Chromatography

Allergènes - matières premières et produits finis : Quelles solutions analytiques

St. MOREAU
SHIMADZU France

CECM – réunion du 21/10/2005

Grasse

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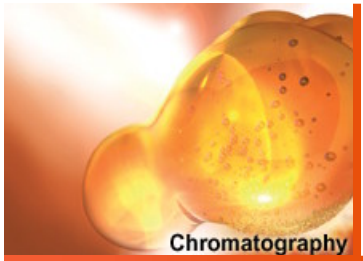

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Sommaire

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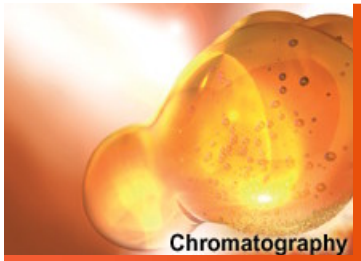


IFRA

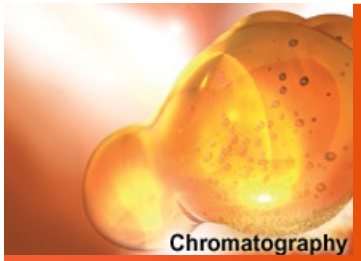
Historique / réglements

Document pointing out usual pitfalls when applying the GC/MS Quantitation method for potential allergens

The document should help to avoid some pitfallsl when using the GC/MS Method for the detection of the 24 'Allergens'.
Pitfalls

Analytical procedure for the GC/MS Quantitation of potential fragrance allergens in fragrance compounds

IFRA IL 683 informed about an analytical method developed by a joint IFRA / EFFA Working Group that allows the determination of the presence of potential allergens in fragrance compounds. This makes reference to the fragrance ingredients defined by the SCCNFP as potential allergens and which are included in the 7TH Amendment of the European Cosmetics Directive for the purpose of labelling in finished cosmetic products. An article extensively describing the method as well as results of testing was accepted for publication in the Journal of Agricultural and Food Chemistry.

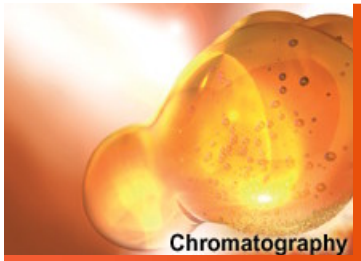


Historique / réglements

Description de la « réglementation »

Version 1 - September 25, 2003
INTERNATIONAL FRAGRANCE ASSOCIATION - ANALYTICAL PROCEDURE

- GC/MS Quantitation of potential fragrance allergens* in fragrance compounds
- *as defined by the SCCNFP in its opinion 0450/01, final and reflected in the 7th Amendment to the European Cosmetics Directive as published in the Official Journal N° L 66 of the European Union on March 11, 2003.
- The above-cited regulation requires indicating the presence of 26 fragrance ingredients in **finished cosmetic products** if exceeding a threshold of 0.01% for rinse-off and 0.001% for leave on products.
- From the **26 materials selected by the SCCNFP** two are natural extracts (oak moss and tree moss) so the method as specified below restricts itself on the **determination of the 24 volatile chemicals.**
- This method is intended to provide a reliable analytical method that can be used as reference in all quality control labs in the fragrance manufacturers industry, the customer industry but also serve the needs of public analysts.
- The method has been developed by the **EFFA / IFRA Analytical Working Group** and has been accepted for publication by the Journal of Agricultural and Food Chemistry (A. Chaintreau, D. Joulain, C. Marin, C-O. Schmidt, M. Vey,GC-MS Quantitation of Fragrance Compounds Suspected to Cause Skin Reaction. Part 1, J. Agric. Food Chem., accepted for publication).



