

# INTRODUCTION AU COUPLAGE CHROMATOGRAPHIE – SPECTROMÉTRIE DE MASSE

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Licence Pro  
1er mars 2024  
Université, Nantes



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# Le LABERCA

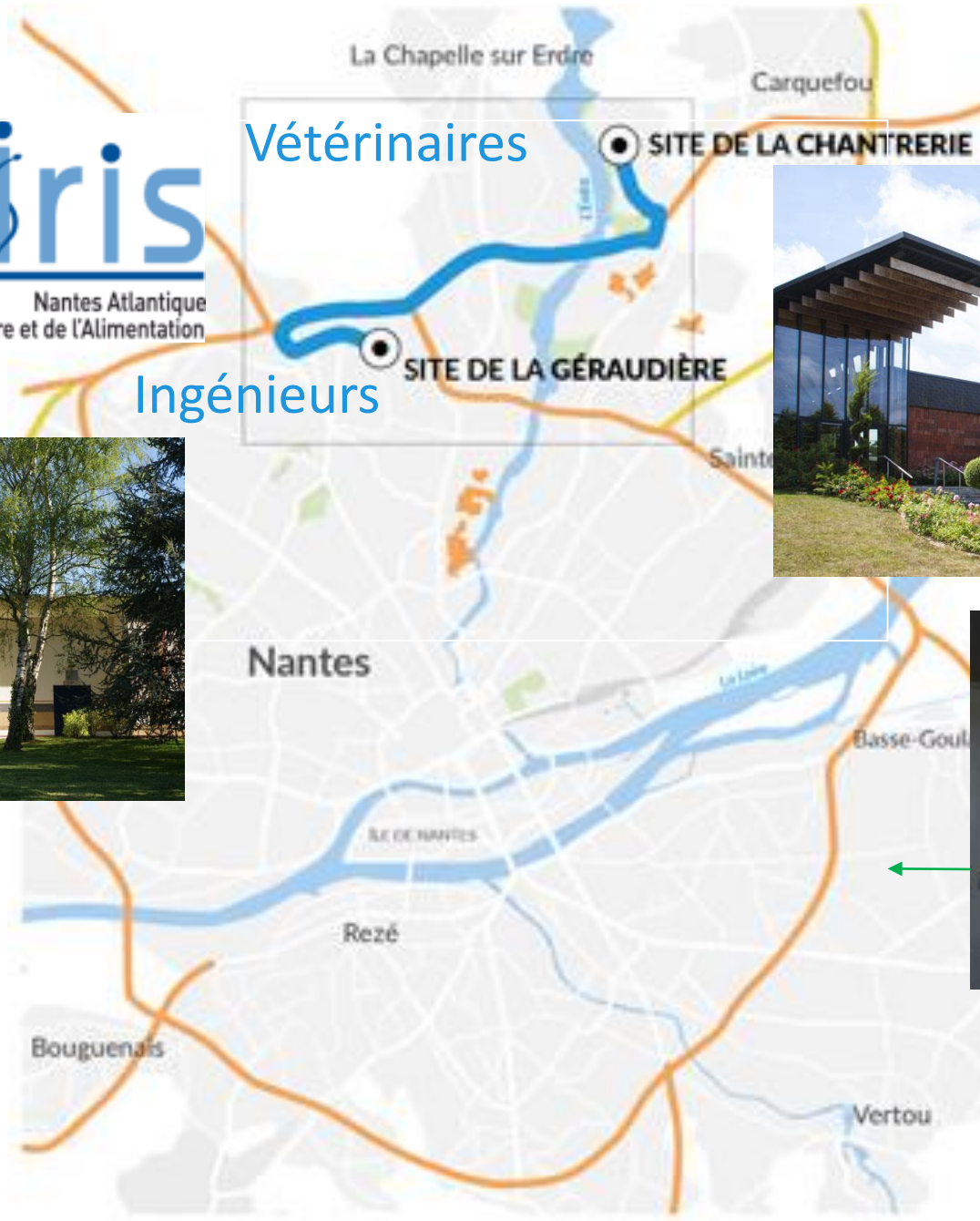


Vétérinaires

SITE DE LA CHANTRIÈRE

Ingénieurs

SITE DE LA GÉRAUDIÈRE



LABERCA

Oniris INRAE

National Reference Laboratory

Mixed Research Unit

PLATFORMS

UPC

UCO

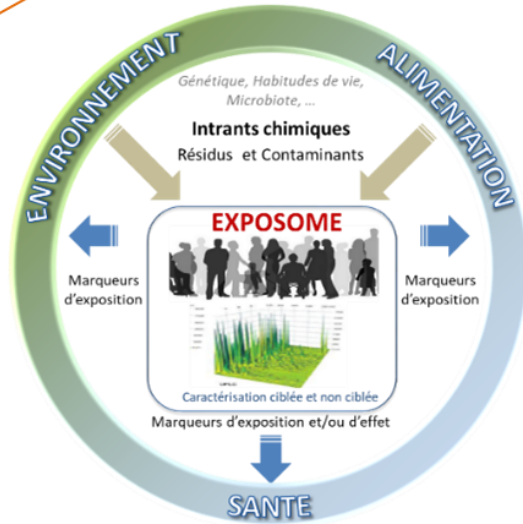
FOOD



HEALTH

HBM

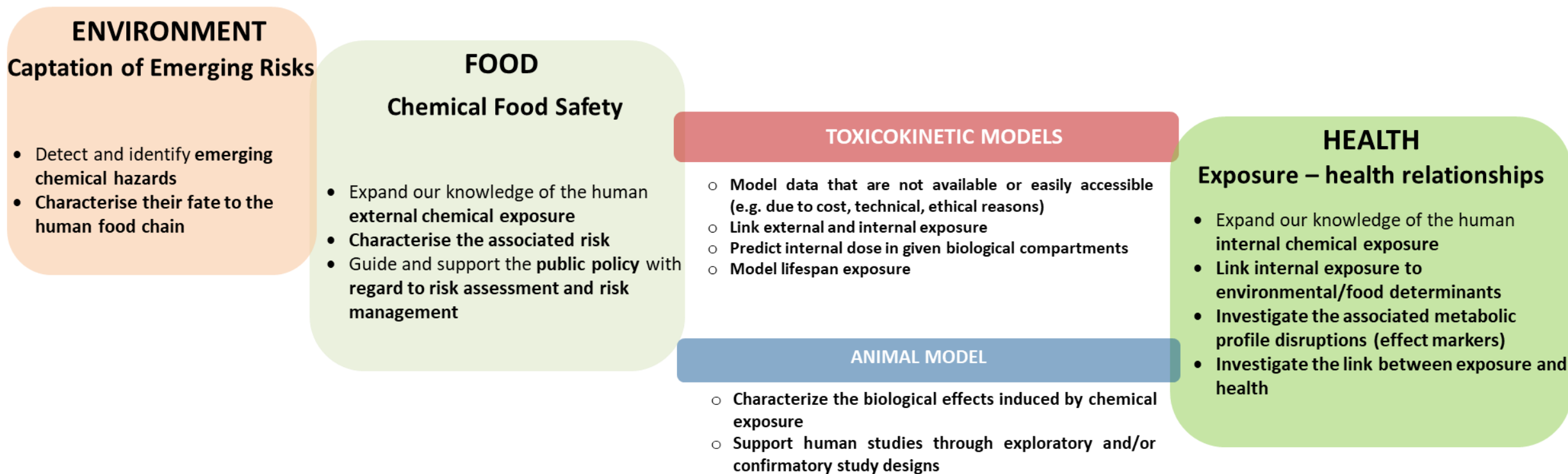
MELISA



- 3 EC
- 4 IR
- 1 CR

- 4 Post-Doc
- 8 PhD
- 4 Master

Global research thematic:  
organic chemical residues and contaminants from their environmental sources to their impact  
on human health through the food chain



LABERCA

MINISTÈRE DE L'AGRICULTURE ET DE L'ALIMENTATION

National Reference Laboratory

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PLATFORMS

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HBM

MELISA

21

ACCREDITATION COFRAC des analyses N° 1-0249 Forêt disponible sur www.cofrac.fr

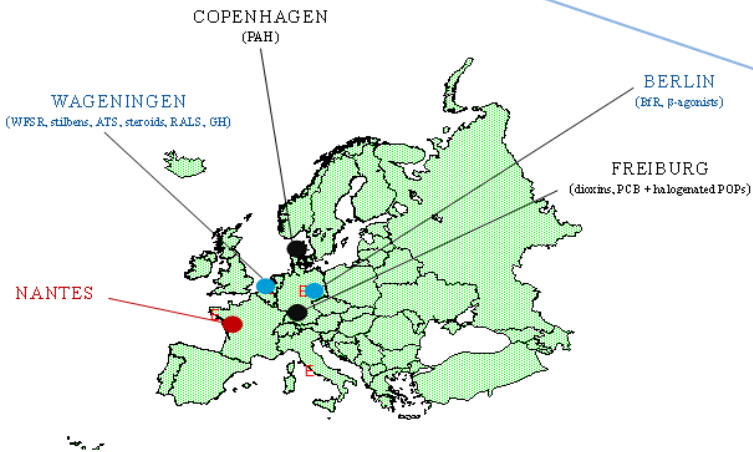


Décrets, arrêtés, circulaires

TEXTES GÉNÉRAUX

MINISTÈRE DE L'AGRICULTURE ET DE L'ALIMENTATION

Arrêté du 26 novembre 2018 modifiant l'arrêté du 29 décembre 2009 désignant les laboratoires nationaux de référence dans le domaine de la santé publique vétérinaire et phytosanitaire

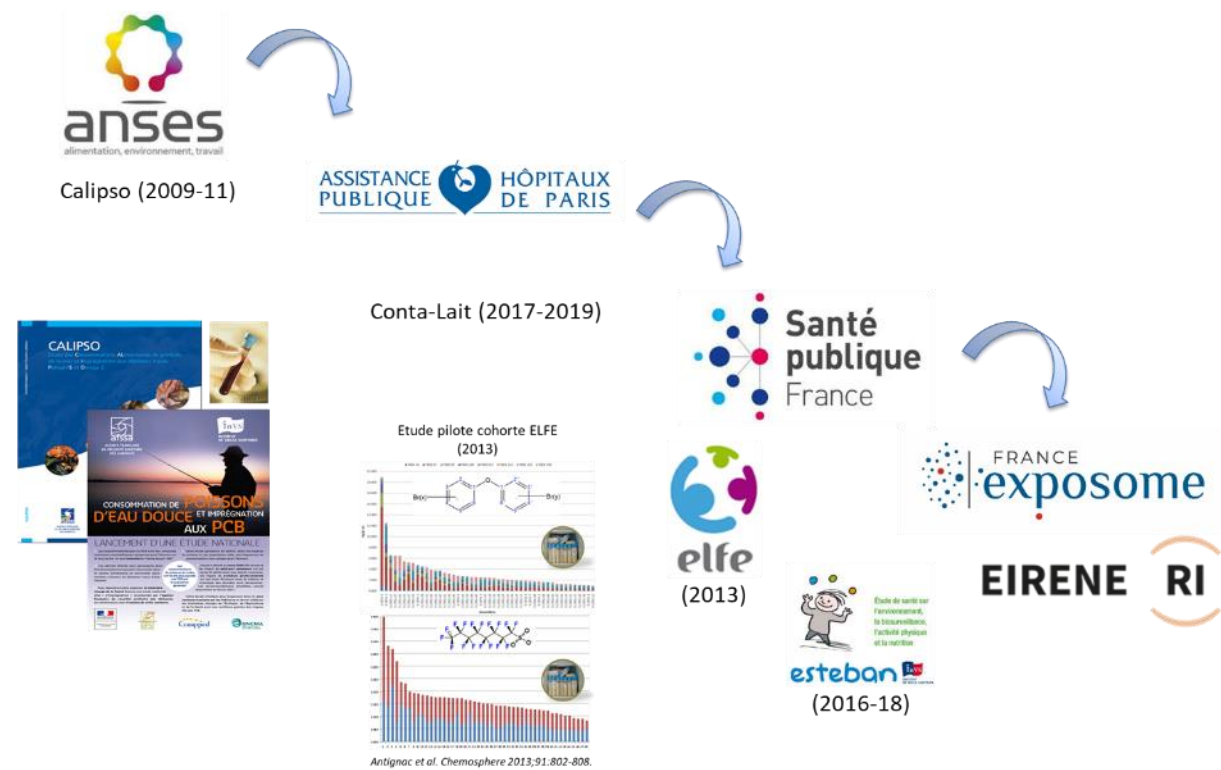


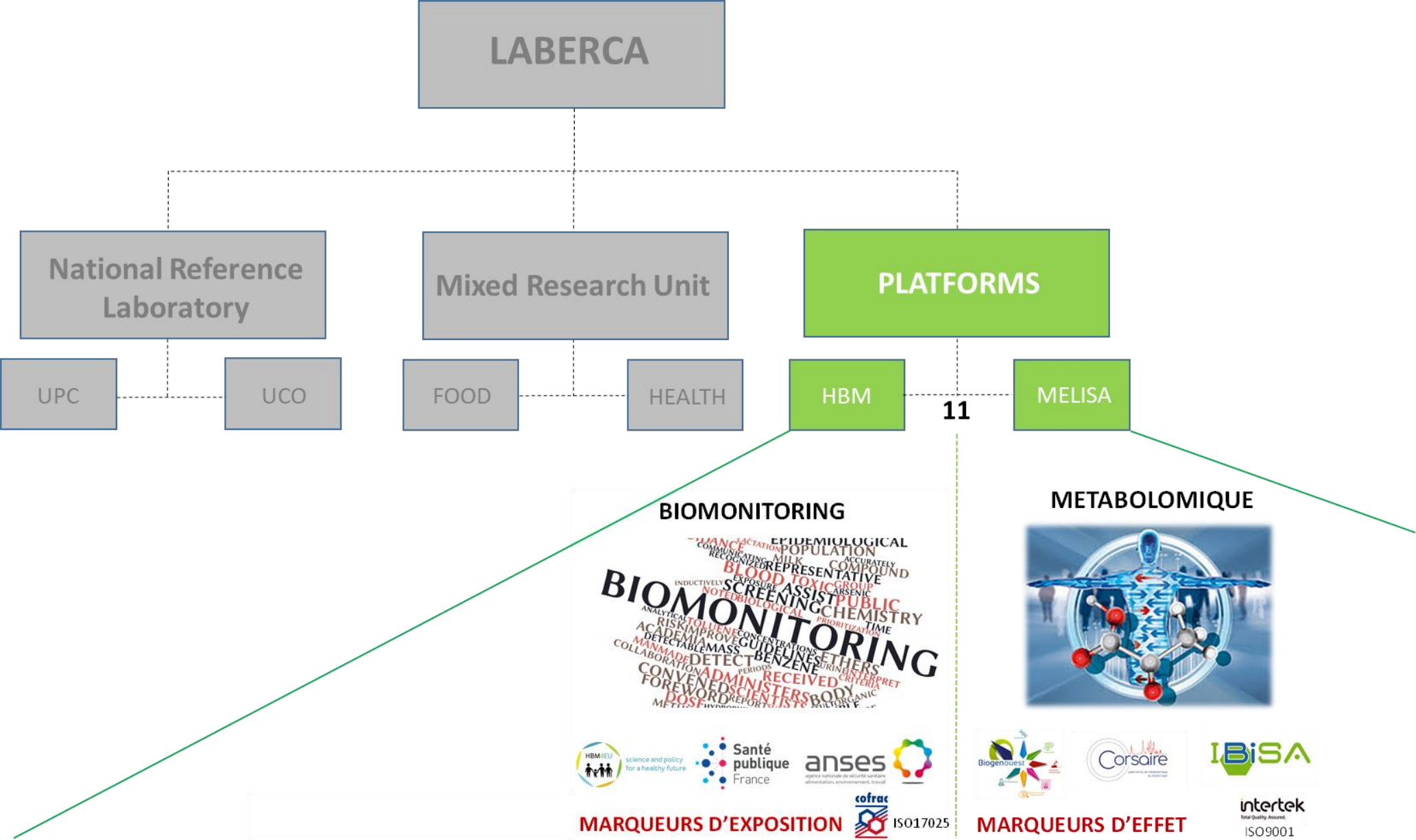


# Characterizing the external (food) exposure e.g. total diet studies



# Characterizing the internal exposure e.g. biomonitoring programs





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FOOD

HEALTH

PLATFORMS

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BIOMONITORING



METABOLOMIQUE



**MARQUEURS D'EXPOSITION**

**MARQUEURS D'EFFET**





LC-QTOF (IMS)



GCxGC-TOF



GC- and LC-HRMS<sup>n</sup> (x 4, Orbitrap systems)



GC-HRMS (x 3, BE)



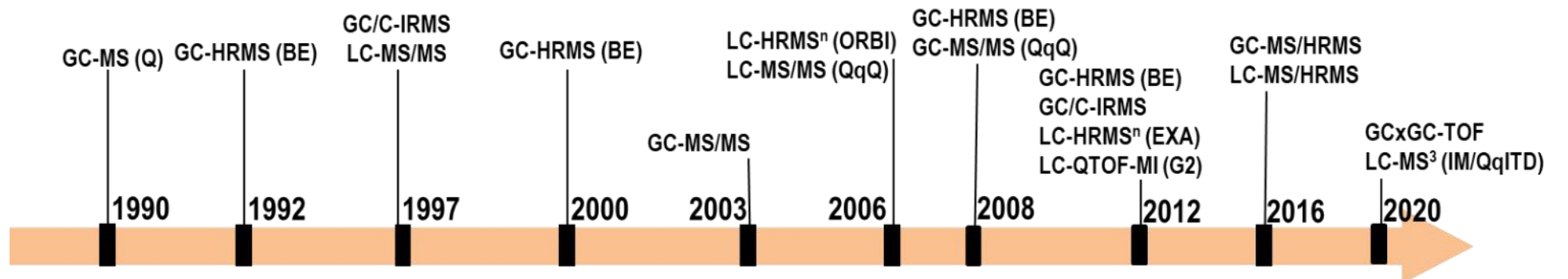
GC-C-IRMS (x 2)

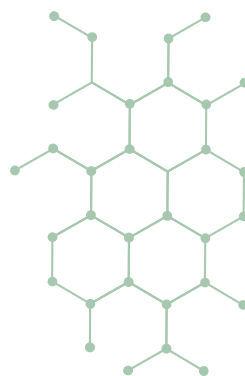
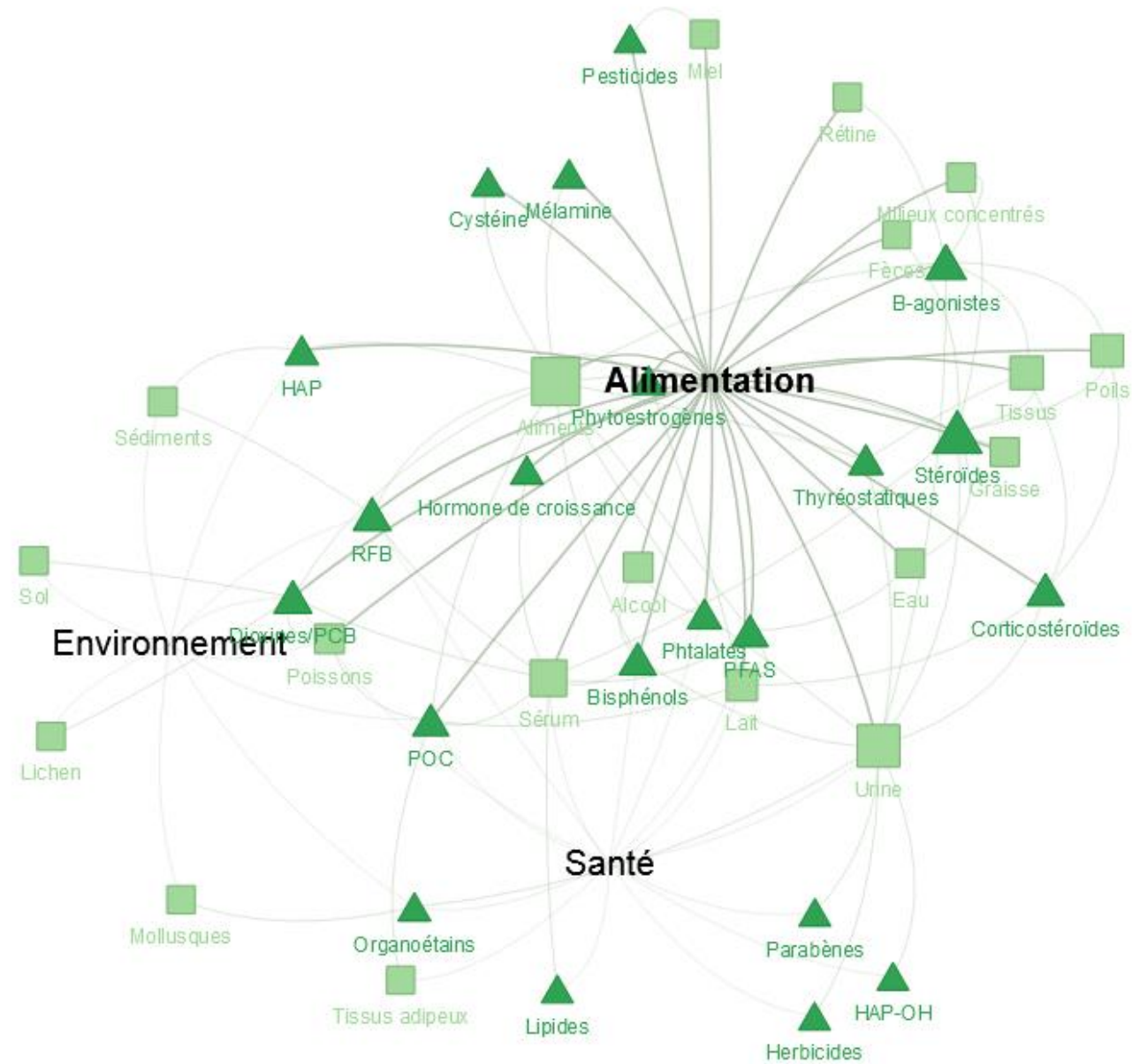


GC-MS/MS (x 3, QqQ)



LC-MS/MS (x 3, QqQ)





### Analyte(s)



Source :  
<http://visite.artsetmetiers.free.fr/instruments.html>

$10^{-9}$  to  $10^{-15}$  grams

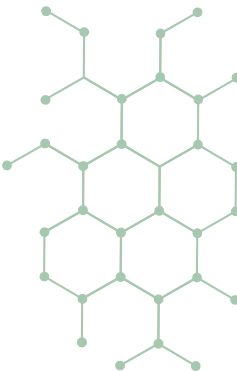


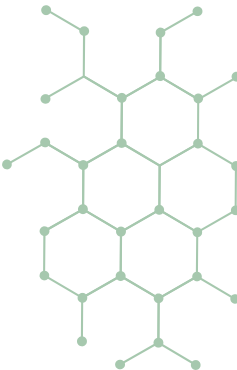
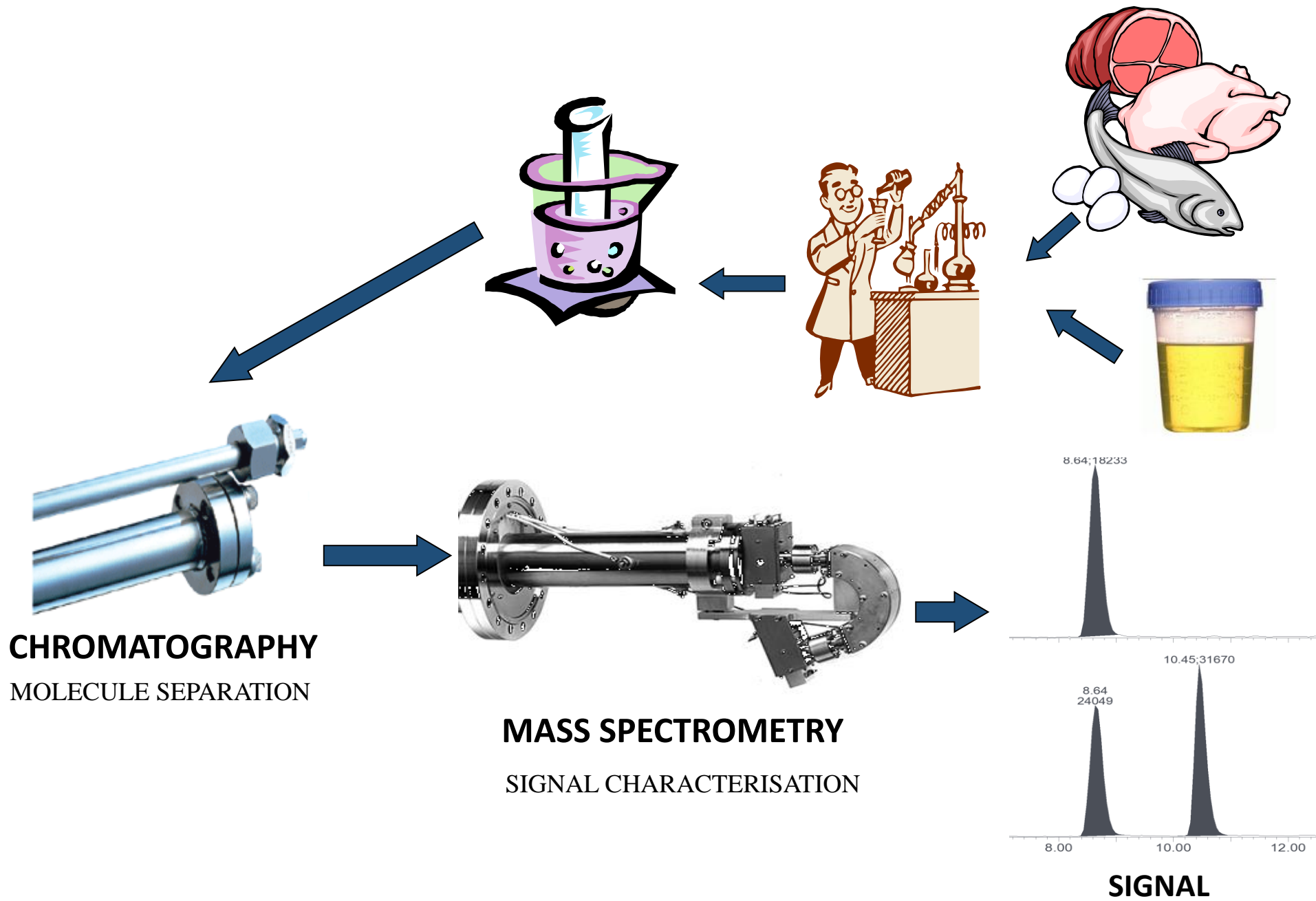
### Matrix

