IFSTL International Food Safety Training Laboratory | parera

INAF INSTITUTE OF NUTRITION AND FUNCTIONAL FOODS

UNIVERSITÉ LAVAL
Faculty of Agriculture and Food Sciences

Methods of Analysis for Organic Chemical Contaminants in Food

Module 4 - Liquid Chromatography

IFSTL International Food Safety Training Laboratory | parera

LESSON 2

Basic Principles of Liquid Chromatography

Modern LC Columns

HPLC

A US FDA chemist in the mid-1950s is shown using a column chromatographic apparatus to separate the constituents in a coal tar chemical analysis.

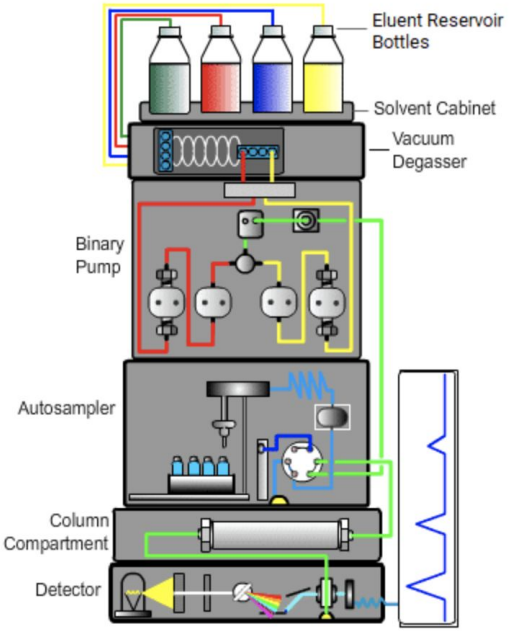
https://www.flickr.com/photos/fdaphotos/albums/72157624615595535/

HPLC Systems

HPLC

- Liquid mobile phase
  - One or more
- Sample in solution
- Solid stationary phase

- ONLY SEPARATION
- Variety of detectors



Recommended: https://www.chromacademy.com/channels/hplc/principles/hplc-introduction/
(free with academic account)

Terminology Liquid Chromatography

HPLC

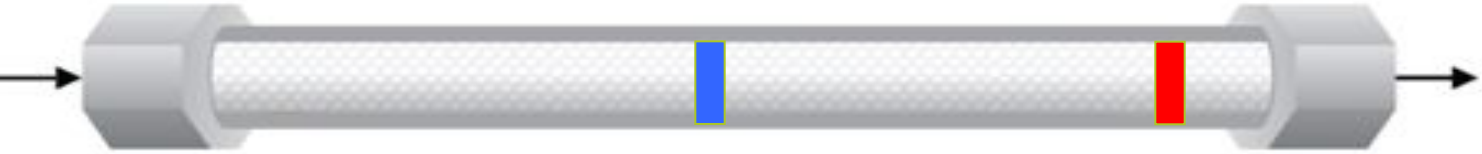
- Mobile phase
  - The solvent that moves the solute through the column. In LC, the mobile phase interacts with both the solute and the stationary phase and, therefore, can have a powerful influence on the separation.

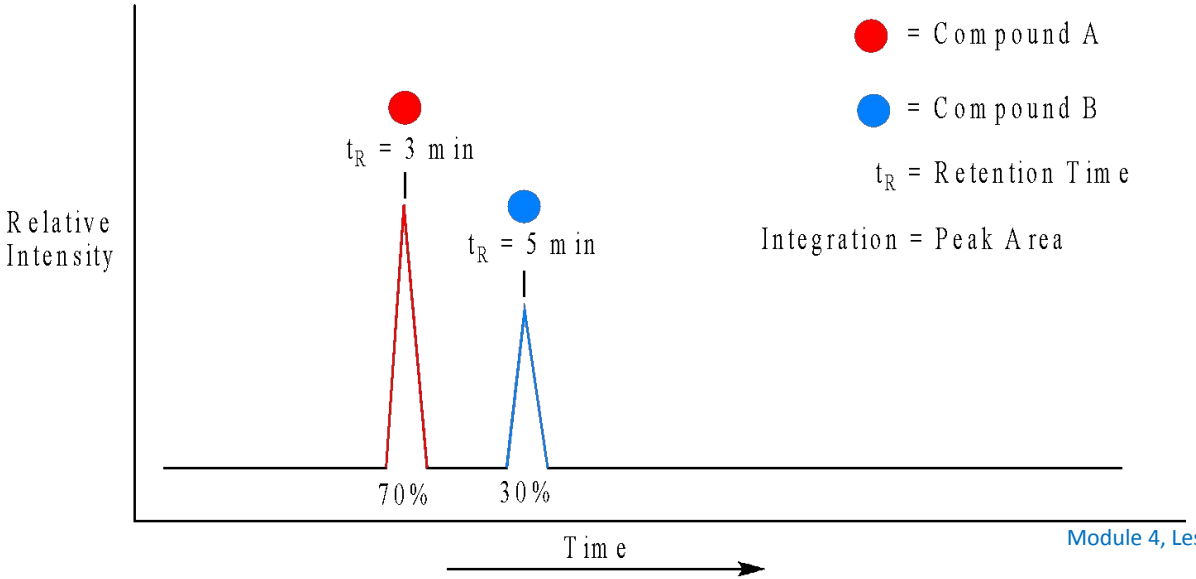
- Stationary phase
  - The chromatographically retentive immobile phase involved in the chromatographic process. The stationary phase in LC can be a solid, a bonded, an immobilized or a coated phase on a solid support or a wall-coated phase. The stationary phase often characterizes the LC mode. For example, silica gel is used in adsorption chromatography and octadecylsilane bonded phase is used in reversed-phase chromatography.