Liste officielle IFRA

INCI (International Nomenclature of Cosmetic Ingredients) names

Historique / réglements

1. Amyl Cinnamal
CAS: 122-40-7
Both the (E) and (Z) isomers. .

2. Benzyl Alcohol
CAS: 100-51-6

3. Cinnamyl Alcohol
CAS: 104-54-1
Both (E)- and (Z)- isomers

4. Citral CAS: 5392-40-5
Both (Z)- (Neral: CAS 106-26-3) and (E)- (Geranial: CAS 141-27-5)

5. Hydroxycitronellal
CAS: 107-75-5
Both (R)- and (S)- enantiomers

6. Eugenol
CAS: 97-53-0

7. Isoeugenol
CAS: 97-54-1
Both (E)- and (Z)- isomers

8. Amylcinnamyl Alcohol
CAS: 101-85-9
Both the (E) and (Z) isomers.

9. Benzyl Salicylate
CAS: 118-58-1

10. Cinnamal
CAS: 104-55-2
Both (E)- (trans- CAS 14371-10-9) and (Z)- (cis- CAS 57194-69-1)

11. Coumarin
CAS: 91-64-5

12. Geraniol
CAS: 106-24-1
The (Z)- isomer (Nerol: CAS 106-25-2) is not included.

13. Hydroxyisohexyl- 3-cyclohexene carboxaldehyde
CAS: 31906-04-4

14. Anise Alcohol
CAS: 105-13-5

15. Benzyl Cinnamate
CAS: 103-41-3
Both (E)- and (Z)- cinnamate isomers

16. Farnesol
CAS: 4602-84-0
Undefined mixture of four isomers (predominantly the (E,E) ("trans, trans-Farnesol": CAS 106-28-5) and (Z,E) ("trans, cis-Farnesol": CAS 3790-71-4) .

17. Butylphenyl Methylpropional (Lilial)
CAS: 80-54-6

18. Linalool
CAS: 78-70-6

19. Benzyl Benzoate
CAS: 120-51-4

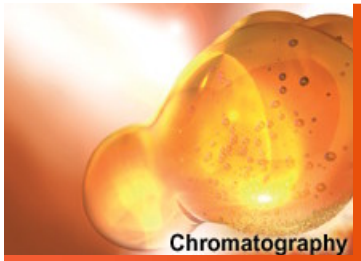
20. Citronellol
CAS: 106-22-9
Both (R)- ((-): CAS 1117-61-9)and (S)- ((+): CAS 7540-51-4) enantiomers are included.

21. Hexyl Cinnamal
CAS: 101-86-0
Both the (E) and (Z) isomers. .

22. Limonene
CAS: 5989-27-5
Both (R)- and (S)- (CAS # 5989- 54-8) enantiomers may have to be taken into account . (See special note on LIMONENE).

23. Methyl 2-Octynoate (Methyl Heptin Carbonate)
CAS: 111-12-6

24. Alpha-Isomethyl Ionone
CAS: 127-51-5
Both (E)- and (Z)- isomers of the specified structural isomer are included in the analysis.

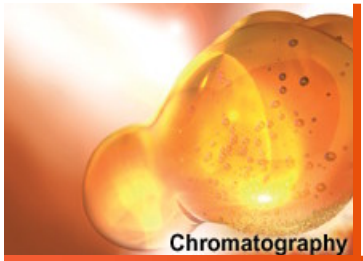


Historique / réglements

remarques

- SPECIAL NOTE ON d-LIMONENE

  Strictly speaking, only the (R)- enantiomer would need be taken into account. Users should however be aware that because chiral differentiation techniques (e.g. Chromatography with chiral columns) are unlikely to be used by verifying laboratories, the (R)- and (S)- enantiomers will not be routinely distinguishable. . For that reason, it is recommended to provide information about the total levels of both enantiomers. If specific analysis has been performed which allows quantitative distinction of each enantiomer, then in addition to the total concentration of both enantiomers, more detailed information may be given.



Plan de travail

Problématiques

Contrôle des matières premières	Coélution Faux positifs / faux négatifs	GCMS-SCAN GCMS-SIM GCMS-Dual column Comprehensive GCMS GCxGC-TOF MDGCMS
Contrôle des compositions	Coélution Faux positifs / faux négatifs	GCMS-SCAN GCMS-SIM GCMS-Dual column Comprehensive GCMS GCxGC-TOF MDGCMS
Contrôle des crèmes, shampoing, émulsions, ...	Coélution Faux positifs / faux négatifs Pb matrice	ALEX-GCMS LINEX-GCMS

conditions chromatographiques

essais

Table 1. GC conditions

Column	Oven program	Time min	Initial gas velocity cm/sec	Co-elutions
DB1, 60m × 0.25mm × 0.25µm	100°-2min; 10°/min; 280°C	25	50	Amylcinnamic ald./1st peak of HMPCC*
DB17, 20m × 0.18mm × 0.18µm	100°-2 min; 10°/min; 280°C	17	60	No co-elution

* The first peak of HMPCC (CAS 31906-04-4) is not the most abundant (# 28 %) and it is not used for its quantitation. Its ions do not interfere with ions of amylcinnamic

Détection MS :

1] scan – limité en sensibilité, coélution

2] SIM – absence de contrôle du spectre

Risque de faux positifs / négatifs

Pb avec les pics larges / fenêtres en SIM

➔ Nouveaux appareils : possibilités en Scan ?