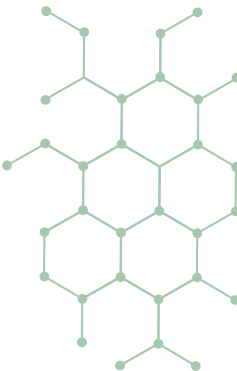
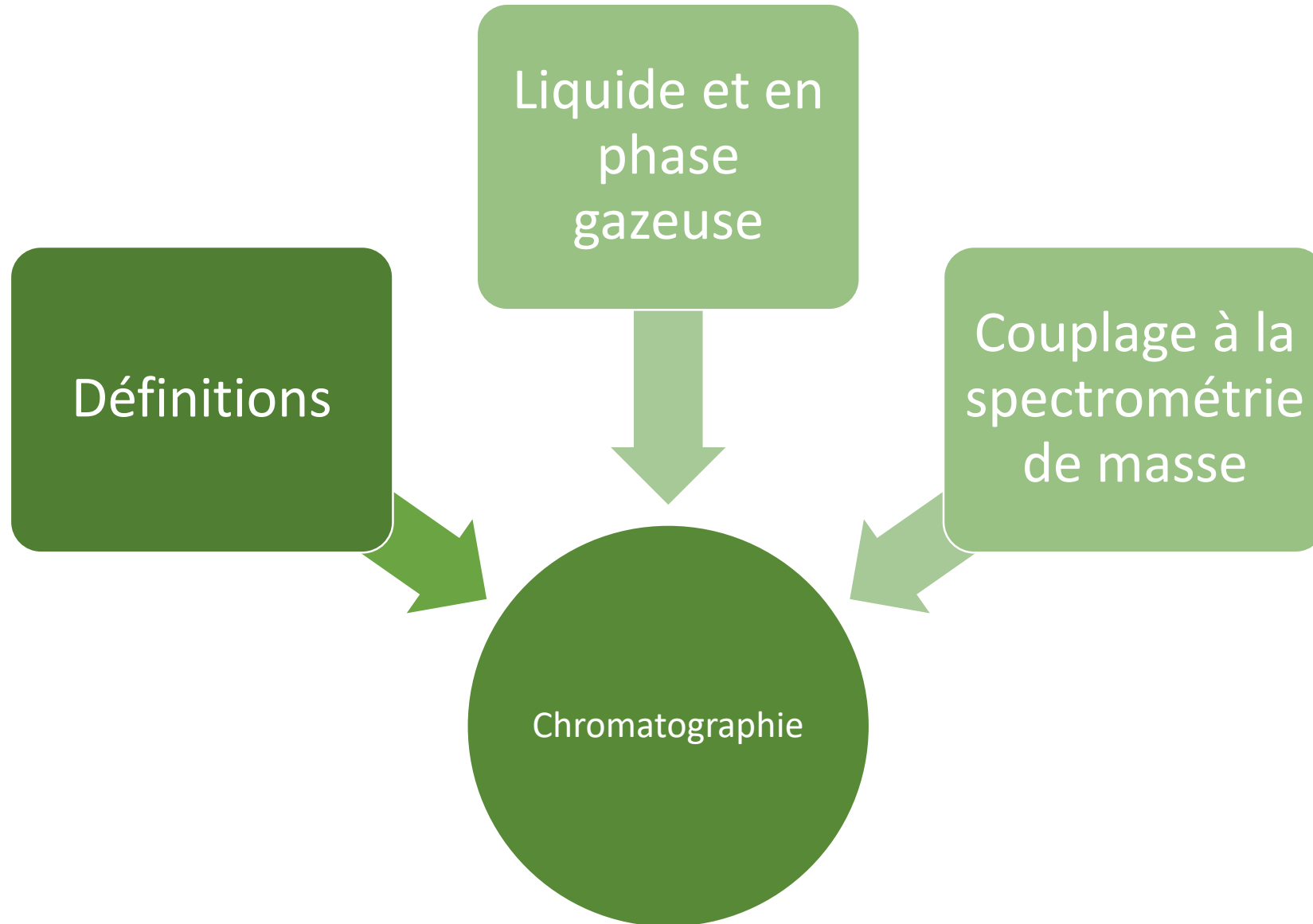


Source :  
<http://visite.artsetmetiers.free.fr/instruments.html>

grams



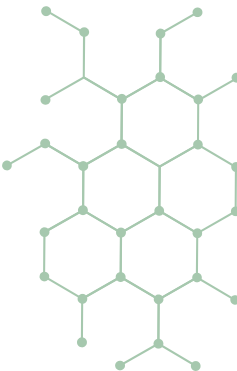




Définitions



Chromatographie

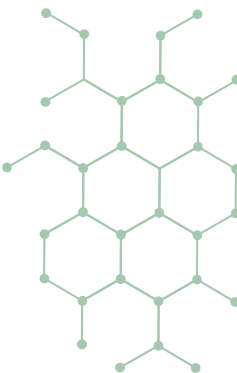
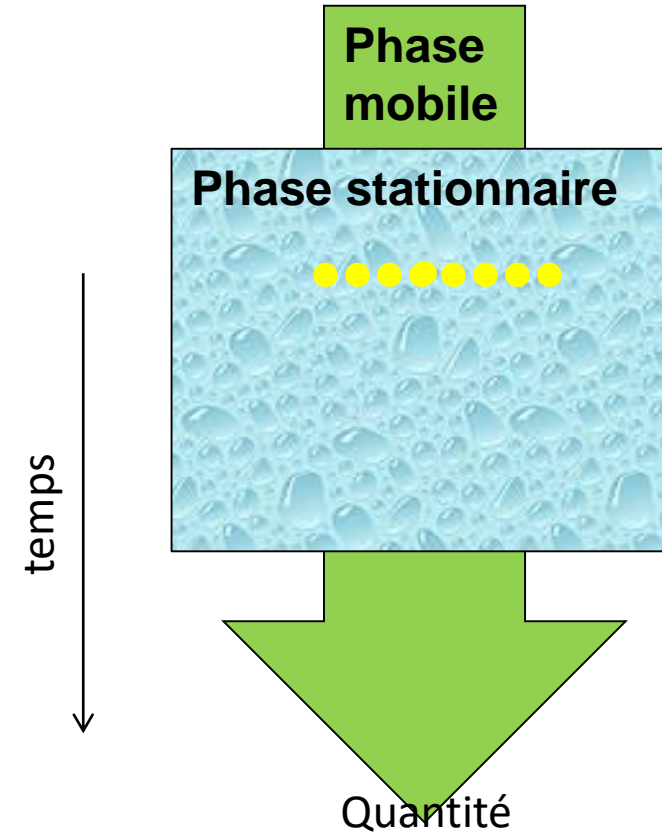
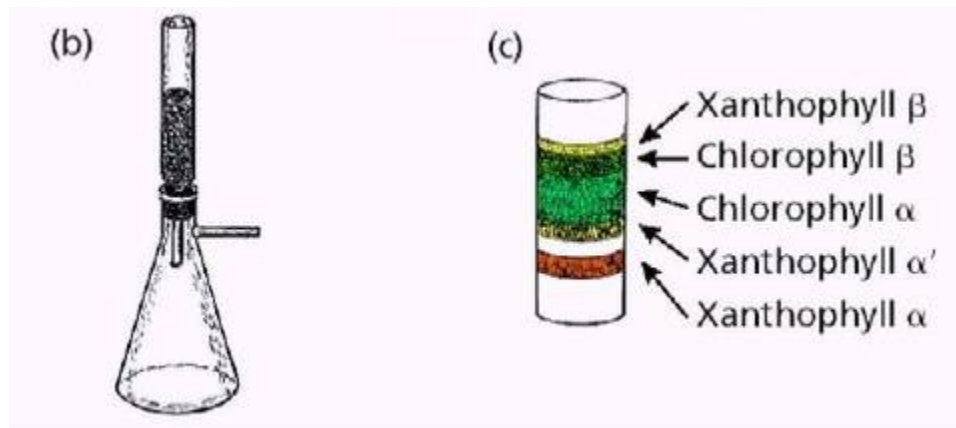


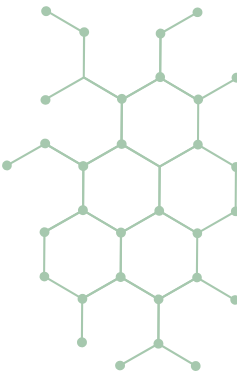
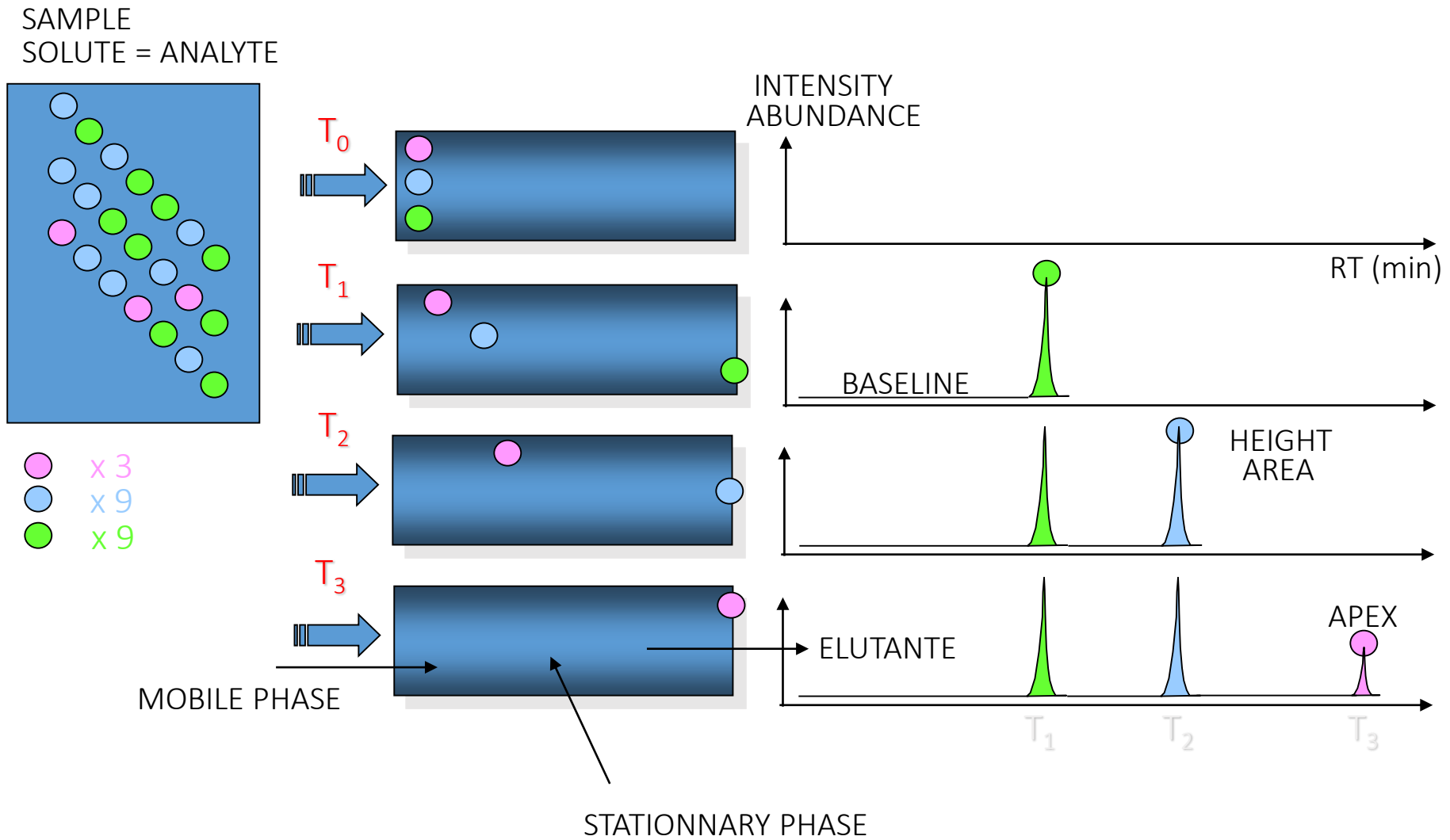


La chromatographie est une méthode **séparative** qui permet **l'identification** et le **dosage** des différents composés d'un mélange.

Le principe est basé sur les différences d'affinité des composés du mélange avec la phase stationnaire et la phase mobile.

Michail Semenovitch  
Tswett – 1903  
Séparation de pigments  
végétaux sur colonne  
remplie de carbonate de  
calcium  
Il sépare la chlorophylle  
des caroténoïdes



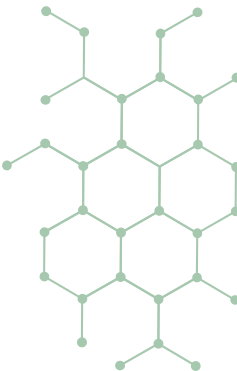
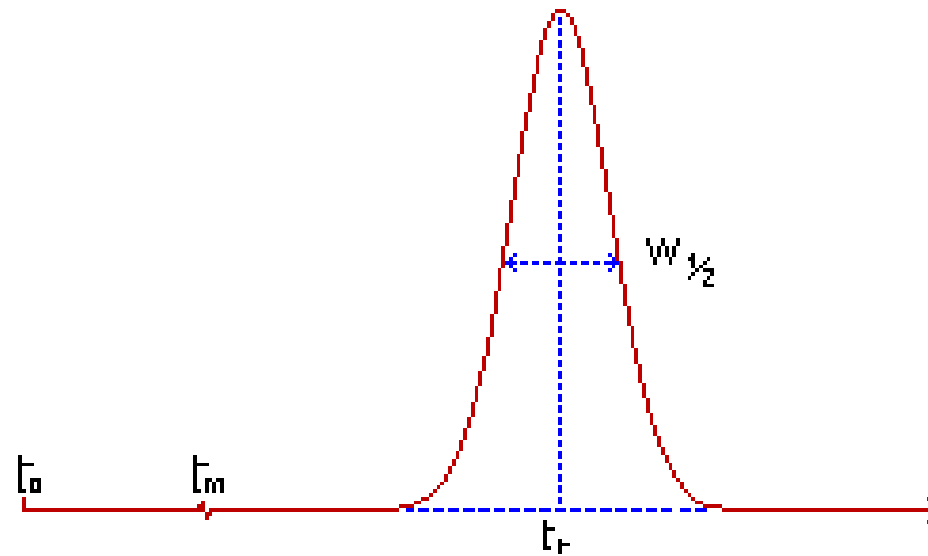


Type de chromatographie

- d'adsorption
- de partage
- d'échange d'ions
- d'exclusion

Phase mobile

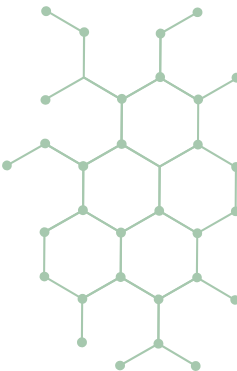
- liquide
- gazeuse
- fluide supercritique

Volume mort ( $V_m$  au  $t_m$ )Temps de rétention ( $t_r$ )Capacité de rétention  $k=(t_r-t_m)/t_m$ 

Chromatographie  
liquide



Chromatographie



# Chromatographie Liquide

**HPLC**



**MS**



<http://autosampvials.kinesis.co.uk/products/vials-caps/kx-lgc-certified-vial-cap-kits-10#.YO3utPmG-FU>

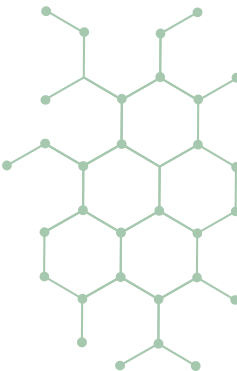
**Passeur d'échantillon**

**Injecteur**

**Four pour colonne**

**Pompe HPLC**

**MS**



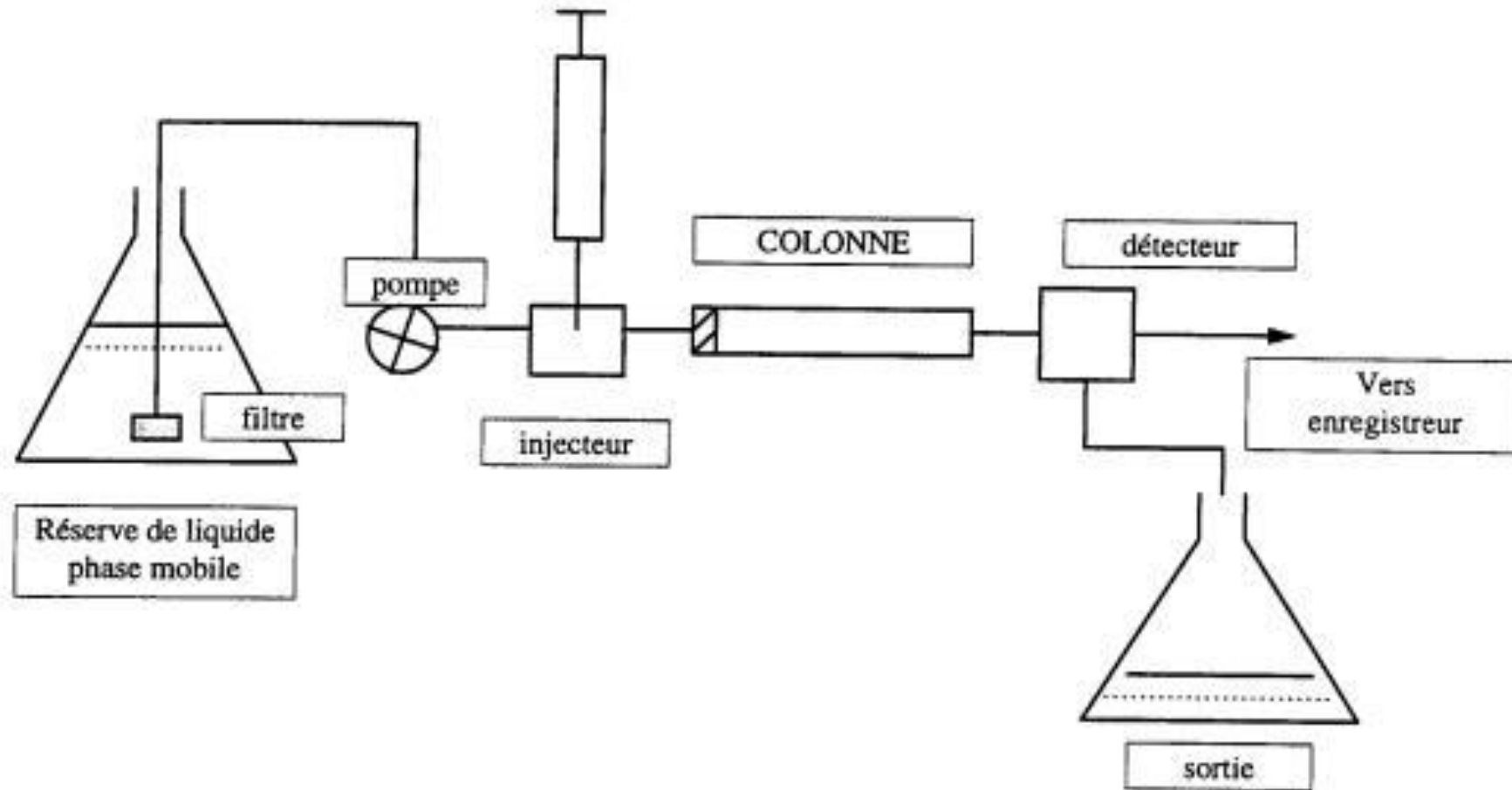
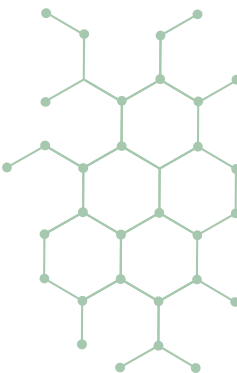


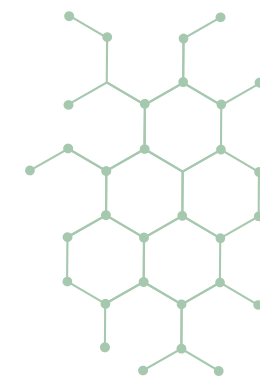
Figure 3 : principe de fonctionnement de l'HPLC





## HPLC Modes

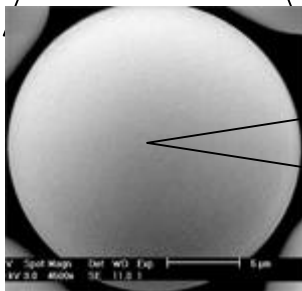
Analytes	Mode	Stationary phase	Mobile phase
Neutrals Weak acids Weak bases	Reversed-phase	C18, C8, Phenyl ... (Apolar)	Water/organic, modified
Ionics Acids Bases	Ion-pairing	C18, C8, Phenyl (Apolar)	Water/organic, <b>ion-pairing reagent</b>
Compounds insoluble in water Organic isomers	Normal phase	Silica, Diol, Amino, Cyano (Polar)	Organic
Ionics	Ion exchange	Ion exchange resin	Aqueous <b>buffer</b>
High MW compounds Polymers	Size exclusion	Polymeric or silica	Aqueous – Gel filtration Organic – Gel permeation
Polar	HILIC	Silica	Organic (ACN) / water



# Chromatographie Liquide

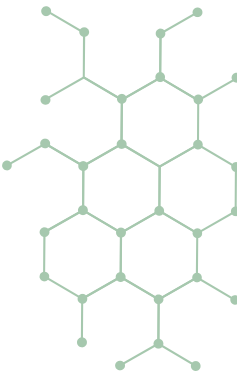
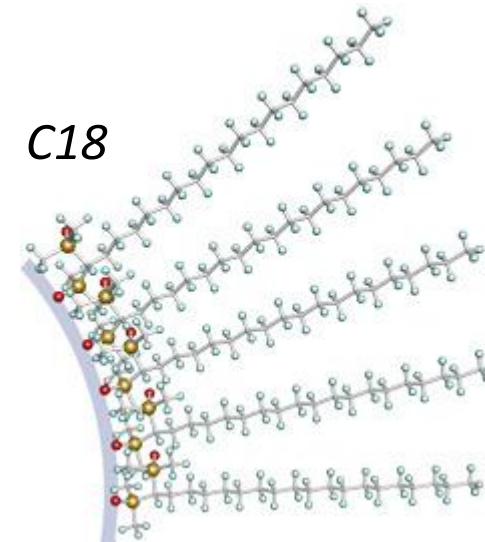
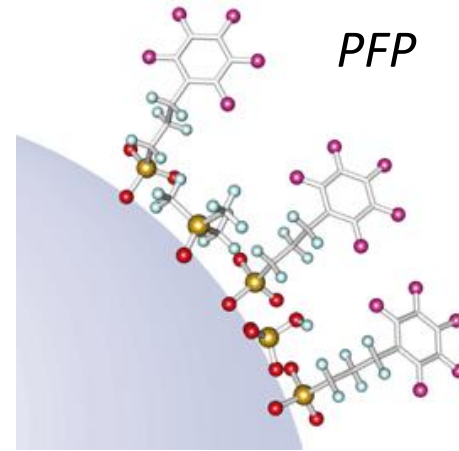


