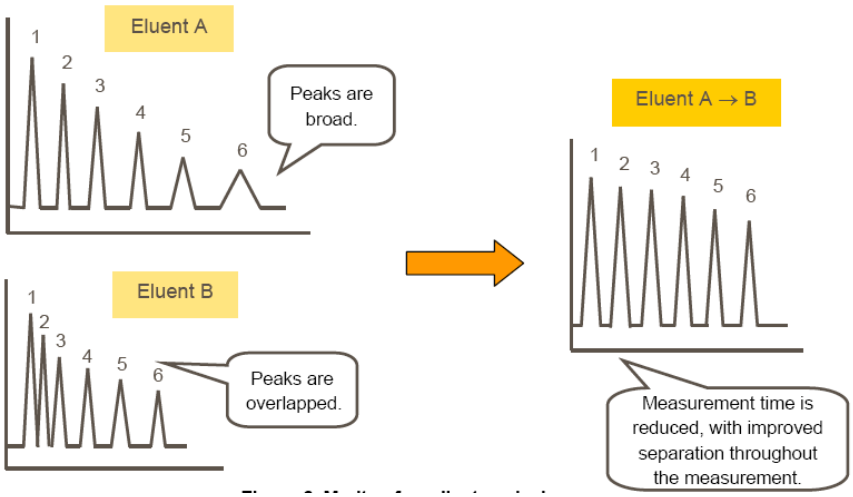


In the measurement of the real sample, if the peak area of the target component is 120000, the concentration of the component is determined to be 46 mg/L from this calibration curve.