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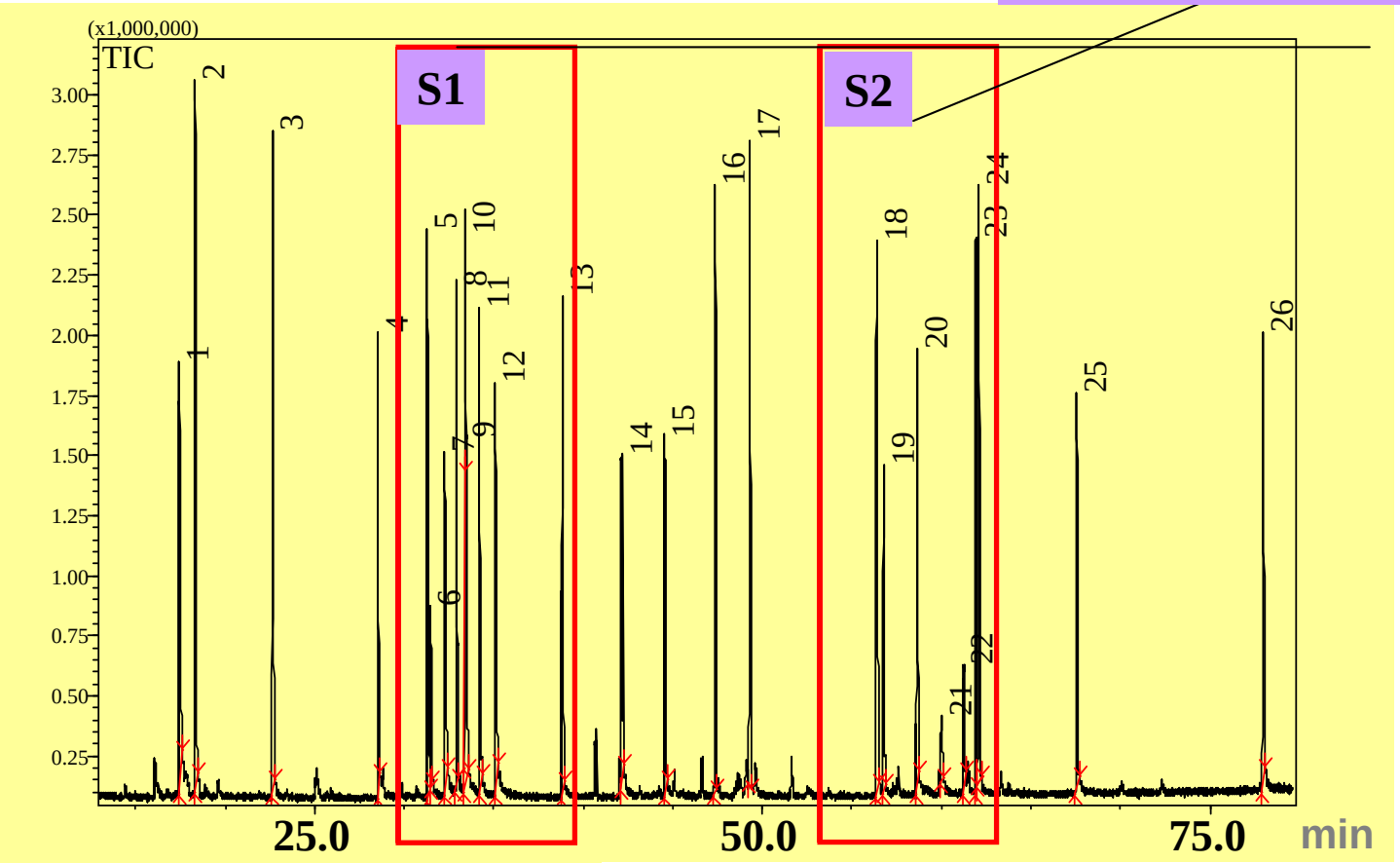
Analyses d'un mélange standard d'Allergènes (26 Composés 400 ppm)

