Theoretical Principles

Origins & evolution of chromatography

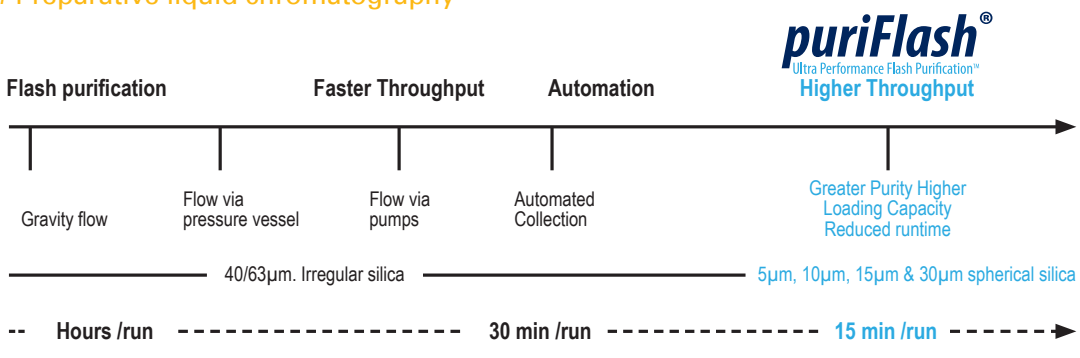
The term "**chromatography**" originated in 1906 thanks to Russian botanist Mikhail Tswett. In 1901, he washed an organic solution of plant pigments through a vertical glass column packed with an adsorptive metal. He discovered that the pigments separated into a series of colored bands on the column, divided by regions entirely free of color.

In 1930, chemists Richard Kuhn and Edgar Lederer used this technique to separate different biologically materials. Since that time, the technique has advanced rapidly and column chromatography is now used widely in many different forms. The column itself has also been refined over the years, according to the type of chromatography, but fulfils the same essential separating function in all forms of column chromatography.

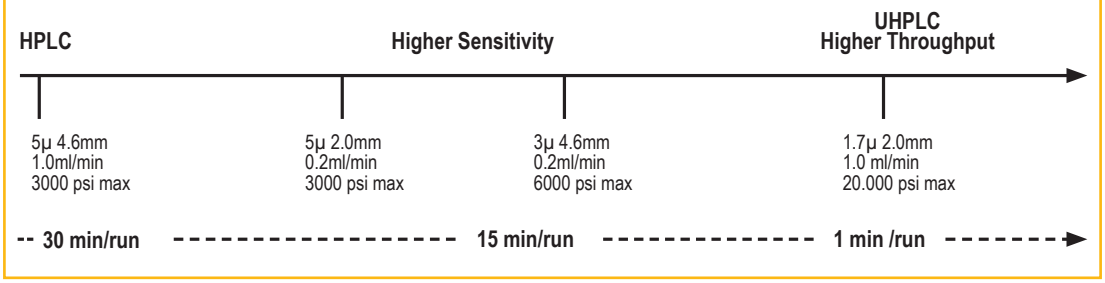
In 1964, the American chemist J. Calvin Giddings refined liquid chromatography to achieve separations of different molecules. This was the origin of the technique now known as High Performance Liquid Chromatography (HPLC), and relied on very small particles size in small diameter columns.

From the mid 80's a number of scientists' as Verzele & Dewaele, Bildlingmeyer, Unger, … published articles dedicated to Preparative Liquid Chromatography on the technique itself, the columns and instruments technology.

Preparative liquid chromatography



Analytical liquid chromatography



Since 1995, Interchim® is an essential actor of the purification market.

In 2008, Interchim® launched puriFlash® a range of advanced automated instruments and consumables supported by the Ultra Performance Flash Purification concept who has revolutionized the purification practices.

Versatile, these systems allow chemists and bio-chemists to work with Flash cartridges and Preparative columns on a single device.

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

In combination with more than 50 different chemistries, 12 puriFlash instruments are available to perform purification of small Organics Molecules, Natural Products, Peptides, and Proteins.

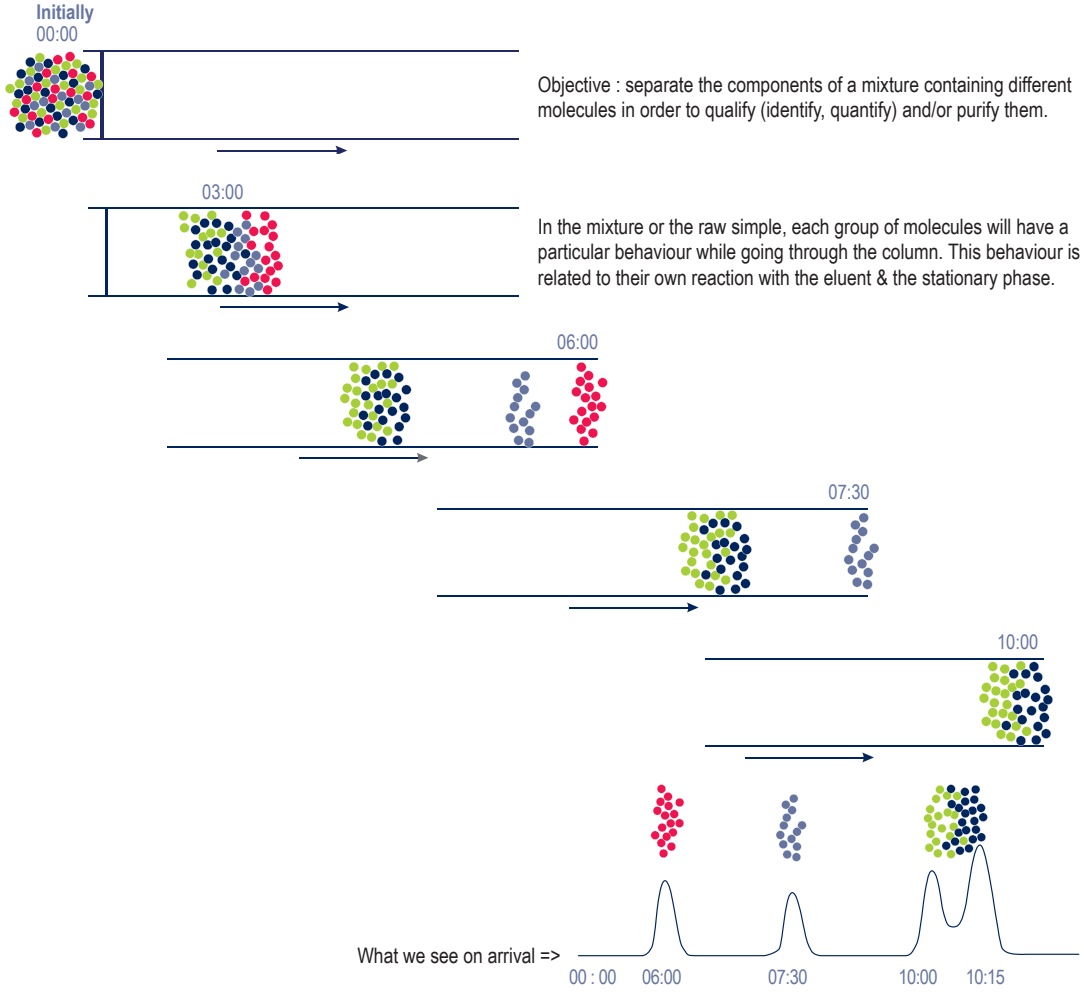
Chromatography principle

Liquid Chromatography is a separation technique.
It can be dedicated to identify and quantify compounds present in a mixture, this is the analytical mode. Very attractive when the goal is to get isolated a pure product from a more or less complex mixture, this technique is then called preparative liquid chromatography and seems to be today the most popular way for purification.

Liquid chromatography manages compromise between multiple parameters and primarily stationary phase, eluents and compounds of interest.

Compounds are eluted by a liquid mobile phase (eluent) in contact with a stationary phase (fixed). The migration speed of the species contained in the sample depends on the interactions with the stationary phase (adsorption or desorption phenomenon), the mobile phase or their solubility and polarity.

4 groups of products in different quantities



What is Purification?

Definition

There are many different purification techniques: distillation, crystallization, filtration, … and chromatography.

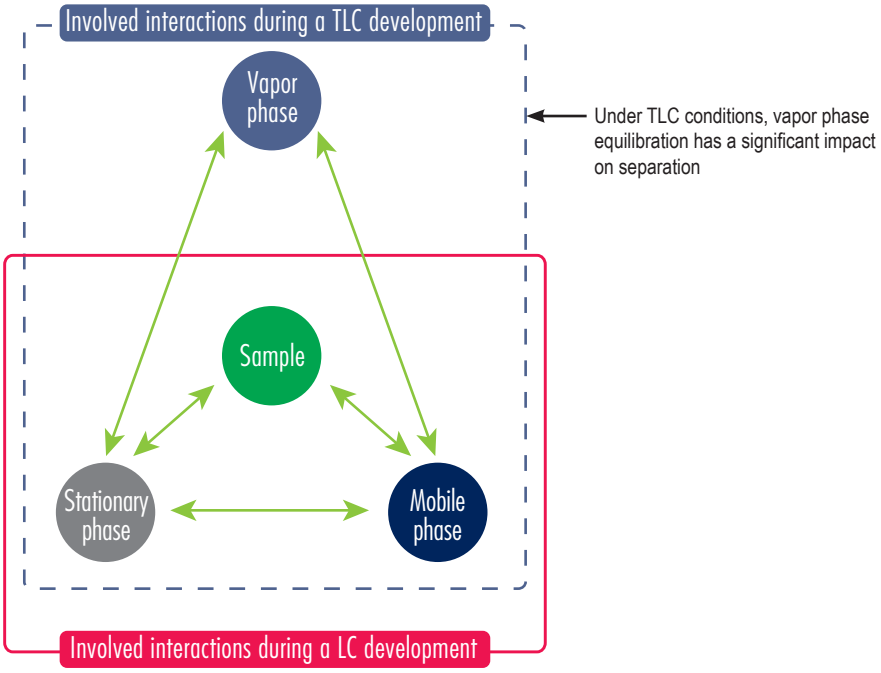
They all have the same goal purify and recover samples, whether an organic or biochemistry synthesis step.

Purification by liquid chromatography is always a challenge and there is often a compromise to obtain the desired purity, loading and throughput. To improve efficiency in delivering pure compounds, chemists may balance between purity, run time and environmental considerations. This delicate balance is often necessary for both crude and final purification.

Principle of purification by Preparative Liquid Chromatography

First of all, the separation of the compound(s) of interest must be developed under analytical experiments. Separation can be obtained if the compound(s) have very different affinities (polar, π- π, hydrophobic, ions exchange interactions) for the mobile phase and stationary phase. The chemist or biochemist has to define the level of purity for its compound(s) of interest.

Depending on the characteristics of the sample, these "analytical"methods developments can be done either by TLC or HPLC.



Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

The mobile phase composition follows eluent strenght rules (Snyder scale - see chapter...) which are present in the whole liquid chromatography principle: TLC, HPLC column, Flash/Prep column. Only TLC have an additional parameter: vapor phase equilibration, involving a correct migration.

Beyond these interactions, other factors are determining in order to obtain quality purifications. As such, the quality of your purifications will depend on the column geometry, the injection technique (liquid injection or solid deposition).

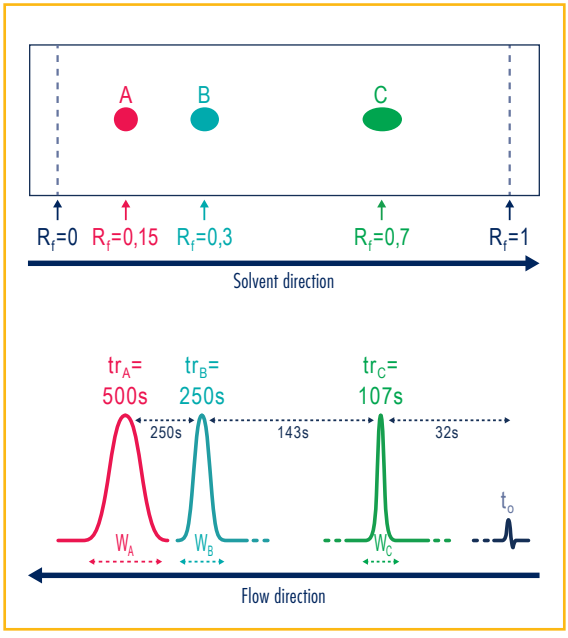
- **Transfer TLC to Purification:**

The TLC can be used as a predictive tool for purification method but users have to take care of the adsorbent features differences between the plate and the column, the difference of the eluent migration capillarity for TLC vs. dynamic for LC, the TLC silica binder.

The ΔRf must be optimized to achieve the best transposition possible.

The purification column size must be linked to crude sample mass.