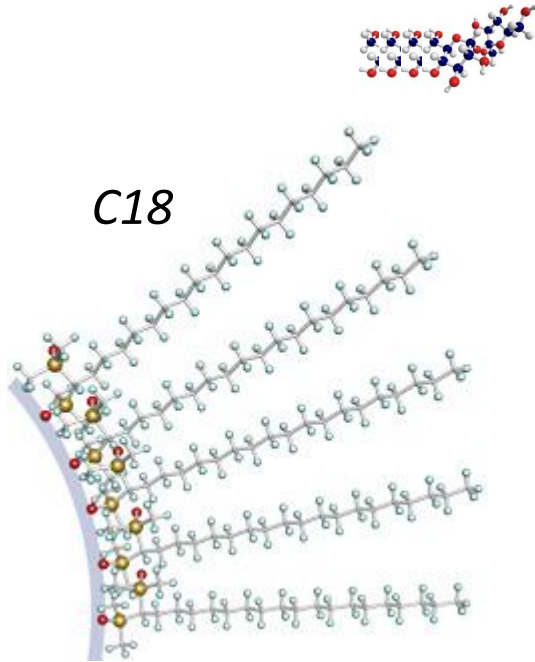
100 x 2 mm



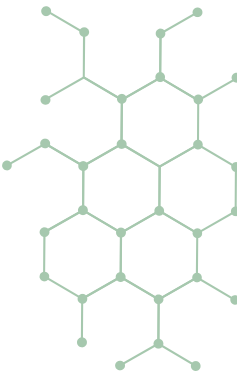
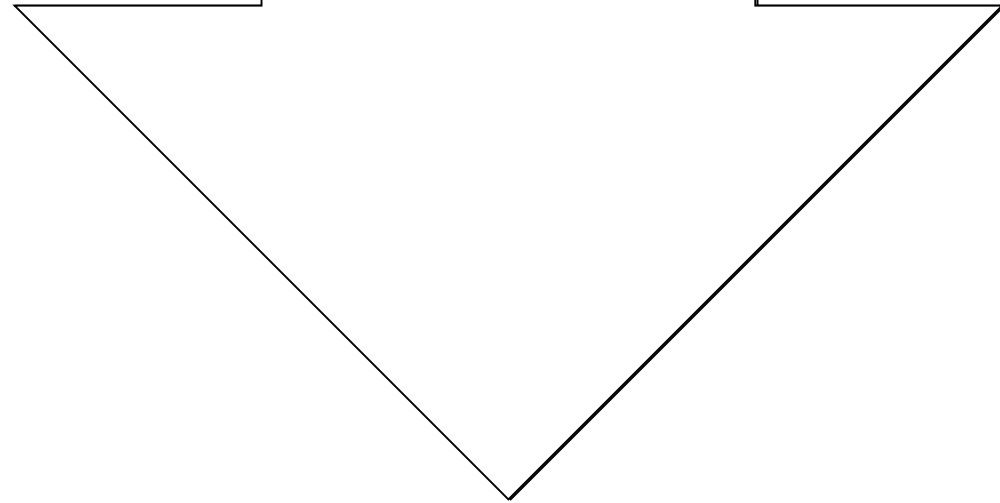
SiOH

2  $\mu\text{m}$





**2-augmentation du pouvoir éluant**



# Chromatographie Liquide

## Force éluante et polarité

Pour les composés **polaires** :

- + la phase mobile sera **polaire**, + elle va entraîner les solutés.
- + la phase mobile sera **apolaire**, - elle va entraîner les solutés.

Pour les composés **peu polaires** :

- + la phase mobile sera **polaire**, - elle va entraîner les solutés
- + la phase mobile sera **apolaire**, + elle va entraîner les solutés.

Solvant A  
Phase aqueuse  
Tampon

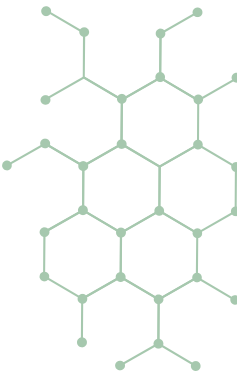


<http://www.sodipro.fr/catalogue/fiche-mat.php?refart=2005591>

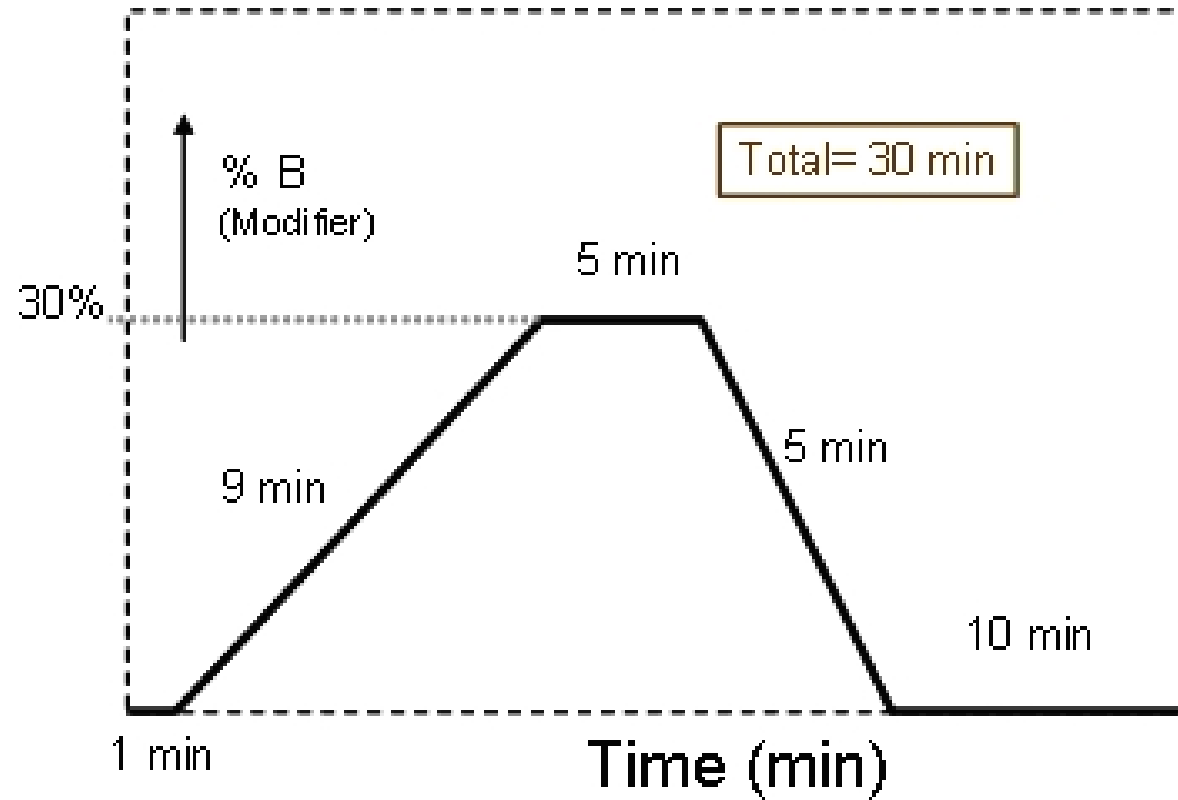


Solvant B  
ACN  
MeOH

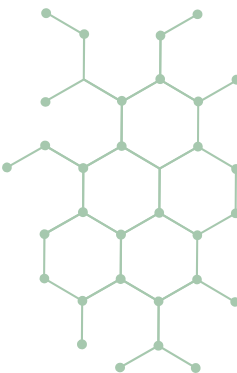
*Vitesse (vélocité) importante pour la séparation : dépend de la colonne*

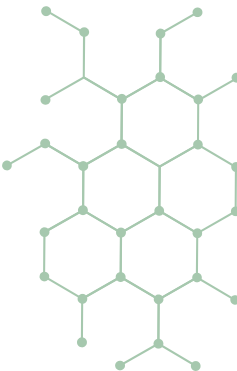
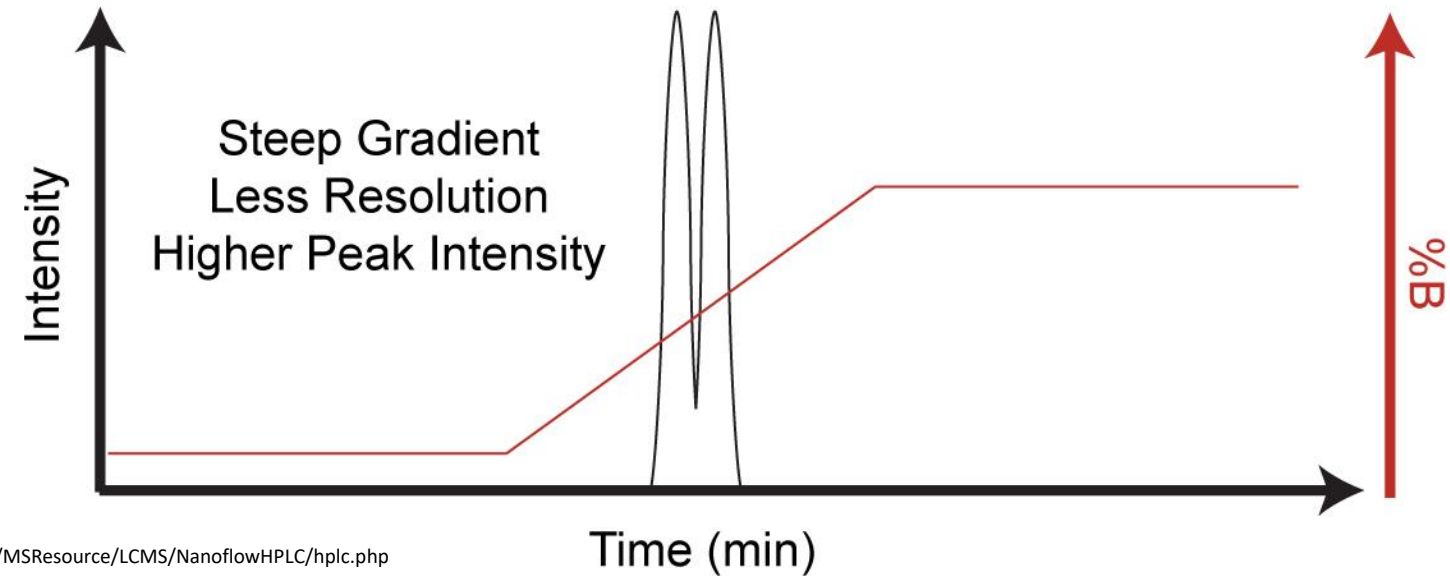
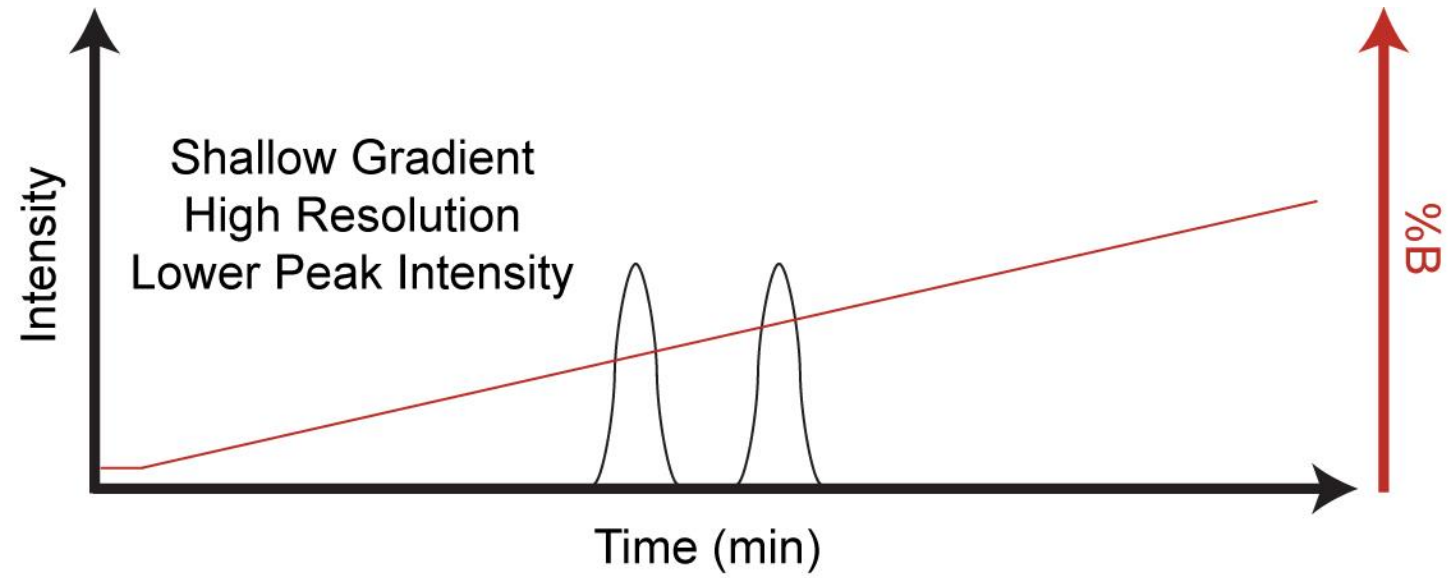


## Gradient et isocratique



[http://www.forumsci.co.il/HPLC/7\\_Isocratic\\_Gradient.html](http://www.forumsci.co.il/HPLC/7_Isocratic_Gradient.html)

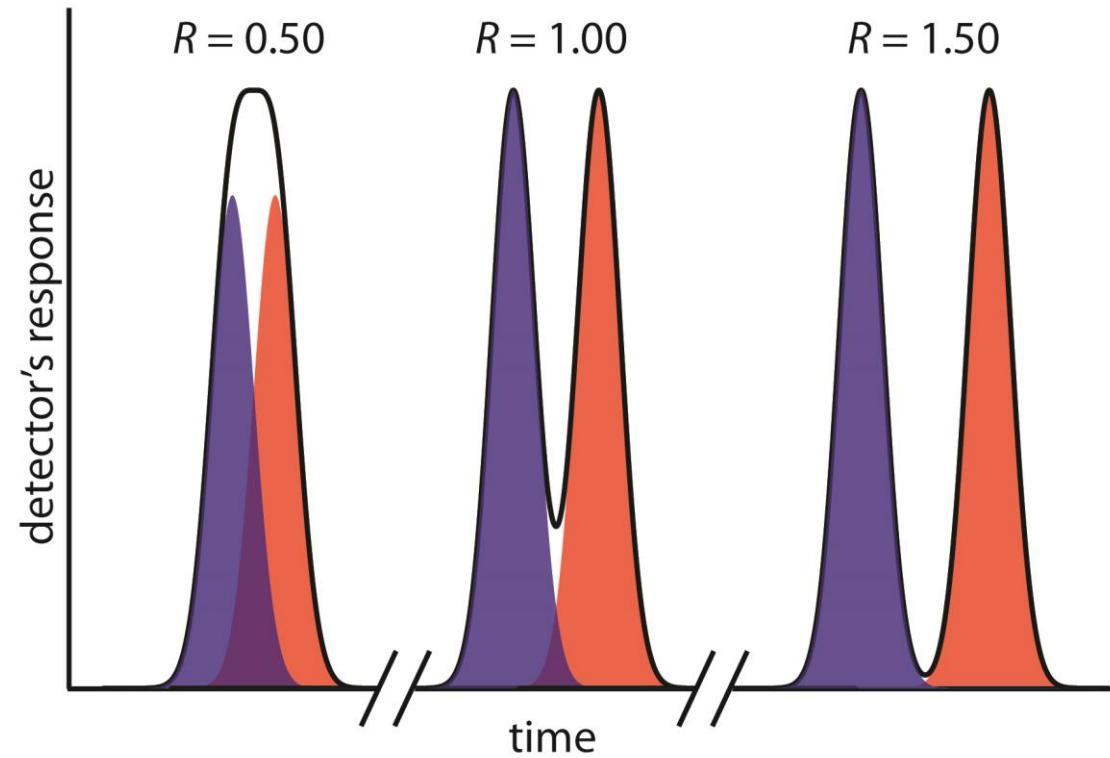
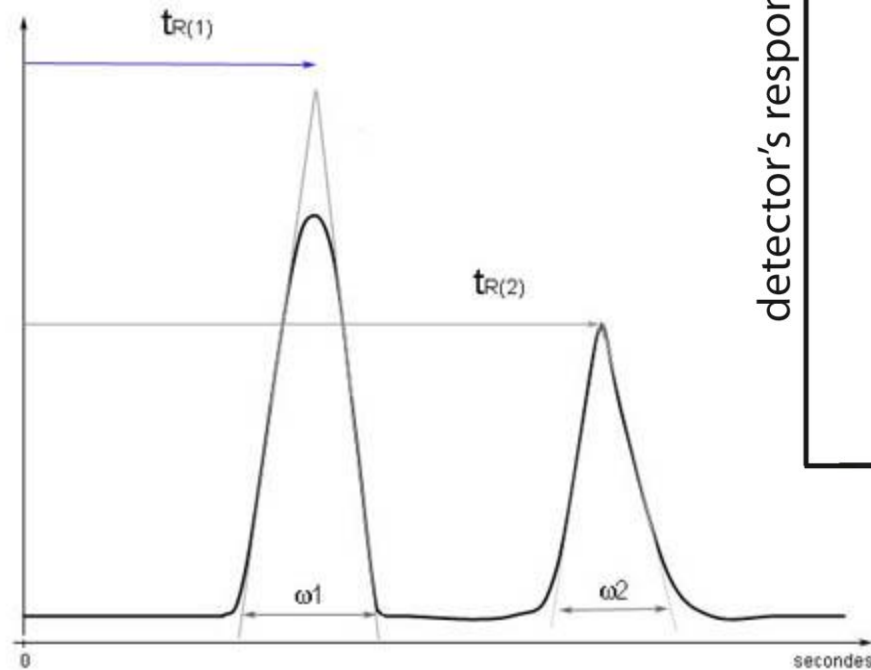




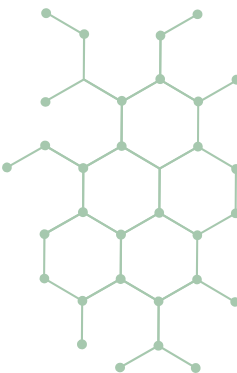


## Résolution chromatographique

$$R = 2 \frac{(t_{r2} - t_{r1})}{(\omega_1 + \omega_2)}$$



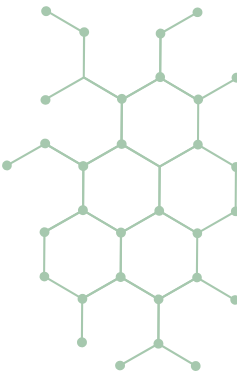
<http://www.lachimie.fr/analytique/chromatographie/facteur-resolution.php>



Chromatographie  
en phase  
gazeuse



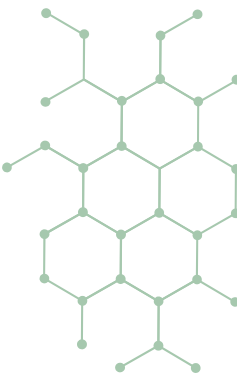
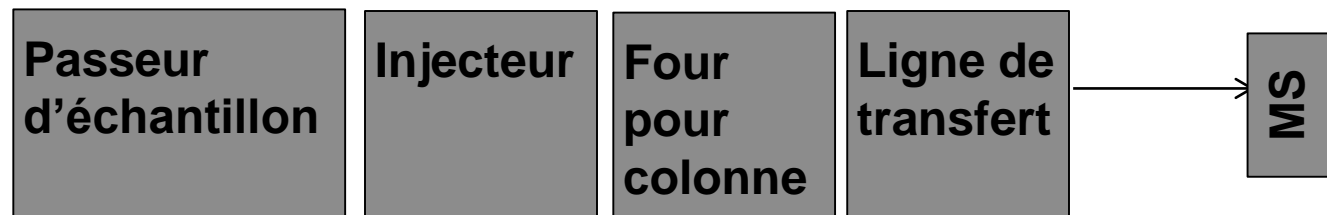
Chromatographie



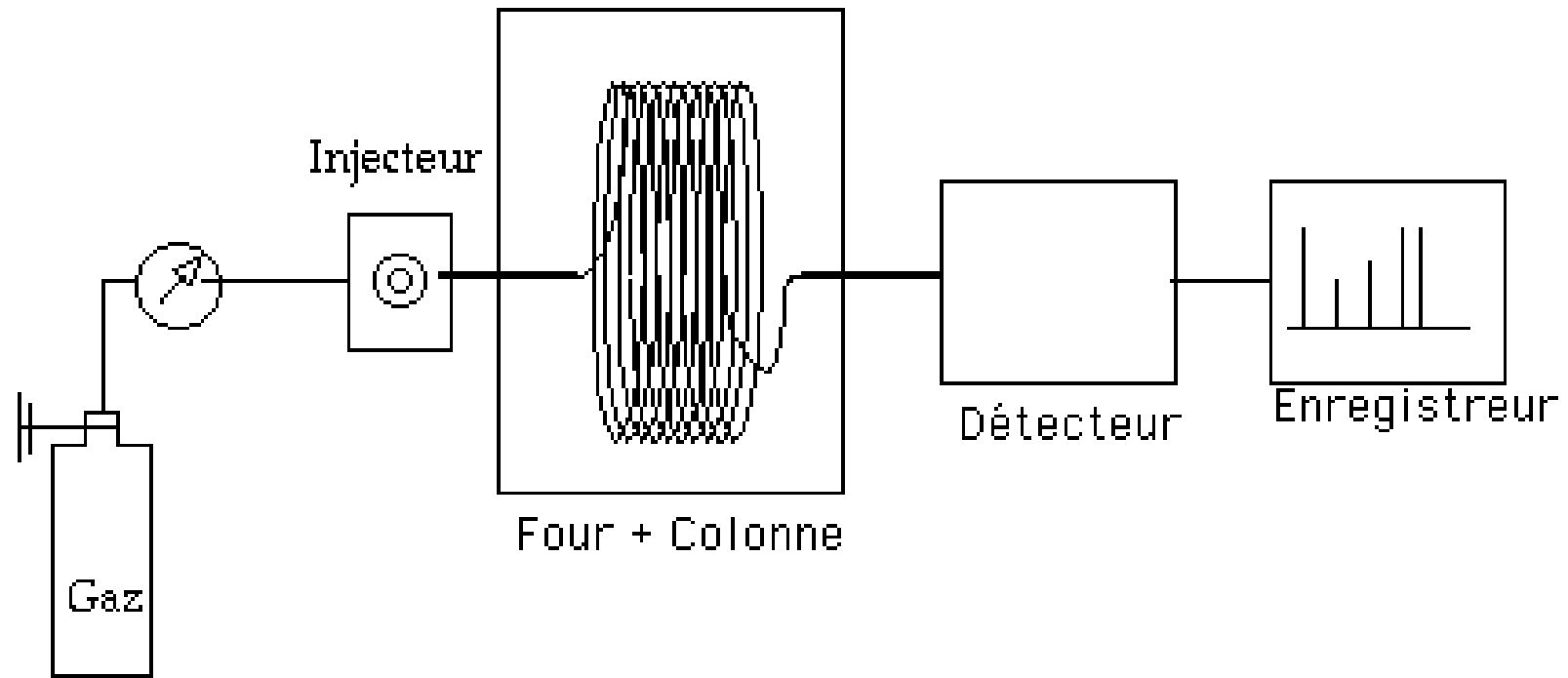
# Chromatographie en Phase Gazeuse



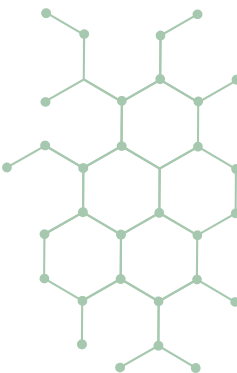
<http://autosamplervials.kinesis.co.uk/products/vials-caps/kx-lcgc-certified-vial-cap-kits-10#.VO3utPmG-FU>