https://www.chromatographyonline.com/view/glossary-hplclc-separation-terms

Simple HPLC in pictures…

HPLC

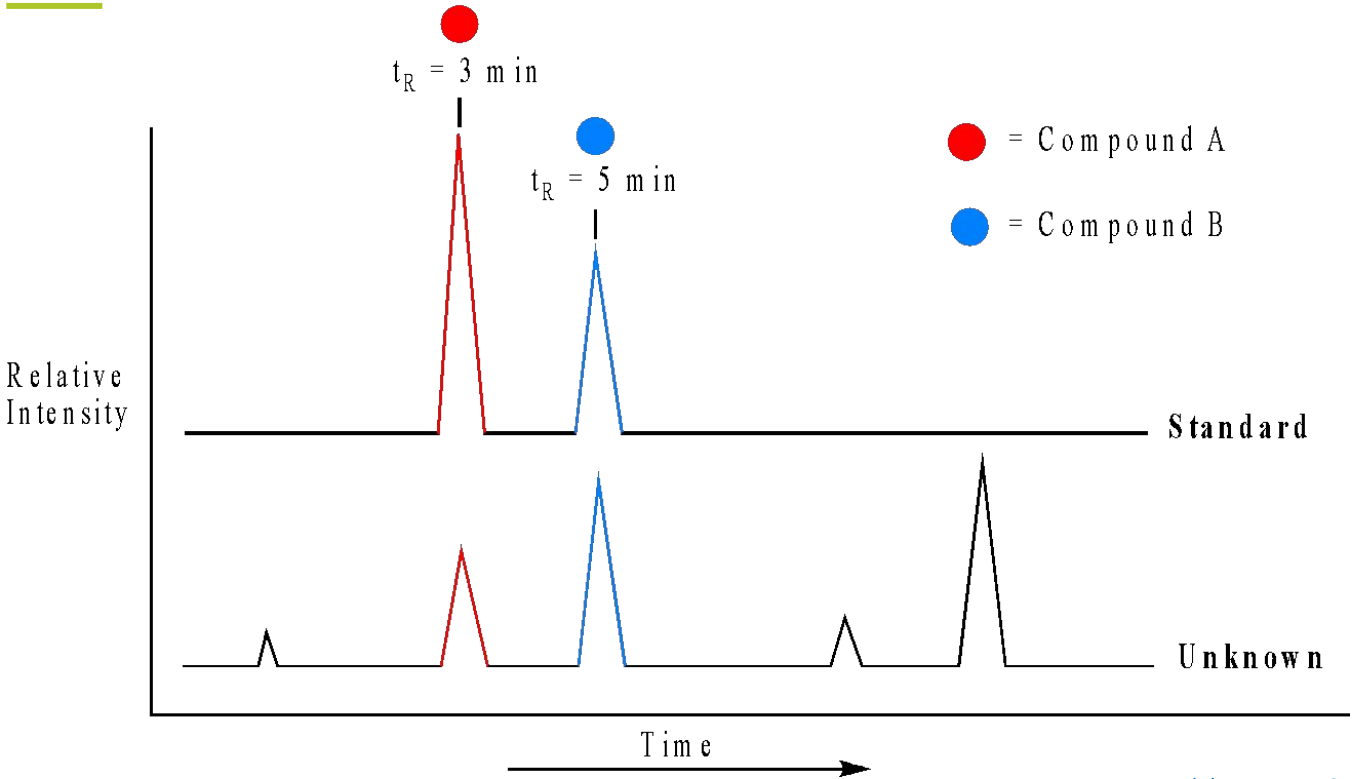




www.waters.com

How we use peaks for identification

HPLC



Terminology Liquid Chromatography

HPLC

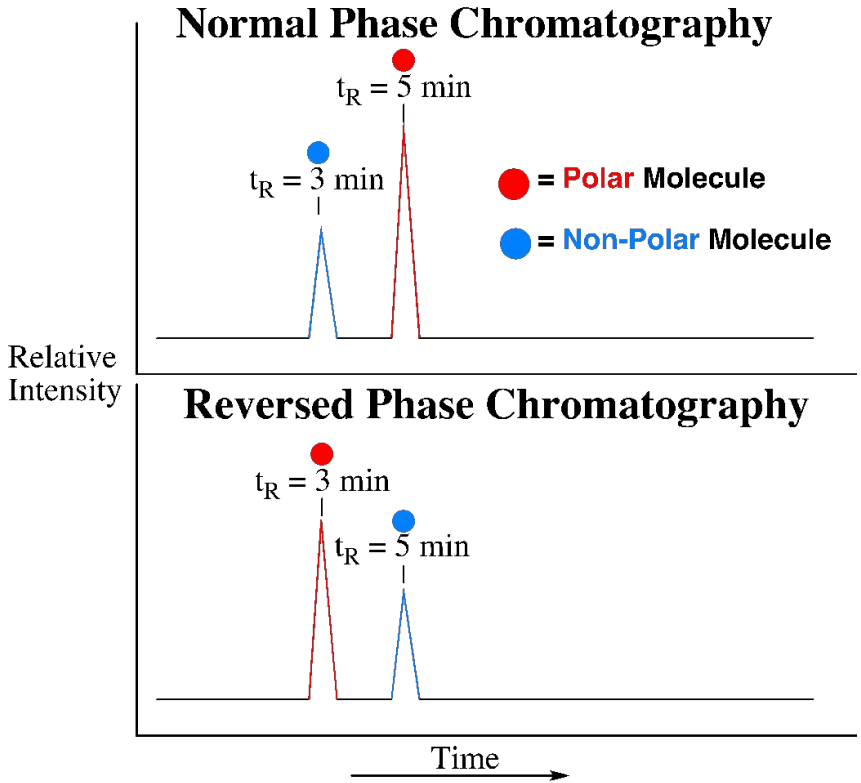
- Normal Phase
  - A mode of chromatography performed when the stationary phase is more polar than the mobile phase.
- Reverse Phase
  - A mode of chromatography performed when the mobile phase is more polar than the stationary phase.
  - The most frequently used mode in HPLC.

Separation Mode	Stationary Phase [particle]	Mobile Phase [solvent]
Normal phase	Polar	Non-polar
Reversed phase	Non-polar	Polar

https://www.chromatographyonline.com/view/glossary-hplclc-separation-terms
https://www.waters.com/waters/en_US/HPLC-Separation-Modes/nav.htm?cid=100490764locale=en_US

Normal and Reverse Phase

HPLC



Mobile Phase Options

HPLC

- Mobile phase is defined by
  - Chemical composition (can be a mixture)
    - Mostly polarity in food safety
  - pH
  - Additives

Stationary Phase Options

HPLC

- Columns are defined by
  - Dimensions: Diameter and length
  - Particle size
  - Particle composition
    - Chemistry of the particle
    - Ligand
    - Ligand density
  - pH range
- Column selection must also consider the detector…

Stationary Phase –Column Dimensions

HPLC

- Column internal diameter
- Affects:
  - Sample loading capacity
  - Mobile phase volume
  - Available pressure
  - Peak height
  - Signal to noise ratio
  - Effective separation (column efficiency, selectivity)

Column Diameter Rules of Thumb

HPLC

Bigger column

- Allow more sample volume
- Require more mobile phase
- Lower pressure
- Separate less effectively
- Produce wider and lower peaks

Smaller column

- Allow only small samples
- Require less mobile phase
- Allow higher pressure
- Separate more effectively
- Produce high, narrow peaks

Column Length

HPLC

Column length:

Affects separation
“adds time”