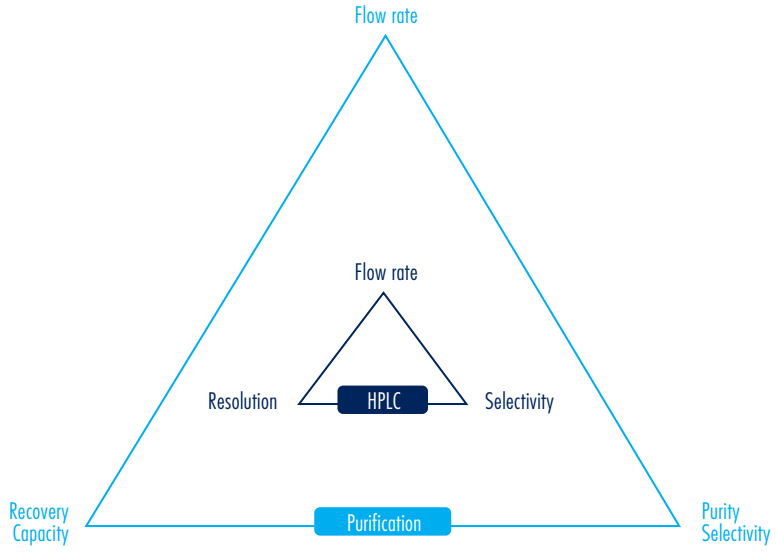
Interchim® TLC mobile app. couple to Genius, our artificial intelligence system, makes a fully automated process from TLC plate image to parameters set-up into the software till a Ready-to-use Purification.



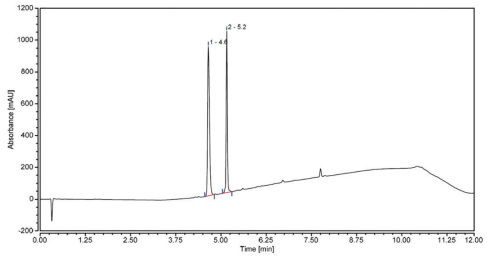
The ΔRf (**frontal ratio**) optimization allows the best transposition on the Flash/Prep column.

- **Transfer HPLC to Purification:**

The transposition HPLC to Purification is direct if the adsorbent features and the elution conditions of the HPLC column are correlated to those of the purification column. The purification column size must be linked to crude sample mass.

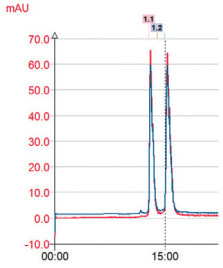


HPLC

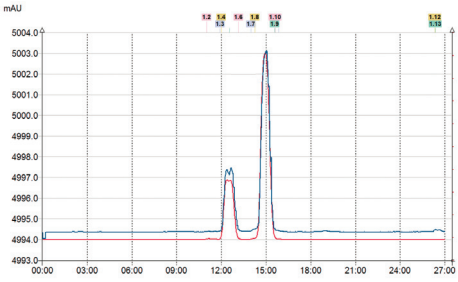


Analytical conditions
HPLC Column: Core-Shell C18 50x2.1mm 2.6µm
Liquid injection 5µL

Purification



Purification
puriFlash® 15µm
C18AQ - F0025
Liquid injection 900µL



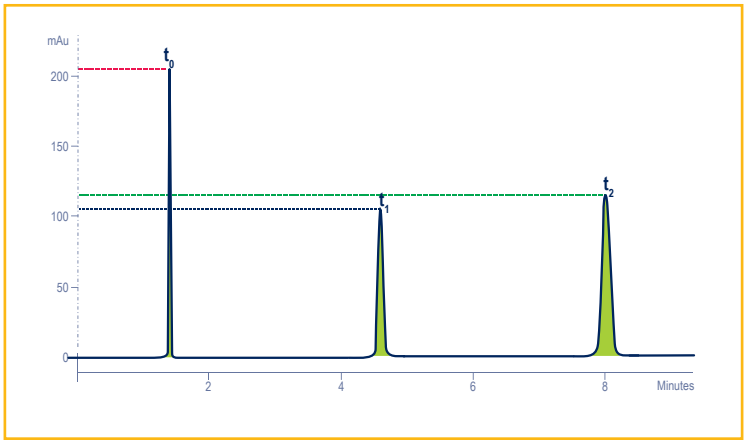
Overload
puriFlash® 15µm
C18AQ - F0025
Liquid injection
2mL

Advantages of Preparative Liquid Chromatography

This technique is really selective, and can lead in a single shot to a collection of a 100% pure compounds.
Versatile, it matches a large number of applications, class of compounds.
It combines numbers of detection techniques to maximize, first the detection of the whole compounds inside the sample, the control of their purity and their identification.

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

Fundamental Notions of Chromatography



Retention time

The time between injection and the appearance of the peak maximum. Corresponding to the time needed by the compound to interact with the stationary phase and the eluent.

t_0 = The elution time of an unretained peak (corresponding to the void volume of the column).

t_1 = retention time of the compound 1

t_2 = retention time of the compound 2

Adjusted retention time

$t'_1 = t_1 - t_0$ $\quad$ $t'_2 = t_2 - t_0$

Retention factor

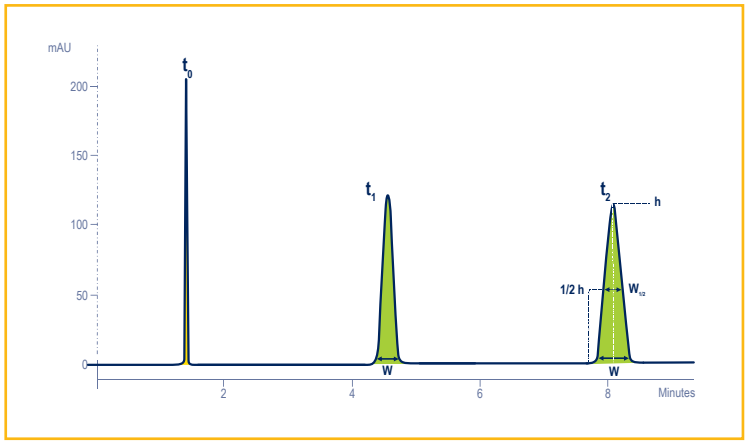
$$k_1 = \frac{t_1 - t_0}{t_0} \qquad k_2 = \frac{t_2 - t_0}{t_0}$$

The rention factor, or capacity factor,
k is the degree of retentivity of a peak compare to an unretained peak.

Selectivity

$$\alpha = \frac{k_2}{k_1}$$

The relative retention value, α, compares the degree of retentivity of one peak with another.



Efficiency

N: Theorical plate number

$$N = 16\left(\frac{t_1}{w}\right)^2 \qquad N = 5.54\left(\frac{t_1}{w_{1/2}}\right)^2$$

The width (W) of the chromatographic band during elution from the column is usually measured at the baseline by drawing tangents to the inflection points on the sides of the Gaussian curve that represents the peak.

H: Height equivalent to a theorical plate

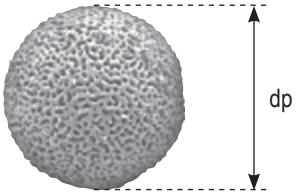
L: Column length

H = L/N

h = H/dp

h: reduced plate heigth
(this value can give an idea of the packing quality of the column)

dp: particle diameter of the stationary phase



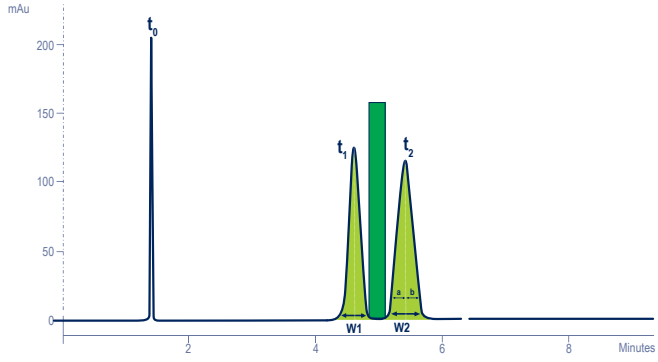
Stationary phase particle

Asymmetry

Asymmetry: $As = \frac{b}{a}$ at 10% of the peak height

Tailing factor: $Tf = \frac{a+b}{2a}$ at 5% of the peak height

Resolution



$$Rs = 2\left(\frac{t_2 - t_1}{w_1 + w_2}\right)$$

This value caracterizes the baseline width from the end of the 1st peak to the beginning of the 2nd.

A value of 1.5 is considered sufficient for baseline resolution for 2 peaks of equal height, but in that case the purity is not 100%.

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

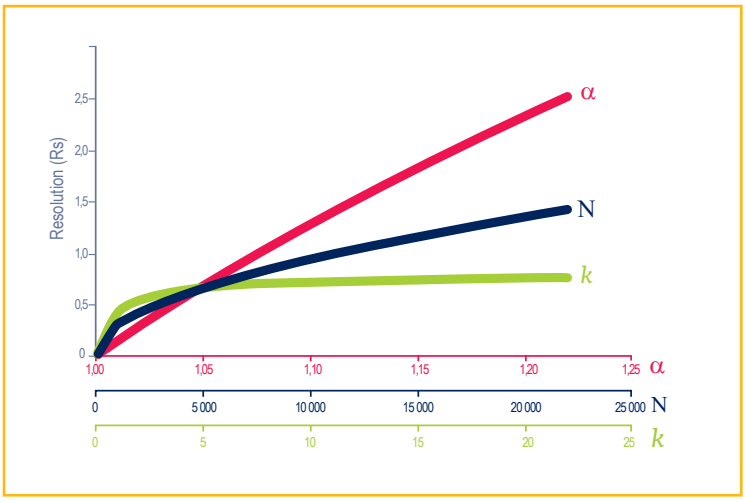
For 2 close peaks $w_1 \approx w_2$

$$Rs = \frac{1}{4}\sqrt{N}\left(\frac{(\alpha - 1)}{\alpha}\right)\left(\frac{k_2}{1 + k_2}\right)$$

Increasing efficiency by using a smaller particle size

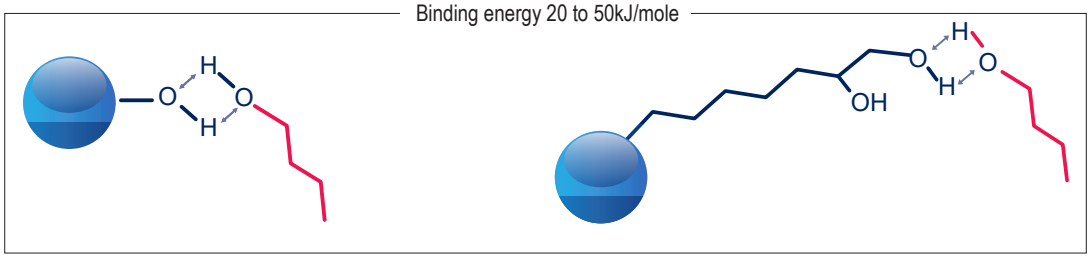
Enhance selectivity by modifying elution conditions and adapting stationary phase

Keep this value betwwen 2 and 10 by adjusting the retention time

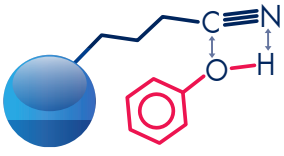


Interaction mechanisms

Polar phase interactions



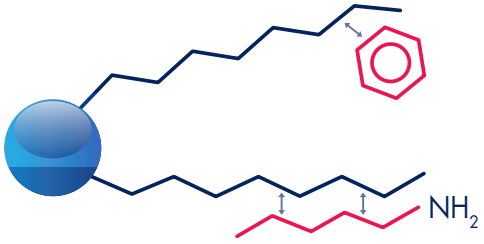
Binding energy 8 to 15kJ/mole



⟷ Dipole-Dipole attraction

Non polar phase interactions

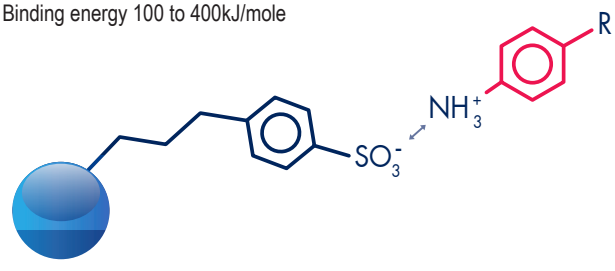
Binding energy 2 to 10kJ/mole

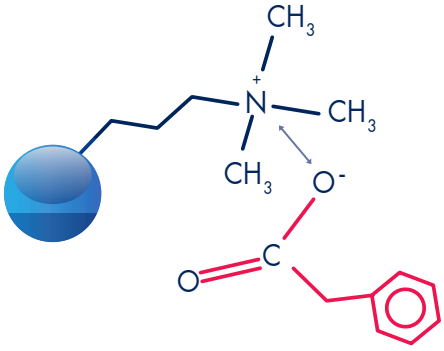


⟷ Dispersion interaction

Electrostatic interactions

Binding energy 100 to 400kJ/mole





⟷ Electrostatic interaction

Adsorbants polarity classification

Normal Phase (NP)			Reverse Phase (RP)	
Si	Silica	↑ POLARITY	CN	Cyano (nitrile)
NH_2	Amino		C1	Methyl
OH	Diol		C4	Butyl
CN	Cyano (nitrile)		C8	Octyl
			C18	Octadecyl (ODS)

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

This chapter was conceived and written in collaboration and under the supervision of Professor A.Tchapla (IUT Orsay - University Paris Sud).