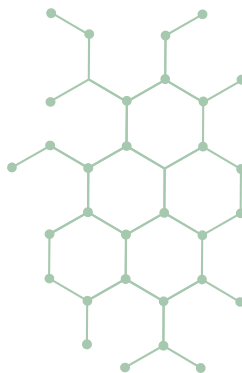
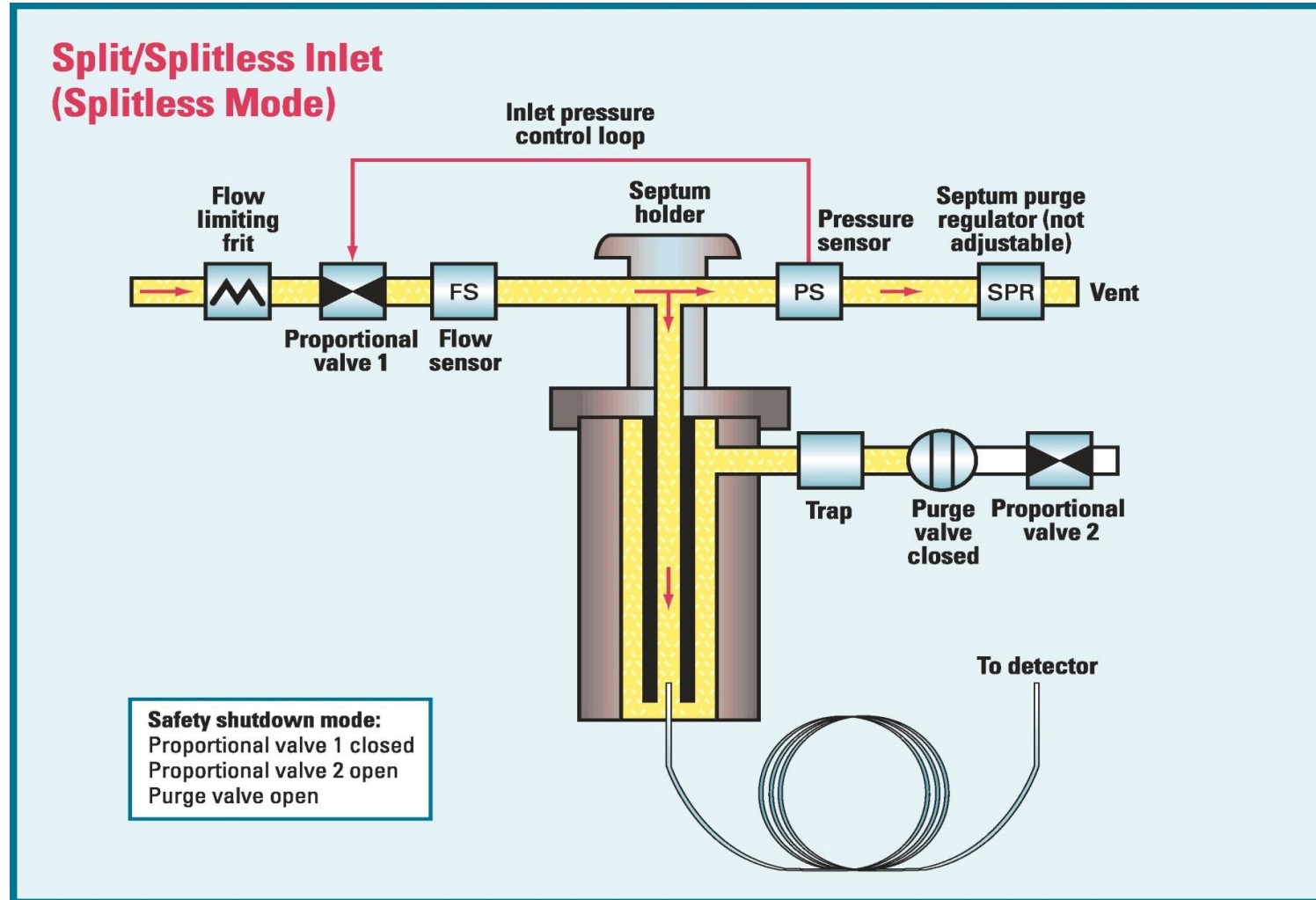
# Chromatographie en Phase Gazeuse

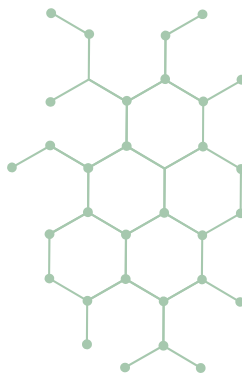
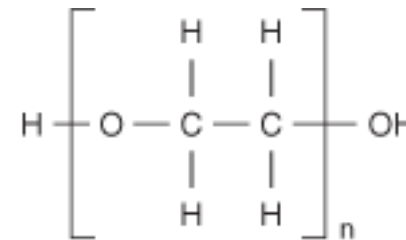
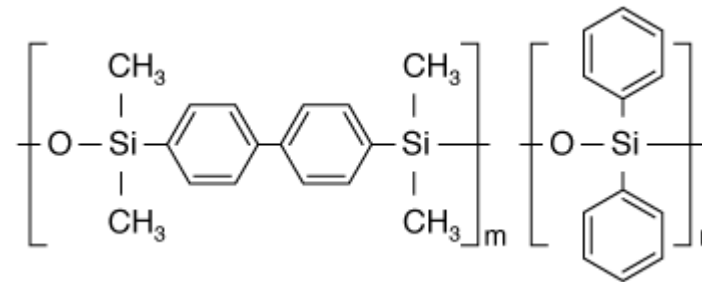
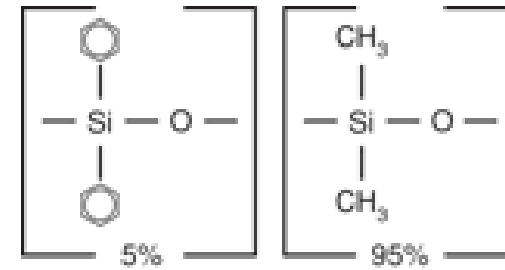
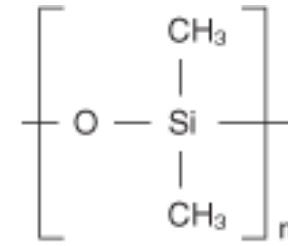
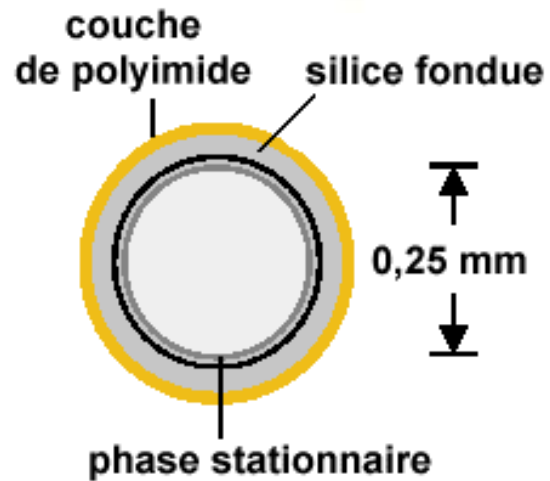


<http://www.snv.jussieu.fr/bmedia/lafont/chromato/A3.html>



# 6890/7890 Splitless Injector







## Etapes

### 1 - Injection avec le passeur automatique

Injecteur à 250 °C, expansion du solvant dans l'insert

### 2 – Dépôt de l'échantillon

Injecteur à 250 °C non splitté pendant 1 min

Four à 90 °C (Toluène)

Condensation en tête de colonne

### 3 – Séparation

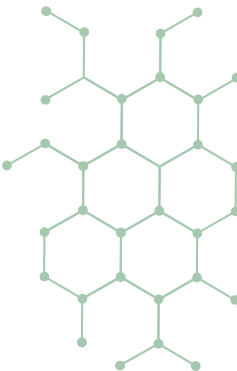
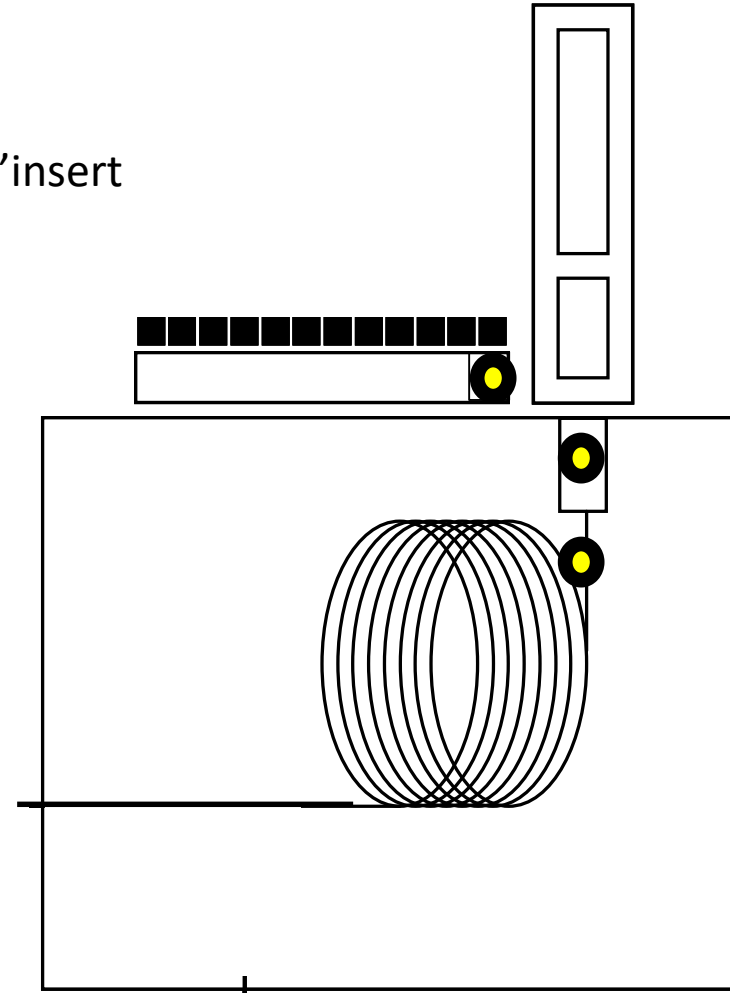
Injecteur à 250 °C en purge après 1 min

Four : T° ↗ : TR toluene

### 4 – Elution

Injecteur à 250 °C en purge après 1 min

Four : T° ↗ : TR Analyte

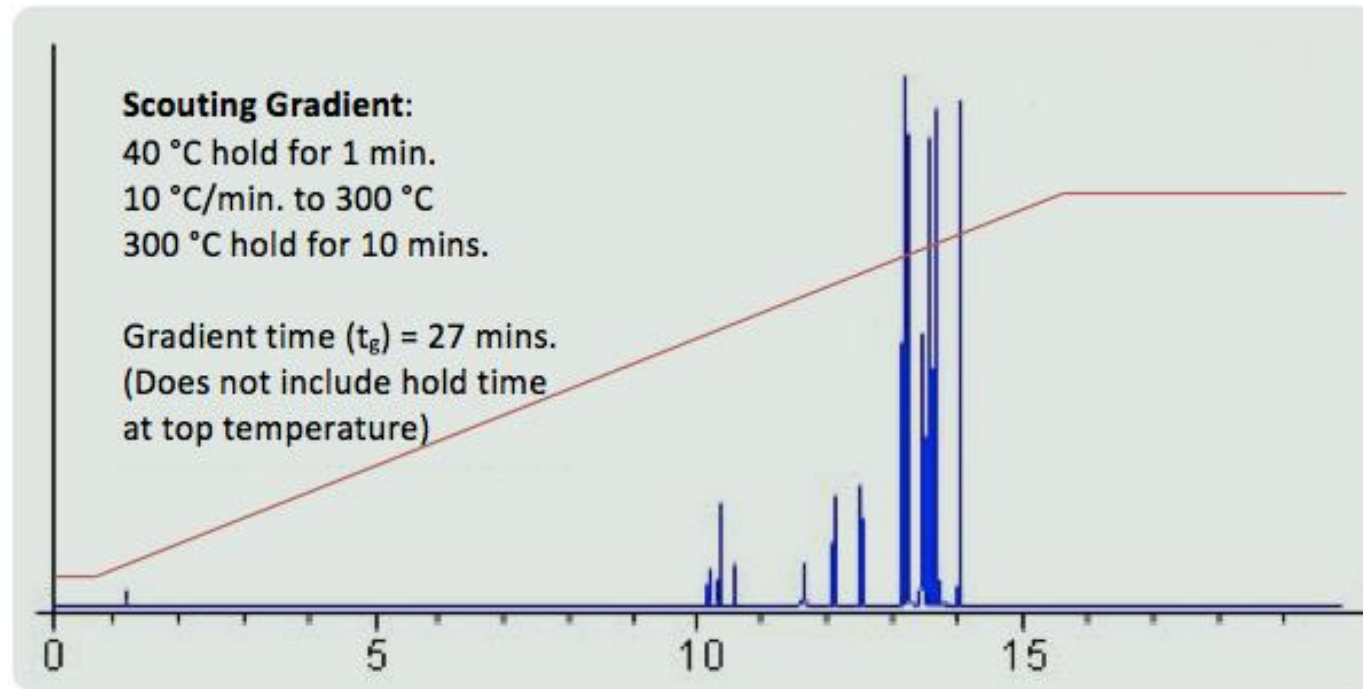


## Elution

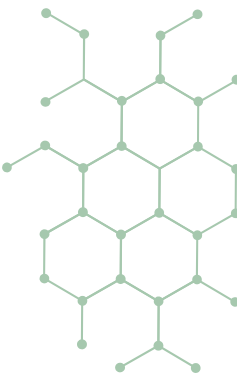
*Gaz vecteur : Helium, H<sub>2</sub> voire N<sub>2</sub>*

*Vitesse (vélocité) importante pour la séparation : dépend de la colonne*

## Gradient et isotherme

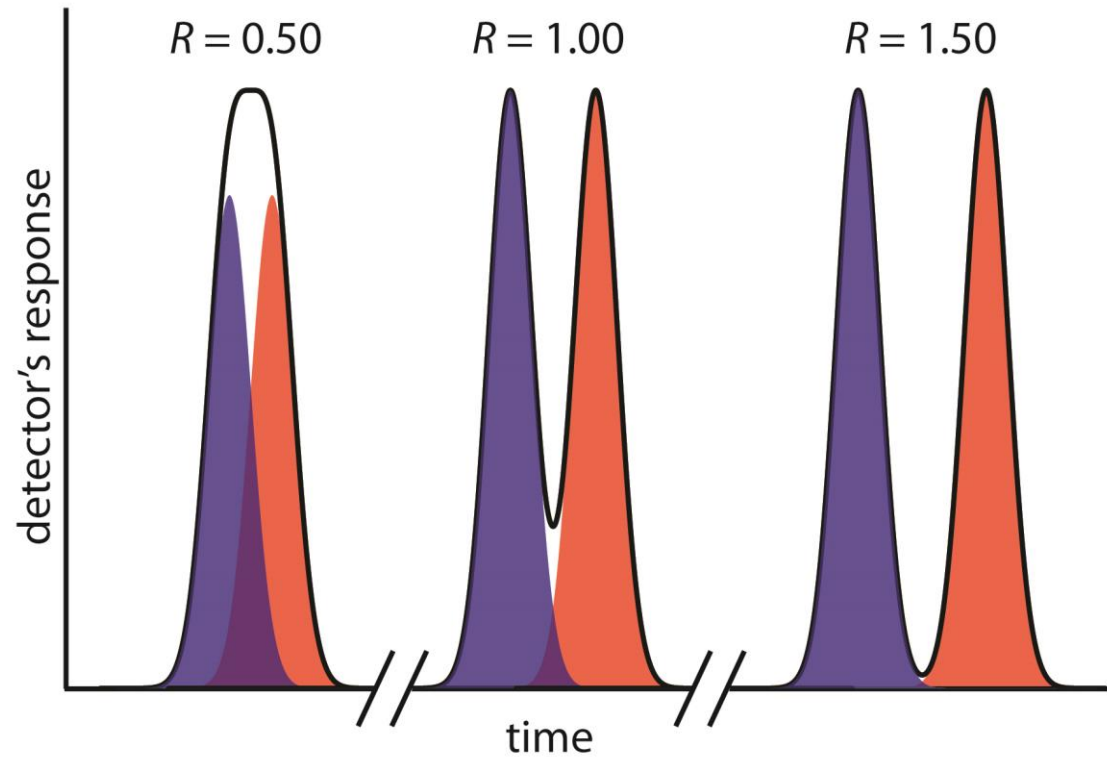
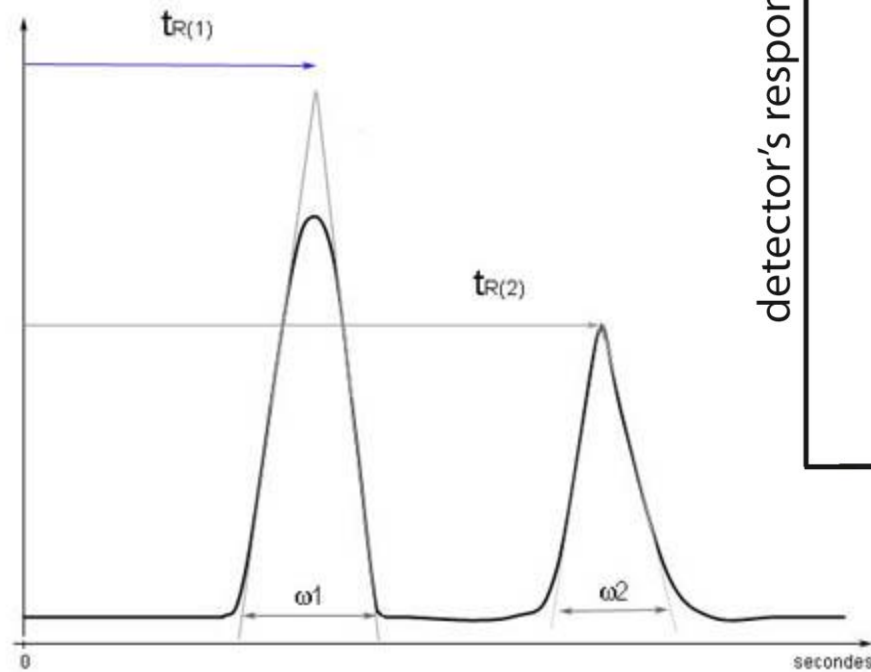


<http://www.chromacademy.com/chromatography-Optimising-GC-Temperature-Programming.html>

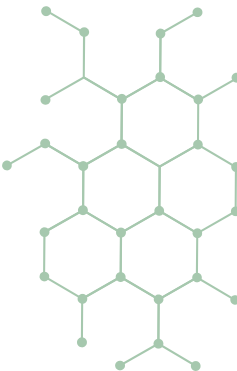


## Résolution chromatographique

$$R = 2 \frac{(t_{r2} - t_{r1})}{(\omega_1 + \omega_2)}$$



<http://www.lachimie.fr/analytique/chromatographie/facteur-resolution.php>



## HPLC

- Organic molecules, biomolecules, ions, polymers
- High pressure pumps
- UV/VIS (Photodiode array (PDA) and Mass spectrometry based)

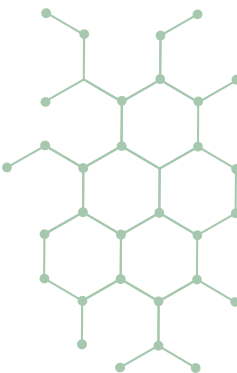
## GC

- Organic  
Inorganic Must be volatile
- Carrier Gas (i.e Helium, Nitrogen)
- FID ,TCD, MS, ECD, FPD, PID

## SFC

- Organic molecules
- Mobile phase = CO<sub>2</sub> + modifier
- High pressure pump + ABPR
- Same detectors as LC

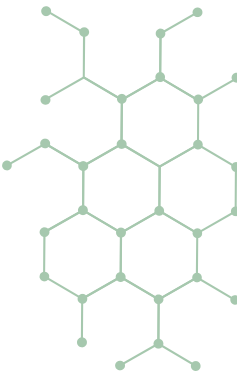
Dans des conditions identiques (colonne, température, débit), TR répétable



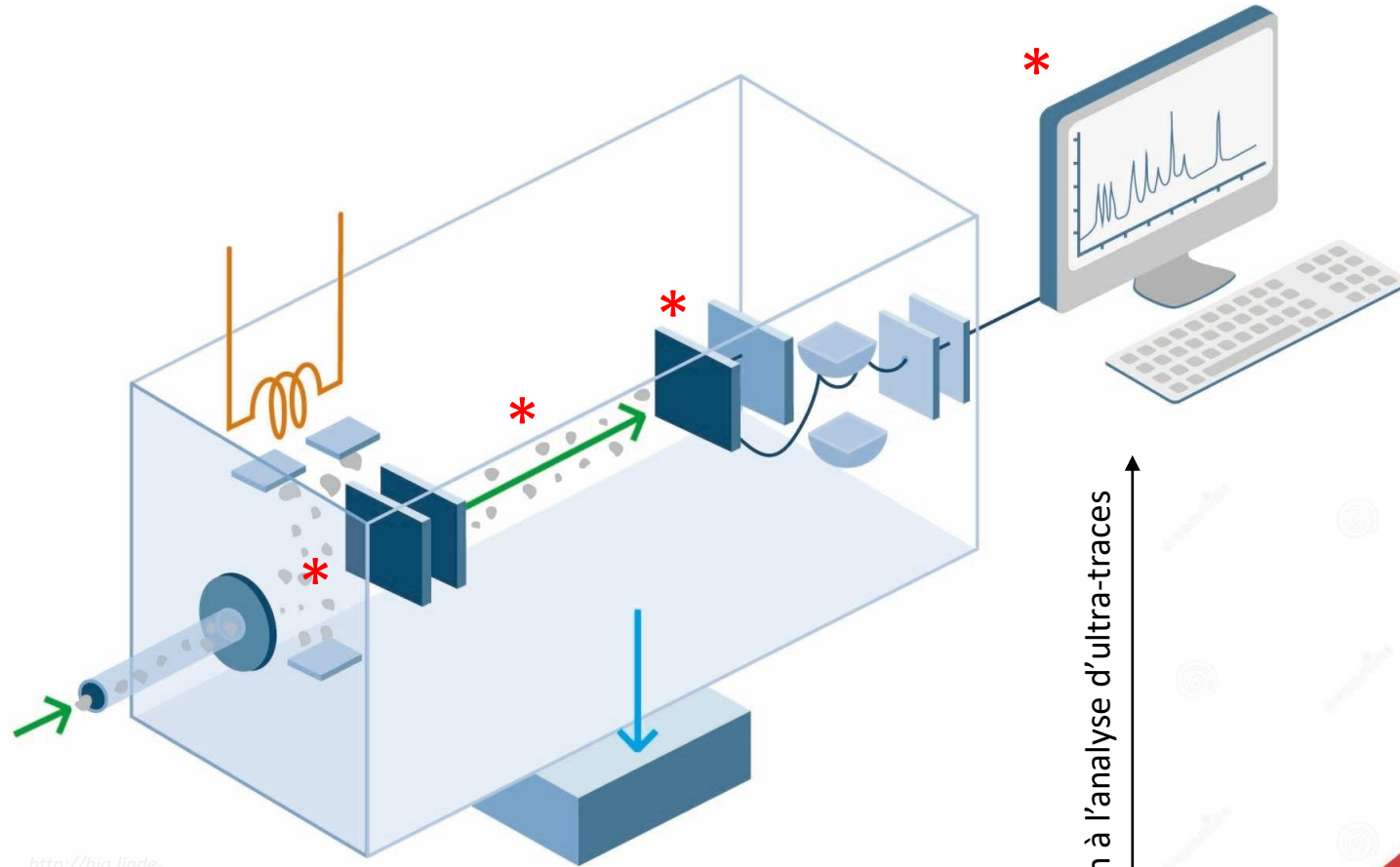
Couplage à la  
spectrométrie  
de masse



