PROGRAMMA

Metodi di screening e di conferma, principi di validazione dei metodi analitici. Sensori elettrochimici, ottici e piezolettrici, biosensori, accoppiamento del materiale biologico al trasduttore di segnale, sviluppo di sensori di affinità, immunosensori. Polimeri, stampo molecolari e aptameri come elementi di riconoscimento. Nanomateriali e sensori .

Accoppiamento tecniche cromatografiche con spettrometria di massa: GC-MS, ionizzazione a impatto elettronico e ionizzazione chimica; LC-MS, ionizzazione electrospray e APCI, analizzatori a quadrupolo, tempo di volo, trappola ionica e trappola orbitale (Orbitrap). Tecniche di frammentazione, acquisizizone massa tandem (MS/MS). Pre-trattamento del campione, tecniche di estrazione e microestrazione, clean-up. Applicazioni nell'ambito delle red biotech. (2CFU)

Approcci analitici targeted e untargeted, spettrometria di massa ad alta risoluzione (HRMS), strumentazione, elaborazione dati e strumenti bioinformatici.

Matrici di sensori, naso e lingua elettronici, tipologia di trasduttori e recettori. Applicazione su analiti di interesse biotecnologico.

Casi studio derivanti da letteratura internazionale basati sulle metodologie analitiche presentate

DECISION 2002/657/CE

Classification of analytical methods

Screening methods

Only those analytical techniques, for which it can be demonstrated in a documented traceable manner that they are validated and have a false compliant rate of < 5 % (β-error) at the level of interest shall be used for screening purposes in conformity with Directive 96/23/EC. In the case of a suspected non-compliant result, this result shall be confirmed by a confirmatory method.

Confirmatory methods

Confirmatory methods for organic residues or contaminants shall provide information on the chemical structure of the analyte. Consequently methods based only on chromatographic analysis without the use of spectrometric detection are not suitable on their own for use as confirmatory methods. However, if a single technique lacks sufficient specificity, the desired specificity shall be achieved by analytical procedures consisting of suitable combinations of clean-up, chromatographic separation(s) and spectrometric detection.

Validation

Establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes

“Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for its intended use.”

There are many reasons for the need to validate analytical procedures. Among them are **regulatory requirements, good science, and quality control requirements.**

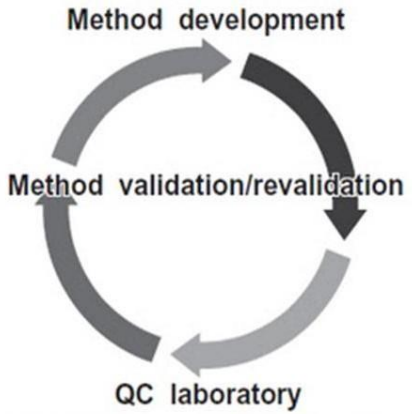


FIGURE 1 Life cycle of analytical method.

Typical validation characteristics which should be considered are:

1) **Accuracy**
2) **Precision**
3) **Specificity**
4) **Linearity**
5) **Range**
6) **Detection Limit**
7) **Quantitation Limit**
8) **Robustness/Ruggedness**
9) **Noise**
10) **Trueness**
11) **Sensitivity**

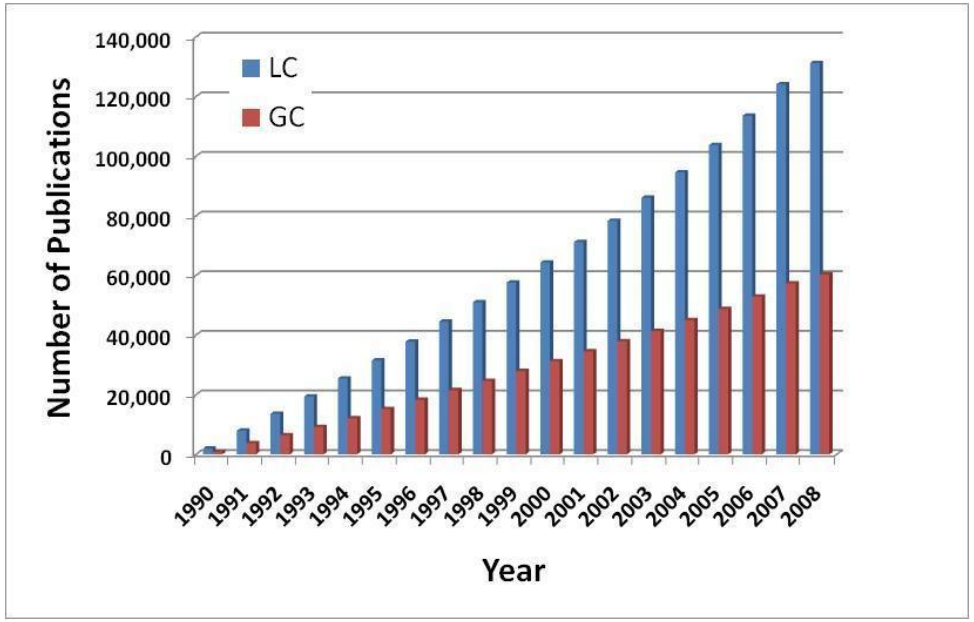
Liquid Chromatography

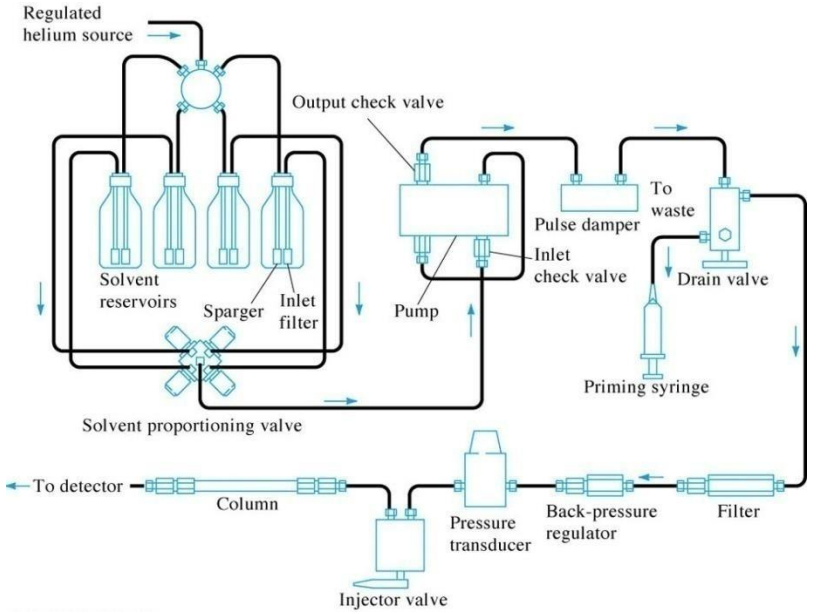
A.) Introduction:

Liquid Chromatography (LC) is a chromatographic technique in which the mobile phase is a liquid.

LC is a much older technique than GC, but was overshadowed by the rapid development of GC in the 1950's and 1960's.

LC is currently the dominate type of chromatography and is even replacing GC in its more traditional applications.





Advantages of LC compared to GC:

1.) LC can be applied to the separation of any compound that is soluble in a liquid phase.

, LC more useful in the separation of biological compounds, synthetic or natural polymers, and inorganic compounds

2.) Liquid mobile phase allows LC to be used at lower temperatures than required by GC

LC better suited than GC for separating compounds that may be thermally labile

3.) Retention of solutes in LC depend on their interaction with both the mobile phase and stationary phase.

, GC retention based on volatility and interaction with stationary phase

, LC is more flexible in optimizing separations → change either stationary or mobile phase

4.) Most LC detectors are non-destructive

, most GC detectors are destructive

, LC is better suited for preparative or process-scale separations

Disadvantage of LC compared to GC:

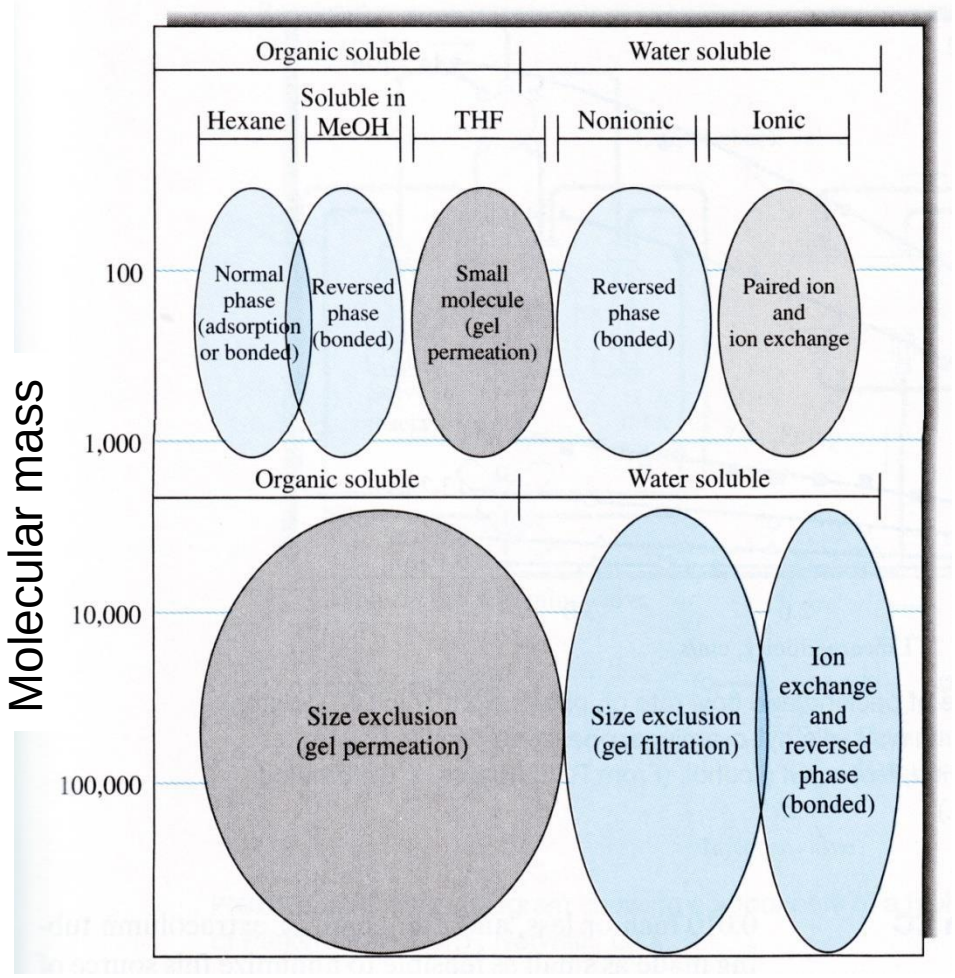
1.) LC is subject to greater peak or band-broadening.

, much larger diffusion coefficients of solutes in gases vs. liquids

B.) Low- and High-performance Liquid Chromatography:

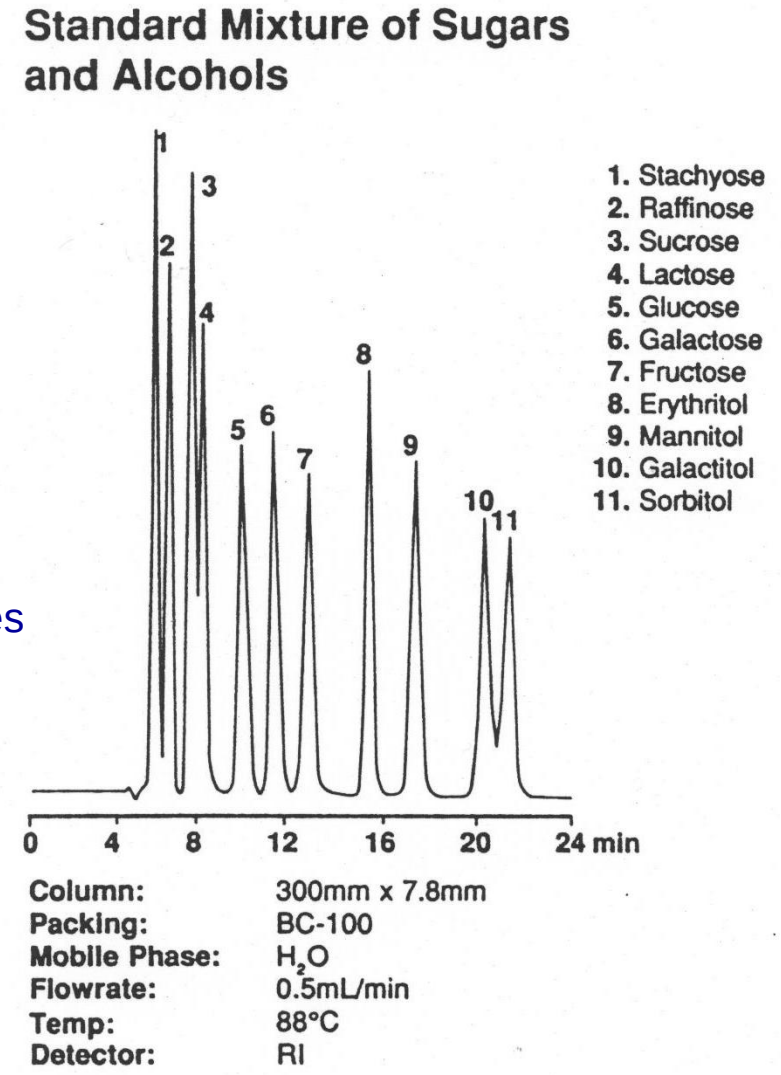
Many types of liquid chromatography are available, based on different stationary phase and mobile phase combinations.