Purpose:
- Determine the chromatographic conditions for the purification of a sample.
- Understand how to choose the solvent which completely solubilizes a sample (for solution or liquid-liquid extraction)

This requires the knowledge of the parameters that lead to the notion of "polarity of molecules". The polarity is the molecular property that allows to evaluate how and with which intensity one molecule attracts another (molecular interactions), and thus to choose the solvent of a sample but also the chromatographic conditions for the purification.

Polarity

Purpose:
- Predicting the polarity of a substance from its molecular structure.

The polarity of an organic molecule is the property that allows either to predict or to evaluate the nature and the strength of the molecular interactions occurring between two molecules whether they are identical (pure material) or different (in mixture) from one to another.
Polarity is the consequence of the development, accessibility and intensity of the totality of the partial electrical charges developing on the surface of organic molecules.
Under which molecular conditions do partial electrical charges develop between the covalently bonded atoms?

Fisrt, we have to define the molecular structure of the analytes, and in which molecular conditions of partial electrical charges develop themselves at the surface of a molecule.

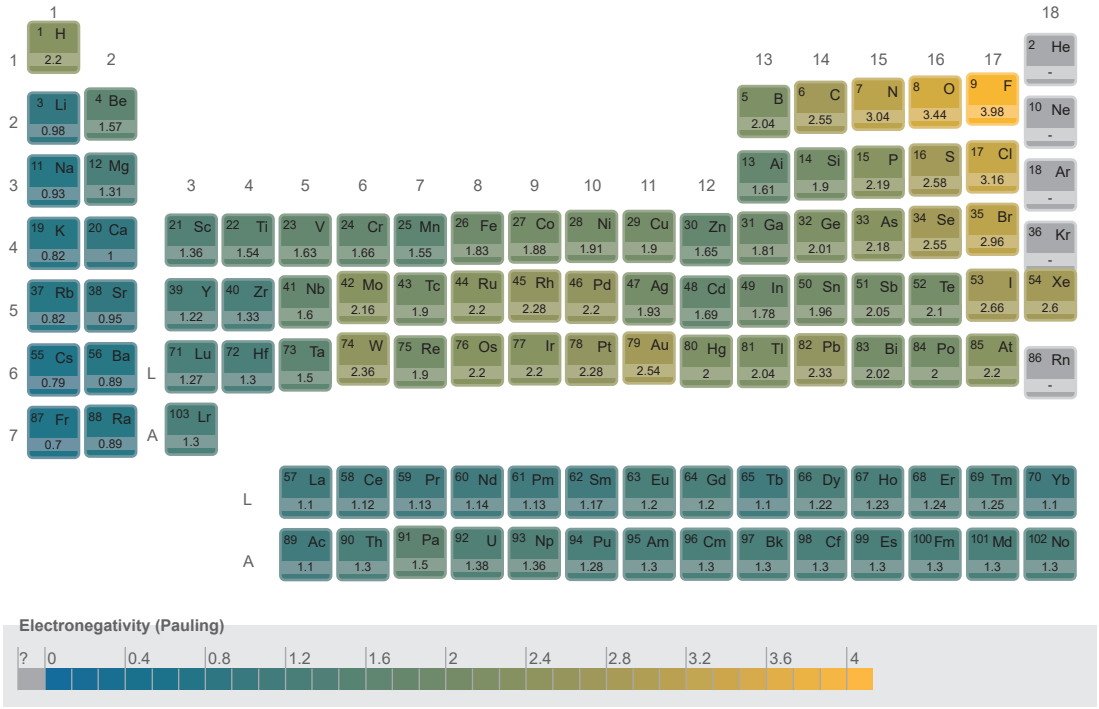
Organics molecules mainly contain: hydrocarbon skeleton at which functionalized groups may be added. This corresponds to two kinds of atoms
- Major atoms (C, H)
- Heteroatoms (O, N, S, P, Halogens)

When 2 atoms are covalently linked, their relative electronic attraction of bonding electrons leads to equal (symmetrical molecular bond) or unequal arrangement of partial charges in the molecule. The charge distribution is marked with the symbols δ^+ and δ^-. Thus, for each covalent bond a dipole is associated, to which correspond a dipolar moment. The vector sum of all dipolar moments leads to the molecular dipolar moment of a given structure.

Predict the nature of partial electric charges which respectively appear on two covalently bonded atoms refers to Pauling electronegativity scale.

The electronegativity (χ) is defined as the attraction power of an atom for an electron. Due to their specific structure, the atomic nucleus and electronic cloud of atoms generate their electronegativity.

In the periodic table of elements, the electronegativity, increases from left to right and from bottom to top.



There are four different ways for developing partial electric charges on a covalent bond. They lead to four different kinds of dipoles.

a) Instantaneous dipole δ_d

For linked atoms having the same electronegativity, binding electrons move quickly from one of the linked atom to the other and none unbalanced permanent charge occurs. However, instantaneously asymmetrical partial electric distribution appears which is immediately reversed.

The emergence of two reciprocal partial electric charges on each of the bonded atoms forms an instantaneous dipole which changes direction at every instant. In the case of organic molecules, these instantaneous partial charges develop on each C-C bond; more particularly in the case of alkanes, but also along the hydrocarbon chains of the functionalized molecules. Their intermolecular attraction potential is characterized by the partial dispersion solubility parameter δ_d which will be defined in the next chapter: solubility.

b) Permanent dipole δ_p

For two different bonded atoms (C-O; C=O; C-N; C-X...), due to their different electronegativity, a permanent unequal repartition of partial electrical charge is created. However, the global environment in which the heteroatom is inserted must, be taken into account: it must not be located in a system of symmetrical links with respect to a center (for example, CO_2 does not have a permanent dipole because it is a linear molecular structure whereas H_2O or Et-O-Et develop a permanent dipole because they are non-linear molecular structures). In the midst of the classes of molecules corresponding to this property are the ketones, the esters, the halogenated compounds, the tertiary amides, the nitriles...

Their potential intermolecular attraction is characterized by the partial dipolar parameters δ_p.

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

c) Peculiar case: notion of dipole causing hydrogen bonding

These interactions appear when in a structure OH, NH or SH functions are present. The very large difference in electronegativity between the heteroatom (O, N or S) and hydrogen leads to the creation of a permanent dipole. The power of attraction between the charge δ^+ on the hydrogen and the charge δ^- on the heteroatom is so strong that it leads to the creation of an intermolecular interaction between the H of a molecule with the heteroatom of the neighboring molecule to form the so-called hydrogen bond.Their intermolecular attraction potential is characterized by the partial hydrogen bond solubility parameter δ_H, which will be defined in the following chapter: solubilit. Among the classes of molecules corresponding to this property are the alcohols, phenols, carboxylic acids, amines I and II, amides I and II, thiol...

d) Induced dipole δ_d

When a polar molecule showing a permanent dipole is close to a neutral but polarizable molecule, its electric field is creating an induced dipole moment on this molecule leading to an unequal repartition of the electric charge.

This case occurs for molecules with multiple bonds C = C and C Ξ C or a carbon bonded to a large polarizable heteroatom, for example C-I in interaction with a polar molecule.

Amidst the molecule classes corresponding to this property, we can find aromatic acetylenic or ethylenic unfunctionalized hydrocarbons.

Their potential molecular attraction is integrated in the partial solubility parameter of dispersion δ_d.

Total resulting dipole δ_T

The total polarity of a molecule is the sum of all the contributions of the partial polarities described above. (vector sum of all the dipole moments of each bond of a molecule) Their intermolecular attraction potential is included in the total solubility parameter δ_T , which will be defined in the next section: solubility

It should be noted that, according to the small difference in electronegativity between hydrogen and carbon, each C-H bond has a very weak dipole moment. In space, thanks to the free rotation around the C-C bonds this effect is canceled out overall. But, this implies that instantly, the longer the chain is, the greater the influence of these instantaneous intermolecular attractions becomes strong. This makes possible to understand that the alkanes, non-polar solutes, can possess a permanent molecular attraction power, because they are liquid at atmospheric pressure from 5 carbons of linear chain up to 15 carbons and then solids above 16 carbons of chain.

For a good interpretation of physical properties of the molecular species, it is necessary to take into account 2 types of interactions:
- Van Der Waals interactions, rely on molecule polarity and polarizability.
- The interactions linked to the intermolecular and some intra molecular hydrogen binding.

Interaction	Mecanism	Molecules types	Involved compounds
Van der Waals	Debye + Keesom + London	Apolar molecules	Alkyl chains, aromatic rings
Dipolar Debye	Permanent dipole - instantaneous dipole	Polar molecules and any molecules	
Dipolar Keesom	Permanent dipole - permanent dipole	2 polar molecules	
Dipolar London	Instantaneous dipole - instantaneous dipole	2 any molecules	
Hydrogen binding		Proton acceptor - proton donor	Alcohols, amines, acids

Solubility

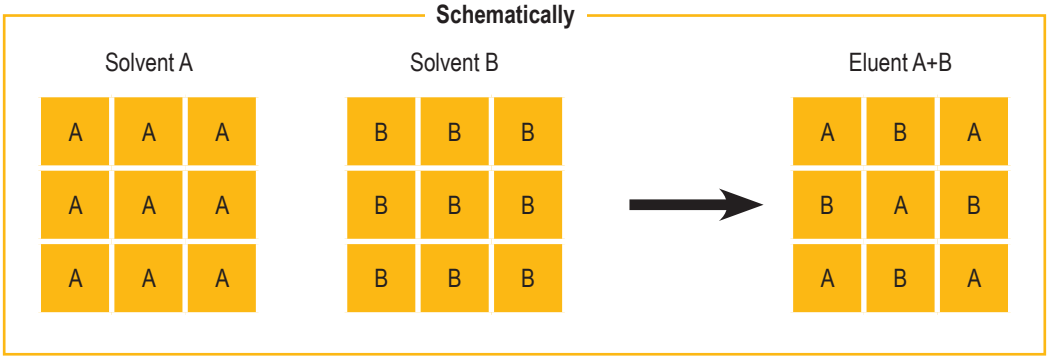
Purpose:
- understand how to choose the solvent that completely solubilizes a sample

In a simplified manner in the majority of situations, two non-electrolyte substances are totally miscible one in the other if:
- they have roughly the same molecular size and polarity.

That means that the ratio between the energies of molecular interactions and the molar volume of the two substances is similar.

From the theoretical point of view this has been modeled by Hildebrand, from which it emerges from the studies that:
- their total solubility parameters δ_T must be approximately identical (+/- 2)
- the nature of their main partial solubility parameter (partial dispersion solubility parameter δ_d or dipolar δ_p or hydrogen bonds δ_H must be identical.

Schematically



In contrast, two solutes with very different total solubility parameters ($\Delta \delta_T > 3$) separate into two distinct phases (demixing). However, in each phase, low concentrations of the other component of the biphasic system are found.

Universal solvents are solvents having total solubility parameters between 10 and 12 and each fractional polarity is close to 33%. They are therefore able to solubilize the majority of the products whatever their polarity. They belong to class E and to a lesser extent to class B as defined in the table and figure below.

The table below shows the values of the total solubility (δ_T) and partial (δ_d , δ_p ,δ_H) parameters of solvents and their fractional polarity parameters (f_d,f_p et f_H) with:

$f_d = (\delta_d / \delta_d + \delta_p + \delta_H) \times 100$
$f_p = (\delta_p / \delta_d + \delta_p + \delta_H) \times 100$
$f_H = (\delta_H / \delta_d + \delta_p + \delta_H) \times 100$

(Particular polymer case: PEG is miscible in water because the molecular interaction energy (binding hydrogen) is the same though the molecular volume is very different).