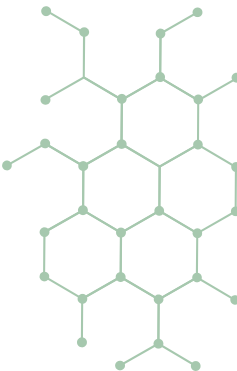
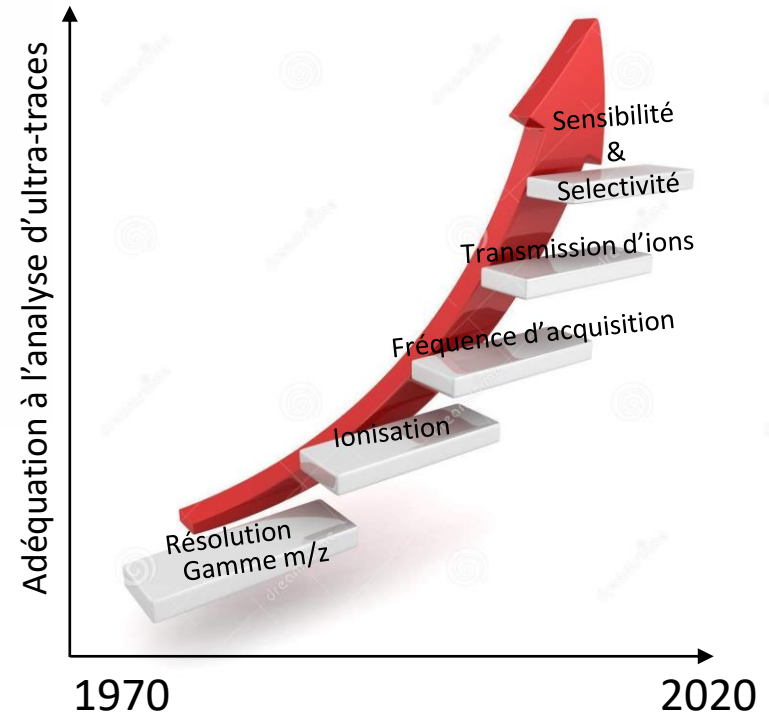
Chromatographie

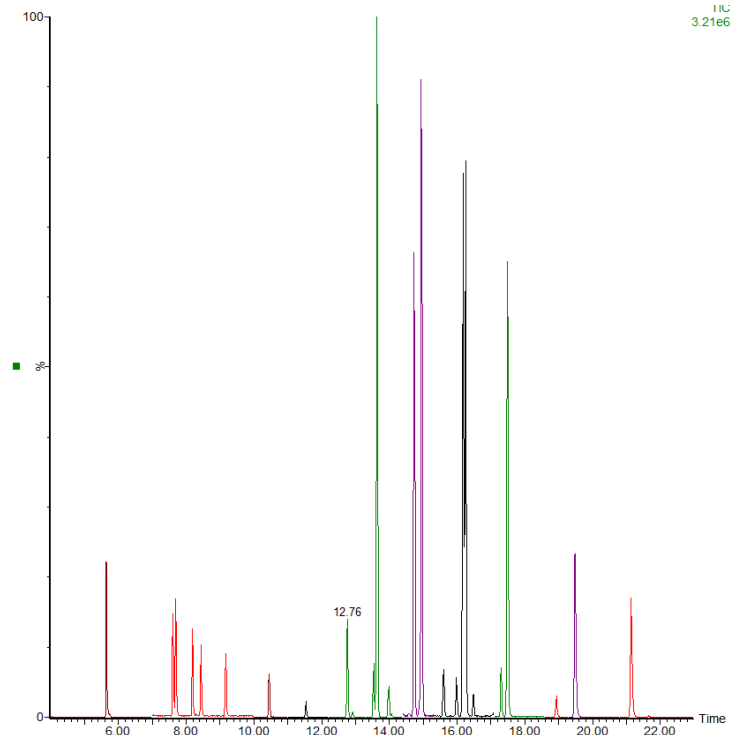
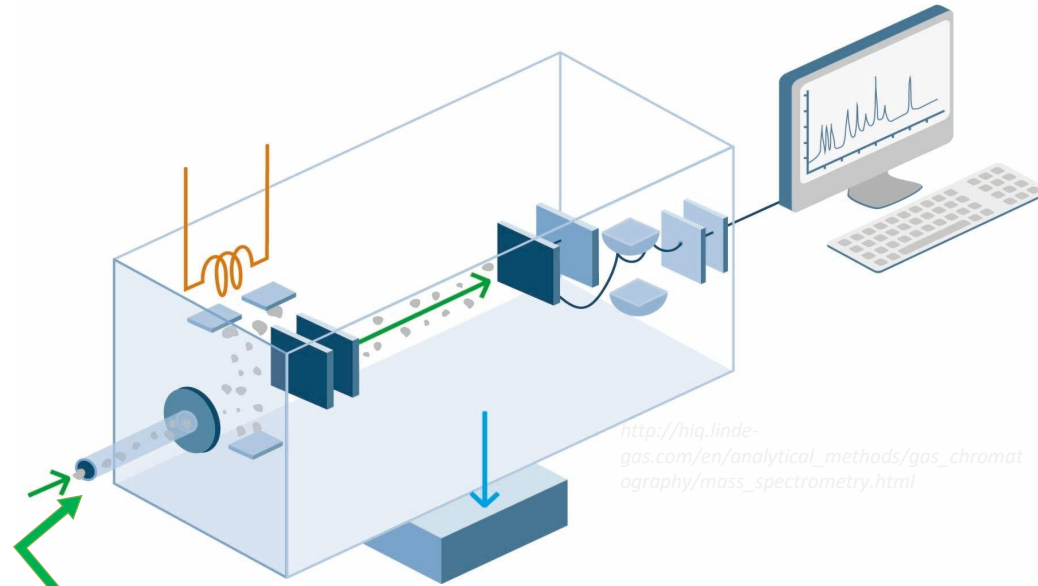


# Spectrométrie de masse



[http://hiq.linde-gas.com/en/analytical\\_methods/gas\\_chromatography/mass\\_spectrometry.html](http://hiq.linde-gas.com/en/analytical_methods/gas_chromatography/mass_spectrometry.html)



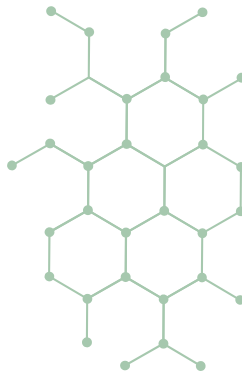


Matrices complexes

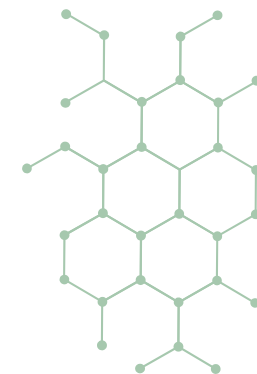
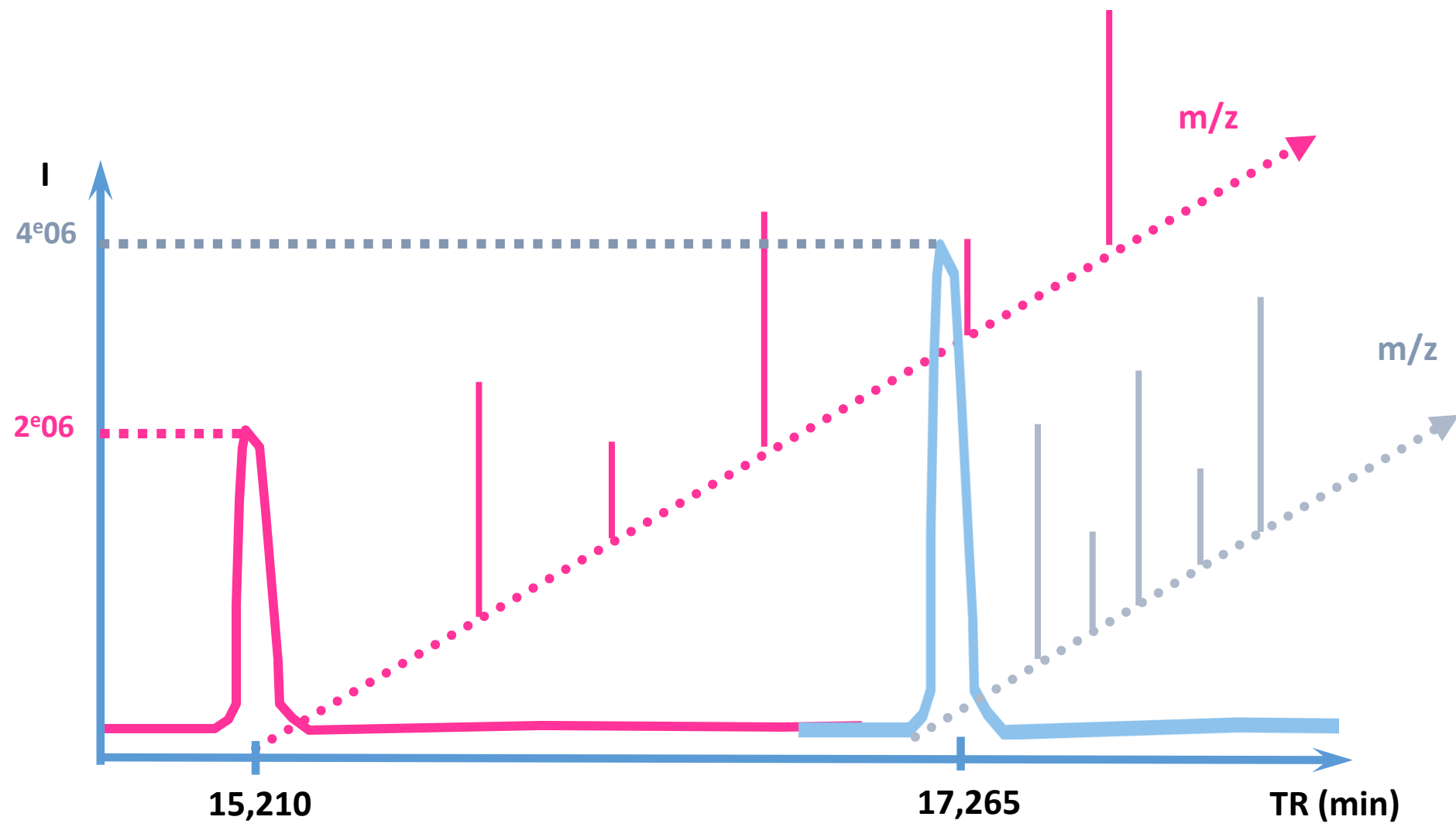
Sensibilité

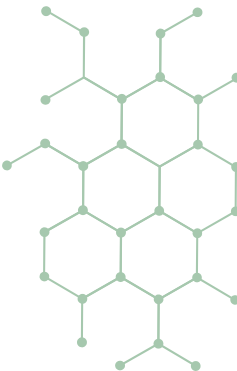
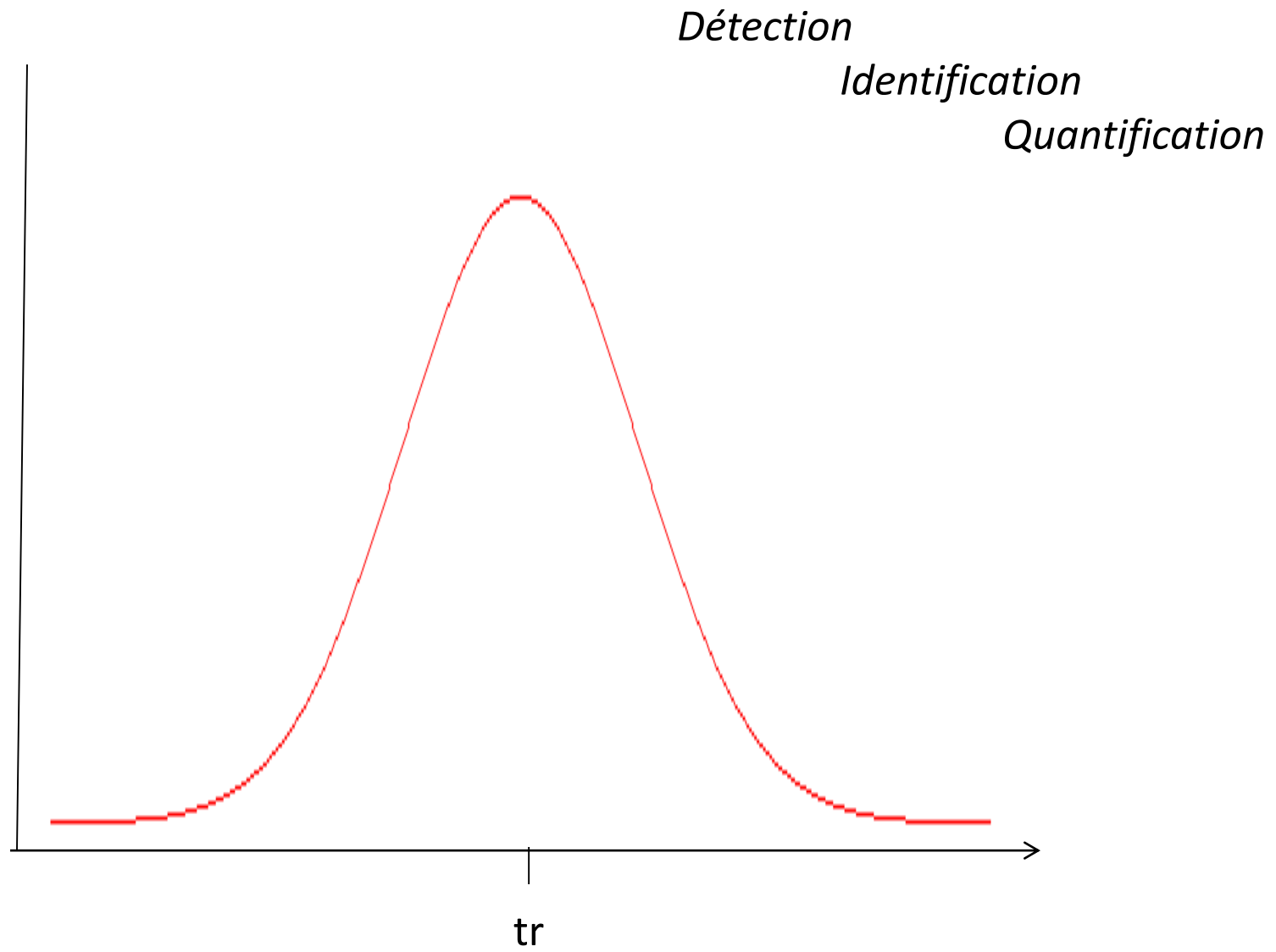


<https://c-marketing.eu/7-etapes-pour-simplifier-un-contenu-complexe/>









Chromatographie

Source d'ionisation

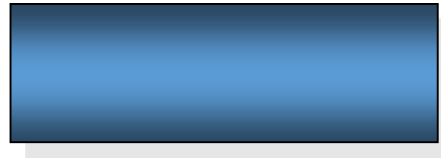
Analyseur

Détecteur

UHPLC

ESI

Quadripole



[M+H]<sup>+</sup>

600 m/z

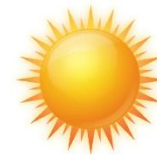
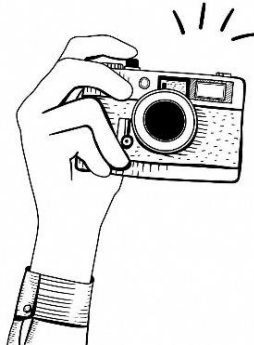
50 m/z

Scan

Ionisation

Chromatographie

Spectrométrie de Masse



Ionisation

UHPLC



Chromatographie

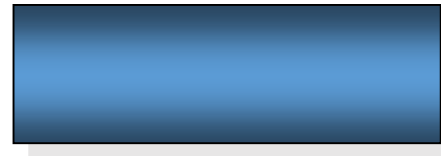
Source d'ionisation

Analyseur

Détecteur

ESI

Quadripole

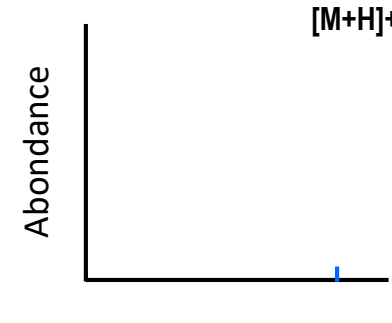
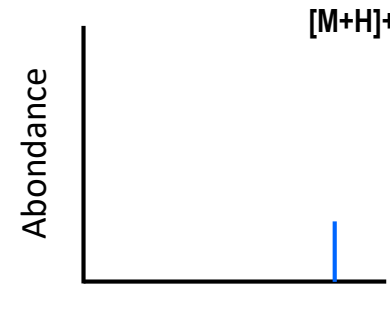
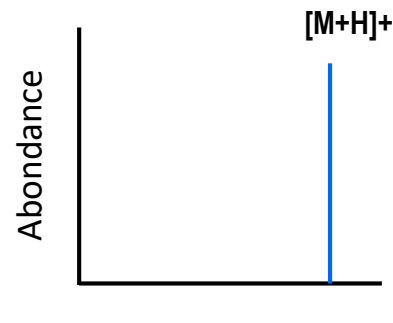
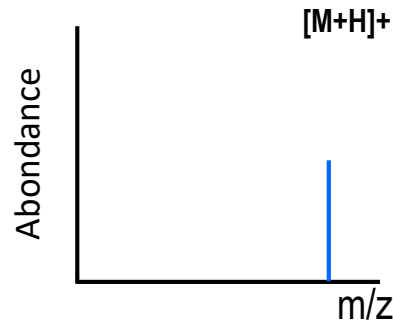
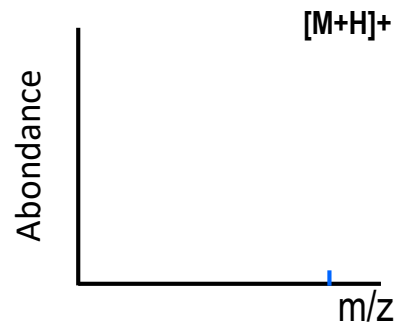
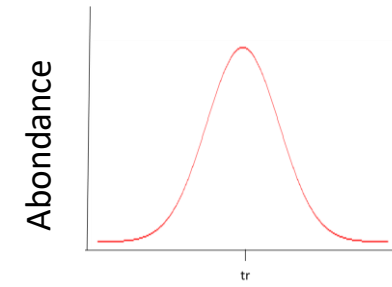
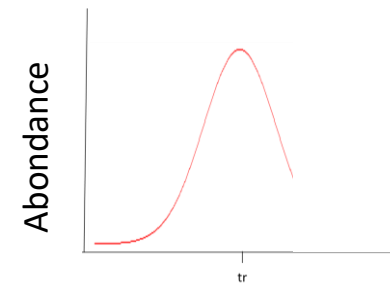
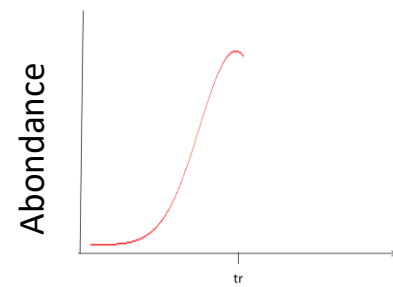
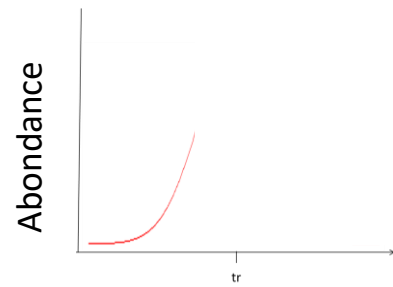
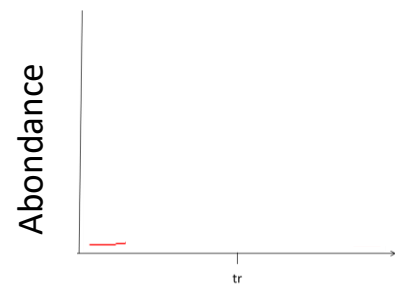


$[M+H]^+$

600 m/z

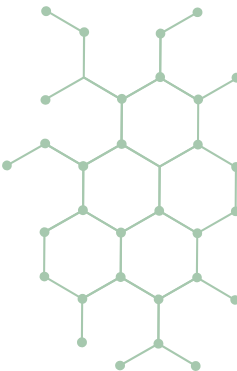
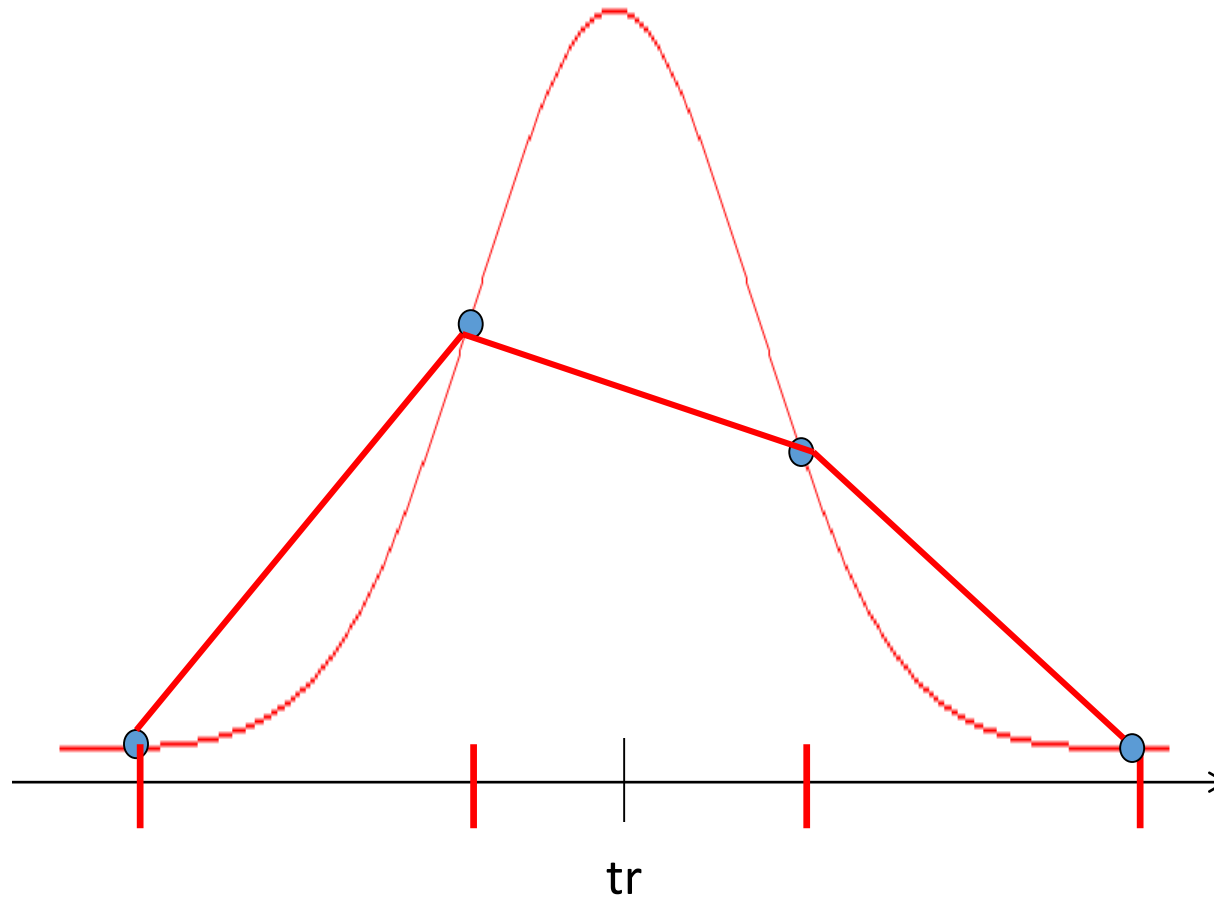
50 m/z