- each type may be further characterized based on its overall efficiency or *performance*



High-performance liquid chromatography (HPLC)

– LC methods that use small, uniform, rigid support material
  , particles < 40 μm in diameter
  , usually 3-10 μm in practice
– good system efficiencies and small plate heights
– such systems have the following characteristics:
  , narrow peaks
  , low limits of detection
  , short separation times
  , columns can only tolerate high operating pressures and faster flow-rates

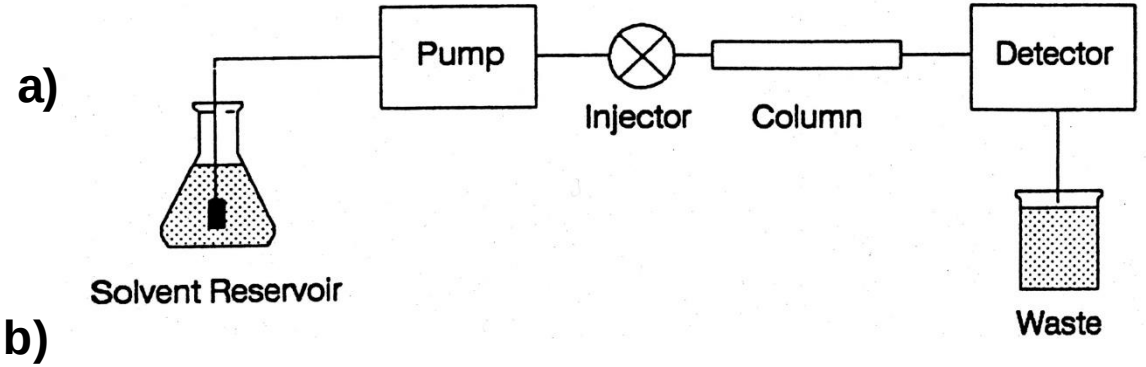


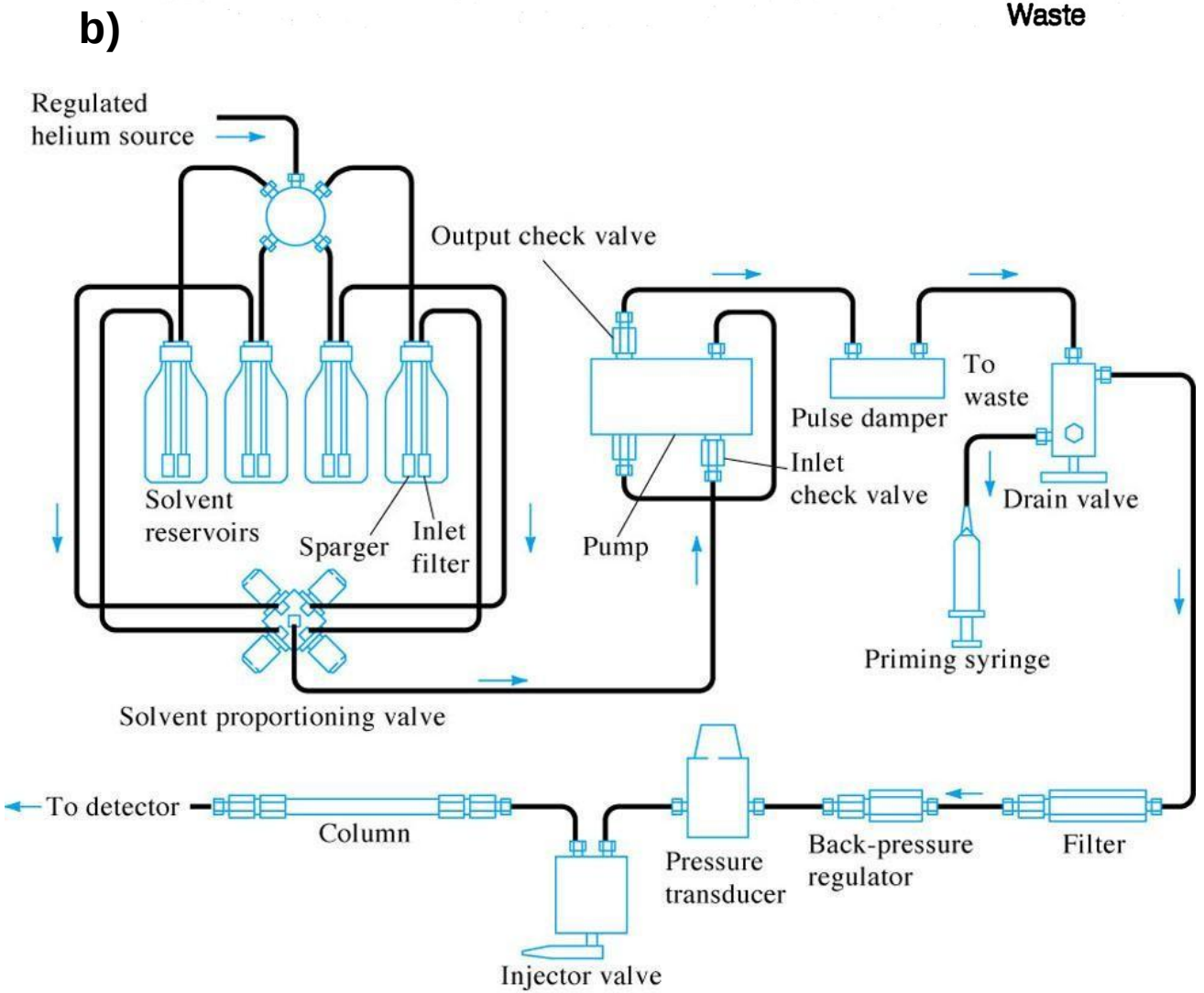
A typical HPLC system:

- Higher operating pressures need for mobile phase delivery requires special pumps and other system components

- Sample applied using closed system (*i.e.*, injection valve)

- detection uses a flow through detector



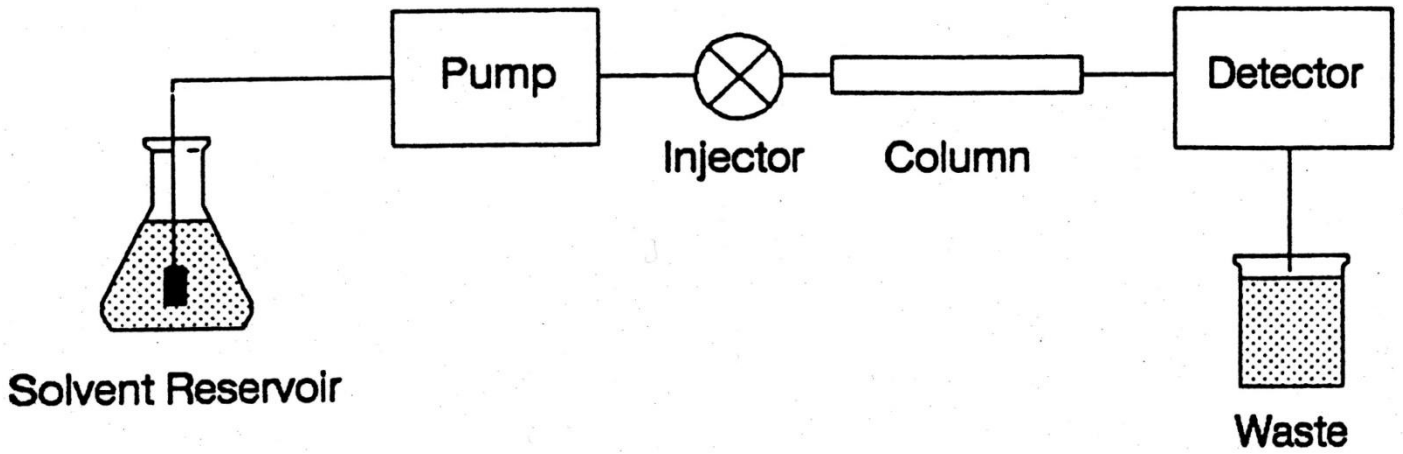


advantages:

– fast analysis time
– ease of automation
– good limits of detection
– preferred choice for analytical applications
– popular for purification work

disadvantages:

– greater expense
– lower sample capacities



C.) Elution:

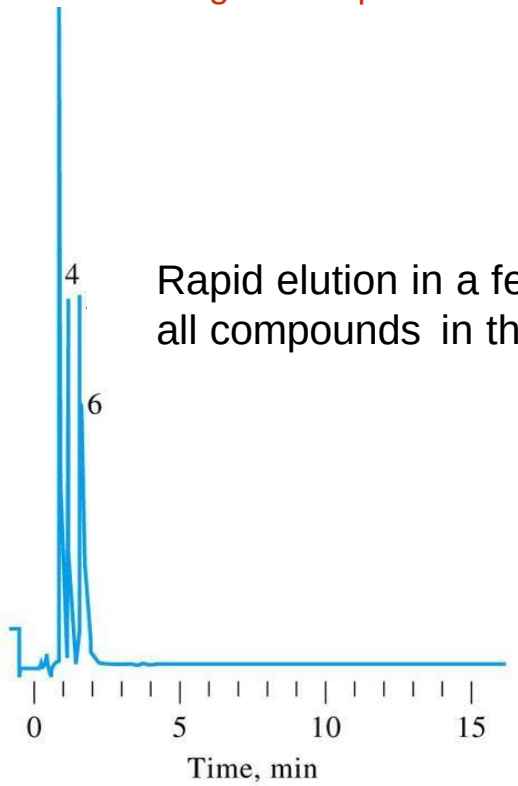
Retention and elution of solutes in LC depends on the interactions of solutes with <u>both</u> the mobile and stationary phases.

- to describe how well solutes are retained on a column with different solvents, the terms *weak mobile phase* and *strong mobile phase* are used.

<u>Strong mobile phase</u>: a solvent that quickly elutes solutes from the column (*i.e.*, small k')

This occurs if the mobile phase is very similar to the stationary phase in its intermolecular interactions with the solutes

- polar solvent would be a strong mobile phase for a column containing a polar stationary phase



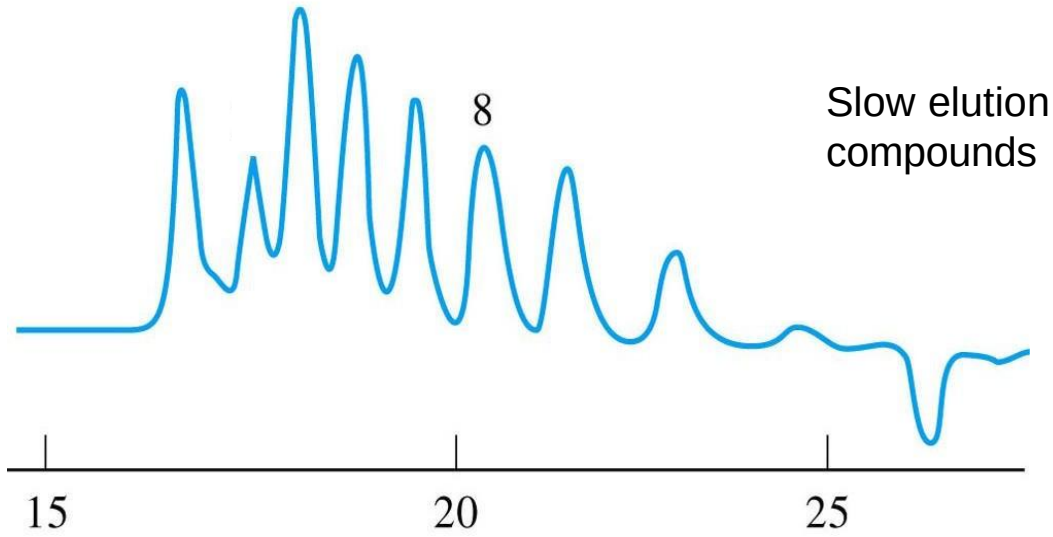
Rapid elution in a few minutes for all compounds in the mixture

C.) Elution:

Weak mobile phase: a solvent that slowly elutes solutes from the column (*i.e.*, high solute retention or large k')

This occurs if the mobile phase is very different from the stationary phase in its intermolecular interactions with the solutes

- a non-polar solvent would be a weak mobile phase for a column containing a polar stationary phase



Slow elution (~ 20 minutes) for all compounds in the mixture

Note: whether a solvent is a weak or strong mobile phase depends on the stationary phase being used. Hexane is a weak mobile phase on a polar stationary phase, but a strong mobile phase on a non-polar stationary phase.