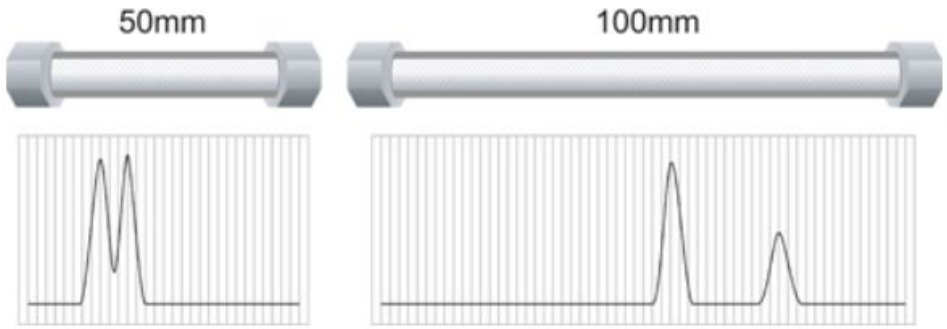
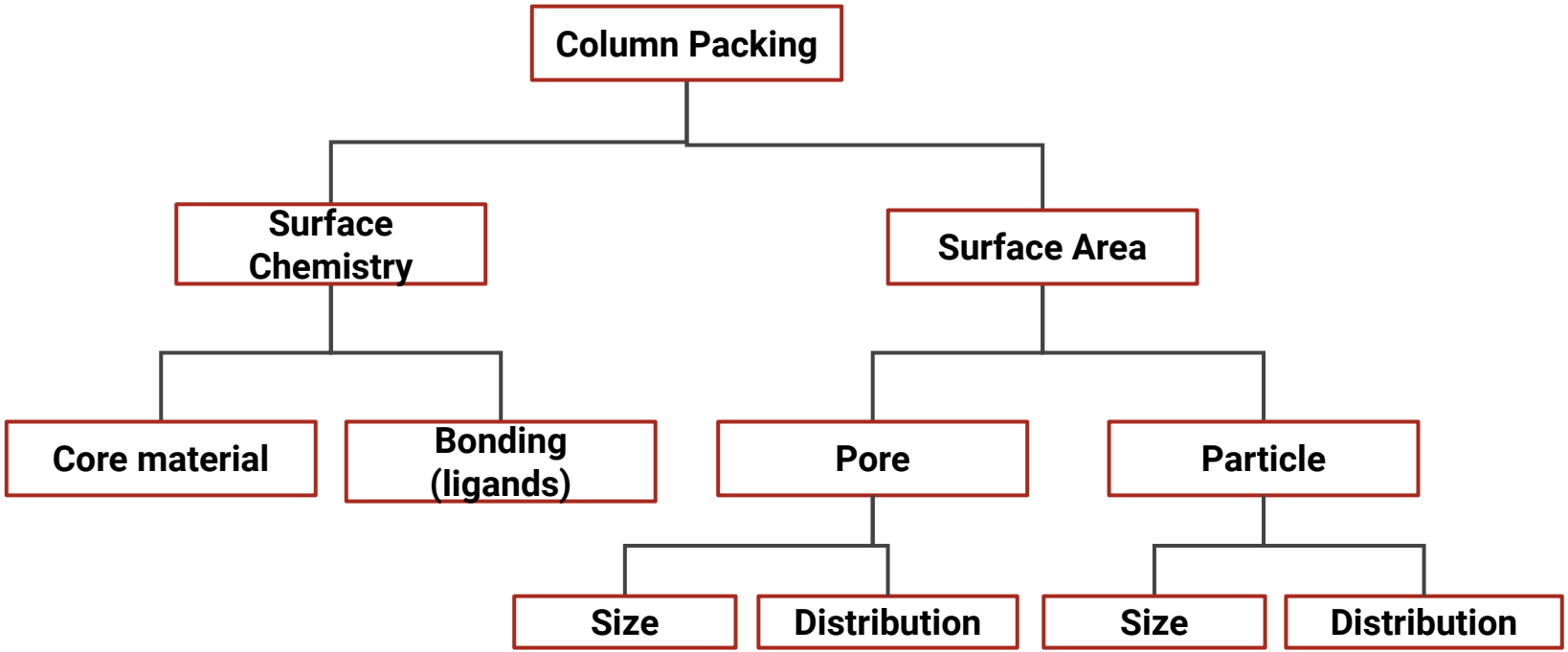


Figure N: Column Length and Mechanical Separating Power [Same Particle Size]

https://www.waters.com/waters/en_US/HPLC---High-Performance-Liquid-Chromatography-Explained/nav.htm?locale=en_US4cid=10048919