Scan



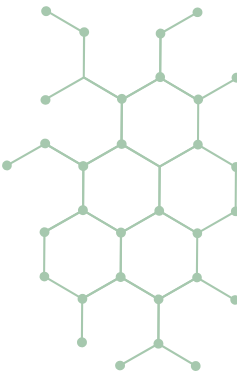
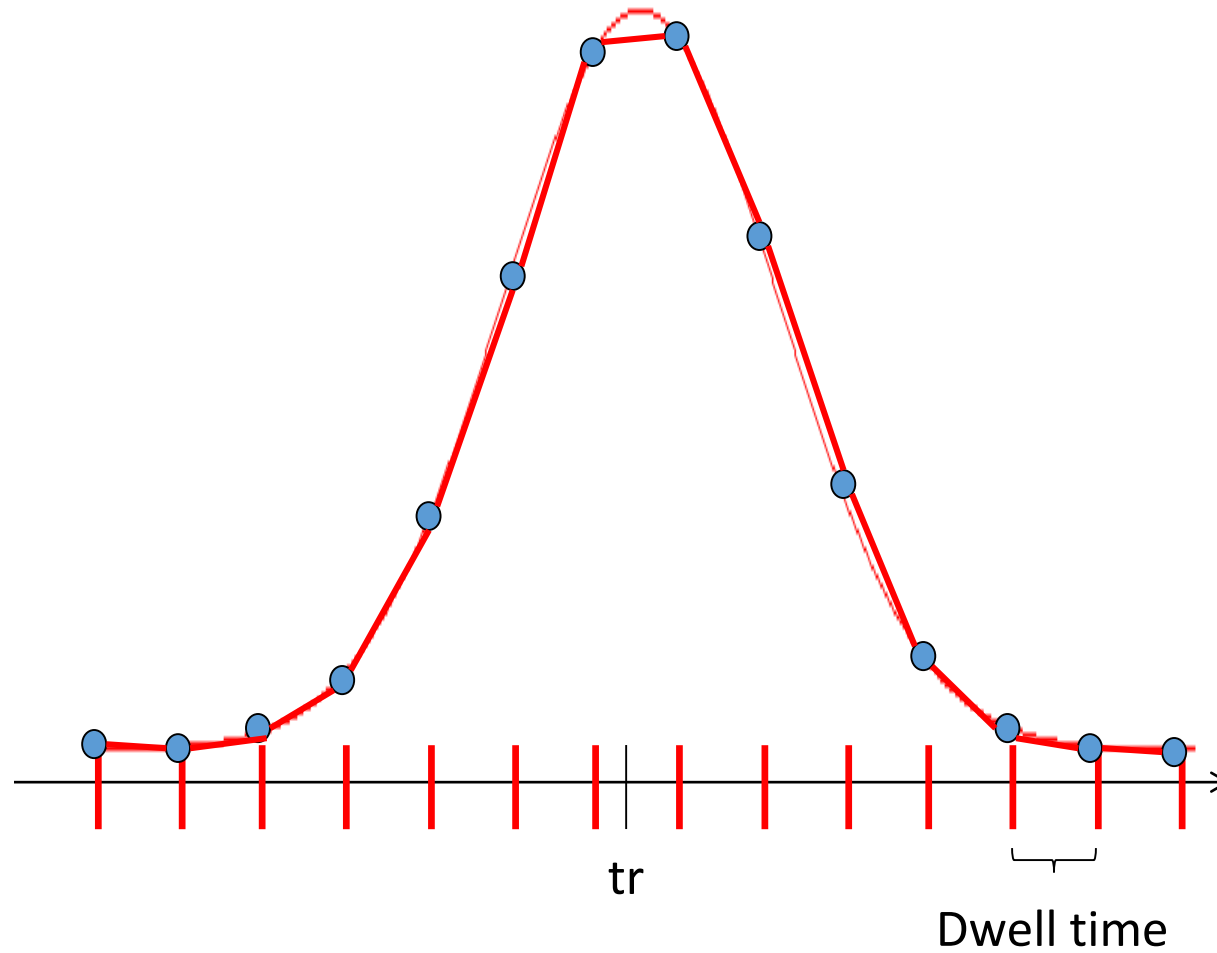
[M+H]<sup>+</sup> 

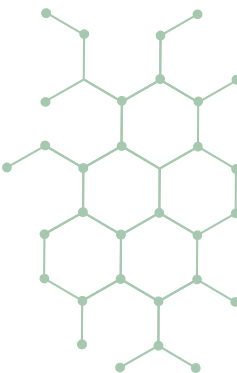
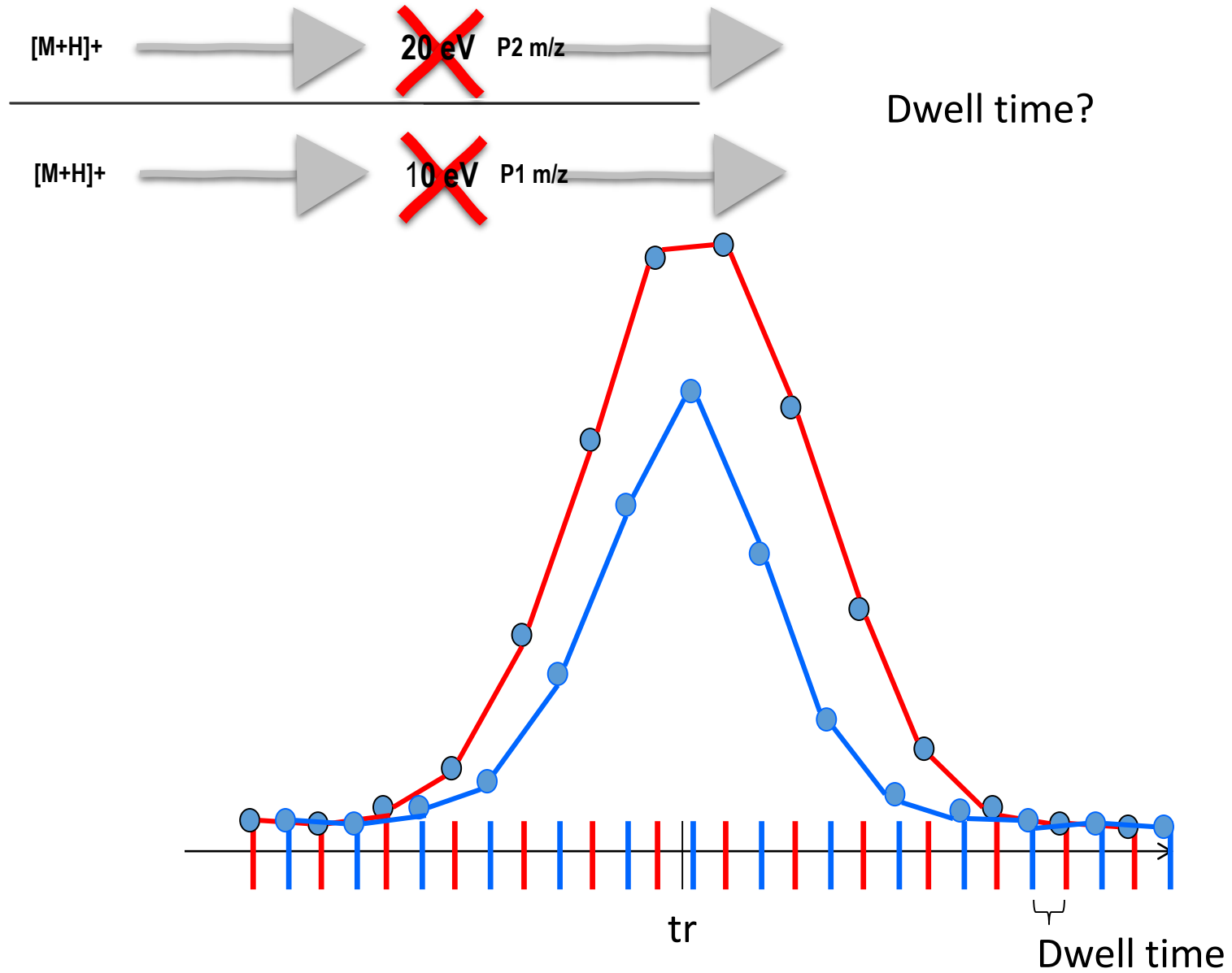
Pendant combien de temps ?



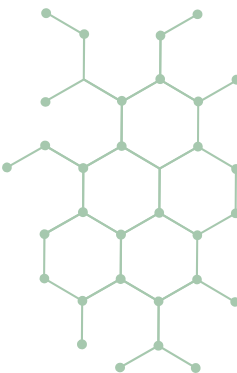
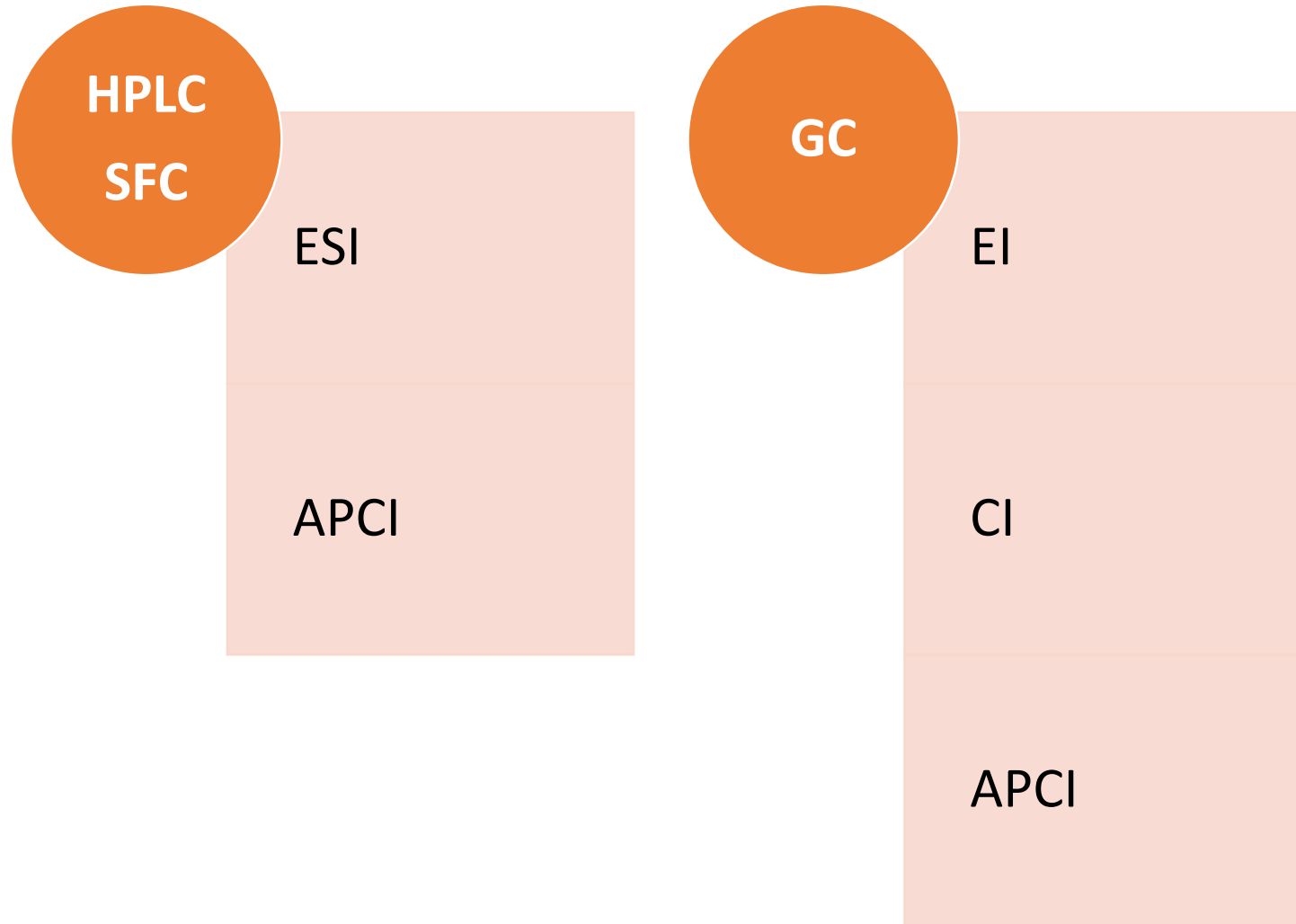
[M+H]<sup>+</sup> 

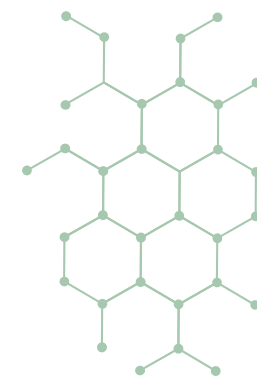
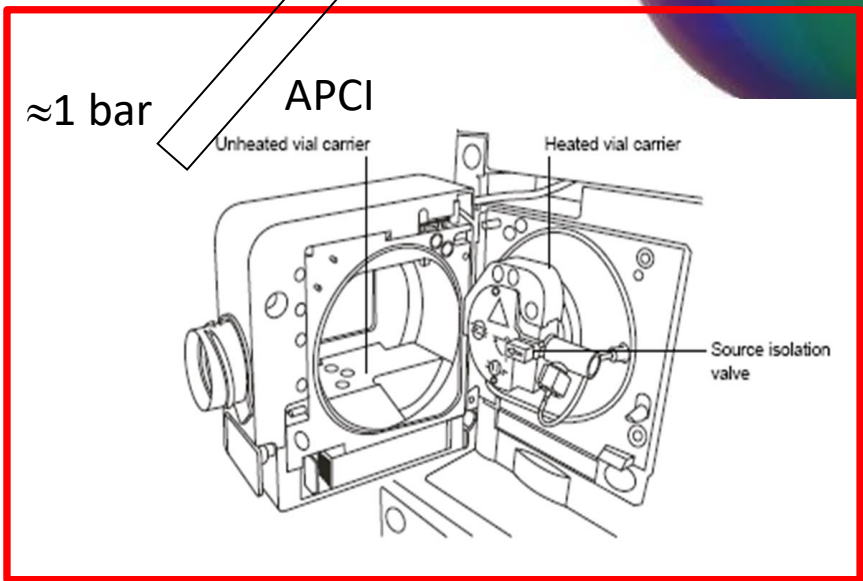
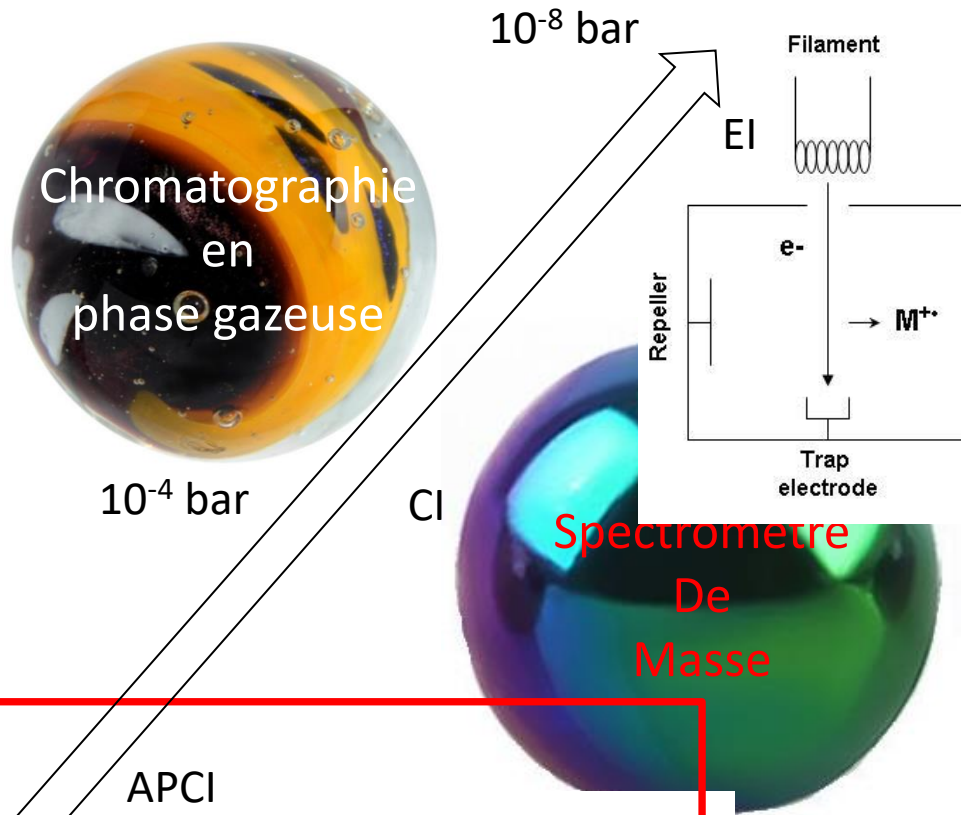
Pendant combien de temps ?







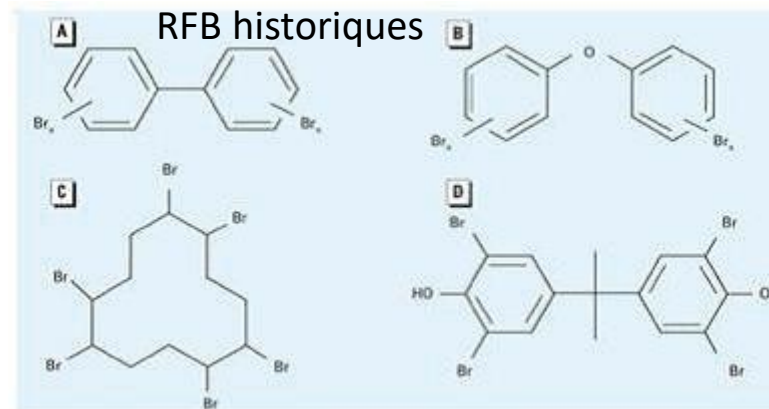




Substances chimiques **ignifuges** visant à inhiber ou retarder le processus de combustion.

Les retardateurs de flamme bromés (**RFB**) représentent **30%** des retardateurs de flammes utilisés en Europe

- Boîtiers électroniques et électriques (plastiques)
- Plaques de circuits imprimés, Fils et câbles
- Mobilier capitonné, matelas
- Tissus d'ameublement
- Transports (train, bateau, avion)
- Constructions
- Textiles
- Jouets

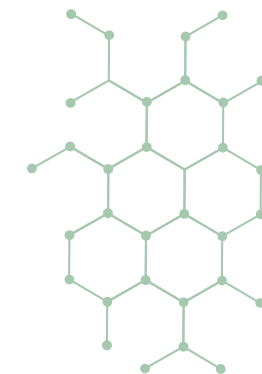
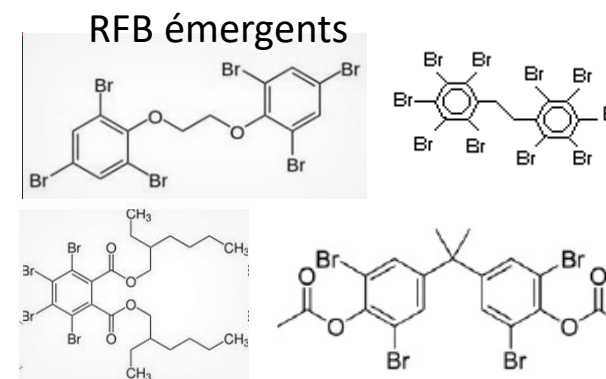


**Contaminants** de l'environnement , **bioaccumulables** dans la chaîne alimentaire

Au niveau alimentaire (données EFSA, 30 mai 2011)

- les poissons et autres fruits de mer ;
- les viandes et produits carnés ;
- les huiles végétales ;
- le lait et produits laitiers ;
- les oeufs et les ovoproduits.

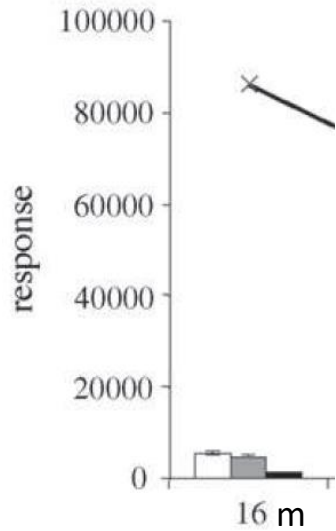
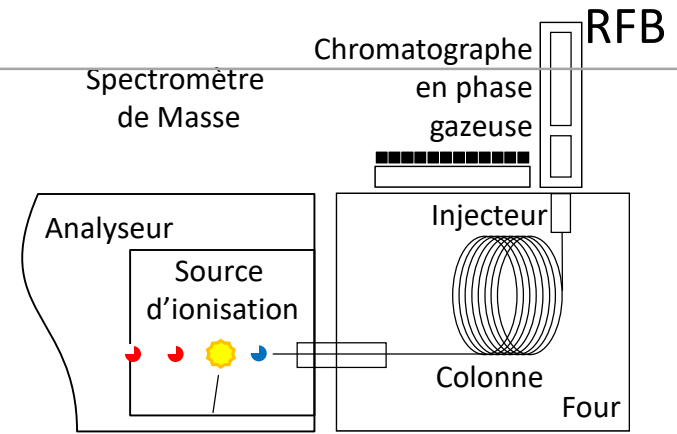
Différents types d'expositions : **alimentaire** et **occupationnelle**



Vers un gain en stabilité des RFB thermolabiles

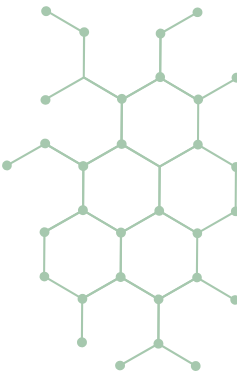
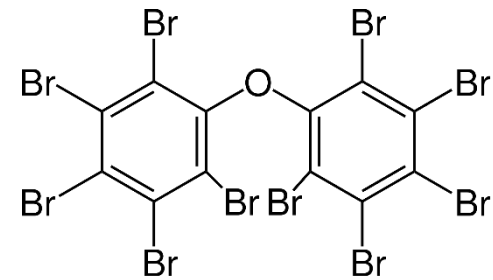
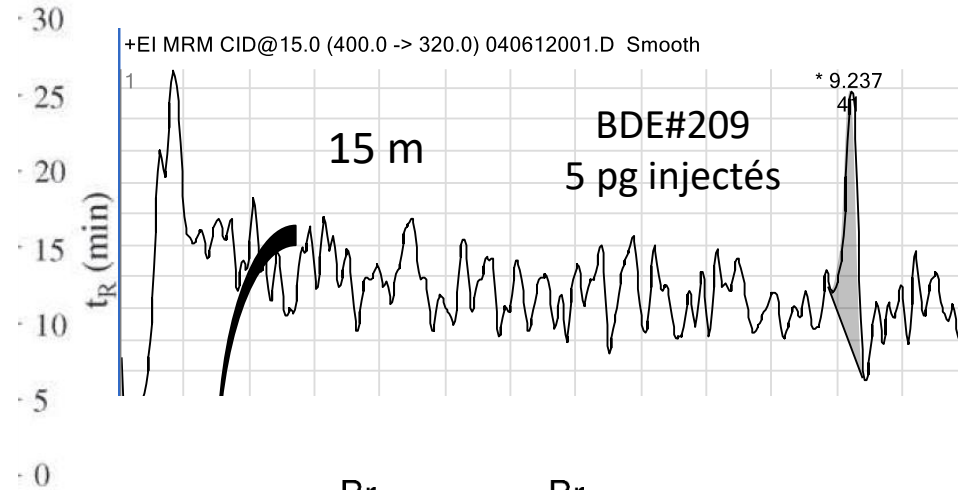
Colonne Rtx-5MS (0.25 mm i.d.; 0.25 µm f.t.)