Similar to GC, solutes can be eluted from a column by using either a constant column conditions or gradient elution

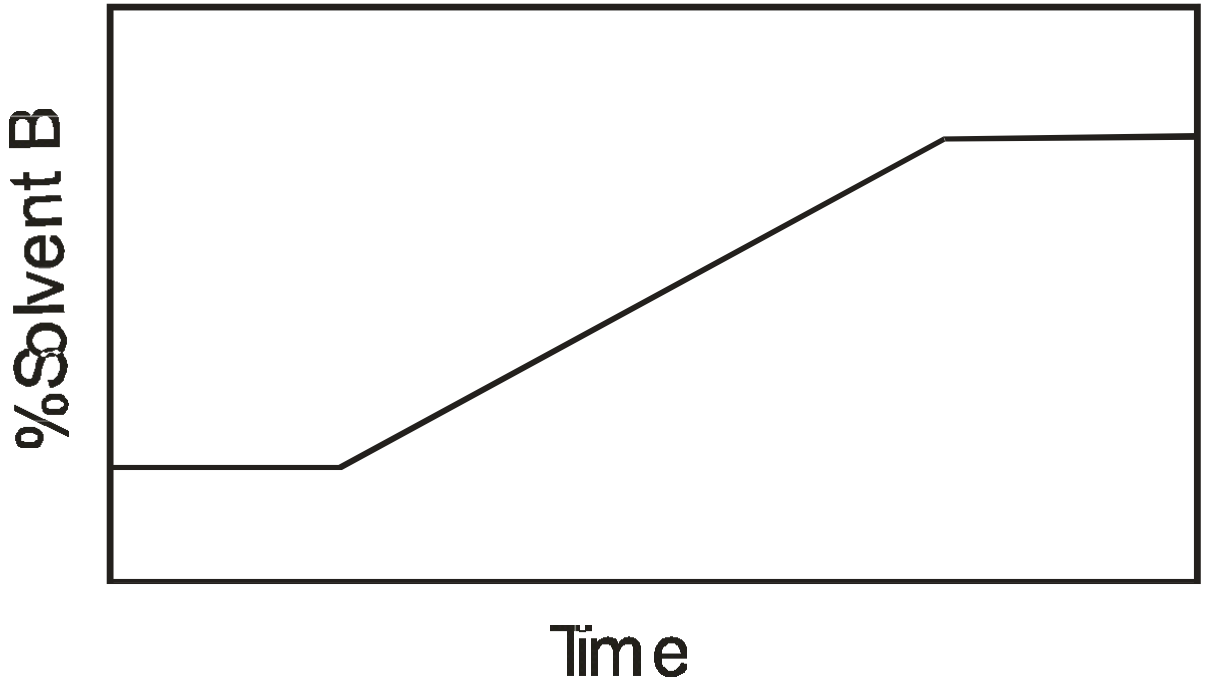
Gradient elution: changing composition of mobile phase with time → *solvent programming*

, going from a weak mobile phase to a strong one.

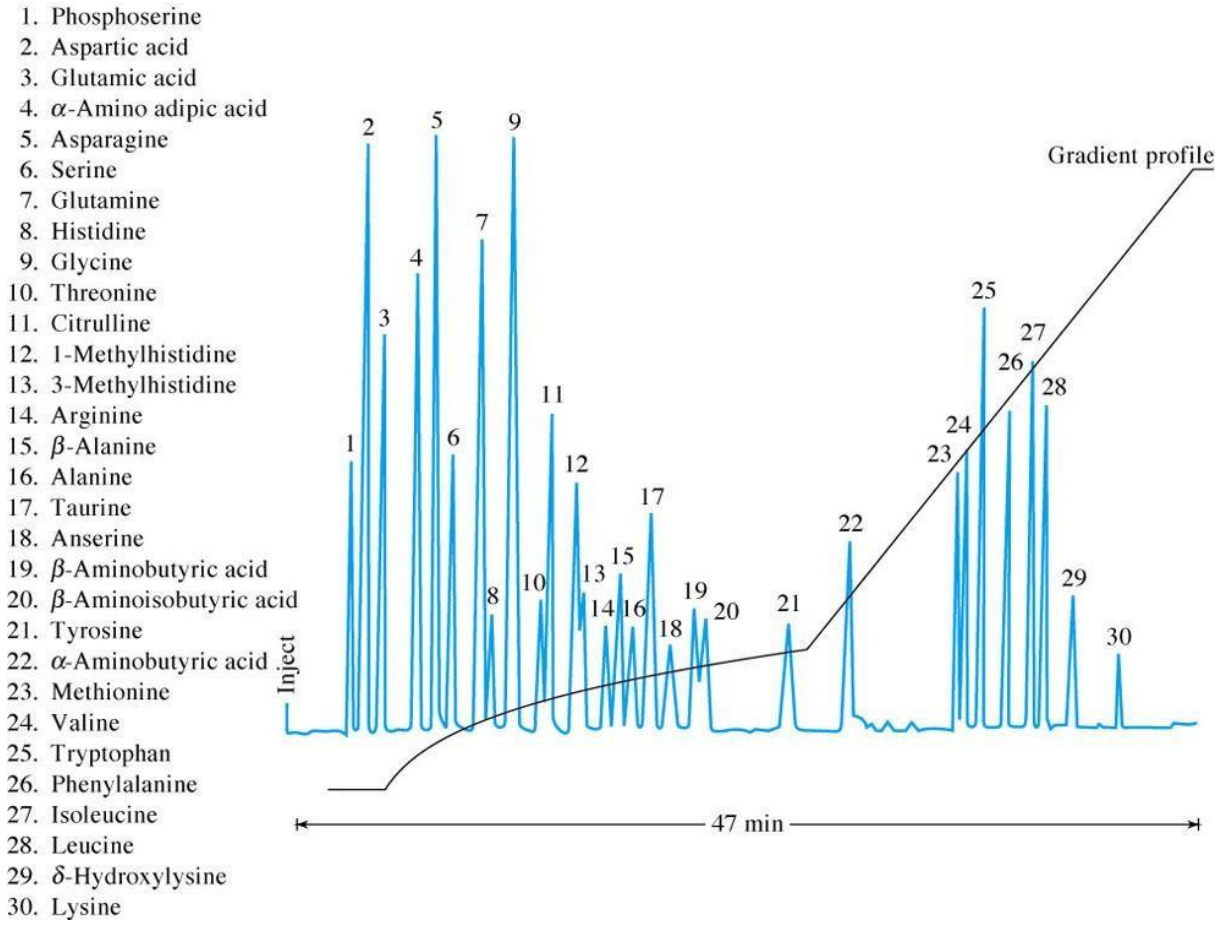
, weak mobile phase → *solvent A*

, strong mobile phase → *solvent B*

, solvent change can be stepwise, linear or non-linear



Gradient elution of mixture of 30 amino-acids

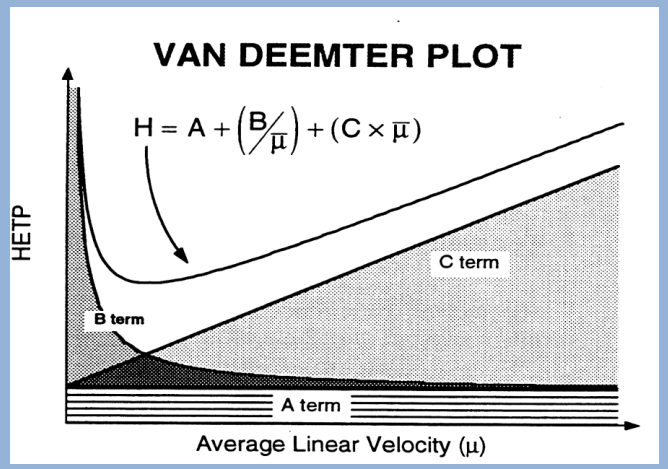


In choosing a mobile phase for LC, several factors need to be considered

– type of stationary phase used
, determines what will be a strong or weak mobile phase
– solubility of the solutes
– viscosity of the mobile phase
– type of detector used and solvent's background signal
– purity of the solvents
– miscibility of the solvents (for gradient elution)

Van Deemter equation: relates flow-rate or linear velocity to H:

$H = A + B/\mu + C\mu$ where:



μ = linear velocity (flow-rate x V_m/L) H = total plate height of the column
A = constant representing eddy diffusion & mobile phase mass transfer
B = constant representing longitudinal diffusion
C = constant representing stagnant mobile phase & stationary phase mass transfer

Plate height (H) may be used to relate these kinetic processes to band broadening to a parameter of the chromatographic system (e.g., flow rate). This relationship is used to predict the resulting effect of varying this parameter on the overall efficiency of the chromatographic system.

Number of theoretical plates(N) $(N) = 5.54\ (t_R/W_h)^2$ *peak width (W_h)*

$H = L/N$

μ optimum

Optimum linear velocity (μ_{opt}) - where H has a minimum value and the point of maximum column efficiency:
μ_{opt} is easy to achieve for gas chromatography, but is usually too small for liquid chromatography requiring flow-rates higher than optimal to separate compounds

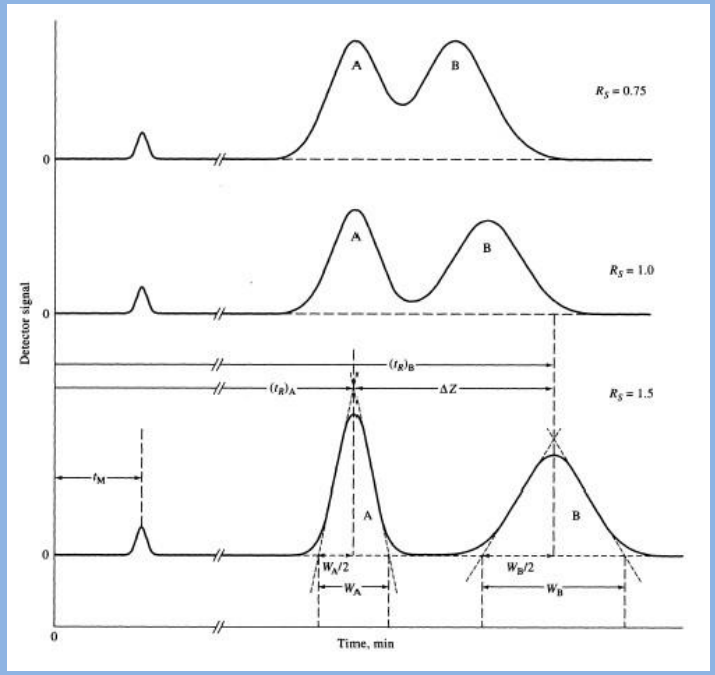
resolution (R_S) – resolution between two peaks is a second measure of how well two peaks are separated:

$$R_S = \frac{t_{r2} - t_{r1}}{(W_{b2} + W_{b1})/2}$$

where:

t_{r1}, W_{b1} = retention time and baseline width for the first eluting peak

t_{r2}, W_{b2} = retention time and baseline width for the second eluting peak



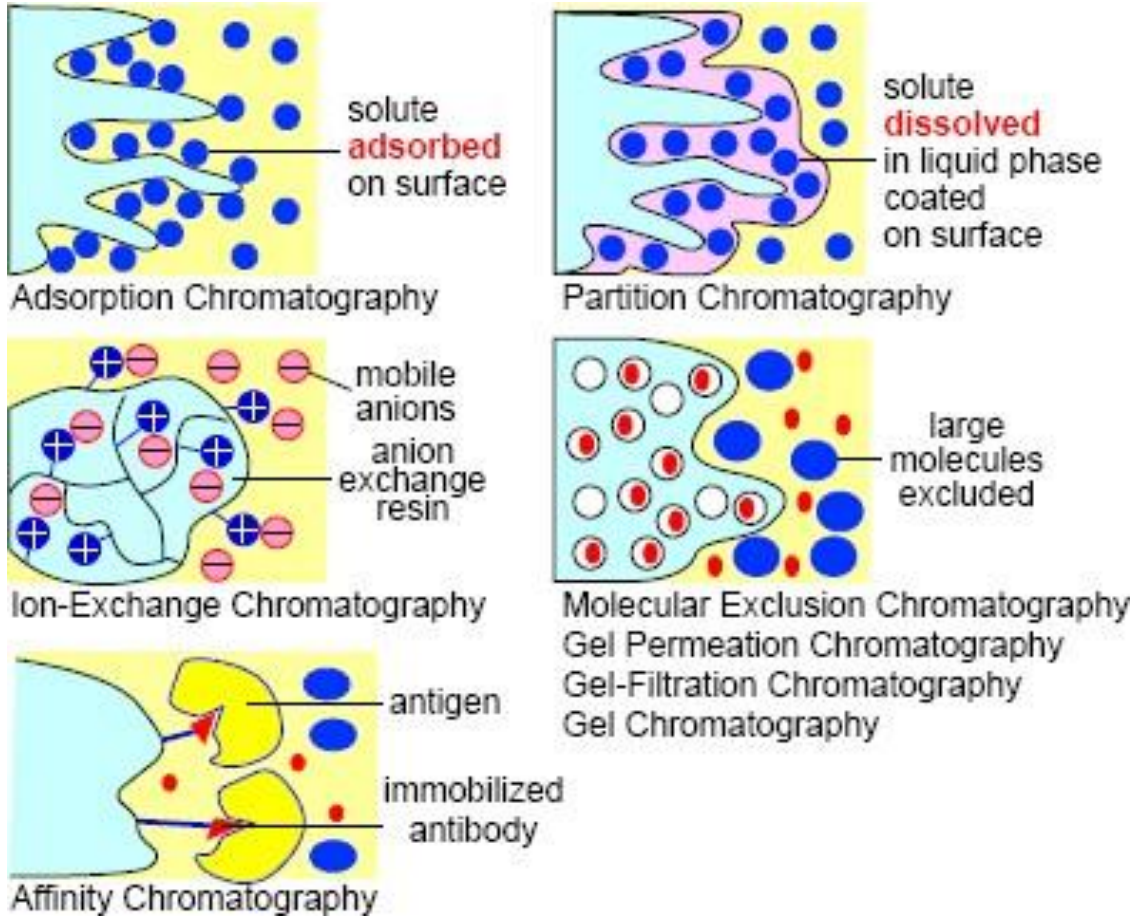
R_s over 1.5 represents *baseline resolution*, or complete separation of two neighboring solutes → ideal case.

R_s over 1.0 considered adequate for most separations.

D.) Types of Liquid Chromatography:

Techniques in LC are classified according to the method of solute separation

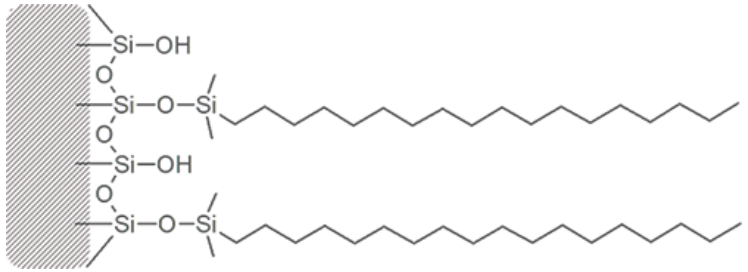
, Adsorption chromatography
, Partition chromatography
,Ion-exchange chromatography
, Affinity chromatography
, Size-exclusion chromatography



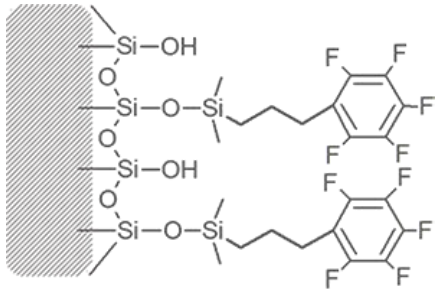
Colonne per HPLC

- Ciascun analita si ripartisce fra la fase mobile e la fase stazionaria.
- Il tempo di ritenzione (t_R) sarà determinato dalla competizione fra le affinità di ciascun analita per la fase mobile (FM) e per la fase stazionaria (FS)

C18



Pentafluorophenyl (PFP)



C18-PFP

E.) LC Detectors:

Common types of LC Detectors

, Refractive Index Detector, Conductivity Detector

UV/Vis Absorbance Detector, Electrochemical Detector

, Fluorescence Detector

As in GC, the choice of detector will depend on the analyte and how the LC method is being used (*i.e.*, analytical or preparative scale)

Detector	Selectivity	Sensitivity	Notes
Refractive Index	**Poor**	**Poor**	Any component that differs in refractive index from the eluate can be detected, despite its low sensitivity. Cannot be used to perform gradient analysis.
UV/Vis	**Moderate**	**Good**	A wide variety of substances can be detected that absorb light from 190 to 900 nm. Sensitivity depends strongly on the component.
Fluorescence	**Good**	**Excellent**	Components emitting fluorescence can be detected selectively with high sensitivity. This is often used for pre-column and post-column derivatization.
Conductivity	**Moderate**	**Good**	Ionized components are detected. This detector is used mainly for ion chromatography.
Electrochemical	**Good**	**Excellent**	Electric currents are detected that are generated by electric oxidation-reduction reactions. Electrically active components are detected with high sensitivity.

2.) UV/Vis Absorbance Detector

Measures the ability of solutes to absorb light at a particular wavelength(s) in the ultraviolet (UV) or visible (Vis) wavelength range.

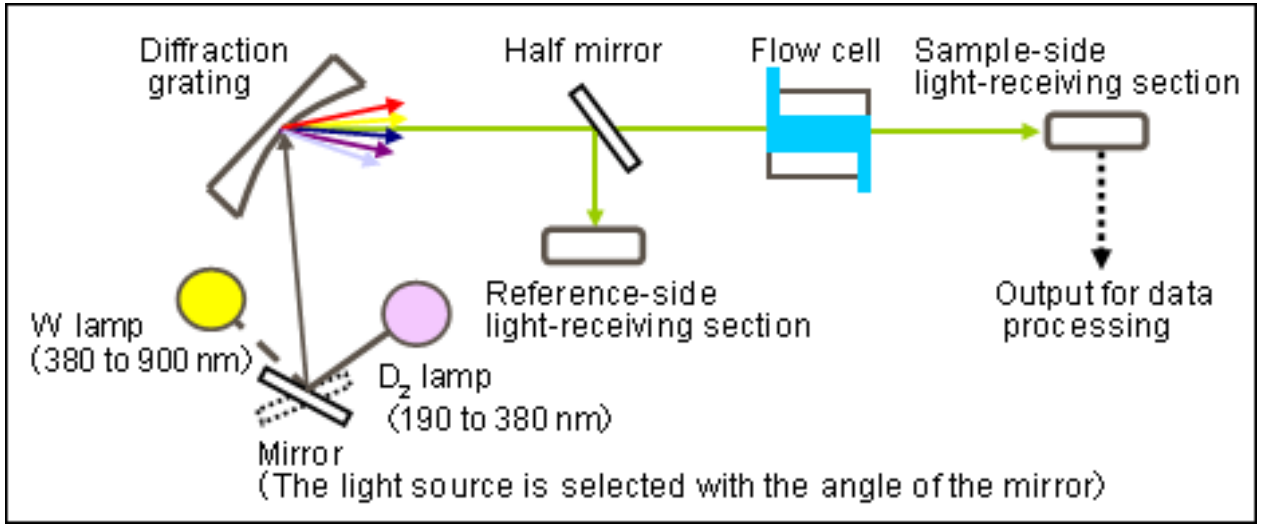
, most common type of LC detector

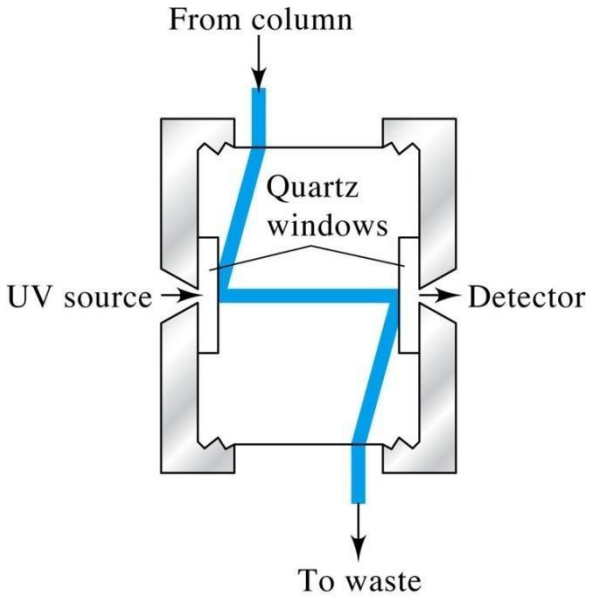
Three Common types of UV/Vis Absorbance Detectors

, Fixed wavelength detectors

, Variable wavelength detectors

, Photodiode array detectors





2.) UV/Vis Absorbance Detector

Fixed Wavelength Detector absorbance of only one given wavelength is monitored by the system at all times (usually 254 nm)

, simplest and cheapest of the UV/Vis detectors

, limited in flexibility

, limited in types of compounds that can be monitored

Variable Wavelength Detector a single wavelength is monitored at any given time, but any wavelength in a wide spectral range can be selected

, wavelengths vary from 190-900 nm.

, more expensive, requires more advanced optics

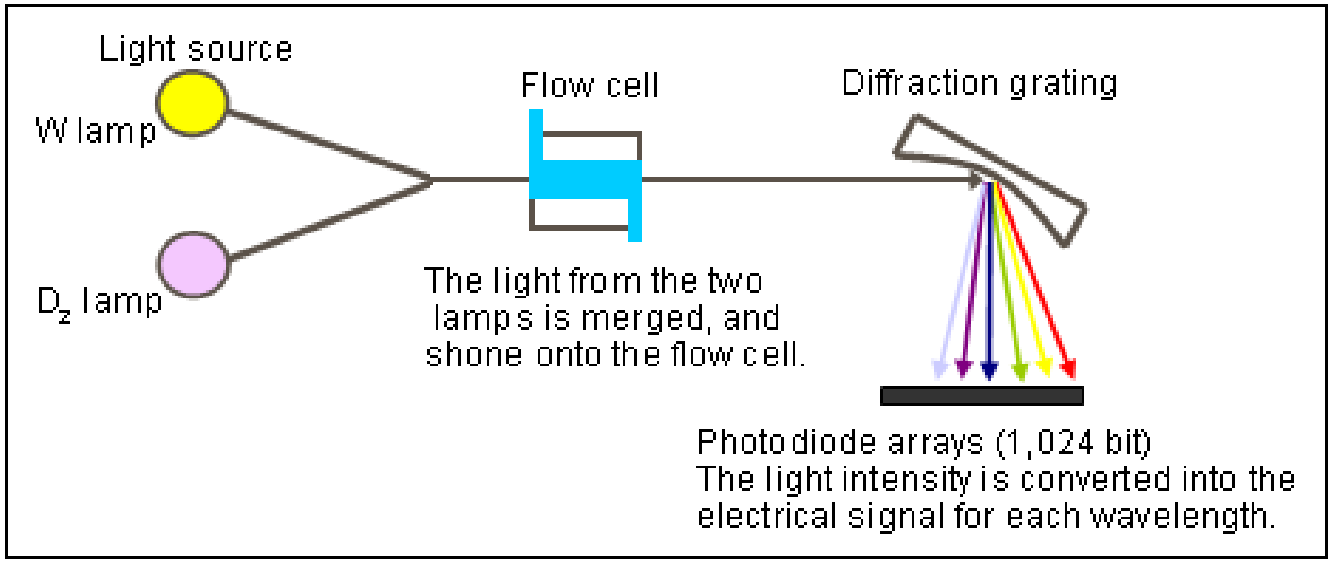
, more versatile, used for a wider range of compounds

Photo Diode Array Detector operates by *simultaneously* monitoring absorbance of solutes at several different wavelengths.

, uses a series or an array of several detector cells within the instrument, with each responding to changes in absorbance at different wavelengths.