Stationary Phase

HPLC



Historical Perspective on Particle Size

HPLC

Year	Particle size (µm)	Resulting N/15 cm
1950's	100	200
1967	57	1,000
1972	10	6,000
1985	5	12,000
1992	3.5	22,000
2003	≤ 2	>30,000

Stationary Phase –Particle Size

HPLC

- Particle or “Beads” inside the column
- Size affects:
  - Sample loading capacity
  - Mobile phase volume
  - Pressure
  - Peak height
  - Signal to noise ratio
  - Effective separation (column efficiency)
- Separates technologies
  - HPLC> 2 µm > UHPLC

Particle Size Rules of Thumb

HPLC

Bigger particles

- Lower pressure
- Separate less effectively
- Produce wider and lower peaks

Smaller particles

- Higher pressure
- Separate more effectively
- Produce high, narrow peaks

Different Ways to Increase Separation

HPLC

Increasing the length of the column and decreasing the size of the particles both increase the resolution

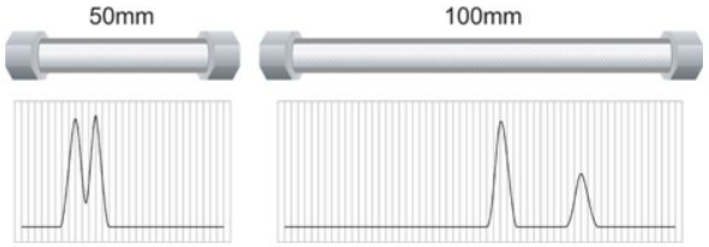


Figure N: Column Length and Mechanical Separating Power [Same Particle Size]

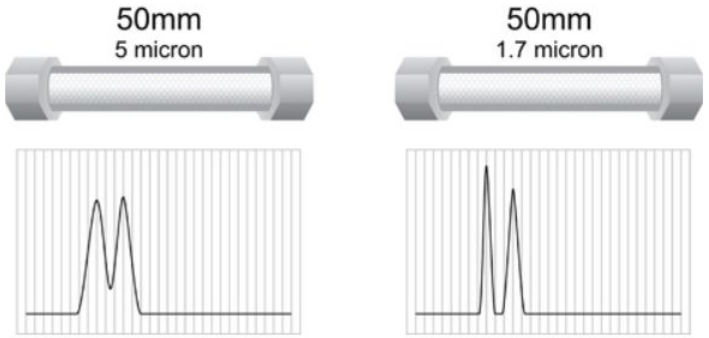


Figure O: Particle Size and Mechanical Separating Power [Same Column Length]

https://www.waters.com/waters/en_US/HPLC---High-Performance-Liquid-Chromatography-Explained/nav.htm?locale=en_US4cid=10048919

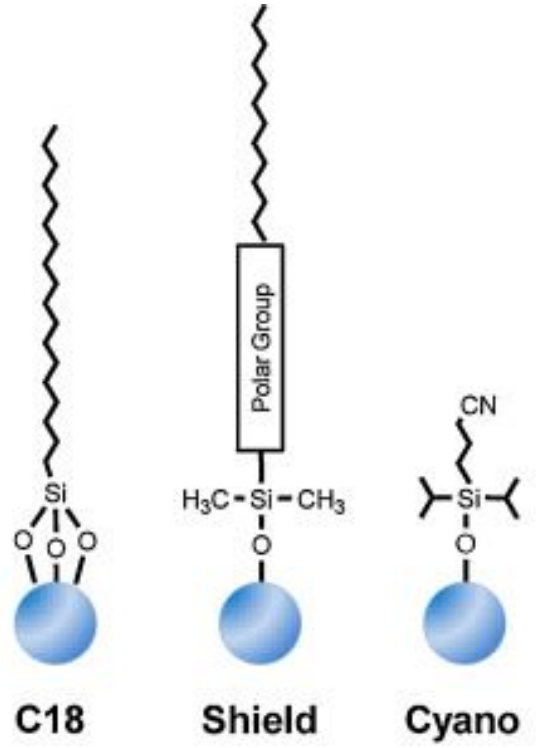
Stationary Phase –Particle Composition and Ligand

HPLC

- "Column Chemistry"
- Determines
  - Chromatography mode
    - Direct *vs* Reverse Phase Chromatography
  - pH range
  - Mobile phase options
  - Sample preparation requirements

