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LAAN-E-LC 215

Fundamentals of HPLC

4 _ Apparatus constituting HPLC (2)

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General features of HPLC detectors

	Target	Sensitivity	Gradient elution applicability
Absorbance	Absorptive compound	ng	Good
Fluorescence	Fluorescent compound	pg	Good
Refractive index	All	μg	None
Evaporative light scattering	Non-volatile compound	μg	Good
Electrical conductivity	Ionic compound	ng	Poor
Electrochemical	Redox compound	pg	Poor

*pg (10^{-12} g) < ng (10^{-9} g) < μg (10^{-6} g)

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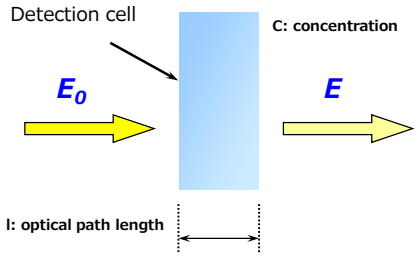
UV detection

- **Absorbance measurement**

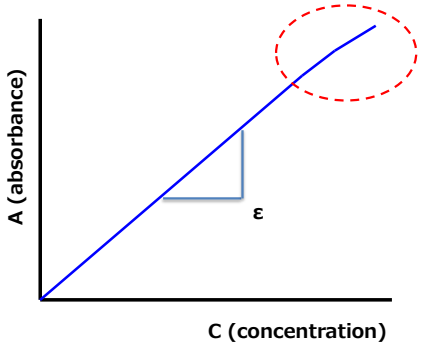


Lambert-Beer's law

$$A = \varepsilon C l = \log (E_0/E)$$

A: Absorbance **ε: Molar Extinction coefficient**

Correct determination could not be done due to lack of linearity at high concentration range



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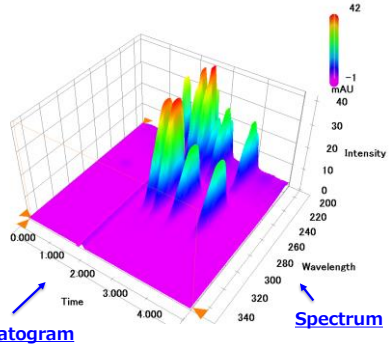
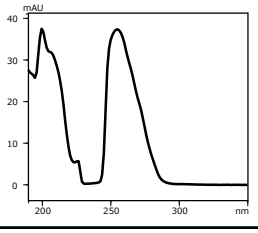
PDA detector data (1)



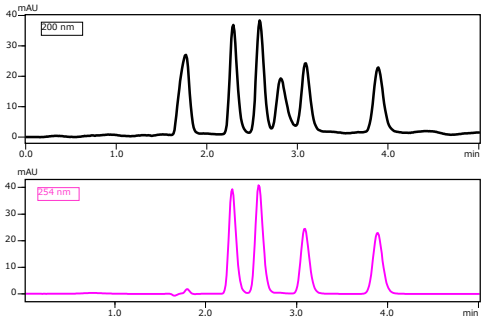
Chromatogram

Spectrum



UV spectrum

Chromatogram at a specific wavelength



In addition to quantitative analysis as an UV-VIS detector, qualitative analysis by UV spectrum can be performed.

Data analysis by changing a monitor wavelength can be performed after the data acquisition.

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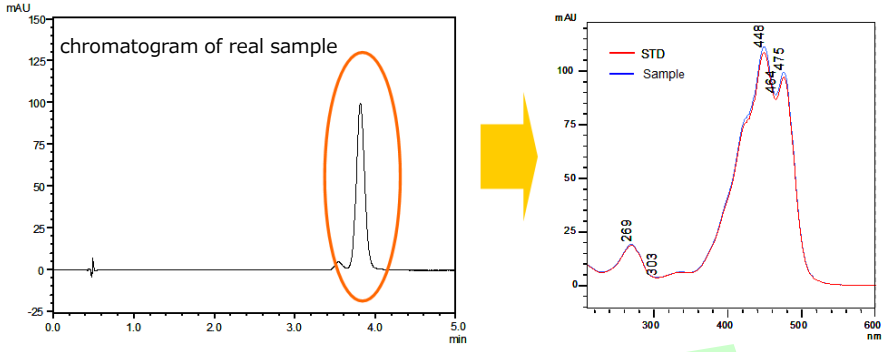
PDA detector data (2)

Purity comparison of standard and real sample spectra can be performed



Spectra comparison could suggest the existence of co-eluted compounds in the real sample

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Pros and cons of absorbance detector

- The most popular detector with HPLC
- Stable and easy to handle
- Useful for organic compounds having UV absorbance in the range of ultraviolet and visible (λ= 190 to 800 nm)
- The sensitivity depends on the molar absorption coefficient of the target compound
- Aromatic ring(s) and conjugated double bond(s) are important
- Difficult to detect sugar and low molecular acid having low absorbance
- The PDA detector provides informative result including qualitative data within a single analysis