A. Binelli et al. / J. Chromatogr. A 1136 (2006) 243–247



- BDE#209
- BB#209
- BDE#183

Colonne raccourcie



## GC/EI/HRMS (BE)



PBDE + nRFB

Run time : 45 min

DB5  
MS30 m x 0.25 mm, 0.25  $\mu$ m

OBIND+DecaBDE

Run time : 7.25 min

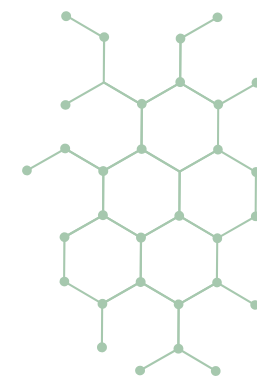
Rtx  
161415 m x 0.25 mm, 0.1  $\mu$ m

PBDE + nRFB + OBIND + DecaBDE

Run time : 13.6 min

Optima  
52.5 m x 0.1 mm, 0.1  $\mu$ m

## GC/APCI/MS/MS



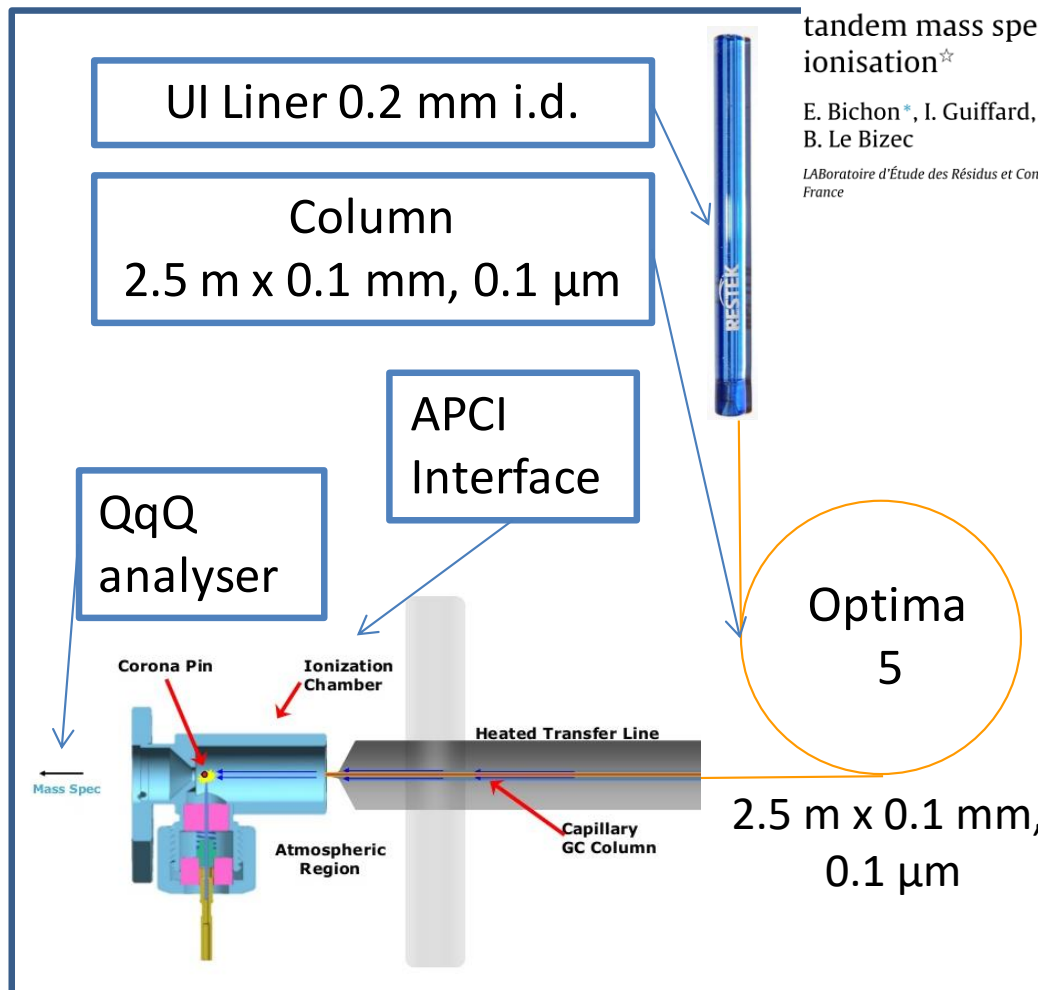


Simultaneous determination of 16 brominated flame retardants in food and feed of animal origin by fast gas chromatography coupled to tandem mass spectrometry using atmospheric pressure chemical ionisation<sup>☆</sup>



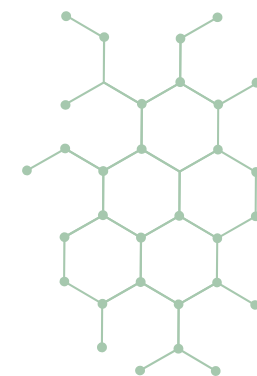
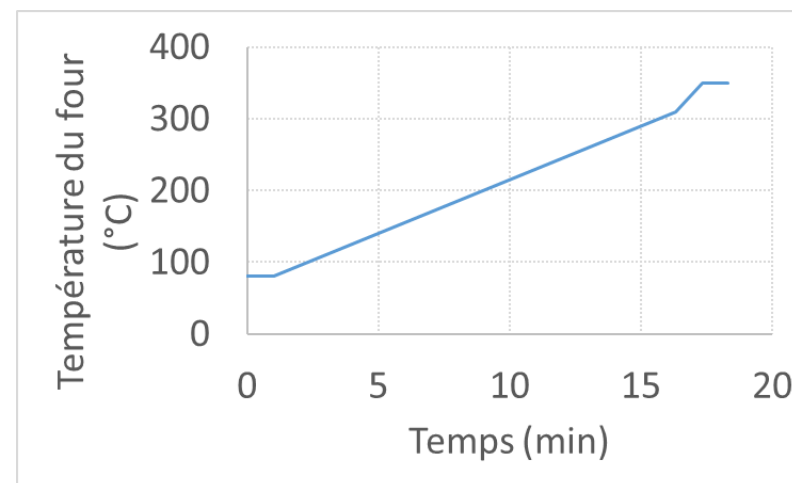
E. Bichon<sup>\*</sup>, I. Guiffard, A. Vénisseau, E. Lesquin, V. Vaccher, A. Brosseaud, P. Marchand, B. Le Bizec

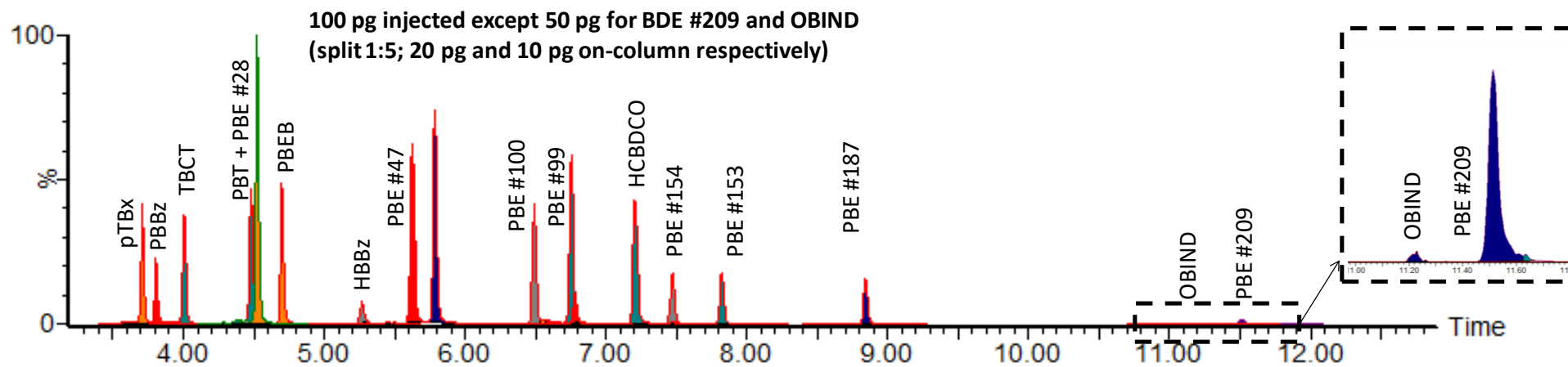
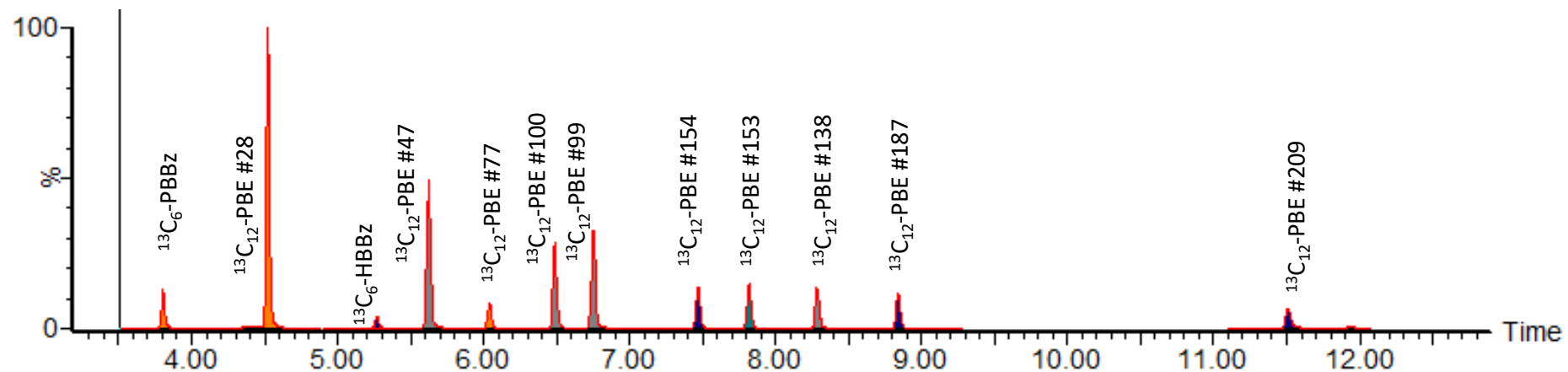
LABoratoire d'Étude des Résidus et Contaminants dans les Aliments (LABERCA), USC INRA 1329, Oniris, LUNAM Université, BP 50707, 44307 Nantes Cedex 3, France



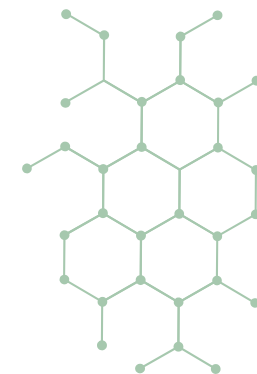
### GC/APCI/MS

Plused Split mode (0,85 min @ 40 psi, split 1:5, 1 μL injecté). T° injecteur: 275 °C  
 Gaz vecteur (hélium) : débit constant (0.48 mL min<sup>-1</sup>).

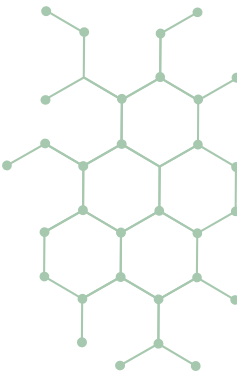
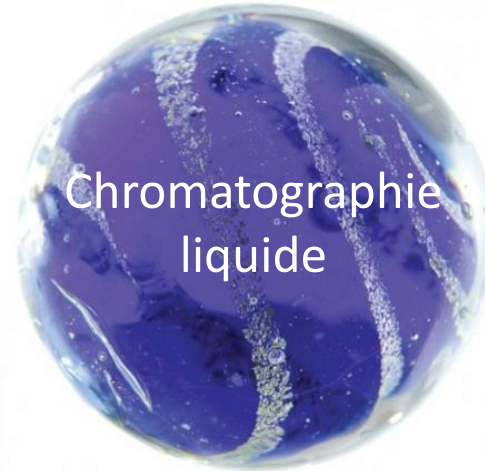




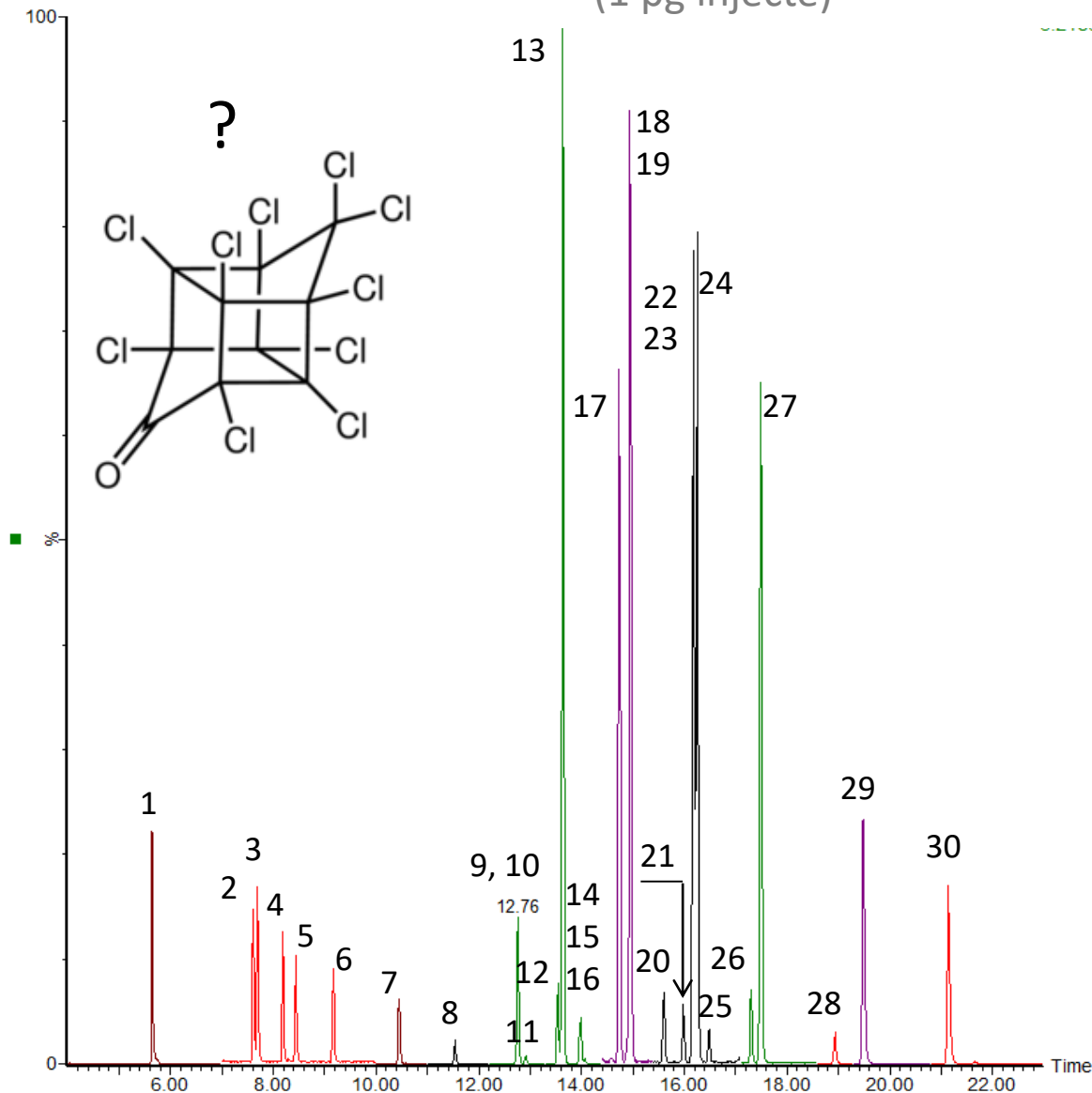
Chromatogramme d'ions extraits d'un mélange de 16 RFB natifs (en bas)  
et de leurs homologues marqués (en haut).



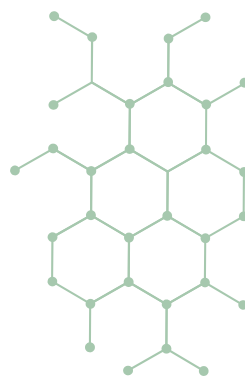


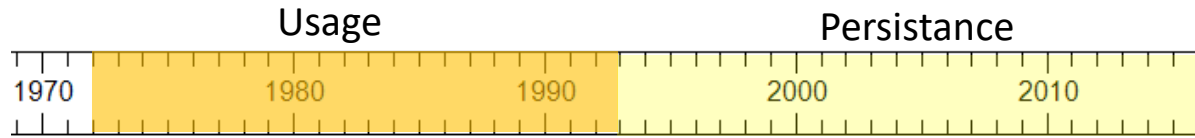


Analyse simultanée de 30 pesticides  
(1 pg injecté)



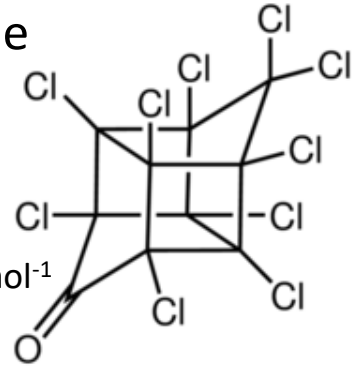
- 1) pentachlorobenzene
- 2)  $\alpha$ -HCH
- 3) HCB
- 4)  $\beta$ -HCH
- 5)  $\gamma$ -HCH (=lindane)
- 6)  $\delta$ -HCH
- 7) heptachlor
- 8) aldrin
- 9) oxychlordane
- 10) heptachlor epoxide cis
- 11) heptachlor epoxide trans
- 12)  $\gamma$ -chlordane (=trans)
- 13) o,p'-DDE
- 14)  $\alpha$ -chlordane (=cis)
- 15)  $\alpha$ -endosulfan
- 16) trans-nonachlor
- 17) p,p'-DDE
- 18) dieldrin
- 19) o,p'-DDD
- 20) endrin
- 21)  $\beta$ -endosulfan
- 22) cis-nonachlor
- 23) p,p'-DDD
- 24) o,p'-DDT
- 25) endrin aldehyde
- 26) endosulfan sulfate
- 27) p,p'-DDT
- 28) endrin ketone
- 29) methoxychlor
- 30) mirex (=perchlordecone)





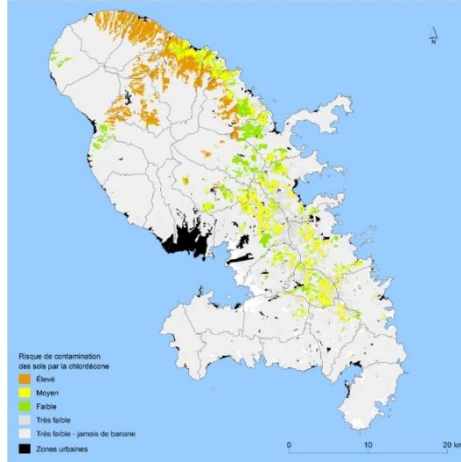
## Chlordécone

$C_{10}H_{10}O$   
 MM = 490,6 g.mol<sup>-1</sup>  
 Log K<sub>ow</sub>=4,5



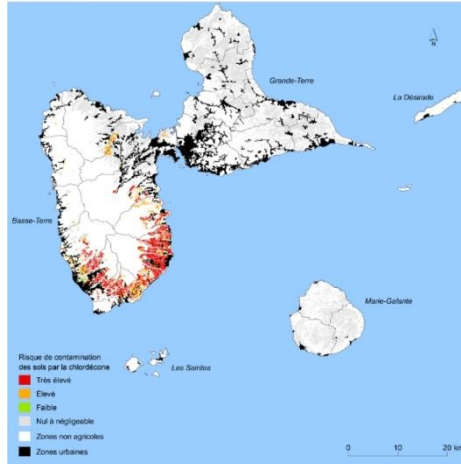
Sensibilité  
 Molécule difficile

Les sols potentiellement pollués par la chlordécone en Martinique

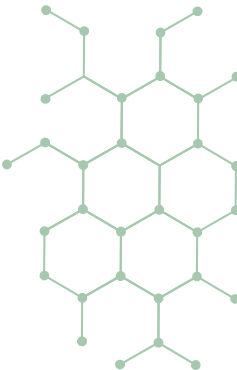


Source : SIG DIREN Martinique – SIG 972 – Chambre d'Agriculture, 2007. Traitements : SOeS, 2013.

Les sols agricoles potentiellement pollués par la chlordécone en Guadeloupe



Source : Source : DAAF Guadeloupe – 2005. Traitements : SOeS, 2013.





RAPID COMMUNICATIONS IN MASS SPECTROMETRY

*Rapid Commun. Mass Spectrom.* 2004; **18**: 1243–1244

Published online in Wiley InterScience (www.interscience.wiley.com)

RCM  
**Letter to the Editor**

To the Editor-in-Chief  
 Sir,

**Detection of chlordecone by liquid chromatography with tandem mass spectrometry**

decone i  
 media and  
 develop :  
 to comple  
 GC. We h  
 method fo  
 chromatog  
 spectromé  
 MS/MS t  
 to simpli  
 ment, and  
 tion of ch  
 samples.

Chlorde  
 Wako P  
 (Osaka. Ie



Journal of Chromatography A, 1408 (2015) 169–177

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: [www.elsevier.com/locate/chroma](http://www.elsevier.com/locate/chroma)

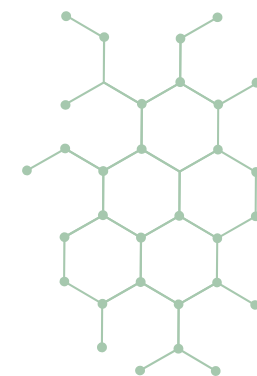
Ultra-trace quantification method for chlordecone in human fluids and tissues

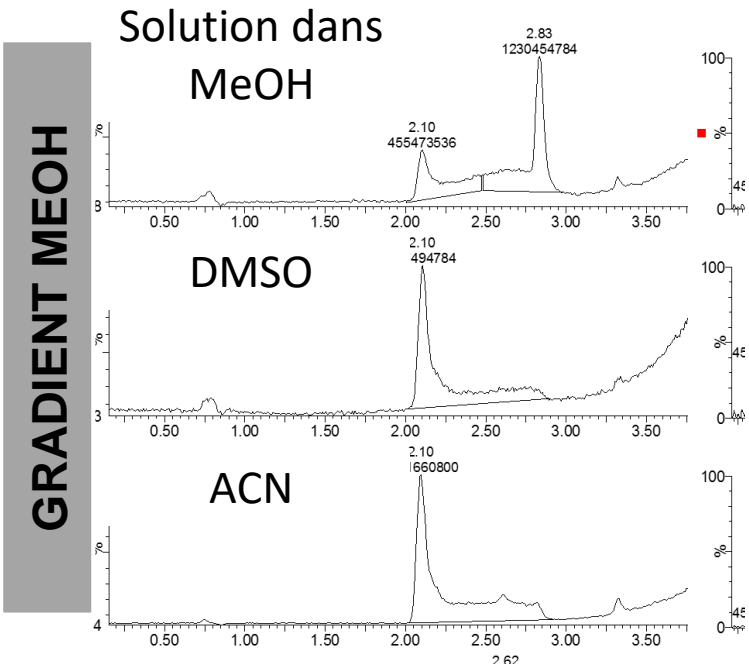
Emmanuelle Bichon<sup>a,\*</sup>, Ingrid Guiffard<sup>a</sup>, Anaïs Vénisseau<sup>a</sup>, Philippe Marchand<sup>a</sup>, Jean-Philippe Antignac<sup>a,b</sup>, Bruno Le Bizec<sup>a</sup>

<sup>a</sup> LUNAM Université, Oniris, Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), Nantes F-44307, France

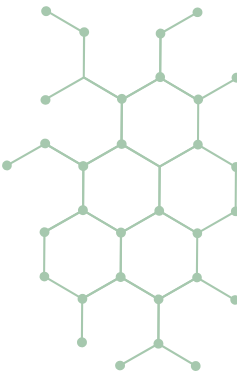
<sup>b</sup> INRA, Nantes F-44307, France

GC column (l. in m, i.d. in mm, f.t.in μm)	Instrument	Pitfalls
RTX-1614 (15/0.25/0.10)	GC-EI-HRMS (JEOL 800D)	Coelution not studied
DB5MS (30/0.25/0.25)	GC-APCI-MS/MS (WATERS APGC)	peak tailing on chlordecone coelution on 3 couples of OCPs
HT8-PCB (60/0.25/0.25)	GC-EI-HRMS (JEOL 800D)	peak tailing on chlordecone coelution chlordecone and cis-nonachlor
Optima-17MS (30/0.25/0.25) and (30/0.25/0.15)	GC-EI-MS (Agilent 5973)	peak tailing on chlordecone coelution chlordecone and endrin