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

Example of total solubility and partial parameter values of some solvents

Solvent	δ_T *	δ_d *	δ_p *	δ_H *	f_d **	f_p **	f_H **	Class
MTBE	6.90	6.90	0.50	?				A
Heptane	7.40	7.40	0.00	0.00	100	0	0	A
Diethylether	7.62				67	23	10	A
Toluene	8.90	8.67	1.00	2.00	74	9	17	A
Thf	9.08	8.22	3.25	3.50	55	22	23	B
Ethyl acetate	9.10	7.44	4.60	2.50	51	32	17	D
Chloroform	9.21				67	10	23	A
Acetone	9.77	7.58	5.70	2.00	50	37	13	D
Dichloromethane	9.93	8.91	3.00	3.10	59	20	21	B
Octanol	10.30				53	6	41	C
Acetic acid	10.35				40	19	41	C
Butanol	11.30	7.81	2.50	7.80	43	14	43	C
Isopropanol	11.50				39	17	44	C
Acetonitrile	11.75				41	43	16	D
Ethanol	12.92	7.73	4.00	9.70	36	19	45	C
Methanol	14.30	7.42	5.50	11.20	31	23	46	C
Water	23.50	7.00	8.00	20.90	19	22	58	
Methylcellosolve	12.06	7.90	4.50	7.90	39	22	39	E
Dimethylformamide	12.14	8.52	6.70	5.50	41	32	27	E
Formic acid	12.15				33	20	47	C
Dimethyl sulfoxyde	12.93				37	33	30	E

*Hansen solubility parameters from J.Roire “Les solvants” EREC (Issy les Moulinwaterx) 1989
** Fractional polarity parameters from J.Roire “Les solvants” EREC (Issy les Moulinwaterx) 1989

Depending on their fractional polarity, the solvents are distributed in 6 different areas of the planar space. Thus they can be grouped into 5 distinct classes plus water that is alone in an area of this space:
A class corresponds to solvents developing mainly nonspecific interactions (f_d majority > 80%).

B class corresponds to intermediate solvents between the three preceding classes (f_d majority with f_H and f_p close to 20%).

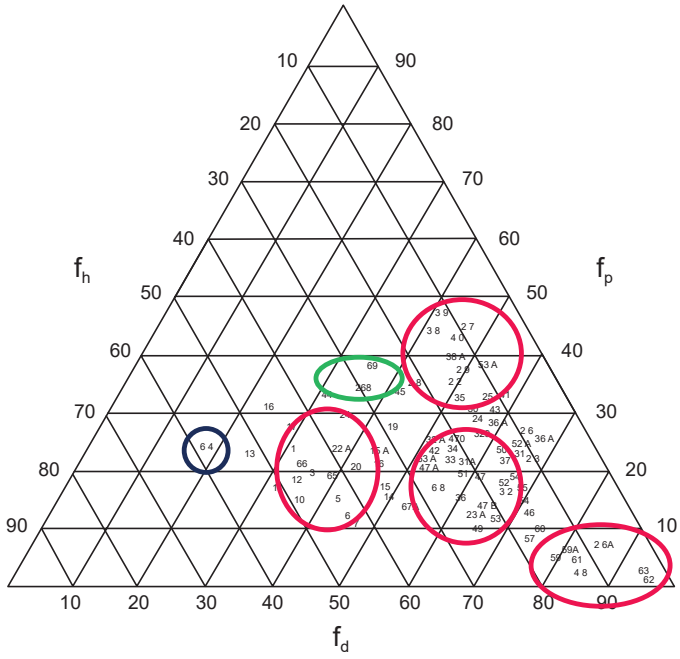
C class corresponds to solvents developing in addition to the dispersion interactions (f_d ~ 40%) some interactions by H bond (f_H > 40%).

D class corresponds to solvents developing in addition to dispersion interactions (f_d ~ 50%) mainly dipolar interactions (f_d > 30%).

E class corresponds to solvents developing in the same way (between 30% and 40%) the three types of interactions.

Finally the water which is apart.

This is shown in the following figure where the zones of solvents of classes A, B, C and D are materialized by red circles or ovals; those of class E by a green oval; finally the water is marked by a small blue circle.



Three-dimensional distribution of some solvents according to their fractional polarity parameter
(from J.Roire "Les solvents" EREC (Issy les Moulinwaterx) 1989)

The total solubility parameter is determined either experimentally or by calculation from the partial solubility parameters. These are obtained from the measurement of the refractive index, the permitivity, the density and the molecular mass of each solvent.

Particular case of the polymers: although their molar volume is very different, PEG is totally miscible in water because their fractional hydrogen binding polarities $(\delta_H/\delta_T)^2$ (or their fractional hydrogen bonding polarity parameter f_H) are close.

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

Liquid-Liquid Extraction of non Electrolytes

Purpose:

- finding the solvent couple allowing the extraction of a non-electrolyte solute in one of the two solvents of the pair with the minimum of steps

Generally one of the both solvents is water. Since the water is polar, the other solvent will necessarily be a non-polar solvent (δ_T very different) in order to obtain two distinct layers. Most often the non-polar solvent will be diethyl ether, chloroform, dichloromethane, ethyl acetate, a hydrocarbon... This choice is made with the knowledge of the total solubility parameters. When one considers, for example, a plant sample in its solid form (leaf, stem, root, bark, fruit, etc.) from which one wants to extract the "active" molecules, it is the water contained in the organism to extract which constitutes one of the two liquid phases of the extraction system. Thus, it is the same theoretical case as above.
Consider now a pure solute. If the solute is polar it will solubilize rather in water and its concentration in the organic phase will be very small. If the solute is apolar it will solubilize preferentially in the organic phase and its concentration in water will be very low. On the other hand, if it is moderately polar, it will partition between the water and the organic phase. The concentration in each of the two phases will depend on its difference in polarity with water and that of the organic solvent.

This has been theoretically described and this equilibrium is characterized by the partition coefficient K_i (ratio of the concentrations of solute i in the two phases). It is expressed mathematically from the three total solubility parameters of solute i, water and the organic solvent chosen, by the following formula

$\ln K_i = 1/RT \times (V_i [(\delta_{Ti} - \delta_{Twater})^2 - (\delta_{Ti} - \delta_{Torga})^2]$ where V_i ($=M_i/r_i$) is the molecular volume of the analyte δ_H must be identical.

Thanks to this formula it is easy to understand that a solute of intermediate polarity between that of the water and that of the chosen organic solvent will lead to the fact that $(\delta_{Ti} - \delta_{Twater}) = (\delta_{Ti} - \delta_{Torga})$ so $\ln K_i = 0$ and $K_i = 1$ therefore that the concentrations of the solute in the two phases are identical. By making two successive extractions with the same volume of organic solvent, 75% of the solute will be extracted, while making 3 extractions 87.5% will be extracted and by making 4 extractions of 93.75% etc.
If now the polarity of the solute leads to a partition of 90% in the organic phase and 10% in the aqueous phase after 2 extractions, 99% of the solute "i" will be extracted and after three extractions the result will reach 99.9% (the aqueous solution will be "exhausted").

Finally, in the case of the extraction of plant matrix: since there is not only one compound in this type of matrix and the compounds polarities and therefore their total solubility parameters are different, each and every be extracted according to its difference $(\delta_{Ti} - \delta_{Twater}) = (\delta_{Ti} - \delta_{Torga})$. Their concentration in each organic solvent is therefore not the same. This explains why, when extracting a plant matrix with hexane, chloroform or ethyl acetate, the same solutes extracted in the three different organic fractions are qualitatively found; only the relative concentrations are changed.
In such a system one can not make selective extraction of a single class of solutes.In this case, each extract must be reprocessed by another separation technique.

Solvents polarity scale in chromatography

Purpose:
- determining the chromatographic conditions for the purification of a sample.

In the chromatographic process, the solvent interacts with the stationary phase.

It enters into competition of interactions with the solute. In order for the solute to be retained, it must develop stronger molecular interactions with the stationary phase than those developing between the mobile phase and the stationary phase. In order for it to be eluted, the mobile phase must develop molecular interactions slightly less strong than those which the solute develops with the stationary phase.

The chromatographic system is thus composed of a stationary phase of opposite polarity to that of the mobile phase. If the stationary phase is polar ($\sum f_p + f_H$ majority) the mobile phase will be rather apolar.
A very apolar solvent will be named "weak".
A very polar solvent will be named "strong" (liquid chromatography with normal phase polarity).
If the stationary phase is apolar (f_d > 80%) the mobile phase will be rather polar. In this case the polar solvents will be named "weak" and the apolar solvent will be named "strong" (liquid chromatography with reversed phase polarity).

Theoretical studies have determined the total solubility parameter of the stationary phases of liquid chromatography, which allows us to understand their mode of operation. We report them in the following chart:

Example of total solubility and partial parameter values of some stationary phases

Stationary phase	d_T	f_d	f_p	f_H
Alumina	~16.0	38	31.0	36.0
Silica	~16.0			
Pyrocarbon	~14.0	100	0.0	0.0
Alkyl bonded silica	~7.0 à 8.0	100	0.0	0.0
Perfluoro-alkyl bonded silica	~6.0	100	0.0	0.0
Cn* bonded silica	~10.5	45	40.5	14.5
Diol* bonded silica	~20.0	26	22.0	52.0
Phenyl* bonded silica	~9.0	80	5.0	15.0

Solubility prameters from P.J. Schoenmakers"Optimization of chromatographic selectivity" J. of chromatography Library vol 35 Elsevier Amsterdam 1986. *Solubility parameters evaluated on the of the silane δ_T

The silica data are partial. It should be noted that they must be close to those of alumina. These two supports are polar. However, alumina, a basic support, is rather a hydrogen-binding acceptor whereas silica, an acidic support, is rather a donor of hydrogen binding. This leads to notable differences in selectivity between these two supports when analyzing mixtures of acidic or basic polar solutes.

In fact, the silica, the alumina and the bonded silicas diol are polar stationary phases. The grafted silicas alkyl, perfluoroalkyl, phenyl and to a lesser extent the cyano grafted silicas are non-polar stationary phases.

In order for the chromatographic phenomenon to be established, it is necessary for the solute to partition neighboringly between the stationary phase and the mobile phase. It must therefore have a polarity place between that of the stationary phase and that of the mobile phase.

According to the same principle as in liquid-liquid extraction described above §A3.3 the solute "i" is divided between the stationary phase and the mobile phase as a function of its polarity.

On the other hand, if the solute develops very strong interactions with the stationary phase and the mobile phase develops much less interactions with the stationary phase, the solute is blocked on the stationary phase and the mobile phase does not elute it. By changing the polarity of the mobile phase by step, the solutes are selectively eluted by class according to their polarity.

If the mobile phase develops very strong interactions with the stationary phase, the solutes are not retained and not separated regardless of their polarity.

These last two cases correspond to the liquid-solid extraction working conditions (SPE) and relate to the step of fixing the solutes and then that of their elution.

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

Normal Phase Liquid Chromatography (NPLC)

In this chromatographic process, the solvent and the solute interact with a polar stationary phase (SIOH, AlOH, MgOH, ZrOH, TiOH).

- The more polar the solute is, the more it will be retained and the more it will be necessary to use a polar mobile phase to elute it.
- If the solute is weakly polar, the mobile phase must be of low polarity.

A relative experimental scale of polarity of the solvents with the adsorption supports was developed by Snyder (in this scale the order of the pure solvents is the same whatever the adsorbent Si-OH, Al-OH, Mg-OH, Zr-OH, Ti-OH). This scale classifies the solvents by increasing polarity as well as that of the total solubility parameters. The sequence is substantially identical, although a few inversions occur since the solubility parameters define the polarity of a pure body and the scale of eluting force that of the same body in interaction with a polar adsorbent.

Thus, by definition, to the least polar solvent has been assigned a zero eluting force ε_0. This does not mean that this solvent is not eluting in particular for nonpolar solutes, but that no solvent having a lower eluent power is known. In the same way in this scale the strongest solvent is the most polar solvent: water. This eluent develops very intense interactions (so strong that it is not possible to quantify the eluting force of the water.) By taking water as a mobile phase, no electrolyte solute is retained on these hydroxylated supports, even goes so far as to create irreversible interactions which deactivate these supports for any subsequent chromatography unless they are subjected to drastic reactivation treatments.