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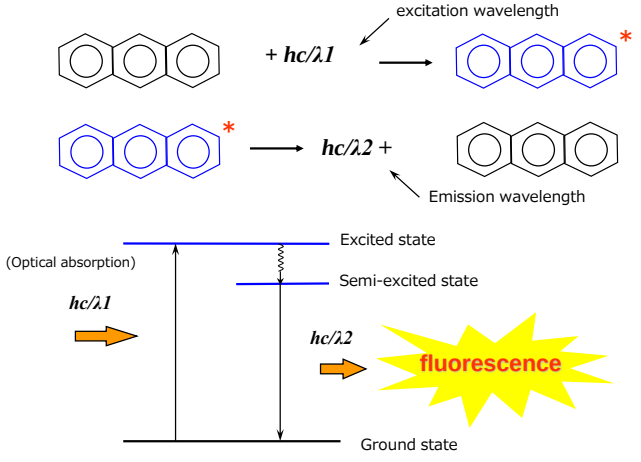
Fluorescence detection

- **Mechanism of fluorescence detection**



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Pros and cons of fluorescence detector

- High sensitivity detection for fluorescent compound
- Highly selective detection using specific excitation and emission wavelengths
- Limited number of detectable compounds
- Useful in combination with derivatization

e.g.

・Dyes, PAHs (polycyclic aromatic)

・Amino acids (OPA derivatization)

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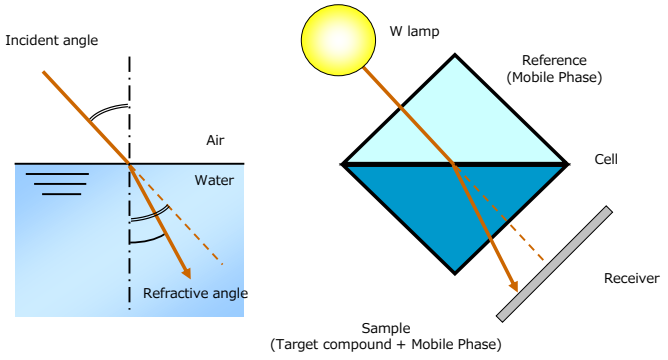
Refractive index detection

- **Measurement of refractive index difference between reference and sample**



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Pros and cons of refractive index detector

- All compounds having different refractive indexes from that of mobile phase can be detected
- Small sensitivity difference depending on target compounds affords rough abundance proportion directly
- Poor detection selectivity
- Gradient elution cannot be employed

e.g.

・Saccharides
(UV absorption is too small to be detected with UV detector)

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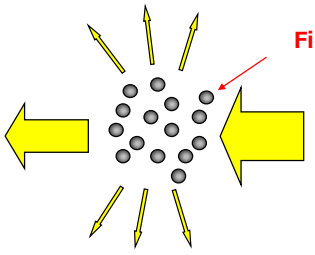
Evaporative light scattering detection

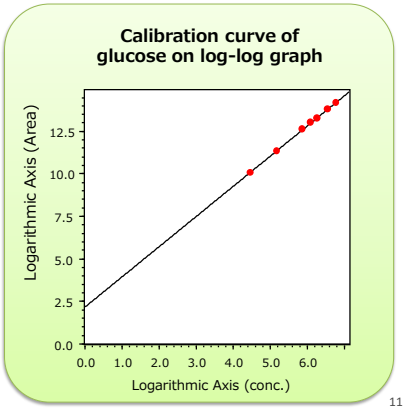
- **Measurement of light scattering caused by the target compound**

Scattered light ⋯▶ Detection



$$I = km^b$$

I :Scattered light intensity
m :Amount of particles
k, b :Constant



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Pros and cons of evaporative light scattering detector

- Measuring scattered light caused by the fine particles remained after evaporating mobile phase
- Almost all nonvolatile compounds can be detected
- Poor selectivity
- Small sensitivity difference depending on target compounds
- Gradient elution method can be employed
- Nonvolatile buffers such as phosphate buffer and high boiling point solvents such as DMSO or DMF cannot be employed

e.g.

・Saccharides
(UV absorption is too small to be detected with UV detector)
・Detergent

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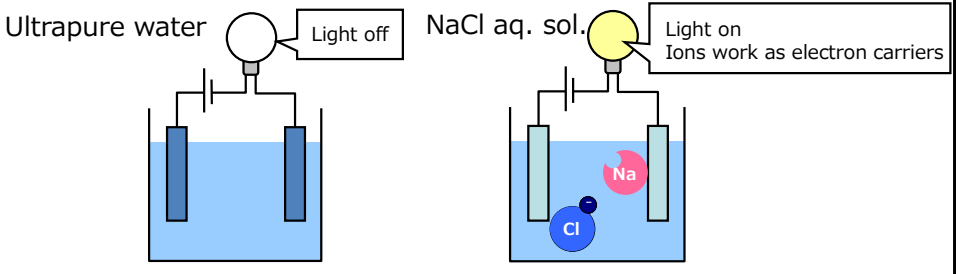
Electrical conductivity detection

- **Measurement of electrical conductivity difference between target compound and mobile phase**



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Pros and cons of electrical conductivity detector

- Ionic compounds can be detected selectively
- Ions in aqueous solution can be detected with high sensitivity
- Non-ionic compounds cannot be detected
- This detector is necessary for ion chromatography
- It is also employed in organic acid analysis systems (pH buffering method)

e.g.

・Cation (Calcium ion, etc.)

・Anion, organic acid (Lactic acid, Acetic acid, Formic acid, etc.)

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Thank you for your attention.

The content of this video is based on the latest information available at the time of publication.