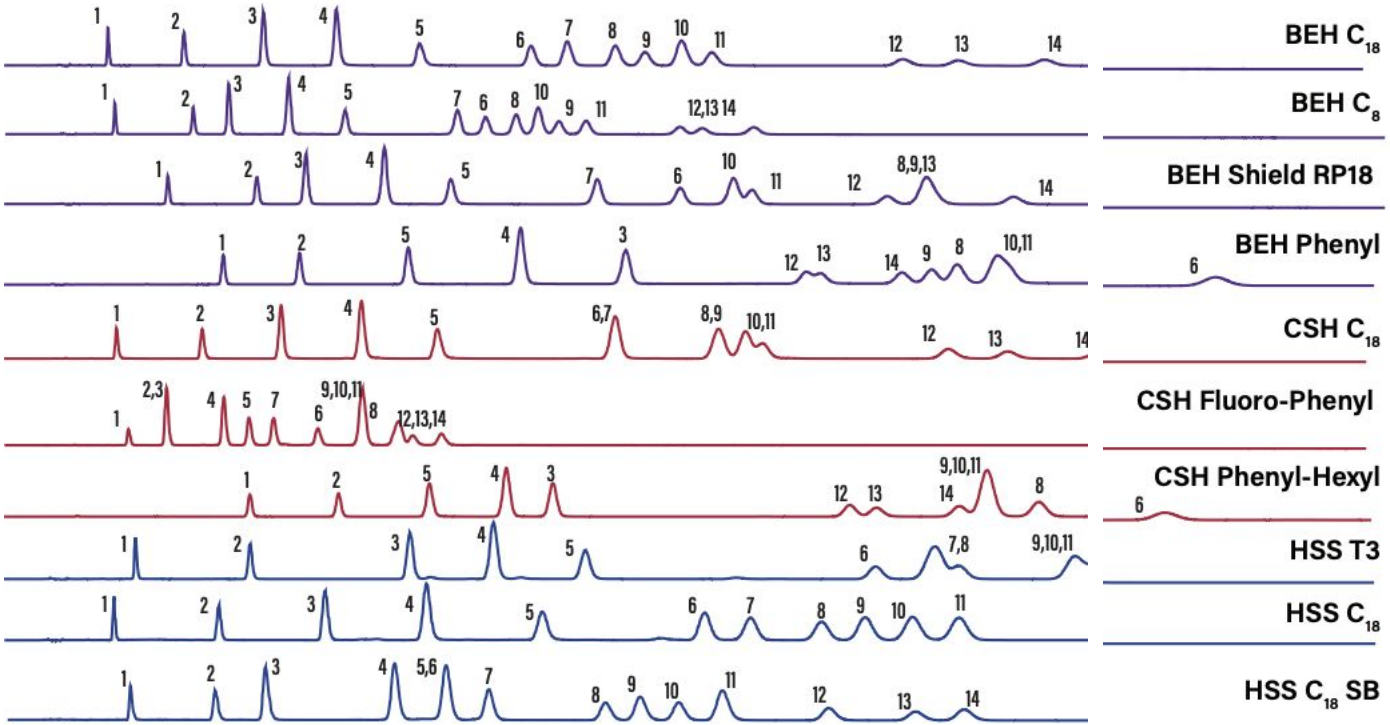
Example of Particle Surface Chemistry

HPLC



Column Selection

HPLC



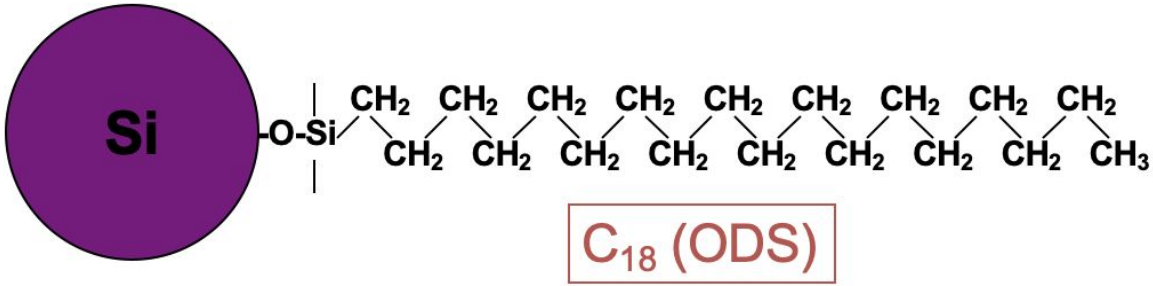
https://www.waters.com/webassets/cms/library/docs/720001983en.pdf