, entire spectrum of a compound can be taken in a minimum amount of time

, useful in detecting the presence of poorly resolved peaks or peak contaminants

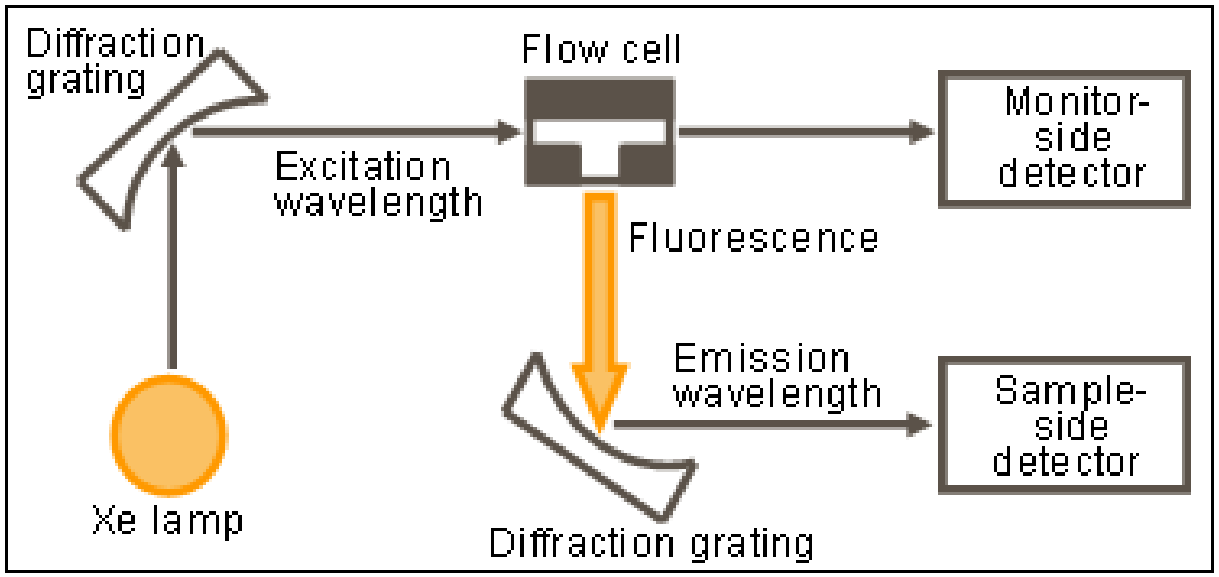


Applications:

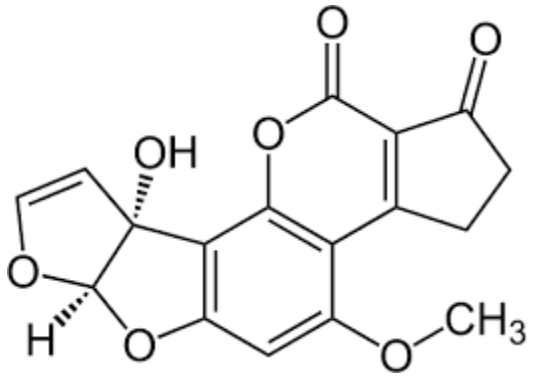
- UV/Vis absorbance detectors can be used to detect any compound that absorbs at the wavelength being monitored
- Common wavelengths:
  - , 254 nm for unsaturated organic compounds
  - , 260 nm for nucleic acids
  - , 280 or 215 nm for proteins or peptides
- Absorbance detectors can be used with gradient elution
  - , wavelength being monitored is above the cutoff range of the solvents being used in the mobile phase
- limits of detection for fixed and variable UV/Vis absorbance detectors are ~ 10^{-8} M
- limits of detection for photodiode array detectors are ~ 10^{-7} M

3.) Fluorescence Detector

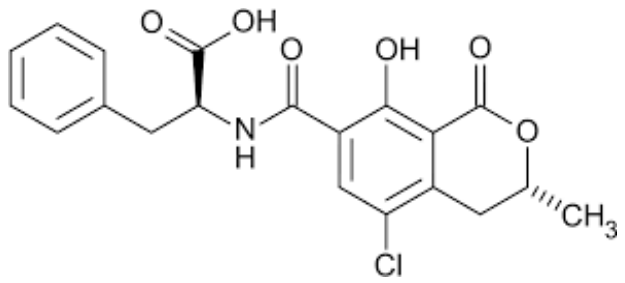
A selective LC detector that measures the ability of eluting solutes to fluoresce at a given set of excitation and emission wavelengths



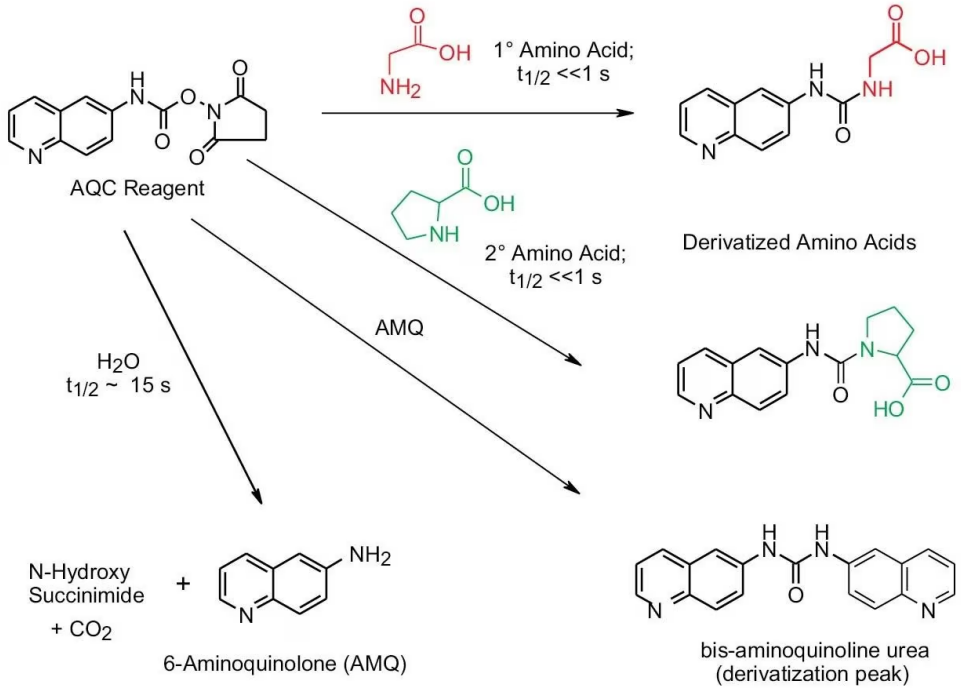
Mycotoxins (aflatoxins, ochratoxin A etc…), derivatised compounds (e.g. aminoacids)



Aflatoxin M1



Ochratoxin A



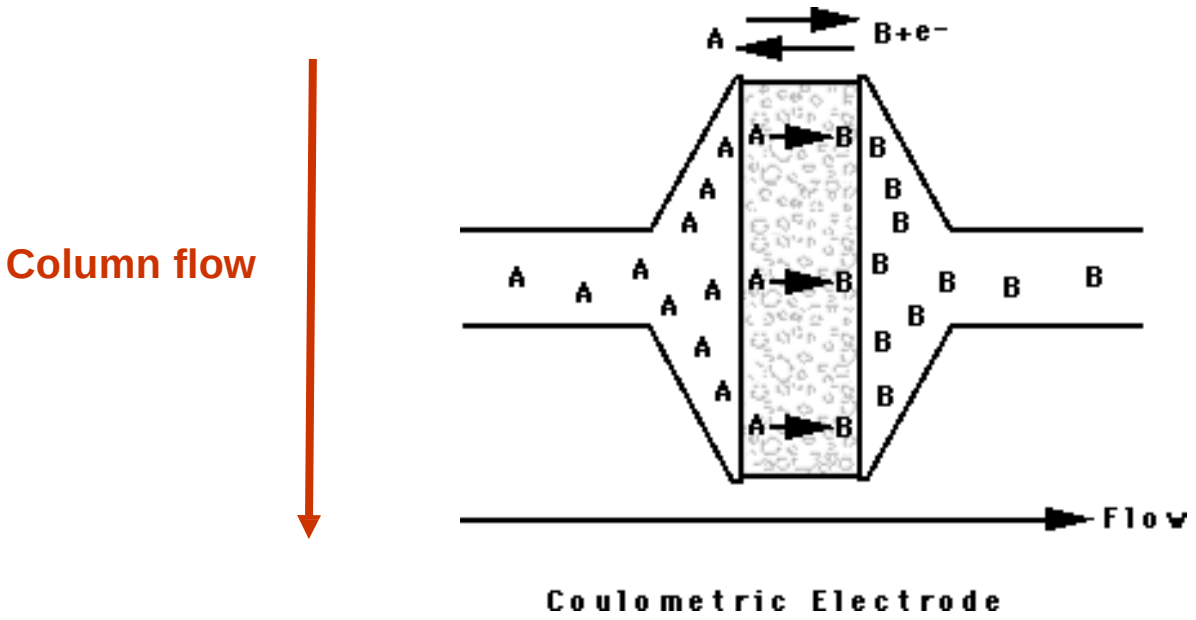
5.) Electrochemical Detector

Used to monitor any compound in the mobile phase that can undergo an oxidation or reduction

, electrochemical detection in liquid chromatography is sometimes referred to as LC/EC

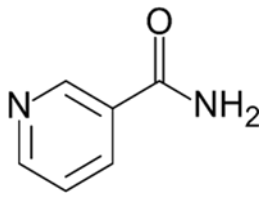
, generally includes two or more electrodes which monitor the current that is produced by the oxidation or reduction of eluting compounds at a fixed potential

, generally electrical output is an electron flow generated by a reaction that takes place at the surface of the electrodes.



Application in food; sugars, any compound that can be reduced/oxidised (not polyphenols!)

Vitamin B3 in Chicken



- Niacin, also known as vitamin B3, is one of the essential human nutrients. Vitamin B3 comprises nicotinic acid, nicotinamide, and numerous enzymatic forms.
- Nicotinamide is a precursor of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), two redox coenzymes primarily involved in the production of energy from dietary proteins, carbohydrates, and fats. They play a key role in several biochemical paths like Krebs' cycle or glycolysis.
- In meat the niacin content is mainly present in the form of nicotinamide: while in living tissues nicotinamide is a component of the coenzymes NAD and NADP, in meat it is found also in the free form because of postmortem hydrolysis of NAD.
- Chicken meat is a really good source of vitamin B3. In particular white meat (breast e.g.) contains a higher amount of niacinamide with the respect of dark meat cuts (thigh and drumstick e.g.).

Extraction procedure

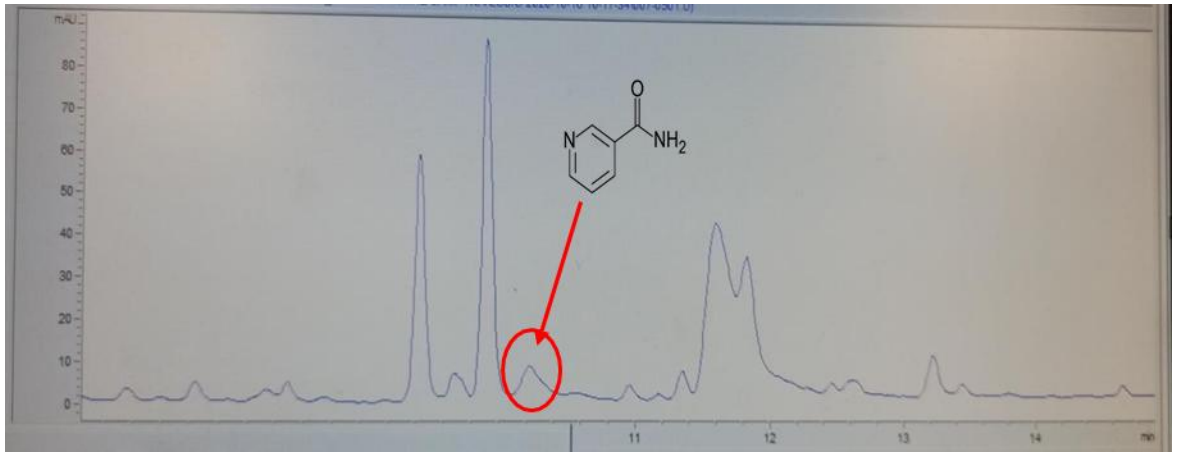
- 0.5 g of samples dissolved in 4.5 mL of HCl 0.1 M
- Homogenization with ultra turrax (1 min)
- Heat treatment (100 °C, 1 h)
- pH correction to 4-4.5, using solution of Sodium Acetate 2.5 M
- Addition of 500 uL of taka-diastase (hydrolysis of phosphorylated forms) 10 % (w/v) solution
- Incubation at 48 °C, 3 h
- Centrifugation at 10000 g, 10 min, 4°C
- Recovery of the supernatant, taken up to volume to 5 mL
- 500 uL of sample in HPLC vial