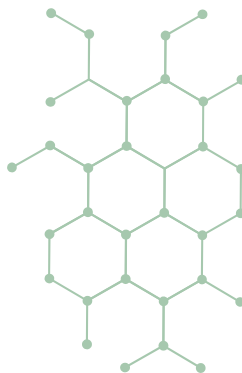
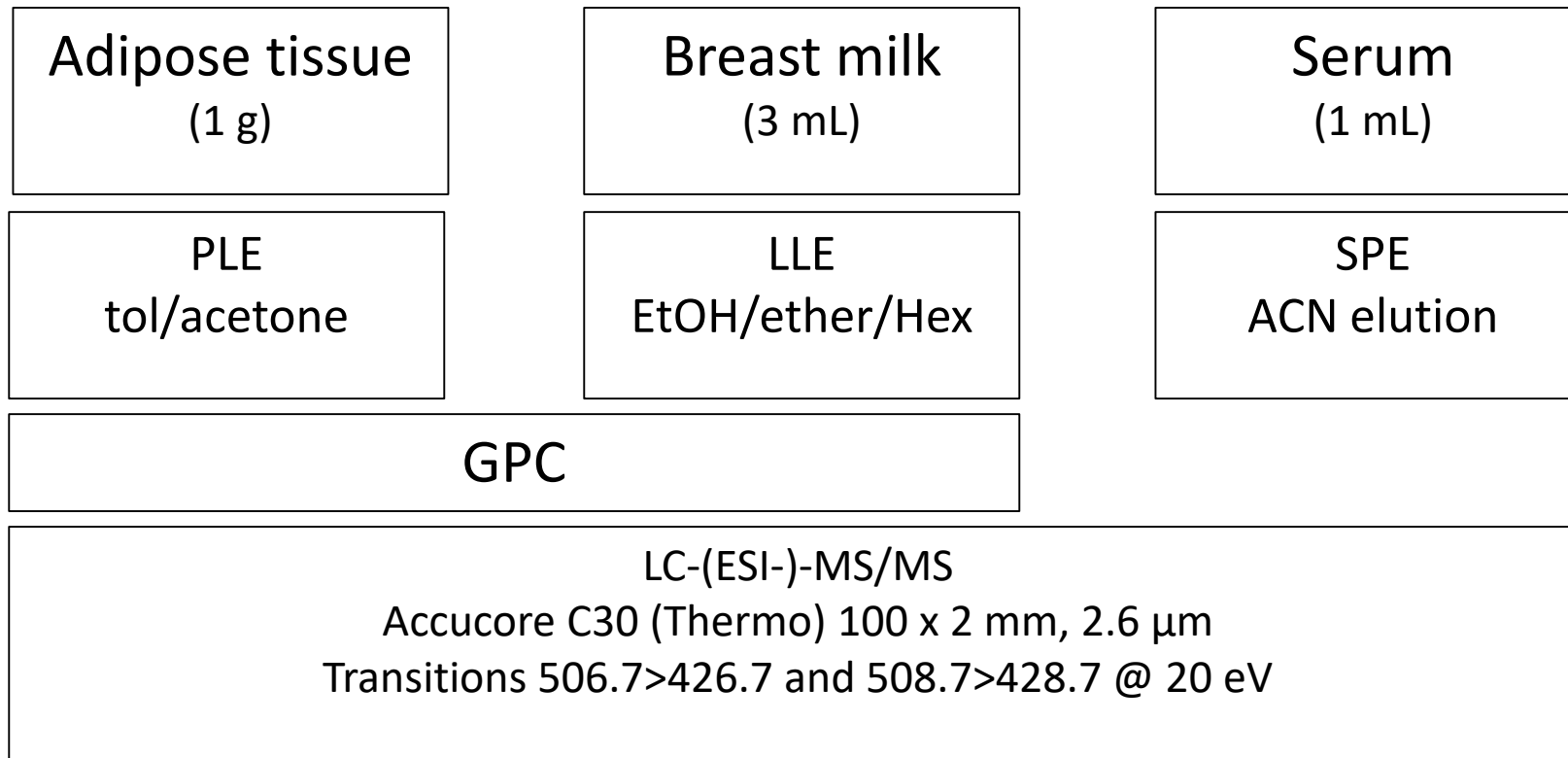


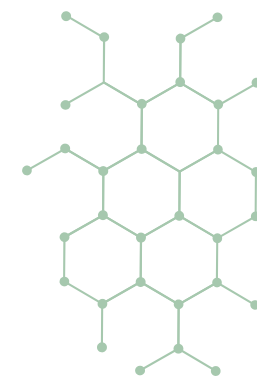
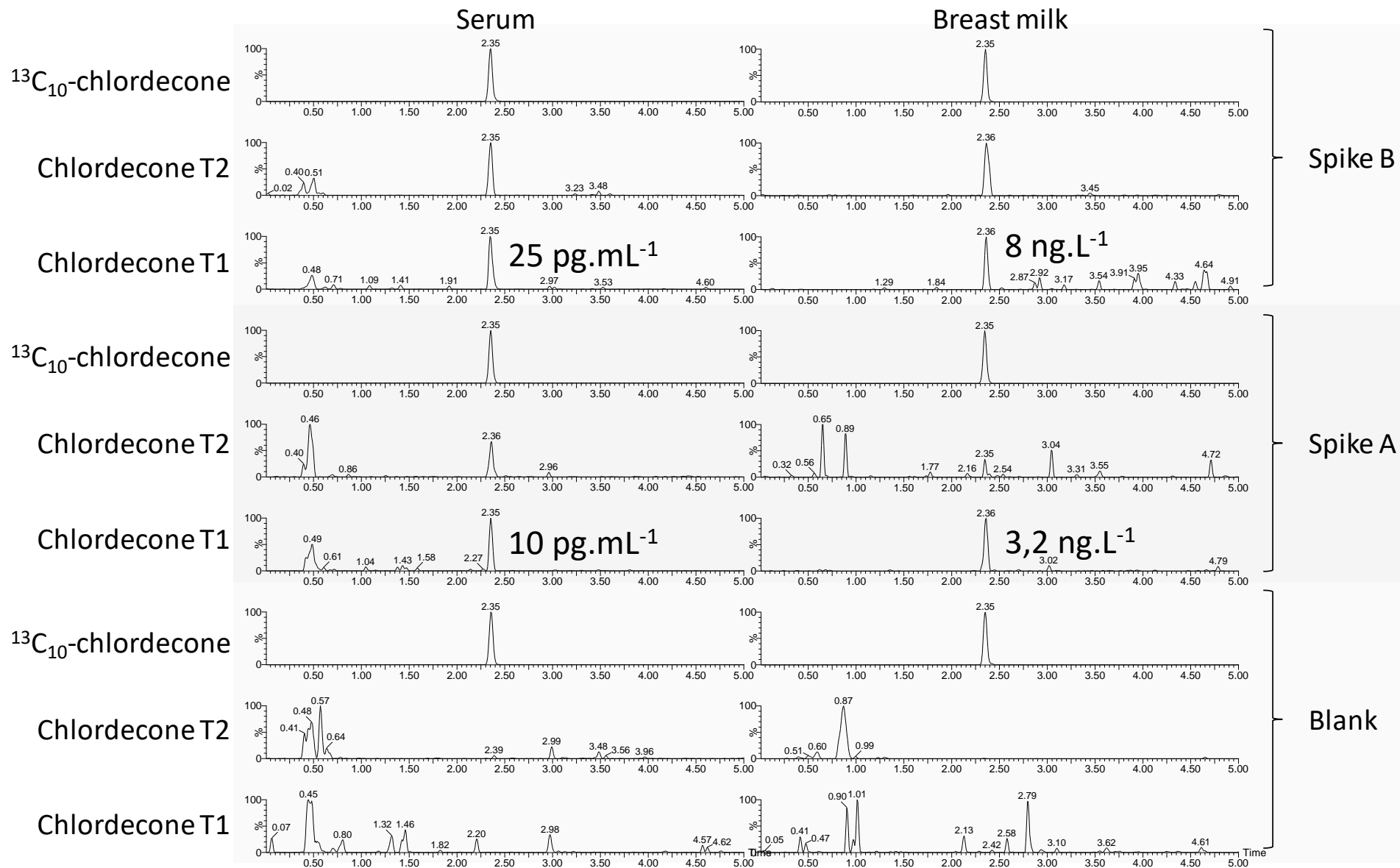


**Colonne C30 Accucore**

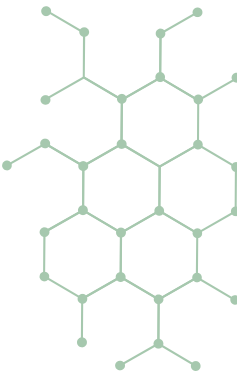
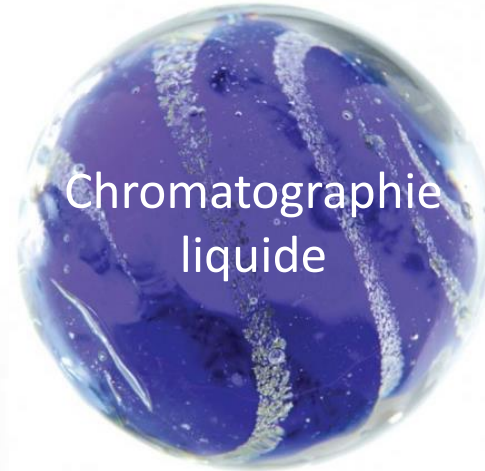


# Préparation d'échantillons



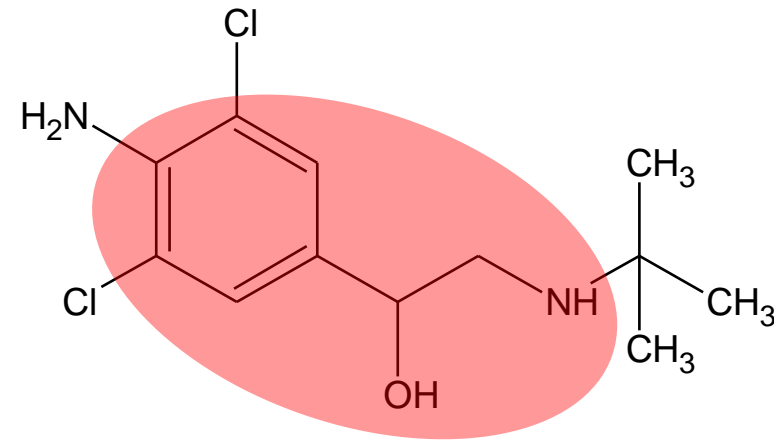


# Quelle chromatographie associée à la spectrométrie de masse ?

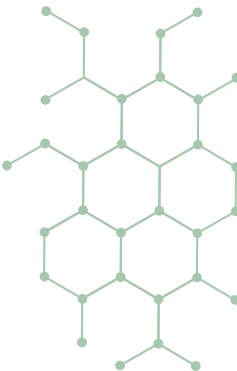


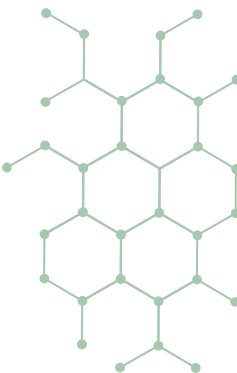
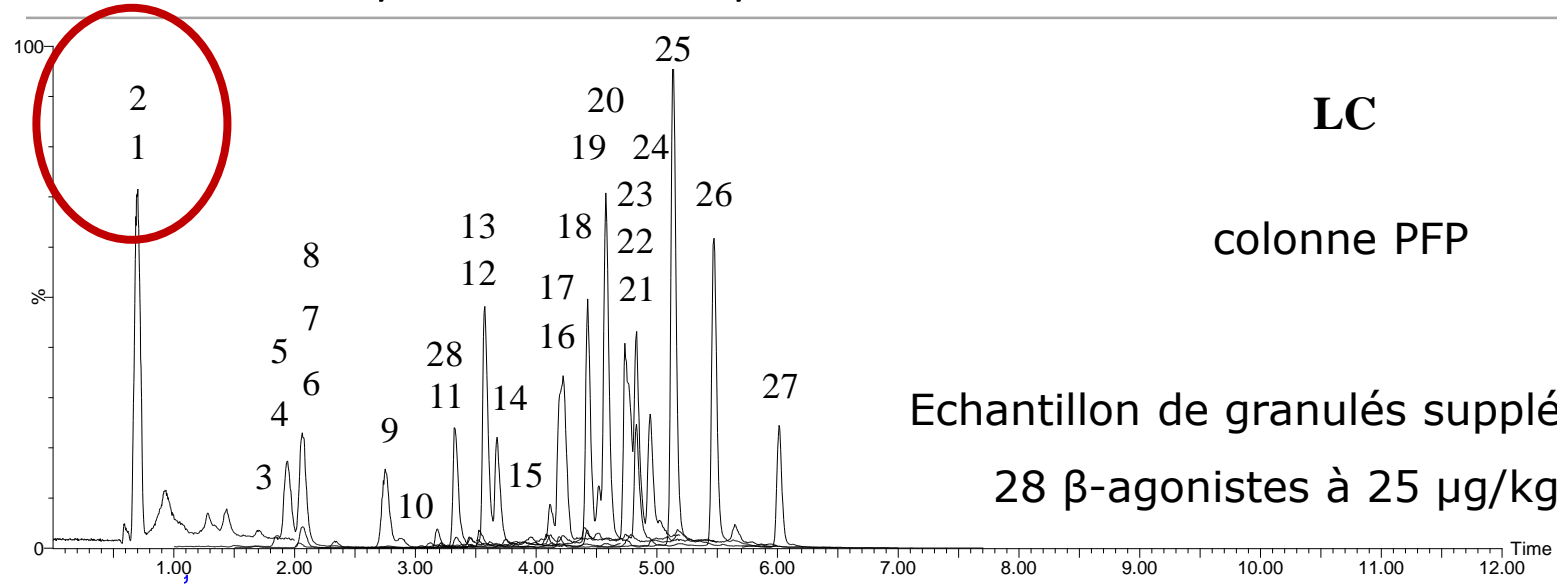


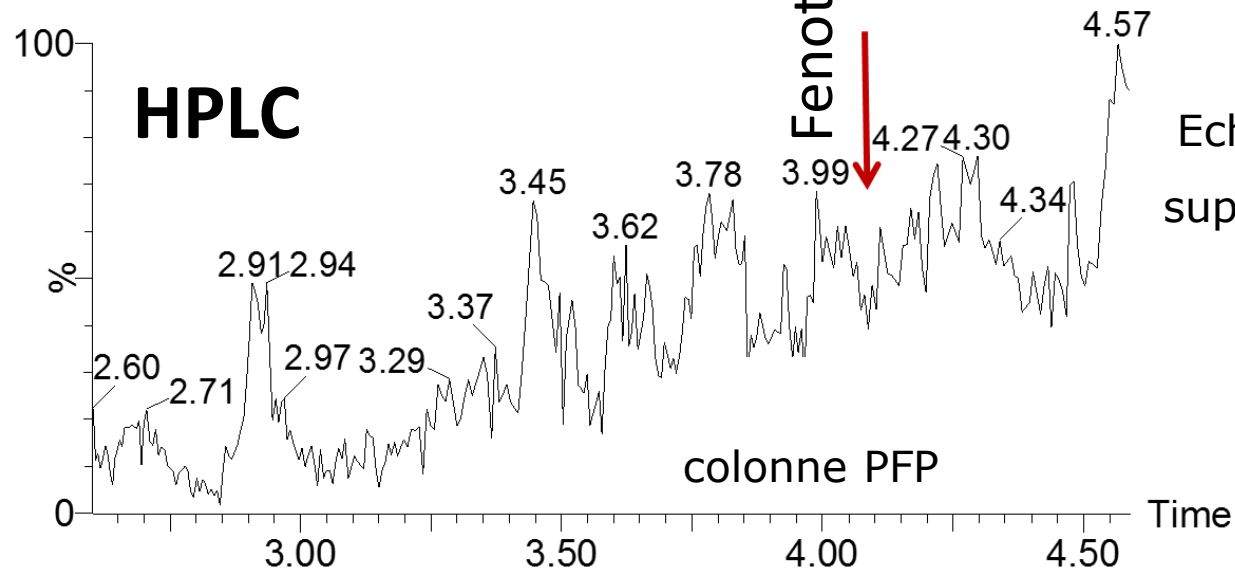
Promoteurs de croissance en élevage



Substances interdites  
(Annexe A directive 96/22/EC)

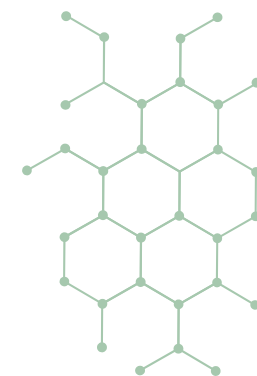
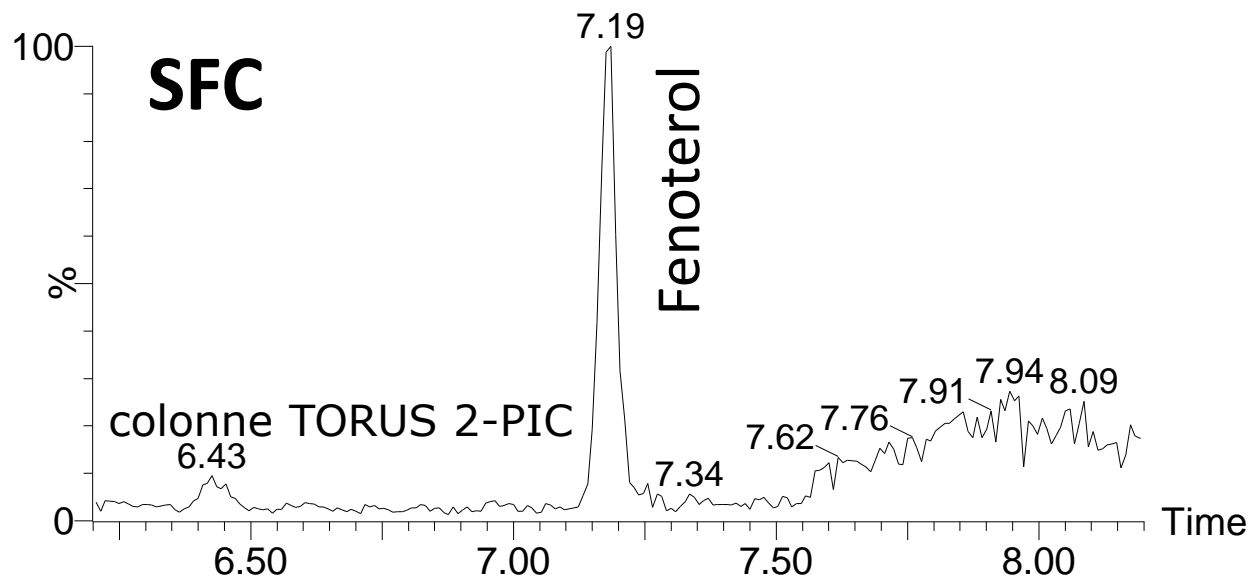


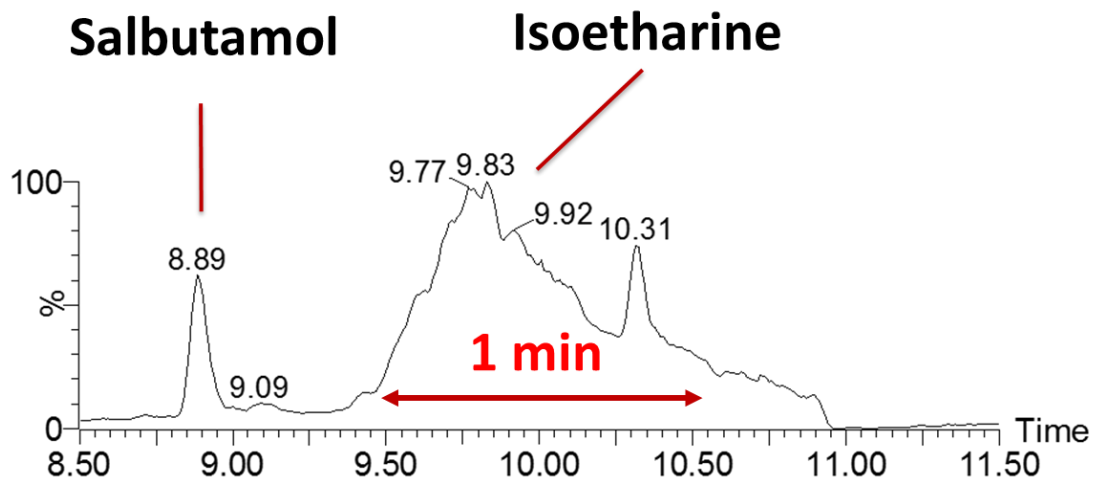
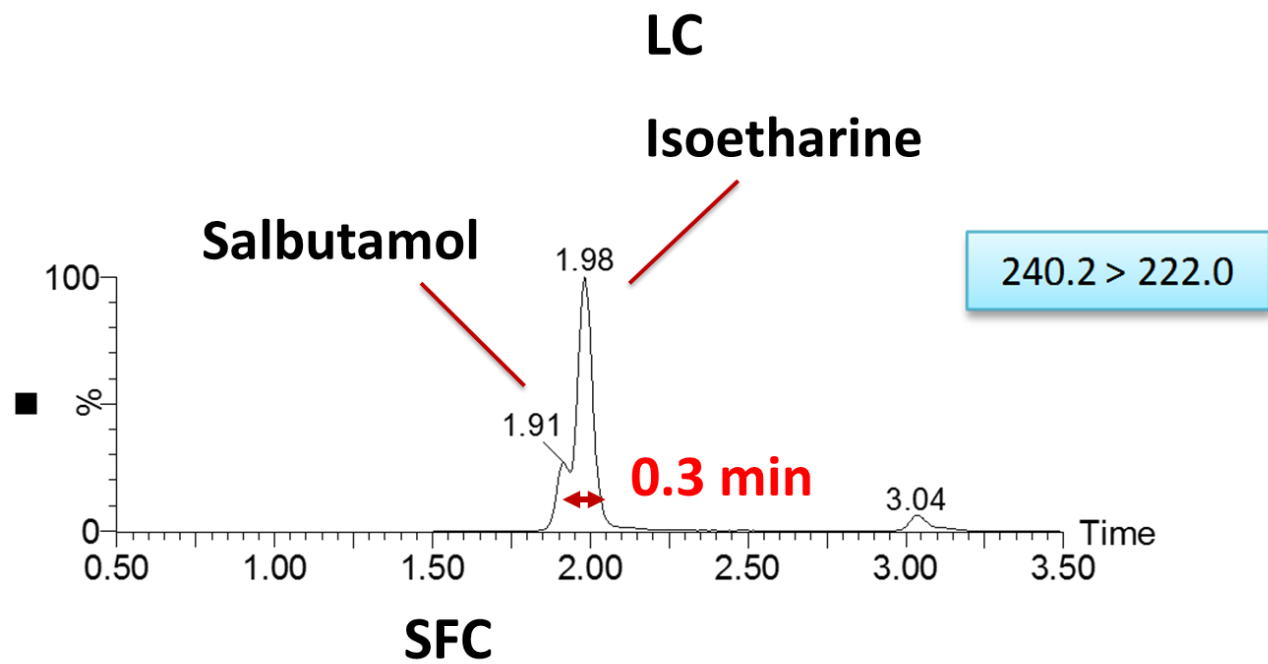




Echantillon de granulés  
supplémenté à 25  $\mu\text{g}/\text{kg}$

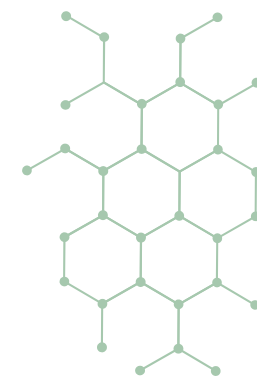
Recommandation  
européenne : **50**  $\mu\text{g}/\text{kg}$





### Gain en Séparation

Au détriment de la  
resolution pour  
l'isoétharine...



Compromis entre résolution/sensibilité chromatographique et résolution/sensibilité MS  
Attention au choix de l'ionisation = visualiser les analytes

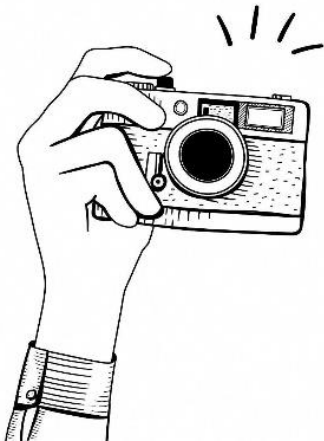


Ionisation

Chromatographie

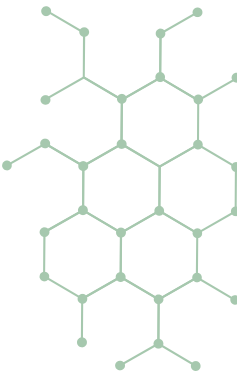


Spectrométrie de Masse

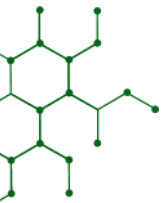
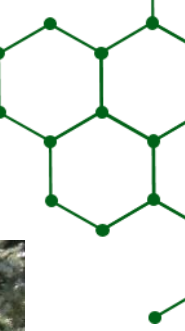


COUPLAGE PERTINENT POUR :

- l'analyse d'ultratraces
- la sélectivité
- les matrices complexes
- la quantification
- le Non-Target Screening







*Merci pour votre attention*