Due to their interaction with the stationary phase the relative positioning of the solvents in this scale is slightly different from that described by the scale of the total solubility parameters. The Snyder scale is given in the following chart:

Eluting strength values of solvents for NonPolarLC

Solvent	δ_T			ε_0 20°C		
		ε_0 (Al2O3)	ε_0 (SiO2)	ε_0 (florisil)	ε_0 (magnesia)	ε_0 (diol)
Perfluoroalkanes	5.6 - 5.8	-0.25	-0.19	-0.13	-0.15	-0.06
N-Pentane	6.99	0.00	0.00	0.00	0.00	0.00
N-Hexane	7.28	0.00	0.00	0.00	0.00	0.00
Isooctane	6.90	0.01	0.01	0.01	0.01	0.00
Petroleum ether	7.85	0.01	0.01	0.01	0.01	0.00
N-Decane	7.80	0.04	0.03	0.02	0.02	0.01
Cyclohexane	8.21	0.04	0.03	0.02	0.02	0.01
Cyclopentane	8.10	0.05	0.04	0.03	0.03	0.01
Diisobutylene		0.06	0.05	0.03	0.03	0.01
1-Pentene		0.08	0.06	0.04	0.05	0.02
1,1,2-Trichlorotrifluoroethane		0.14	0.11	0.07	0.08	0.03
Carbon disulfide	9.97	0.15	0.12	0.08	0.09	0.03
Carbon tetrachloride	8.80	0.18	0.14	0.09	0.10	0.04
1,1,1-Trichloroethane	7.72	0.19	0.15	0.10	0.11	0.04
Tert-Butyl methyl ether	6.90	0.20	0.15	0.10	0.12	0.05
1-Chloropentane		0.26	0.20	0.14	0.15	0.06
1-Chlorobutane		0.26	0.20	0.14	0.15	0.06
Xylene	8.90	0.26	0.20	0.14	0.15	0.06
Diisopropyl ether	7.00	0.28	0.22	0.15	0.16	0.06

Solvent	δ_T			ε_0 20°C		
		ε_0 (Al_2O_3)	ε_0 (SiO_2)	ε_0 (florisil)	ε_0 (magnesia)	ε_0 (diol)
2-Chloropropane		0.29	0.22	0.15	0.17	0.07
Toluene	8.90	0.29	0.22	0.15	0.17	0.07
1-Chloropropane		0.30	0.23	0.16	0.17	0.07
Chlorobenzene		0.30	0.23	0.16	0.17	0.07
Benzene	9.14	0.32	0.25	0.17	0.19	0.07
1-Bromoethane		0.37	0.28	0.19	0.21	0.09
Diethyl ether	7.62	0.38	0.29	0.20	0.22	0.09
Diethyl sulfide		0.38	0.29	0.20	0.22	0.09
Chloroform	9.21	0.40	0.31	0.21	0.23	0.09
Dichloromethane	9.93	0.42	0.32	0.22	0.24	0.10
Isobutyl metyl ketone	9.04	0.43	0.33	0.22	0.25	0.10
Tetrahydrofuran	9.08	0.45	0.35	0.23	0.26	0.10
1,2-Dichloroethane	9.43	0.49	0.38	0.25	0.28	0.11
Ethyl methyl cetone	9.63	0.51	0.39	0.27	0.30	0.12
1-Nitropropane		0.53	0.41	0.28	0.31	0.12
Acetone	9.77	0.56	0.43	0.29	0.32	0.13
1,4-Dioxane	8.90	0.56	0.43	0.29	0.32	0.13
Ethyle acetate	9.10	0.58	0.45	0.30	0.34	0.13
Methyle acetate		0.60	0.46	0.31	0.35	0.14
1-Pentanol		0.61	0.47	0.32	0.35	0.14
Dimethyl sulfoxyde	11.78	0.62	0.48	0.32	0.36	0.14
Aniline		0.62	0.48	0.32	0.36	0.14
Diethylamine		0.63	0.49	0.33	0.37	0.15
Nitromethane	12.71	0.64	0.49	0.33	0.37	0.15
Acetonitrile	11.75	0.65	0.50	0.34	0.38	0.15
Pyridine	10.61	0.71	0.55	0.37	0.41	0.16
2-Butoxyethanol		0.74	0.57	0.38	0.43	0.17
Isopropanol	11.50	0.82	0.63	0.43	0.48	0.19
1-Propanol	11.88	0.82	0.63	0.43	0.48	0.19
Ethanol	12.92	0.88	0.68	0.46	0.51	0.20
Methanol	14.30	0.95	0.73	0.49	0.55	0.22
Ethylene glycol	17.06	1.11	0.85	0.58	0.64	0.26
Acetic acid	10.35	high	high	high	high	high
Water	23.46	very high	very high	very high	very high	very high

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

The determination of the best polarity of a mobile phase for a separation problem on these supports is determined experimentally by choosing step by step the solvents of increasing eluting force and by evaluating each time the retention of all the compounds of a mixture and their separation. This can be done by TLC, in which case it will be necessary to find a mobile phase placing all the solutes with RF ranging between 0.09 and 0.3. This can also be done in HPLC by finding the mobile phase composition placing all the solutes in the range of retention factors between 2 and 10 (15, strictly speaking). If no mobile phase composition allows this, it will be necessary to work in elution gradient or to find another chromatographic support.

The differences in polarity (eluting force) between two successive pure solvents are sometimes sufficiently important that in switching from one to the other the elution becomes too fast.Snyder has therefore proposed a progressive polarity scale of various binary mixtures of solvents which can be used to control this drawback. In order to choose the mobile phase of good composition, given that the eluting force variation of the solvent binary mixtures is not linear, it is necessary to use the Snyder nomogram which proposes the successive use of the binary mixtures:

CH_2Cl_2-hexane	(3.5% to 100%)	($0.05 < \varepsilon_0 < 0.30$)
MTBE-hexane	(0.2% to 84%)	($0.10 < \varepsilon_0 < 0.45$)
Ethyl acetate-hexane	(0.3% to 75%)	($0.10 < \varepsilon_0 < 0.45$)
MTBE -CH_2Cl_2	(30% to 88%)	($0.35 < \varepsilon_0 < 0.45$)
MeCN-CH_2Cl_2	(12% to 88%)	($0.10 < \varepsilon_0 < 0.5$)
MeOH-CH_2Cl_2	(3.5% to 95%)	($0.40 < \varepsilon_0 < 0.9$)

On the other hand, it frequently happens that, although all the solutes are eluted between $0.09 < R_f < 0.3$ so $2 < k < 10$ two or more solutes are poorly separated.

When two solutes are poorly separated in a mobile phase of a given composition (ε_0 fixed), we must choose, for the new mixture, a solvent having a total solubility parameter (δ_T) identical or very close to the most polar solvent of the mixture used, but having a different dominant solubility partial parameter (these binary mixtures therefore consist of a weak solvent belonging to class A mixed with a strong solvent chosen either in class B or C or D).

This leads to subtly modify the solute-solvent molecular interactions in order to increase the separation while keeping the retention close to the same value. This notion led Snyder to define eluotropic series composed of solvents of different polarity (Snyder's eluotropic series) (see, for example, LR Snyder, Chapter 6, JJ Kirkland, "Modern Pratice of Liquid Chromatography," J Wiley and Sons, New York 1971).

Basis on Rohrschneider's work on the polarity of gas chromatography stationary phases, Snyder has developed an empirical model for the expression of the solvent polarity by a value called polarity parameter P' which unfortunately does not use the concept of solubility parameter. The polarity P' index isn't sufficient enough to judge the total interactions in a liquid state.

This model is built on the determination of the chromatographic behavior of three control solutes of very different polarities:
- ethanol (as representative of a molecule which predominantly gives hydrogen binding interactions when considering the binding donor power H)
- para dioxane as representative of a molecule which predominantly gives rise to hydrogen binding interactions by considering the binding acceptor strength H)
- nitromethane (as representative of a molecule that predominantly gives dipolar interactions).

Having determined P' as the sum of these three properties exactly as was done by Hildebrand define for the fractional polarity parameter of the solvents, Snyder then makes the ratio of a property on the sum of the three to define the acceptor polarity parameters of proton (Xe), that of proton donor (Xd) and that of dipole-dipole interaction (Xn) of each of the pure solvents tested.The mixtures of solvents showing similar retention but giving different selectivities are composed of a weak solvent of group I to which a group II or group VI or group VII solvent is mixed (because the solvents of these 3 groups have different fractional polarities parameters) and remembering that chloroform gives particular selectivities but is often eliminated nowadays for environmental reasons.

Example of Snyder total polarity and fractional parameters values of some solvents

Solvent	P' *	X_e **	X_d **	X_n **	Group
Heptane	-0.09				non classifiable
Mtbe	~2.30	0.41	0.19	0.40	I
Diethylether	2.80	0.53	0.14	0.33	I
Octanol	3.23	0.58	0.17	0.25	II
Isopropanol	3.92	0.57	0.17	0.26	II
Ethyl acetate	4.24	0.34	0.23	0.43	VI
Thf	4.28	0.38	0.20	0.42	III
Dichloromethane	4.29	0.27	0.33	0.40	VII
Chloroform	4.31	0.31	0.35	0.34	isolated
Ethanol	4.40	0.52	0.19	0.29	II
Acetone	5.10	0.35	0.23	0.42	VI
Methanol	5.10	0.48	0.22	0.31	II
Acetonitrile	5.64	0.31	0.27	0.42	VI
Acetic acid	6.13	0.41	0.30	0.30	IV
Water	10.20	0.37	0.37	0.25	VIII
Toluene	68.00	0.25	0.28	0.47	VII
Methylcellosolve	5.71	0.41	0.22	0.36	III
Dimethylformamide	6.31	0.40	0.21	0.39	III
Formic acid					IV
Dimethyl sulfoxyde	7.29	0.39	0.22	0.39	III

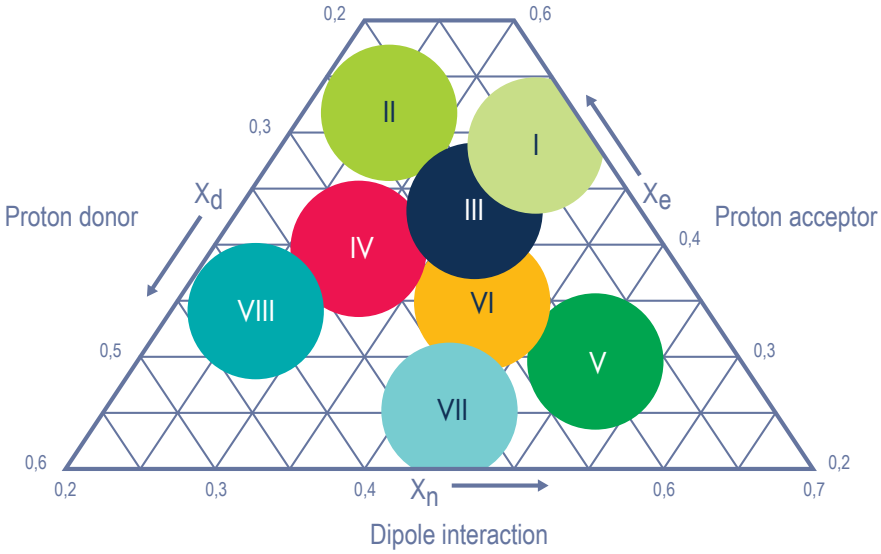
* Snyder polarity parameters from V.R. Meyer "Practical High Performance Liquid Chromatography'" J,WILEY and Sons (Chichester) 1988
** Snyder fractional polarity parameters from V.R. Meyer "Practical High Performance Liquid Chromatography'" J,WILEY and Sons (Chichester) 1988

Each of the three values is then plotted on a ternary diagram and the solvents are distributed in this plane space in 10 different zones where the solvents are grouped by 8 groups of partial polarity.

Each group gathers solvents according to their own relative specificities described by three fractional polarity parameters:
- X_e for their hydrogen bond acceptor power
- X_d for hydrogen bonding
- X_n for the dipolar interactions

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

G I: aliphatic ethers (MTBE, diethyl ether, etc.)
G II: aliphatic alcohols (Methanol, isopropanol, etc.)
G III: Pyridine derivates, methyl cellosolve, THF, N,N-Dimethylformamide...
G IV: acetic acid, glycols, (propylen glycol...)
G V: dichloromethane, 1-2 dichloroethane
G VI: (a) aliphatic ketones (Acetone, MEK), esters (ethyl acetate), dioxane, nitriles (acetonitrile)
G VII: aromatic hydrocarbons, aromatic compounds, nitromethane
G VIII: water, tetrafluoropropanolAnd: isolated chloroform (surrounded on the figure by a green circle) non-classifiable saturated hydrocarbons



The space occupation is different from that of the partial parameters of solubility triangle insofar as the solvents are not classified by the same properties.

Here, the interaction power due to the instantaneous dipoles is not taken into account (thus characterizing the very important non-specific interactions due to the hydrocarbon skeleton of the molecules). On the other hand, the interactions due to the hydrogen bind are divided into acceptor (X_e) and donor (X_d) of Hydrogen links, which is judged globally in the δ_H parameter of Hildebrand.