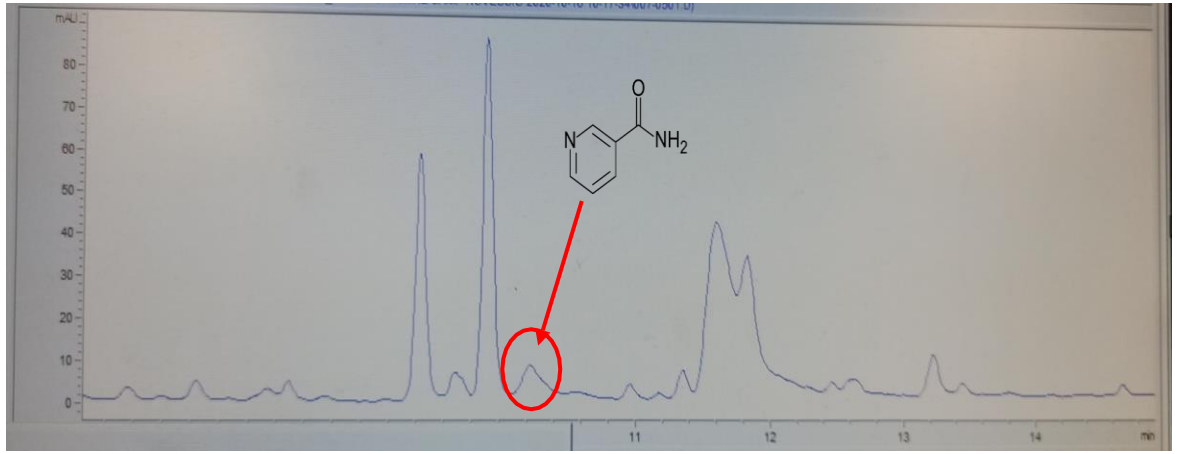
Ultra turrax homogenizer

Chromatographic method

- Instrument: HPLC Perkin Elmer sieres 200 equipped with autosampler, pump and Uv-vis detector.
- Column:C18 Luna Omega Polar 3 um 4.6x150.
- Mobile Phases
  - A: ammonium acetate 0.01 M; methanol 0.01 %
  - B: methanol
- Gradient: from 100 % to 30 % in 30 min
- Wavelength: 260 nm

HPLC system

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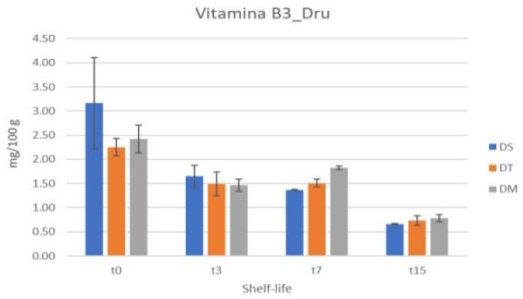
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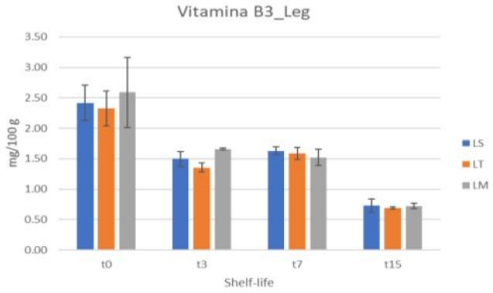
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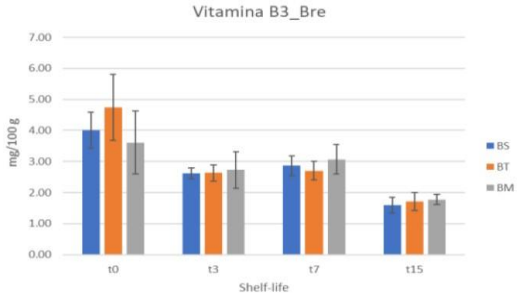
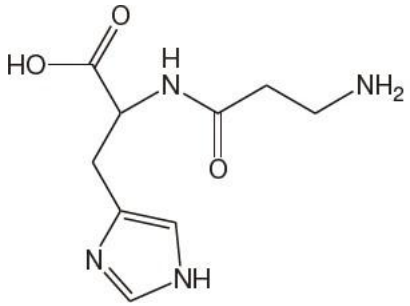
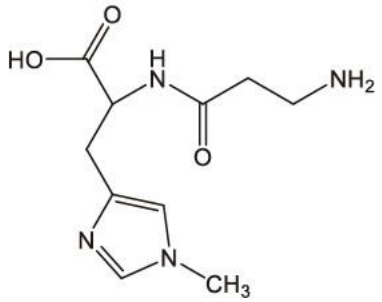
LT
LM


Vitamina B3_Bre
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
mg/100 g
t0
t3
t7
t15
Shelf-life
BS
BT
BM

ANSERINE



CARNOSINE



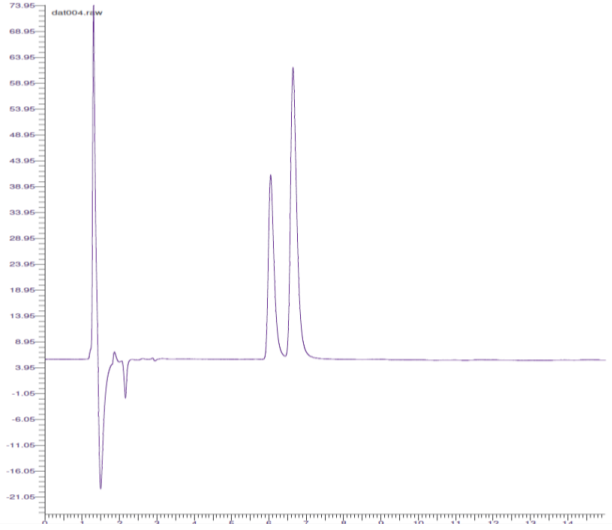
DIPEPTIDES PRESENT IN POULTRY MEAT IMPORTANT FOR

- ANTIOXIDANT ACTIVITY
- ANTI-AGING ACTIVITY
- BUFFER CAPACITY
- ANTI TUMORAL AND ANTIINFLAMMATORY ACTIVITY

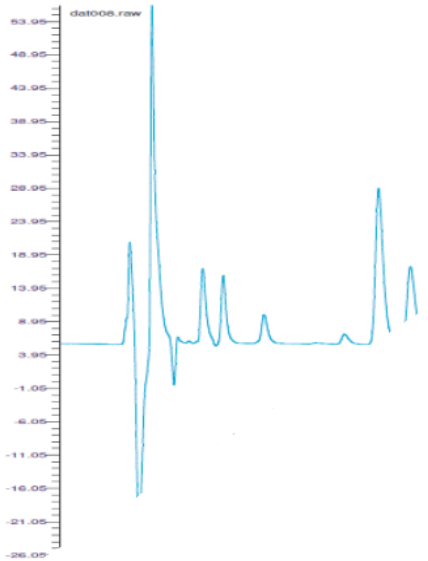
Sample	Anserine mg/100g	Carnosine mg/100g	C/A	Reference
Breast extract		71.8		Intarapichet and Maikhunthod 2005
Thigh extract		10.4		
Chicken- turkey burger 25-75	287.9	66.6	0.23	Gil-Agustì et al., 2008
20 wk breast	336.7	71.2	0.21	Kim et al., 2012
20 wk drumstick	116.5	39.5	0.34	
20 wk wing	259.4	38.4	0.15	
90 wk breast	291.5	55.3	0.19	
90 wk drumstick	69.3	14.2	0.20	
90 wk wing	226.4	35.9	0.16	
Breast	1170	700	0.60	Peiretti et al., 2011
Breast	1670	920	0.55	
Breast	1460	760	0.52	
Breast	710	255	0.36	Mori et al., 2015
Leg	220	106	0.48	
Fillet	608	157	0.26	
Breast (Conv)	92.6	63.16	0.68	Kim et al., 2020
Breast (Welfare)	117.54	65.30	0.56	

After elimination of fat and connective tissue 0.5±0,05 g treated with trichloroacetic acid (0.07%) and homogeneised. After centrifugation at 4000 rpm (15 min) 2 mL of supernantant then and centrifuged again (10000 rpm 15 min). Dilution 1:20 mobile phase 20 uL injected in HPLC.

Column Agilent Zorbax Bonus RP 3.5µm, 4.6x150mm (densely packed diisopropyl-C14 groups). Mobile phase is 10 mM phosphate buffer in 5% acetonitrile and 5 mM octansulfonic acid **(octanesulfonate is an ion pairing reagent that is used in HPLC, notably in the analysis of small organic compounds. The anionic sulfonate counterion permits the separation and resolution of positively charged analytes).** 15 min isocratic at 1 mL/min flow rate, UV detector at 224 nm.



Standards



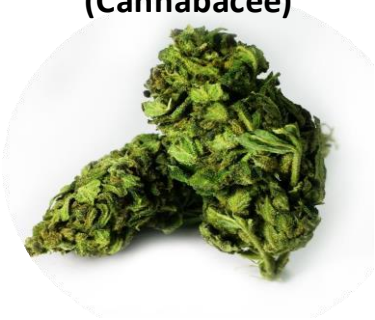
Sample

Chemical characterization of Polyphenols in different extracts of :

***Coriandrum sativum*L.**
(Apiaceae)

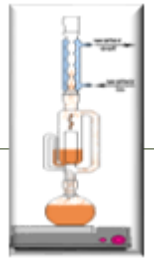
***Thymus vulgaris* L.**
(Lamiaceae)

***Cannabis sativa* L.**
(Cannabacee)



Extraction Techniques

(UAE)
Ultrasound Assisted Extraction



Soxhlet

(RSLDE)
Rapid Solid-Liquid Dynamic Extraction
(Naviglio extractor)

Maceration

Extractionparameters

Solvent: Ethanol
Extraction Time:

- 30 days for Maceration
- 20 minutes for Ultrasounds
- 2 hours and 6 hours for RSLDE and Soxhlet

The weight of the matrix subjected to extraction was calculated maintaining the same ratio for the different extraction techniques

HPLC-UV Analysis parameters

HPLC Perkin-Elmer series 200
Phase A (Inorganic): 1% acetic acid in water
Phase B (Organic): acetonitrile
Column:Kinetex C18 (dimensions: 250 x 4.6 mm, particle size: 5 µm, pore size: 110 Å)

UV-Vis detector Perkin Elmer LC 240 set at 280 nm

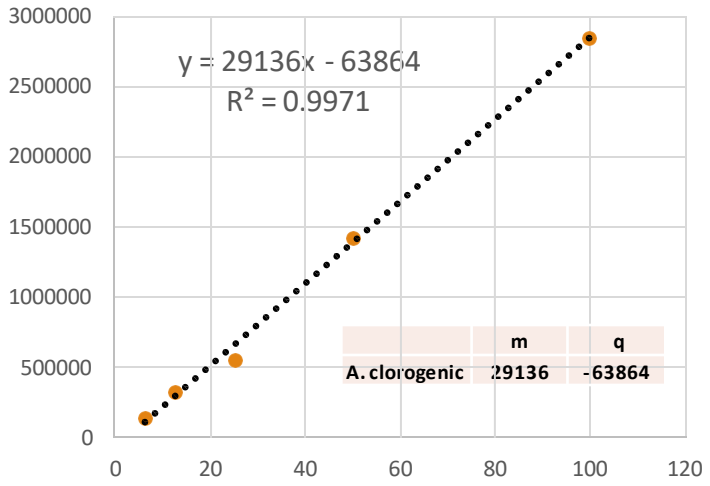
Polyphenols analyzed

The polyphenols chosen for the analysis are: gallic acid, p-OH benzoic acid, apigenin, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, ferulic acid, rosmarinic at start. The working standard mixtures were prepared by appropriate dilution of the standards in methanol at concentration of 100 ppm

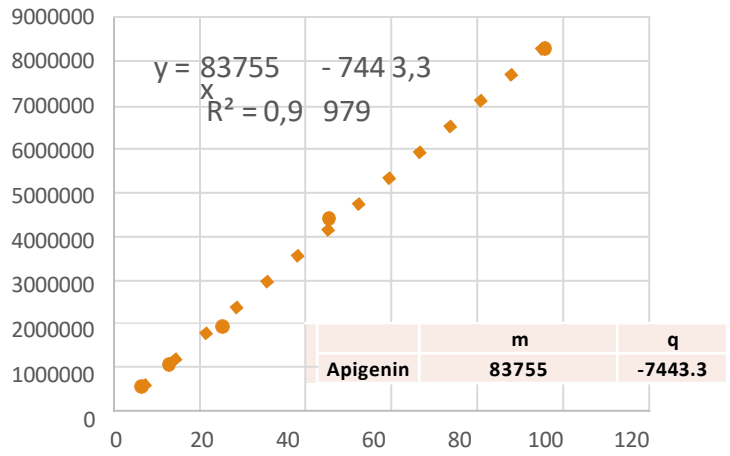
Quantitative Analysis

The quantification for each analyte was performed by means of a calibration line, starting from the mix of polyphenols at 100 ppm, a 6-point line (1-6.25-12.5-25-50-100 ppm)