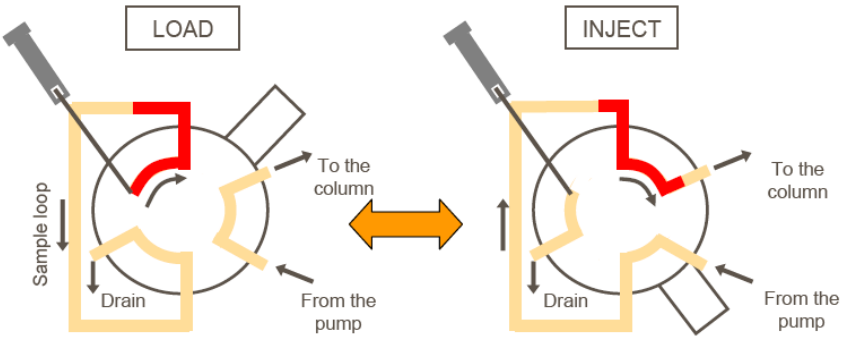
2. Configuration of an HPLC system



Merits of gradient analysis



Sample-injection unit

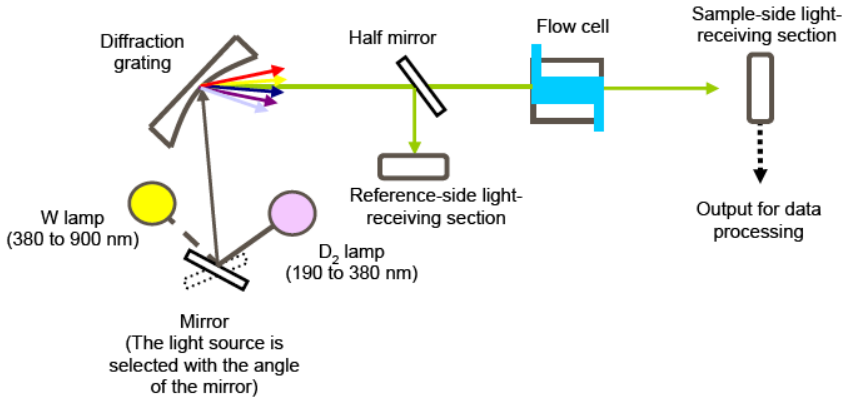


Separation unit

Detection unit

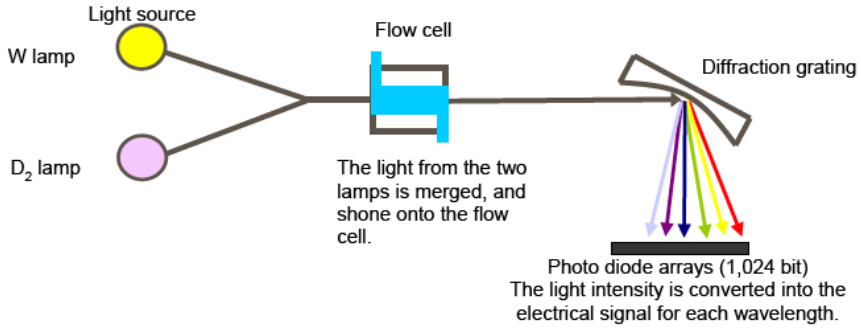
UV detector	The light source is a D_2 lamp. This detector is used mainly to detect components having an absorption wavelength of 400 nm or less in the ultraviolet region.
UV-VIS detector	A D_2 lamp and a W lamp are used as the light source. This detector is effective in the detection of coloring components such as dyes and stains because of coverage of the visible light region.
Diode array detector (DAD)	Data on the spectrum from the ultraviolet to visible light range is also collected.
Fluorescent light (FL) detector	Fluorescent substances can be detected specifically with high sensitivity.
Differential refractive index (RI) detector	Change in the refractive index is detected. Components absorbing no ultraviolet light can also be detected despite low sensitivity.
Conductivity detector	Mainly inorganic ions are detected by monitoring the conductivity.

UV/UV-VIS detectors



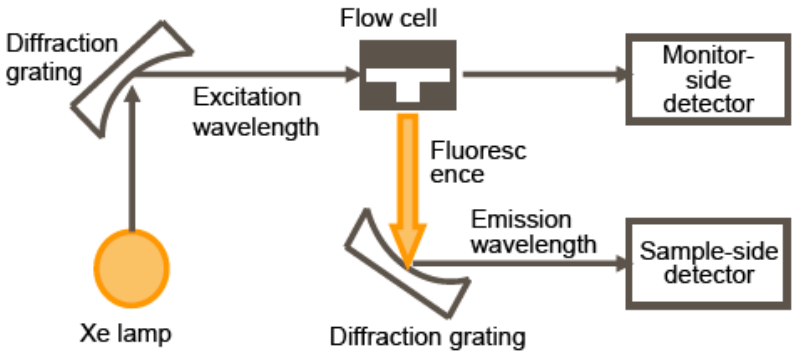
Diagrammatic illustration of a UV-VIS detector optical system

Diode array detector
(DAD, PDA: Photo Diode Array Detector)



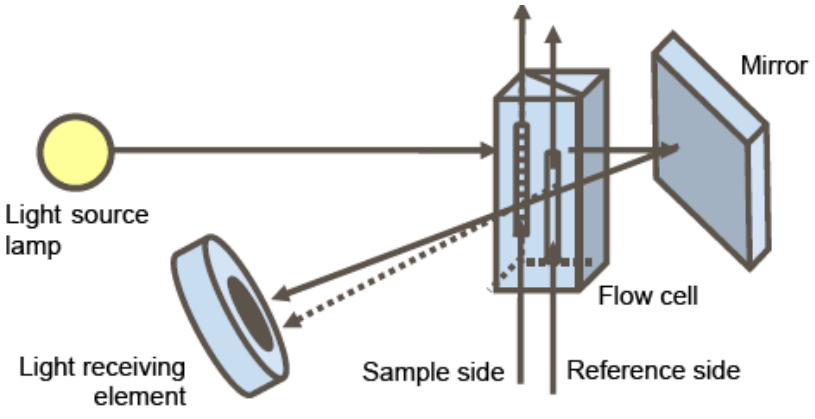
Diagrammatic illustration of a DAD optical system

Fluorescence (FL) detector



Diagrammatic illustration of a FL detector optical system (a patent of HITACH)

Differential refractive index (RI) detector



Diagrammatic illustration of a RI detector optical system

Data-processing unit

Ending

Thank you for your listening

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