Reversed phase liquid chromatography (RPLC)

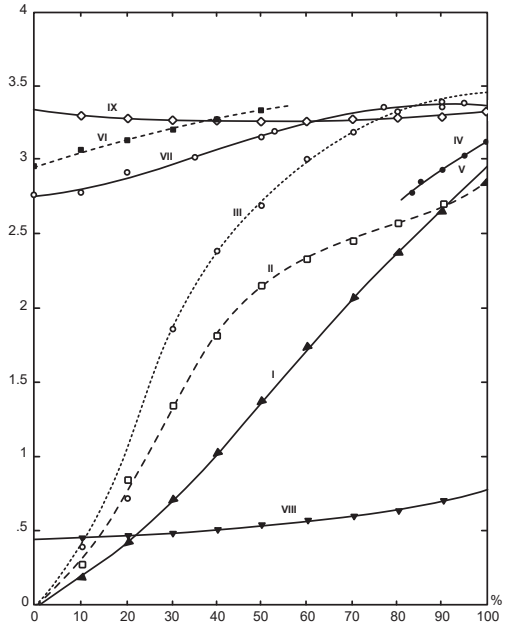
In this chromatographic process, the solvent and the solute interact with a non-polar stationary phase (alkyl bonded silicas, hydrocarbon coated supports, porous graphitized carbon, aromatic polymers).

- The more the solute is apolar the more it will be retained and the more it will be necessary to use an apolar mobile phase to elute it.

- If the solute is "weakly apolar" the mobile phase must be also weakly apolar. The more polar the solute is, the more polar must be the eluent.

In this chromatographic mode, water, the most polar solvent, is the weak solvent (the most retentive). The strong solvents (the most eluting) used must be completely miscible with water and show a different dominant partial polarity (the respective partial solubility parameters or fractional dominant Snyder polarity parameters are different). This leads to the selection of three solvents: Methanol, acetonitrile and THF which belong to classes (D, C and B) or different groups (II, VI and III) in each of the two three-dimensional polarity spaces.

By analogy with the phenomena of molecular interactions developing in NPLC, Snyder proposed a progressive polarity scale of the various Methanol-water binary mixtures based on the measurement of methylene selectivity (selectivity between two homologous solutes whose length difference of alkyl chain is one carbon). By definition he gave the eluent strength value ε_0=0 to the methylene selectivity in pure water cause none other pure solvent has lower eluting power than water in this chromatographic mode. In this scale pure Methanol has an eluting force ε_0=2.9.This is shown in the Colin-Guiochon eluent force diagram below:



Solvent eluotroppic strength of various mobile phase systems (A-B): the composition is given in volume percent of solvent B.

I	A=H_2O	B=MeOH	B=MeOH	(△)
II	A=H_2O	A=MeCN	A=MeCN	(□)
III	A=H_2O	A=THF	A=THF	(○)
IV	A=H_2O	A=EtoH	A=EtoH	(▲)
V	A=MeOH	A=A_c	A=A_c	(●)
VI	A=MeOH	A=THF	A=THF	(■)
VII	A=MeOH	A=Et.Ac	A=Et.Ac	(○)
VIII	A=(20MeOH + 80H_2O)	A=(20THF + 80H_2O)	A=(20THF + 80H_2O)	(▽)
IX	A=(80THF + 20H_2O)	A=(50THF + 50MeOH)	A=(50THF + 50MeOH)	(◇)

In this polarity scale, the eluting force variation of the water-Methanol binary mixtures as a function of the % of Methanol (φ) is approximately linear. (this is not the case for mixtures of water-acetonitrile and water-THF for which this variation has a convex shape).

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

The determination of the best composition of a mobile phase for a separation problem on these RP sorbents is established experimentally by choosing step by step the solvents of decreasing eluting force (increase of water % by 10% in 10%) and by evaluating each time the retention of all the compounds of a mixture and their separation. This is done in HPLC by finding the mobile phase composition placing all the solutes in the range of retention factors included between 2 and 10 (15, strictly speaking). If no mobile phase composition allows this it will be necessary to work in elution gradient or to find another chromatographic support.

The weak and medium polar solutes will be eluted with mobile phases of intermediate composition. The polar solutes (δT>13) will be eluted with high ratio of weak solvent mobile phases (high water ratio). The non-polar solutes (δT <8) will be eluted with very eluting mobile phases composed of high ratio of organic solvents (NonAqueousRP mode).

When two solutes are poorly separated with a Methanol-water mobile phase of given composition (fixed ε0), it is necessary to replace the Methanol with acetonitrile or THF, while keeping the solutes retention near the same values. The change of organic modifier most often leads to the selectivity modification of poorly separated peak pairs in a given binary mixture.

The simplest equivalence rules (iso-eluence) are as follows:
By using solubility parameters, we find:
φMeCN = 0.78 φMeOH
φTHF = 0.62 φMeOH
φTHF = 0.80 φMeCN

By using the Snyder polarity parameters, we find:
φMeCN = 0.82 φMeOH
φTHF = 0.58 φMeOH

These two very similar results therefore lead substantially to the same equivalences and one can use either one or the other or the average of the two estimates without this being detrimental from the point of view of the method and of the final result.

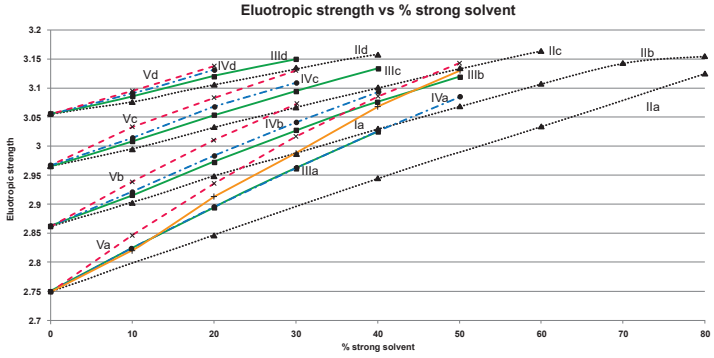
Non Aqueous RP mode

For non-polar solutes (δ_T<8 to 9) (triglycerides, ceramides, hydrocarbons, carotenoids, anthraquinoides, PAH...) which are insoluble in water or water-organic mixtures, binary mixtures are made up of:

- as weak solvents: acetonitrile or much more rarely Methanol
- as strong solvents: the solvents most often having an fd>50%, then chloroform, dichloromethane, Acetone, ethyl acetate, THF, diethyl ether, MTBE or a saturated hydrocarbon, making sure they are completely miscible with the weak solvent.

Considering their respective values of f_p and f_H or their belonging to different classes in the Hildebrand polarity representation, these strong solvents will give different selectivities for poorly separated pairs of solutes in a given binary composition.

Their similar eluting strength was recently reported by the following Heron-Tchapla diagram for the "green" binary blends Acetonitrile-CH2Cl2, Acetonitrile-Acetone, Acetonitrile-isoPropanol, Acetonitrile-Ethyl Acetate and Acetonitrile-Butanol at 4 different temperatures.



1. Solvent eluotropic strength of various mobile phase systems (MeCN/Strong solvent). The composition is given in volume percent of strong solvent.
(I) MeCN/CH_2Cl_2 (in orange); (II) MeCN/AcMe (in black); (III) MeCN/iPrOH (in green); (IV) MeCN/AcOEt (in blue); (V) MeCN/BuOH (in red).
(a) T = 25°C; (b) T = 43°C; (c) T = 63°C; (d) T = 85°C

Miscibility chart with polarity and solubility parameters

solvant	classe d	famille polarité	e0 20°C					Perfluoroalkanes	n-Pentane	n-Hexane	Isooctane	Pétroleum ether	n-Decane	Cyclohexane	Cyclopentane	Diisobutylene	1-Pentene	1,1,2-Trichlorotrifluoroethane	Carbon disulfide	Carbon tetrachloride
			e0 (Al2O3)	e0 (SiO2)	e0 (florisil)	e0 (magnesie)	e0 (diol)	A	A	A	A	A	A	A	A	A	A	A	A	A
Perfluoroalkanes	A	NC	-0.25	-0.19	-0.13	-0.15	-0.06													
n-Pentane	A	NC	0	0	0	0	0													
n-Hexane	A	NC	0	0	0	0	0													
Isooctane	A	NC	0.01	0.01	0.01	0.01	0													
Petroleum ether	A	NC	0.01	0.01	0.01	0.01	0													
n-Decane	A	NC	0.04	0.03	0.02	0.02	0.01													
Cyclohexane	A	NC	0.04	0.03	0.02	0.02	0.01													
Cyclopentane	A	NC	0.05	0.04	0.03	0.03	0.01													
Diisobutylene	A		0.06	0.05	0.03	0.03	0.01													
1-Pentene	A		0.08	0.06	0.04	0.05	0.02													
1,1,2-Trichlorotrifluoroethane	A		0.14	0.11	0.07	0.08	0.03													
Carbon disulfide	A	NC	0.15	0.12	0.08	0.09	0.03													
Carbon tetrachloride	A	VII	0.18	0.14	0.09	0.1	0.04													
1,1,1-Trichloroethane	A		0.19	0.15	0.1	0.11	0.04													
tert-Butyl methyl ether	A	I	0.2	0.15	0.1	0.12	0.05													
1-Chloropentane	A		0.26	0.2	0.14	0.15	0.06													
1-Chlorobutane	A		0.26	0.2	0.14	0.15	0.06													
Xylene	A	VII	0.26	0.2	0.14	0.15	0.06													
diIsopropyl ether	A	I	0.28	0.22	0.15	0.16	0.06													
2-Chloropropane	B		0.29	0.22	0.15	0.17	0.07													
Toluene	A	VII	0.29	0.22	0.15	0.17	0.07													
1-Chloropropane	B		0.3	0.23	0.16	0.17	0.07													
Chlorobenzene	A	VII	0.3	0.23	0.16	0.17	0.07													
Benzene	A	VII	0.32	0.25	0.17	0.19	0.07													
1-Bromoethane	A		0.37	0.28	0.19	0.21	0.09													
Diethyl ether	A	I	0.38	0.29	0.2	0.22	0.09													
Diethyl sulfide	A	NC	0.38	0.29	0.2	0.22	0.09													
Chloroform	A	peculiar	0.4	0.31	0.21	0.23	0.09													
diChloroMethane	A	VII	0.42	0.32	0.22	0.24	0.1													
Isobutyl methyl ketone	B	VI	0.43	0.33	0.22	0.25	0.1													
Tetrahydrofuran	B	III	0.45	0.35	0.23	0.26	0.1													
1,2-Dichloroethane	B	V	0.49	0.38	0.25	0.28	0.11													
Ethyl methyl ketone	D	VI	0.51	0.39	0.27	0.3	0.12													
1-Nitropropane	D	VII	0.53	0.41	0.28	0.31	0.12													
Acetone	D	VI	0.56	0.43	0.29	0.32	0.13													
1,4-Dioxane	B	VI	0.56	0.43	0.29	0.32	0.13													
Acétate d'Ethyle	D	VI	0.58	0.45	0.3	0.34	0.13													
Methyl acetate	D		0.6	0.46	0.31	0.35	0.14													
1-Pentanol	C	II	0.61	0.47	0.32	0.35	0.14													
Dimethyl sulfoxyde	E	III	0.62	0.48	0.32	0.36	0.14													
Aniline	B	VI	0.62	0.48	0.32	0.36	0.14													
Diethylamine	B		0.63	0.49	0.33	0.37	0.15													
Nitromethane	D	VI	0.64	0.49	0.33	0.37	0.15													
Acetonitrile	D	VI	0.65	0.5	0.34	0.38	0.15													
Pyridine	B	III	0.71	0.55	0.37	0.41	0.16													
2-Butoxyethanol	C		0.74	0.57	0.38	0.43	0.17													
isopropanol	C	II	0.82	0.63	0.43	0.48	0.19													
1-Propanol	C	II	0.82	0.63	0.43	0.48	0.19													
Ethanol	C	II	0.88	0.68	0.46	0.51	0.2													
Methanol	C	II	0.95	0.73	0.49	0.55	0.22													
Ethylene glycol	C	II	1.11	0.85	0.58	0.64	0.26													
Acetic acid	E	IV	high	high	high	high	high													
Water	peculiar	VIII	very high	very high	very high	very high	very high													
N,N-Dimethylformamide	E	III																		
								Perfluoroalkanes	n-Pentane	n-Hexane	Isooctane	Pétroleum ether	n-Decane	Cyclohexane	Cyclopentane	Diisobutylene	1-Pentene	1,1,2-Trichlorotrifluoroethane	Carbon disulfide	Carbon tetrachloride

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM


A 1,1,1-Trichloroethane
A tert-Butyl methyl ether
A 1-Chloropentane
A 1-Chlorobutane
A Xylene
A diisopropyl ether
B chloro 2 Propane
A Toluene
B 1-Chloropropane
A Chlorobenzene
A Benzene
A 1-Bromoethane
A Diethyl ether
A Diethyl sulfide
A Chloroform
A diChloroMethane
B Isobutyl methyl ketone
B Tetrahydrofuran
B 1,2-Dichloroethane
D Ethyl methyl ketone
D 1-Nitropropane
D Acetone
B 1,4-Dioxane
D Acétate d'Ethyle
D Methyl acetate
C 1-Pentanol
E Dimethyl sulfoxyde
B Aniline
B Diethylamine
D Nitromethane
D Acetonitrile
B Pyridine
C 2-Butoxyethanol
C Isopropanol
C 1-Propanol
C Ethanol
C Methanol
C Ethylene glycol
E Acetic acid
isolé Water
E dimethyl formamide

Detectors distinguish compounds from the mobile phase according to their physical properties. The collected fractions are cutted based on the dectector signal. Collect can be done relatively to a threshold and/or a slope value. Detection sensitivity is different from a detector to another, and is linked to compounds concentration or not. To maximize the dectection potential it is recommanded to couple different devices ie. UV+ELSD, UV+ELSD+MS,...