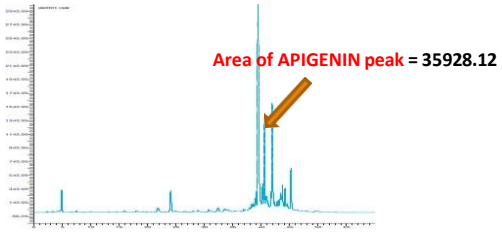
A. chlorogenic



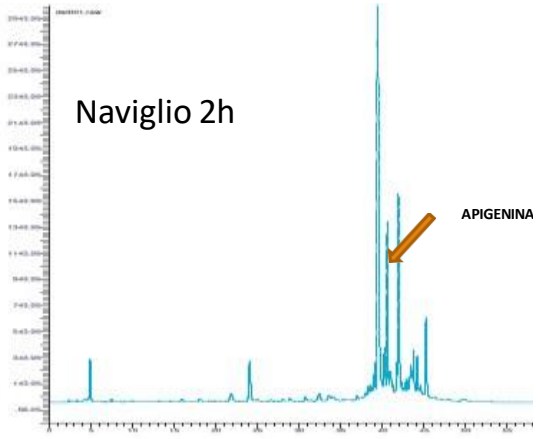
Apigenin

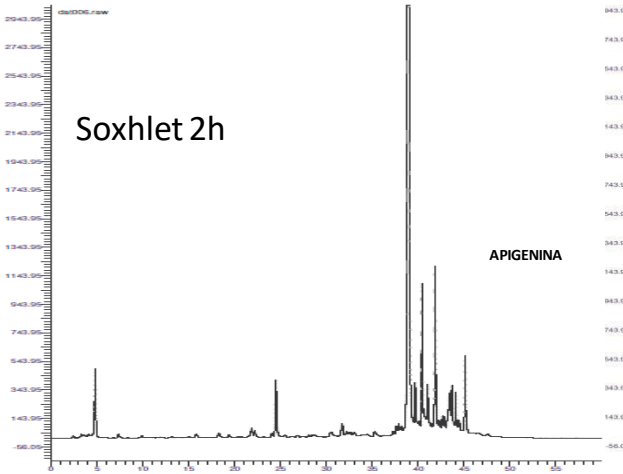


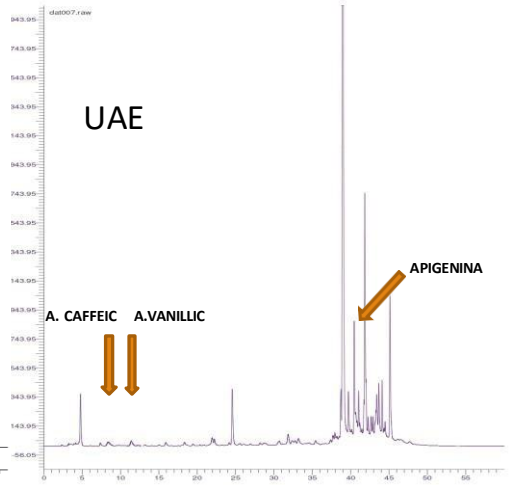
The concentration of the analyte present in the sample is calculated through the calibration line:
(Area of my peak - q) / m
The result are expressed in ppm .

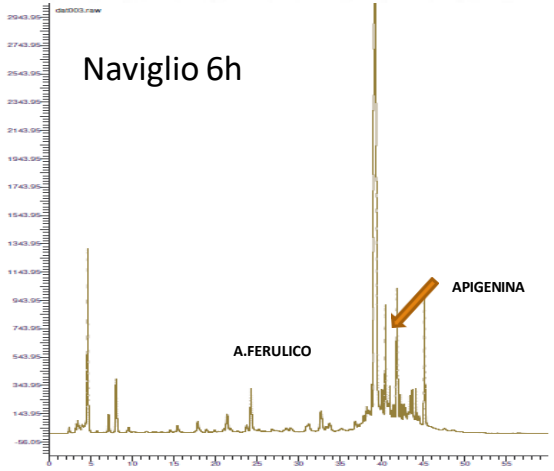


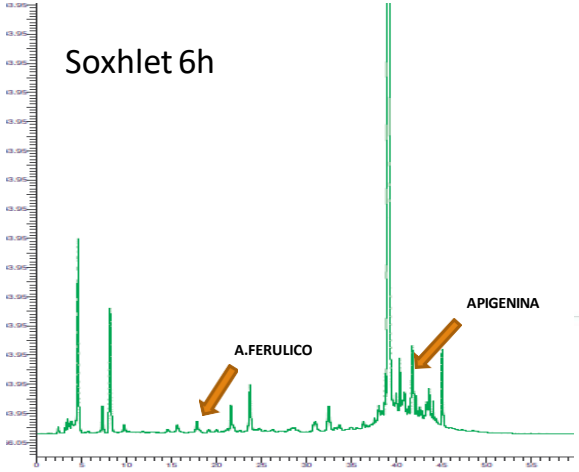
Chromatograms of Thymus extracts:

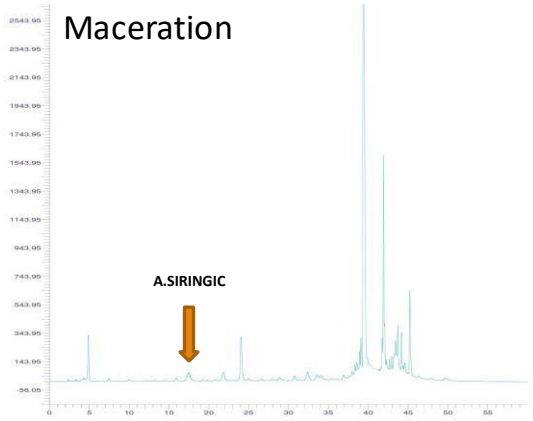












UHPLC – Ultra High Performance Liquid Chromatography

La **cromatografia liquida a ultra alta prestazione** (UHPLC, Ultra High Performance Liquid Chromatography) è una recente implementazione che sfrutta l'avanzamento tecnologico nella costruzione dei componenti strumentali tipici della classica HPLC, come la possibilità di produrre colonne contenenti una fase stazionaria con diametro delle particelle molto minore oltre a pompe e parti meccaniche in grado di operare a pressioni di esercizio ancora più elevate.

La tecnica UHPLC permette di ottenere una separazione delle sostanze eluite caratterizzata da una maggiore efficienza e in tempi notevolmente ridotti, utilizzando come fase stazionaria particelle dal diametro solitamente **<3 µm** e pressioni che possono superare i **1000 bar**. Altra caratteristica di non secondaria importanza è il ridotto volume di campione iniettato (la sensibilità è nettamente maggiore) e il risparmio di eluente che si ottiene con questa tecnica. L'unico difetto è che la vita delle colonne si abbassa nettamente.

HPLC vs UHPLC

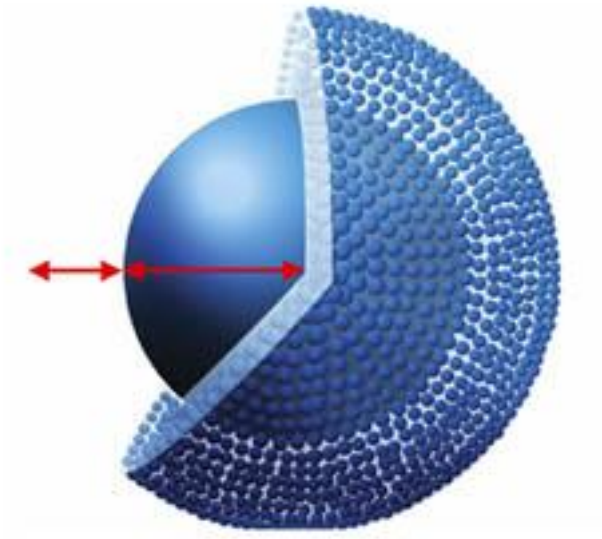
HPLC

- Impiego di colonne con fase stazionaria con diametro delle particelle tra i 3 e i 10 µm
- Massima pressione di esercizio 300-400 bar
- Contropressione: pressioni massime di 400-600 bar (HPLC)
- Maggiore volume di campione iniettato (sensibilità minore) e aumento eluente necessario.
- Portata di flusso: 0.01-5 mL/min
- Volume di iniezione: 5 µL
- Minor efficienza

UHPLC

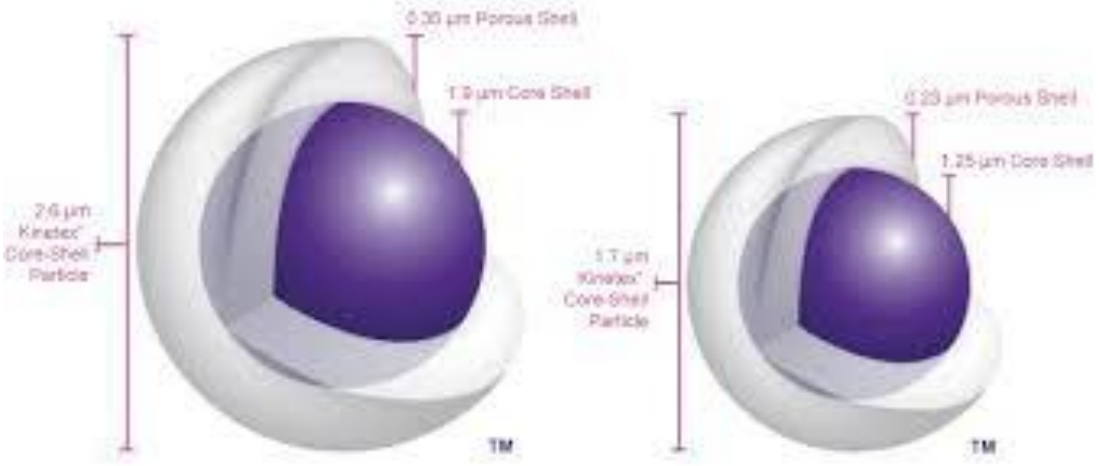
- Impiego di colonne con fase stazionaria con diametro delle particelle <3 µm
- Massima pressione di esercizio >1000 bar
- Contropressione: pressioni massime fino a 1500 bar (UHPLC)
- Ridotto volume di campione iniettato (maggiore sensibilità) e il risparmio di eluente necessario.
- Portata di flusso maggiore
- Volume di iniezione: 2 µL
- Elevata efficienza

Particelle Core-Shell



Fully Porous		Kinetex Core-Shell	Average Efficiency Gain with Kinetex*
5 µm	vs	5 µm	90 % Higher
3 µm	vs	2.6 µm	85 % Higher
1.7 µm	vs	1.7 µm	20 % Higher
1.7 µm	vs	1.3 µm	50 % Higher

* May not be representative of all separations.

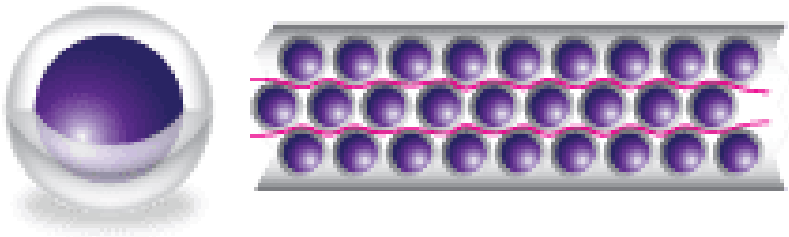


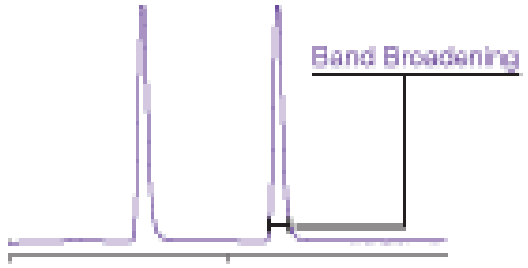
Le particelle core-shell hanno un nucleo di silice non porosa circondato da un sottile strato di silice porosa.

La diminuzione della superficie interfacciale fra fase stazionaria e mobile, rispetto alle particelle porose, è compensata dalla diminuzione del contributo del termine CM nell'equazione di Van Deemter, con conseguente incremento dell'efficienza.