Common Column Chemistry for Food Safety

HPLC

- C_{18} (ODS) type
- C_8 (octyl) type
- C_4 (butyl) type
- Phenyl type
- TMS type
- Cyano type

ODS: Octadecyl group-bonded silica gel



Terminology Mobile Phase

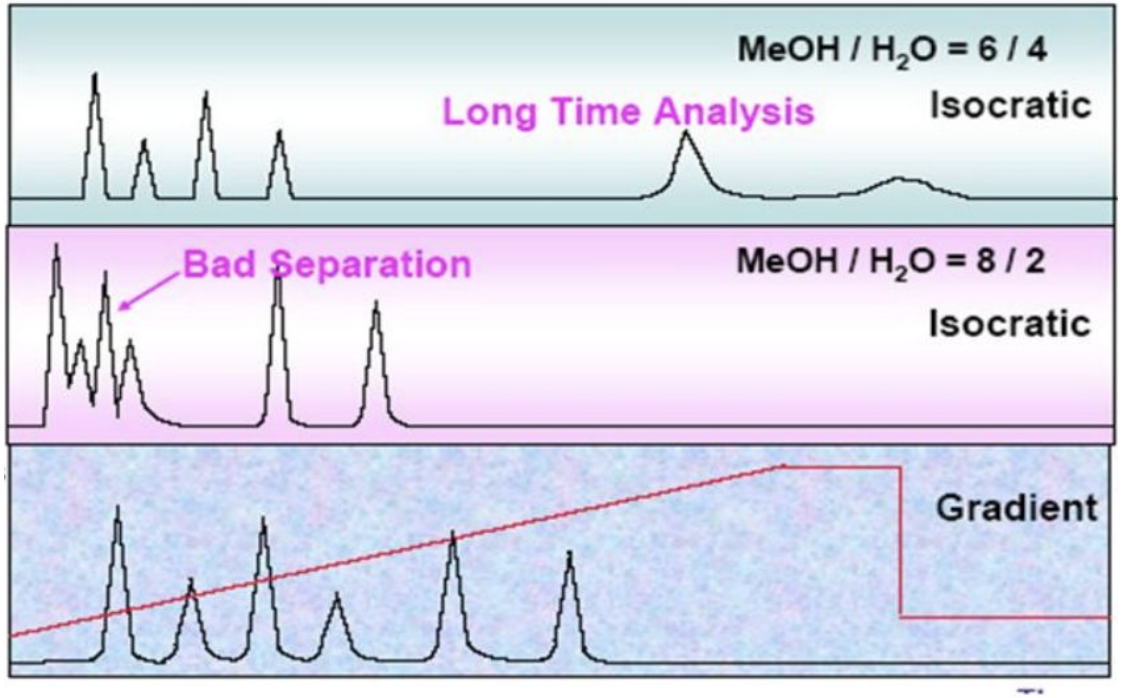
HPLC

- Isocratic
  - Using a time invariant–eluent composition in LC
  - Only one mobile phase
- Gradient
  - A process to change solvent strength as a function of time (normally solvent strength increases) thereby eluting progressively more highly retained analytes. Typically gradients can be binary, ternary, and quaternary solvent mixtures in which solvents are blended to achieve the proper strength.

https://www.chromatographyonline.com/view/glossary-hplclc-separation-terms

An example of the effect of a gradient

HPLC



https://www.waters.com/webassets/cms/library/docs/720001983en.pdf

Image by Suzanna Ross

Terminology Mobile Phase

HPLC

Additive: A substance added to the mobile phase to improve the separation or detection characteristics

Examples:

- Acids/bases/buffers to adjust pH
- Competing base to negate the effects of silanols
- Chelating agent to block metal sites
- UV-absorbing compound for indirect photometric detection

https://www.chromatographyonline.com/view/glossary-hplclc-separation-terms

Examples of Additives

HPLC

Mobile-Phase Chemical	pK_a	Buffer Range
Acetic Acid (glacial)	4.8	—
Ammonium Acetate pK_a 1	4.8	3.8-5.8
Ammonium Acetate pK_a 2	9.2	8.2-10.2
Ammonium Bicarbonate	9.2, 10.3	(8.2-11.3)
Ammonium Formate pK_a 1	3.8	2.8-4.8
Ammonium Formate pK_a 2	9.2	8.2-10.2
Ammonium Hydroxide	9.2	—
Ammonium Phosphate, Dibasic	7.2, 9.2	(6.2-10.2)
Formic Acid	3.8	—