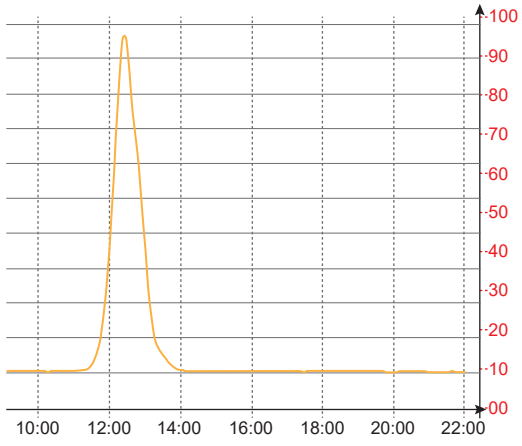
UV Visible Detector - Diode Array (DAD) Technology

When subjected to a light radiation, certain functional groups may be the seat of electronic excitation corresponding to an energy absorption at a specific wavelength. The signal collected correspond to a light absorption.

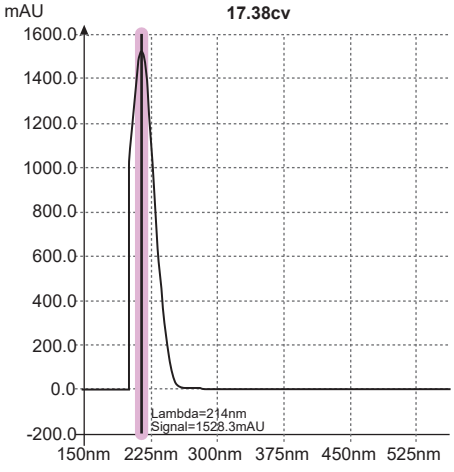
The UV detectors are not universal detectors. To be detected a compounds must have a chromophore in its molecular structure (i.e substances with aromatic ring, with at least 2 conjugated double bonds, with a double bond adjacent to an atom with ion electron pairs, with carbonyl groups, or containing bromine, iodine, or sulfur).

Different UV detectors are commonly used:

- Detector with a fixed wavelength, managed by a specific lamp. In this case, compounds response must be verified.
- Detector with variable wavelength allowing to choose between several wavelengths. This leads to a maximum of sensitivity.
- Diode Array Detector (DAD), uses hundreds of diodes to scan a range of wavelengths and gives a 3D representation (time, absorbance, wavelength) of the signal. This detector allows the acquisition of the UV spectrum that give indication of the purity of each detected compound.



Absorbance in mAU in function of the time or column volume.



UV spectrum showing the wavelength with the maximum absorbance at a given time.

- **All wavelength detection:**
  When the maximum absorbance of molecule is unknown, the scan function of the detector is the right solution. This scan signal corresponds to an average absorbance based on wavelengths within the range selected.

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

Solvent	UV (nm) Cutoff @1AU
Acetone	330
Acetonitrile	190
Dimethylformalmide	268
Dimethyl sulfoxyde	268
1,4-Dioxane	215
Ethanol	210
Isopropanol	120
Methanol	205
Tetrahydrofuran	215
Water	200
Benzene	280
n-Butanol	254
Carbon Tetrachloride	263
Chloroform	245

Solvent	UV (nm) Cutoff @1AU
Cyclohexane	200
1,2-Dichloroethane	235
Dichloromethane	235
Ethyl Acetate	260
Diethyl ether	220
Heptane	200
Hexane	200
Iso-octane	215
Methyl tert-butyl ether	210
Butanone	329
Pentane	200
Toluene	285
Xylene	290

- **Limits of detection:**

The mobile phase can also interact in the detection and can absorb at a specific wavelength => solvent cut off.

Every compounds have their own molecular extinction coefficient.
Due to this property, the apparent equivalent absorbance of 2 compounds should lead to 2 different concentrations.
According to the Beer-lambert law, absorbance of each molecule will be linked to its own concentration.

$$A = \varepsilon C l$$

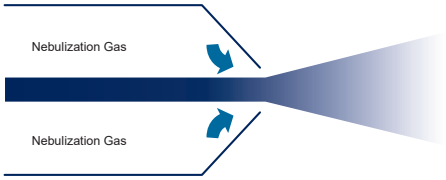
C = concentration in mol/L
l = cell length in cm
ε = molecular extinction coefficient (L.mol-1.cm-1)

The absorbance is improved when the cell path is increased.

Evaporative Light Scattering Detector (ELSD)

Principle:

ELSD detection is a three steps process:



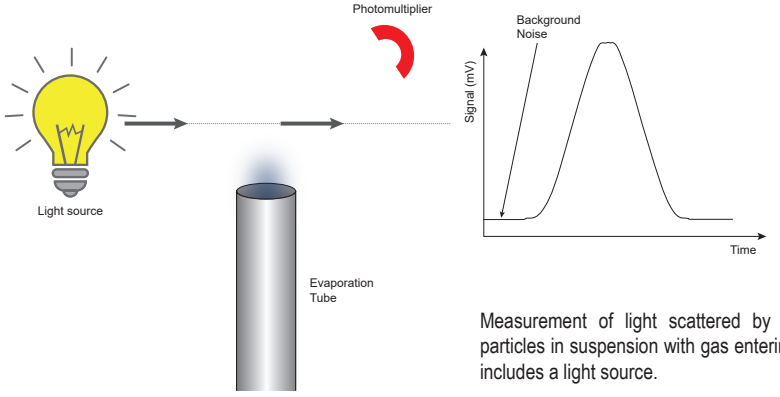
- **Nebulization**

The nebulization is done, in a nebulization chamber, with a venturi nebulizer that generate droplets of mobile phase containing the compound of interest, the largest droplets are eliminated. Compressed dry air or Nitrogen are used as nebulization gas.

- **Evaporation**

The evaporation is set in a drift tube. The nebulized eluent goes through a heated drift tube to evaporate the mobile phase. The temperature is optimized in function of the nature of the solute and the mobile phase. For low or non volatile compounds, the temperature of evaporation is increased to improve the detection.

- **Detection of light diffusion using a Photomultiplier or a Photodiode:**



Measurement of light scattered by a stream of solid particles in suspension with gas entering a flow cell which includes a light source.

- Detection is obtained by the measure of the intensity of the scattered light.
- A significant difference of volatility between the mobile phase and the compound is necessary.
  Caution must be consider for semi and highly volatile compounds detection as the signal is only generated by non-evaporated compounds.

Mobile Phase	Solute
100% Volatile	Non-Volatile
↓	↓
No Signal	Signal (Peak)

- Both isocratic or gradient mode can be used
- There is no solvent restriction as long as it can be evaporated before detection and except: phosphate, sodium, sulphate, potassium, HCl and H_2SO_4 buffers that are forbidden.
- ELSD response has not a linear reponse with the concentration of the compound.

Caution:

The ESLD is a destructive method of detection for the sample.
As for purification recovery is the one of main goal, the lowest quantity possible of sample has to be sent into the ELSD.

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

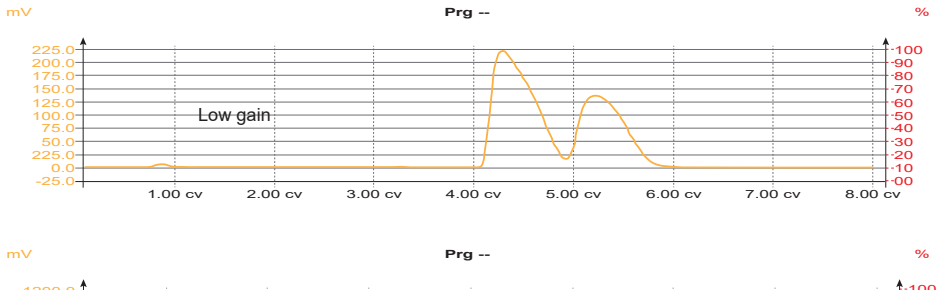
SAGA Function

Interchim® developed with Sedere an innovative automatic gain (SAGA: Sedex Automated Gain Adjustement). This technology adapt the gain to avoid saturation while continuing the detection of small quantity of products. ELSD becomes almost unsaturable without an impact on sensitivity.

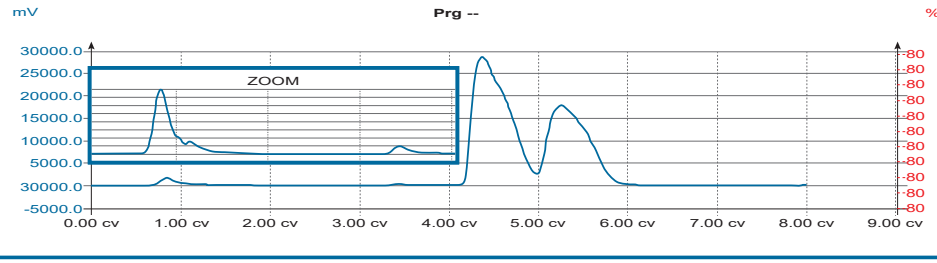
Application: Separation of 2 diastereo-isomers

Injection of 5mL (625mg of each compound)
Column: PF-15SIHP-F0025
Flow rate: 15mL/min

Without Interchim® SAGA



With Interchim® SAGA

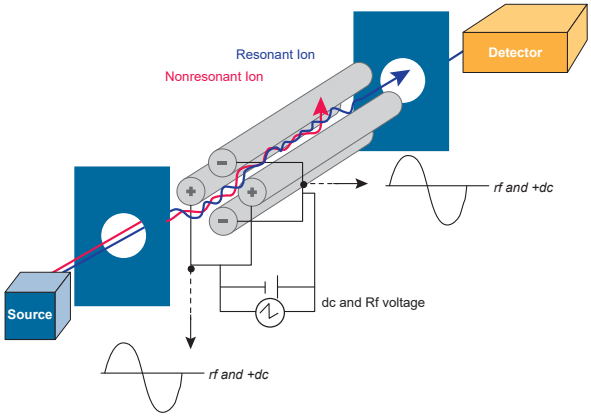


This advanced technology allows to detect all compounds regardless the quantity to purify.
Impurities can be seen even if a concentrated peak is close.

- Simplicity => automatic gain adjustment according to the sample load.
- Flexibility => manage both small quantity (2mg) and higher sample loading (up to 20%) within the same run.
- Confidence => numbers of class of compounds can be detected.

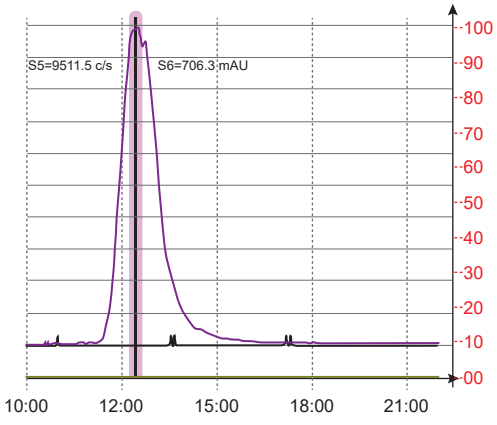
Mass Spectrometer Detectors (MS)

A Mass Spectrometer measures the mass to charge ratio (m/z), so it converts sample compounds into ions. The ions fly under vacuum are sorted and separated according to their mass to charge ratio under the influence of an electrical and or a magnetic field. The detection system measures the amount of ions.

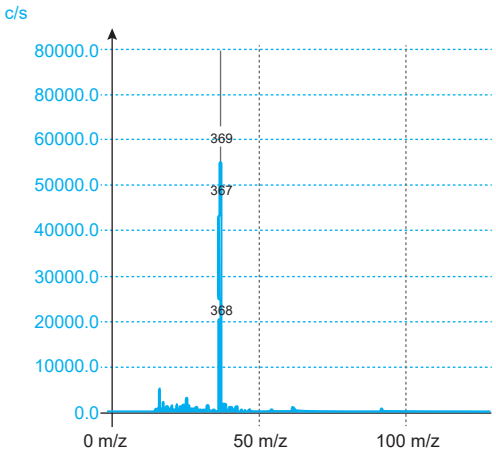


A mass spectrometer measures the spectrum over time (a sequence of spectra) to produce chromatograms

- **TIC** - Total Ion Chromatogram - adds all the masses together and shows how the entire mass spectrum varies with time - like a UV signal
- **XIC** (or EIC) - Extracted Ion Chromatogram - show how one mass varies with time, allows you to identify where your peak of interest elutes.