HIGH EFFICIENCY HPLC

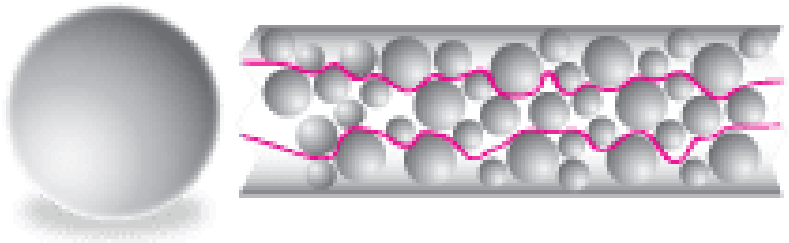
Kinetex Core-Shell

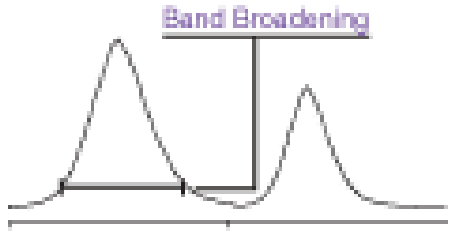




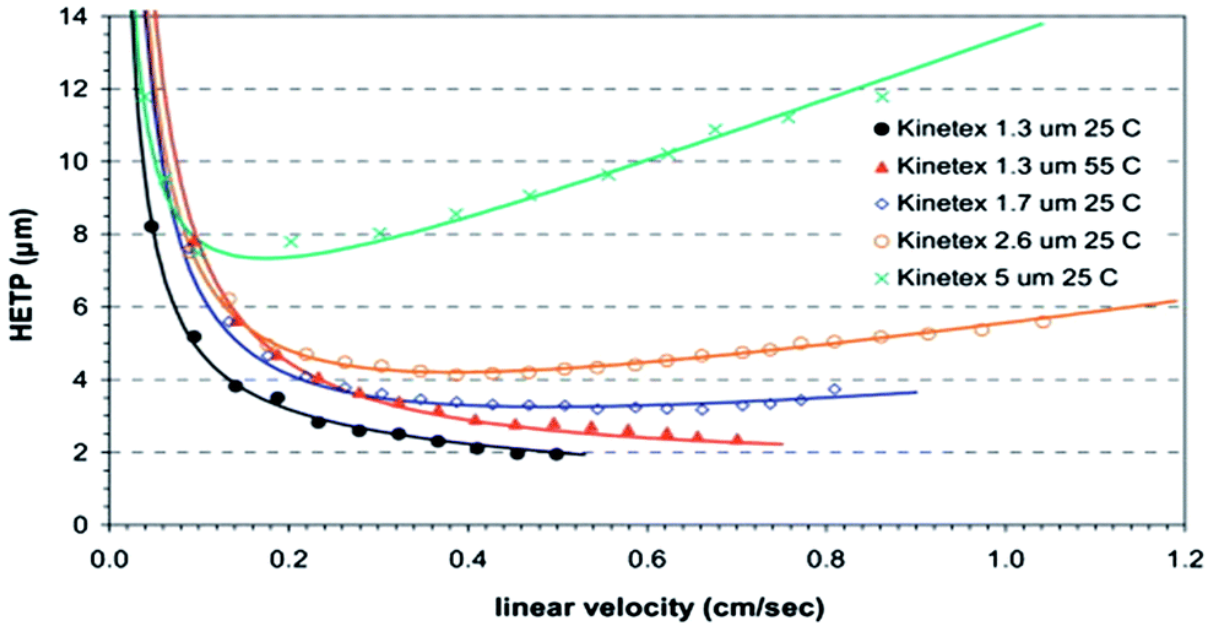
- Il core solido riduce tutti i contributi all'allargamento di banda in cromatografia liquida (diffusione molecolare longitudinale, resistenza al trasferimento di massa e percorsi multipli)
- Maggiore efficienza
- Per UHPLC

Fully Porous





- Particelle di silice totalmente porose
- Basse contropressioni
- Favorisce allargamento della banda in cromatografia liquida a fase inversa
- Minore efficienza
- Per HPLC



Teoria dell'allargamento della banda: equazione di Van Deemter

Percorsi multipli (eddy diffusion)

- Le molecole eluiscono in t diversi ed è indipendente da u

- Dipende da:
  - Granulometria e dall'impaccamento della FS
  - Diametro medio delle particelle del riempimento

$$H = A + B/u + C_S u + C_M u$$

Coefficiente di **trasferimento di massa** nella fase stazionaria (cinetica di adsorbimento/desorbimento):
Film di liquido su particella solida - direttamente proporzionale a (spessore)^2 , inversamente proporzionale a D_S nel film
Fase stazionaria solida – direttamente proporzionale al tempo adsorbimento/desorbimento

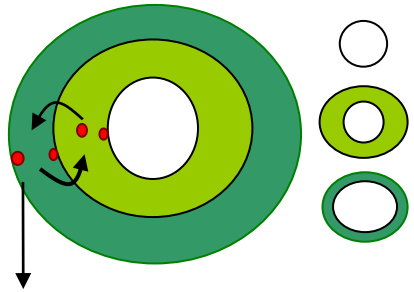
- Dipende da:
  - Tempo di assorbimento e desorbimento dell'analita dalla FS

Diffusione longitudinale:

- Indipendente dalle dimensioni delle particelle

- Dipende da:
  - Formazione gradienti di concentrazione nella FM
  - L'analita diffonde da zone a concentrazione maggiore (al centro) a zone a concentrazione minore

Direttamente proporzionale al coefficiente di diffusione più importante in GC che in LC



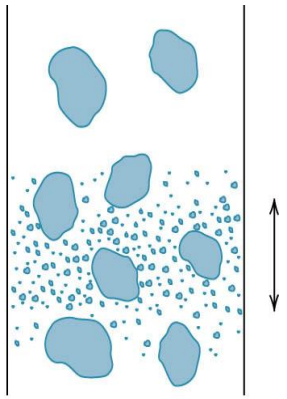
Supporto

Fase stazionaria liquida o solida

Fase mobile

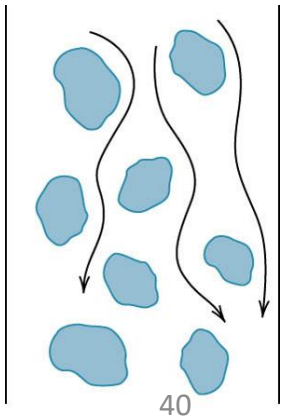
Coefficiente di **trasferimento di massa** nella fase mobile:
inv. prop. a D_M, funzione di (diametro particelle)^2 (diametro colonna)^2 e u

Diffusione turbolenta

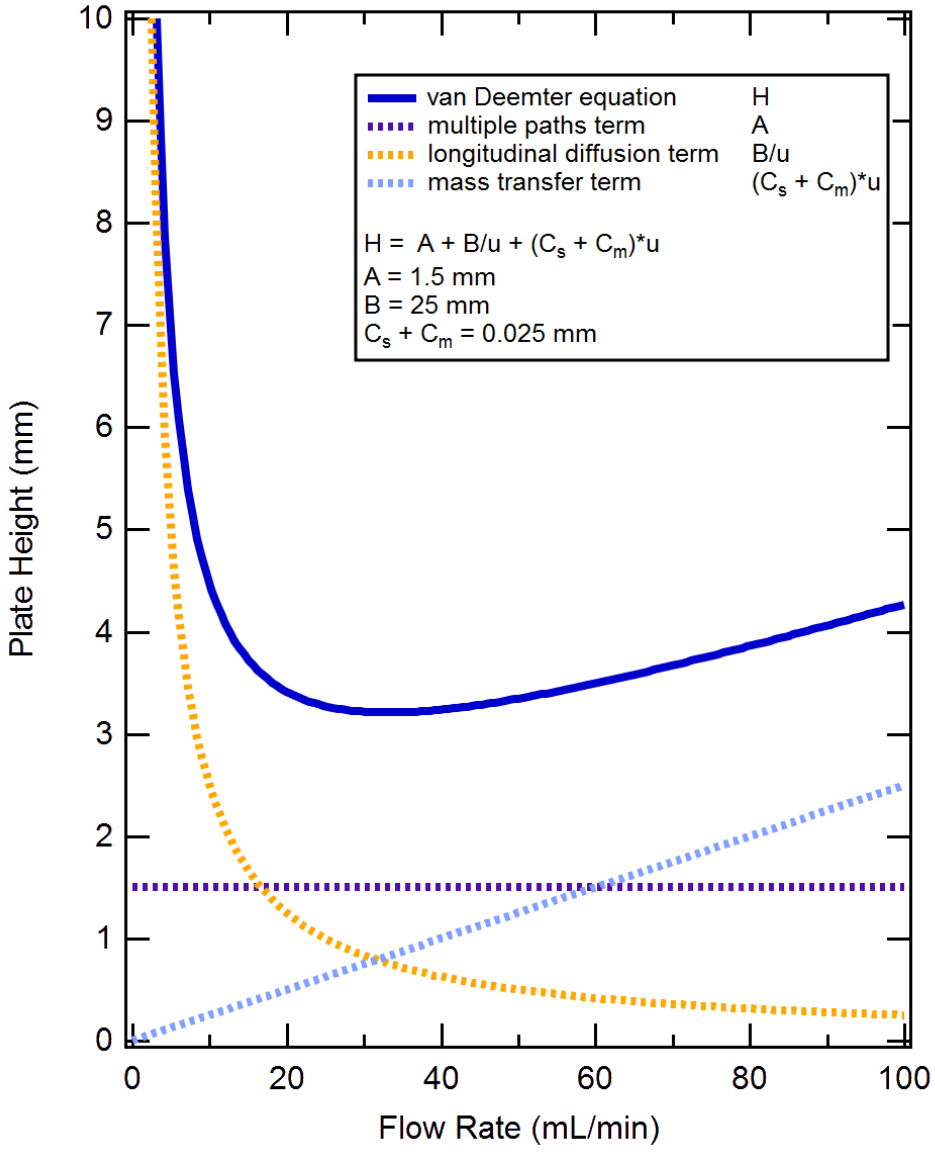


Molecular diffusion

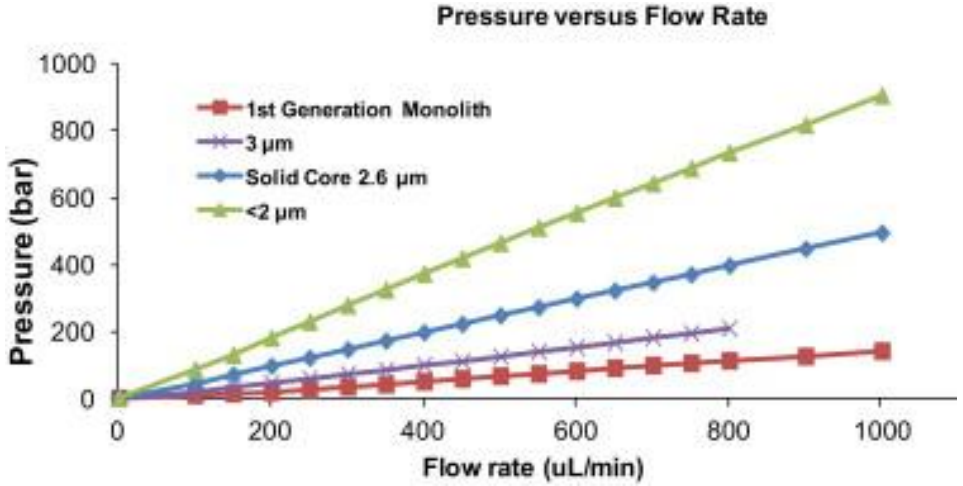
La diffusione turbolenta diminuisce a flussi bassi di fase mobile
L'allargamento di banda può essere minimizzato dall'utilizzo di particelle piccole e regolari (p.es. sfere)



Eddy diffusion



$$H = A + B/u + C_S u + C_M u$$



- Piccole d_p danno HETP più bassi
- Il flusso ottimale è maggiore con d_p più piccolo
- Colonne con particelle piccole hanno minor perdita di efficienza a flussi alti

Quando il flusso dell'eluente è forzato in colonna, esso genera una contropressione.
La relazione tra questa contropressione ΔP e le altre variabili cromatografiche è data da:

$$\Delta P = \frac{\eta L v}{\Theta d_p^2}$$

Dove: η = *viscosità della fase mobile*
v = *velocità lineare della fase mobile*
L = *lunghezza della colonna*
d_p = *diametro medio delle particelle*
Θ = *costante*