https://www.waters.com/webassets/cms/library/docs/720001983en.pdf

Terminology

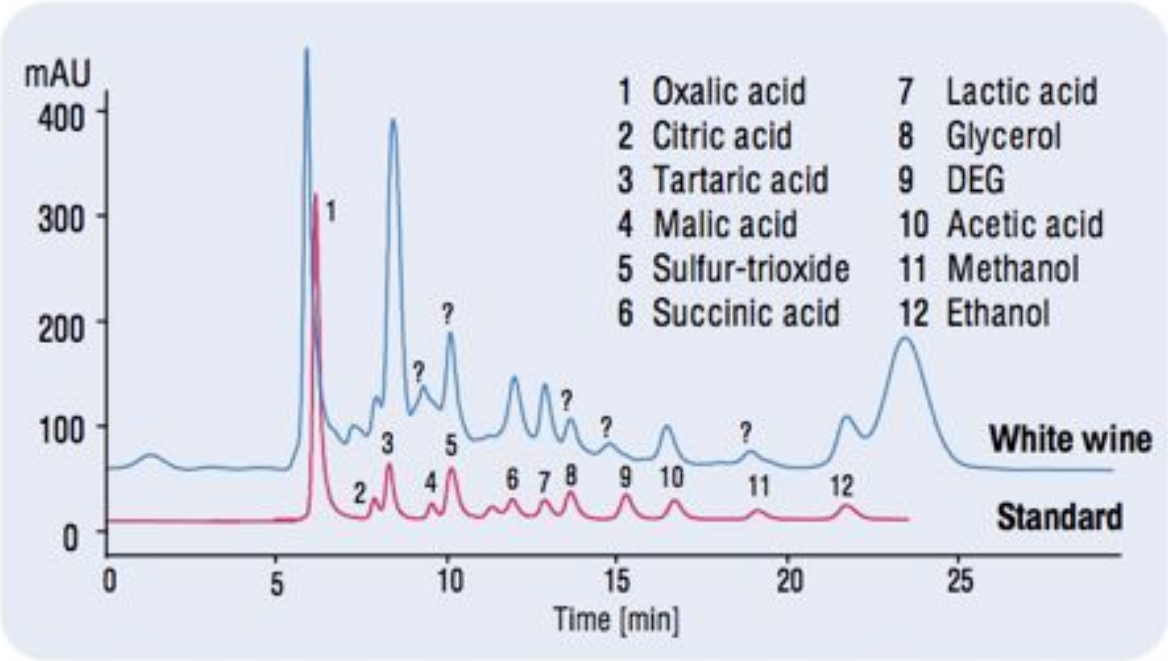
HPLC

- Standard
  - A sample which contains known quantities of the compounds of interest.
  - Standards are used to help identify sample peaks by comparing the time in which they elute to the retention times obtained through the injection of the sample under the same conditions.

https://www.chromatographyonline.com/view/glossary-hplclc-separation-terms

Example of Using a Standard: White Wine

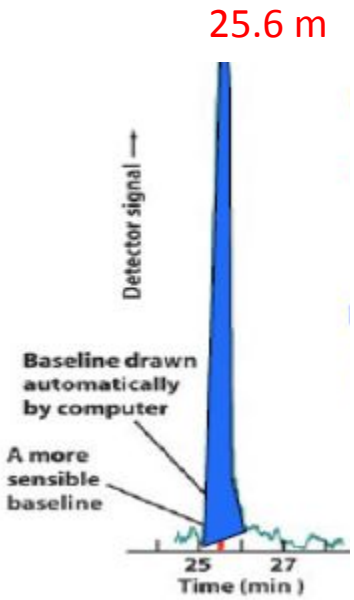
HPLC



Gratzfeld-Hüsgen, A. and Schuster, R., 2001, *HPLC Food Analysis*, Agilent Tech. Co. Germany, 3 p.

Qualitative and Quantitative Information

HPLC



Retention time: 25.6 min

Tells us about the identity of the compound = Qualitative

Peak area or height

Tells us about how much is present = Quantitative

Terminology Standards (US FDA*)

HPLC

- Internal Standard
  - A chemical added to the sample, in known quantity, at a specified stage in the analysis to facilitate quantitation of the analyte.
- Calibration Standard
  - A known amount or concentration of analyte used to calibrate the measuring/detection system. May be matrix matched for specific sample matrices.

Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products (2019)
Available at: https://www.fda.gov/media/81810/download

IFSTL International Food Safety Training Laboratory | parera

LESSON 2
END