TIC (total ion chromatogram)
or XIC (extracted ion chromatogram) in function of the time or column volume



Mass spectrum corresponding showing the m/z ratio with the maximum intensity

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

The MS detection is applicable to a broad range of organic compounds. As well as providing chromatographic data it definitively identifies the compound by confirming its mass.

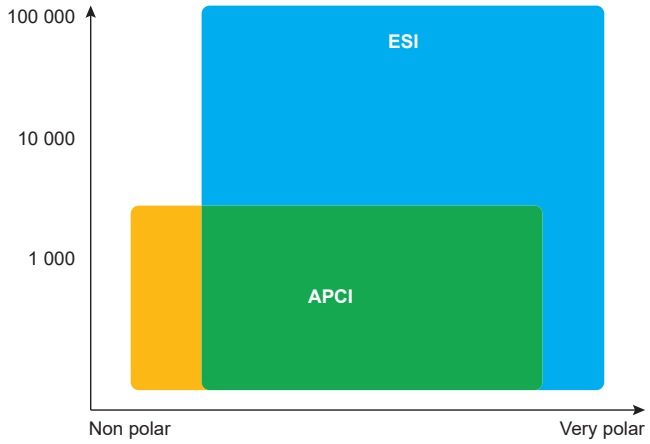
The ionization of the compounds is made by a source.

No Ion Source is universal, selection depends on the compound to be analyzed and the mass spectrometer type.

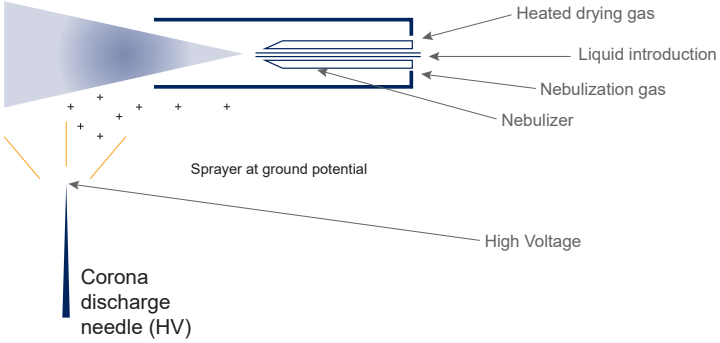
Two of the most common Ion Sources are Electro spray and Chemical Ionization and they can only be used with gas source (nitrogen).

APCI and ESI generally ionize by proton transfer:

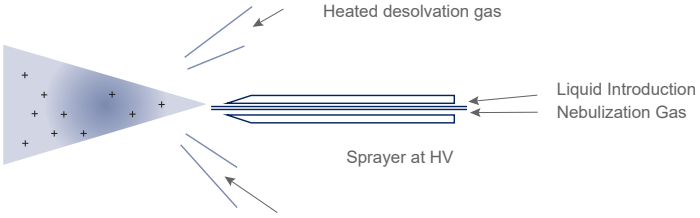
- Acceptance of a proton to produce [M+H]+ in positive ion
- Abstraction of a proton from the analyte to produce [M-H]- in negative ion. Ionization may also occur by forming adducts with other species
- e.g. NH_4^+ (+18), Na^+(+23), K^+(+39), Methanol (CH_3OHH^+, +33), Acetonitrile (CH_3CNH^+, +42), Acetic Acid (CH_3COOHH^+, +61)



Atmospheric Pressure Ionization Source (APCI)



Electrospray Ionization (ESI)



Electrospray is produced by applying a strong electric field to a liquid passing through a capillary electric field to a liquid passing through a capillary tube with a weak flux.

Desolvation by gas flow (N_2) heating (100-300°C).
Ions are preformed in solution before nebulization

Ion Sources: APCI & ESI

Electrospray (ESI)	**APCI**
Volatibility not mandatory	Volatibility needed
Technique adapted to heat-labile compound	Compounds must be thermally stable
Ions formed in solution	Ions are formed in the gas phase
Can form multi-charged ions	The ions are singly charged

Why choose APCI for purification ?

- Better for neutral compounds than ESI
- Simpler spectra than ESI: more often the molecular M+H ion, less adducts, fragments dimers, No multiple charging
- Generally 'Easy To do': less sensitive to operating conditions that ESI
  less solvent dependence as ionization occurs in the gas phase
  less matrix suppression
- Can accept higher sample concentrations
- More sensible than ESI: less noise at high flow rate (>750 mL/min)
  However, samples must be sufficiently volatile to be vaporized, & thermally stable to 130 - 150 °C
  ESI might be considered for Reverse Phase and is essential for biological molecules.

Applications vs. Sources

Proteins, peptides, RNA, sugars, carbohydrates, PPG's...

Most drugs, metabolites, aromatic compounds containing at least one ionizable functional groups taht can be protonated / deprotonated like NH_2, CO_2H, SO_3H, Ph-OH.

Small (<1.000u) volatile, polar and neutral molecules, steroids.

Neither Ion Source works for:

Napthalene, biphenyl, PAH's with no heteroatoms, hydrocarbon waxes, resins and glues

The Mass spec. is a destructive method of detection for the sample.
As for purification recovery is the one of main goal, the lowest quantity possible of sample has to be sent into the MS.

Interchim developed a **Unique interface "Split & Dilution in a box"** to avoid saturation of the MS spectrometer whatever the concentration of sample injected is. It does not generate additional backpressure even at high flow rate.

It is combined to a normalized scale of MS signals, UV, ELSD by Interchim software.

Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

Triple Detection: UV-ELSD-MS

To Sum-up the detectors are compounds dependent.

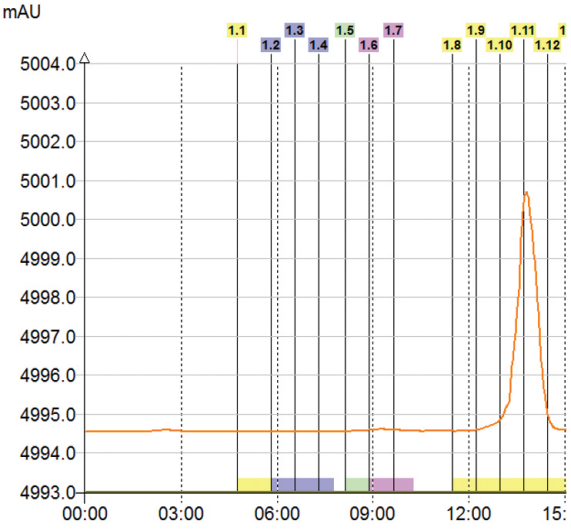
	UV	ELSD	MS
Detection	Chromophore	Non volatile at the working temperature	Ionizable Gives an indication on the compounds structure

To be sure to detect and collect all the compouds a combination of several detectors is advised.

A triple detection UV-ELSD-MS can be easily used with a puriFlash® system.

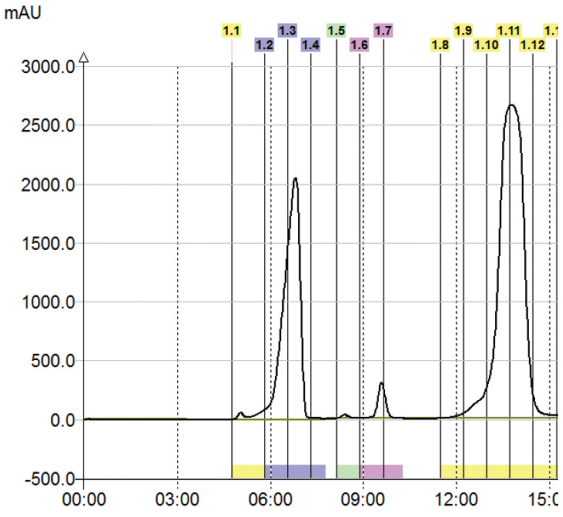
Application example: customer mixture using PF system with triple detection

UV Signal



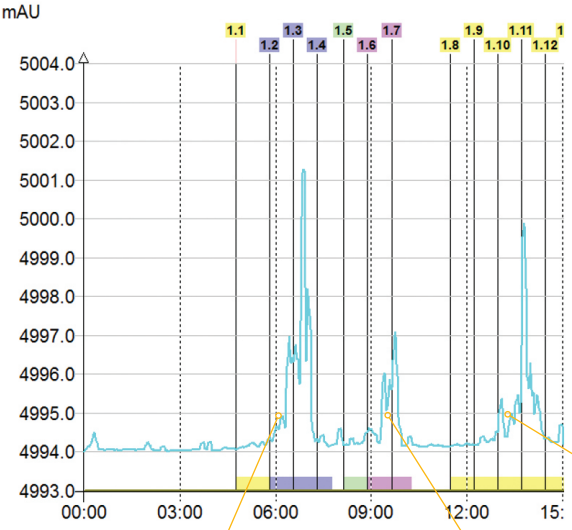
Only 1 compound detected

ELSD Signal



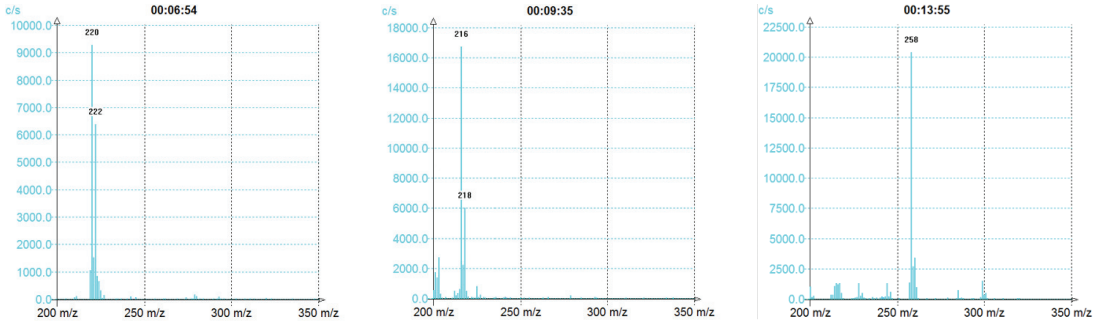
3 compounds detected

MS TIC Signal



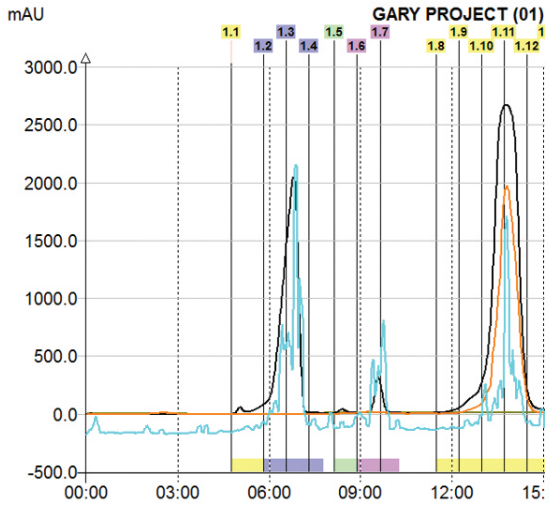
3 compounds detected

With information
of the m/z for each peak



Tel.: +33 4 70 03 88 55 | Hotline: +33 4 70 03 73 01 | E-mail: interfine@interchim.fr | Fax: +33 4 70 03 82 60 | WWW.INTERCHIM.COM

Triple Detection



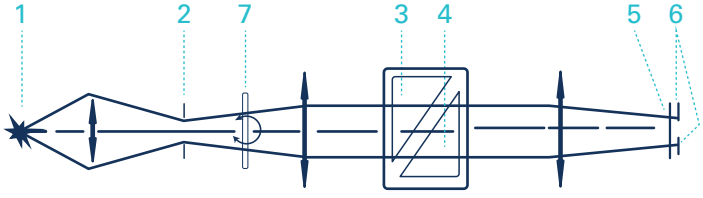
In this example with only UV detection customer would have collected only one product instead of 3 with the triple detection. Moreover, with the MS he was able to identify his interest compounds.

Refractive Index Detector (RI)

The RI detector measures the refractive index of an analyte relative to the solvent.

A light beam crosses a 2 compartments cell in which one is filled with the solvent and the other with the column effluent. So, this is a difference of refractive index of the 2 liquids which is measured.The greater the RI difference between sample and mobile phase, the larger the imbalance will become so the sensitivity will be higher. There is no detection if the refractive index of the compound is too close to the solvent refractive index.

Optical system



1: AsGa diode 940nm
2: Input slit
3: Reference cell
4: Sample cell
5: Mask
6: Photocells
7: Optical zero adjustement

Limits of Detection:

RI detector is a pure differential instrument, and any changes in the eluent composition require the rebalancing of the detector. This factor is severely limiting RI detector application in the analyses requiring the gradient elution, where mobile phase composition is changed during the run.