Segments à problèmes



RTX-5 50m, 0.25 mm ID,0.25 µm

50°C, 1min, 2°C/min 210 °C, 10°C/min 280°C,10 min
He Velocity 34.4 cm/sec, split 300:1
MS SCAN 30-300 amu

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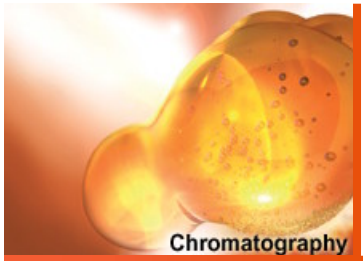

Approches

essais

- 1] Utiliser les performances des nouveaux GCMS avec pompes turbomoléculaires pour connecter deux colonnes dans le détecteur de Masse.
- 2] Etudier le gain en fast GCMS
- 3] Essais en Comprehensive GC-qMS
- 4] Produits finis

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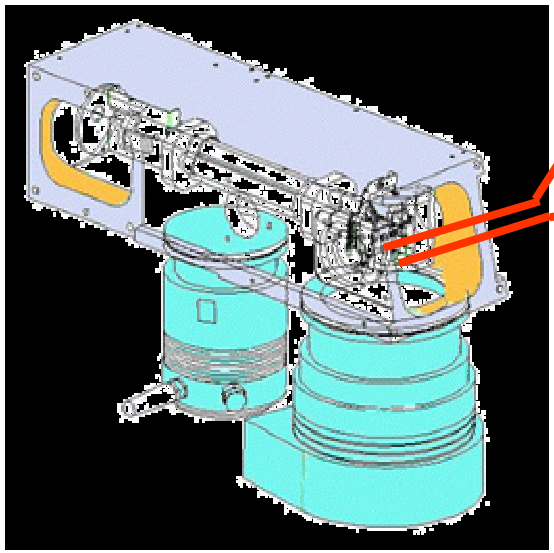
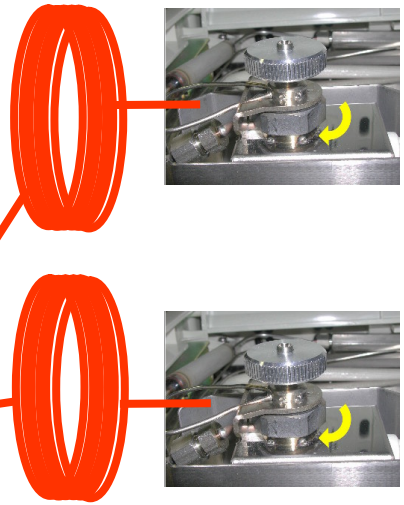
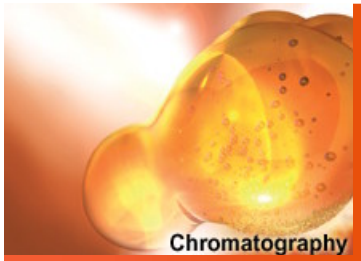
essais

1ère approche : 2 colonnes dans le détecteur MS







Avantages / Contraintes

essais

- <u>2 colonnes dans l'interface :</u>

  - Economie d'un GCMS

  - Souplesse analytique (SPB5 – WAX)

  ➔ confirmation, résolution coélution

  - réduction du temps d'analyse

  ➔ travail en scan : pas besoin de réinjecter pour éliminer les faux positifs / négatifs

  - identification par le spectre complet avec recherche en librairie et confirmation avec Indice de rétention si nécessaire

  - élimine le problème des fenêtres en SIM dans le cas des pics larges (« shift » du temps de rétention)

Avantages / Contraintes

essais

- Matériel nécessaire :
  - Pompes turbomoléculaires avec système à double pompage (220 L/s + 65L/s)
  - Capacité de 15 ml/mn au niveau de la source
  - 2 colonnes débitent environ 4 ml /mn (limite des appareils mono-pompe)
  - Deuxième pompe pour un vide plus poussé et une meilleure sensibilité en Scan.
  - Injecteur automatique capable d'injecter sur les deux voies

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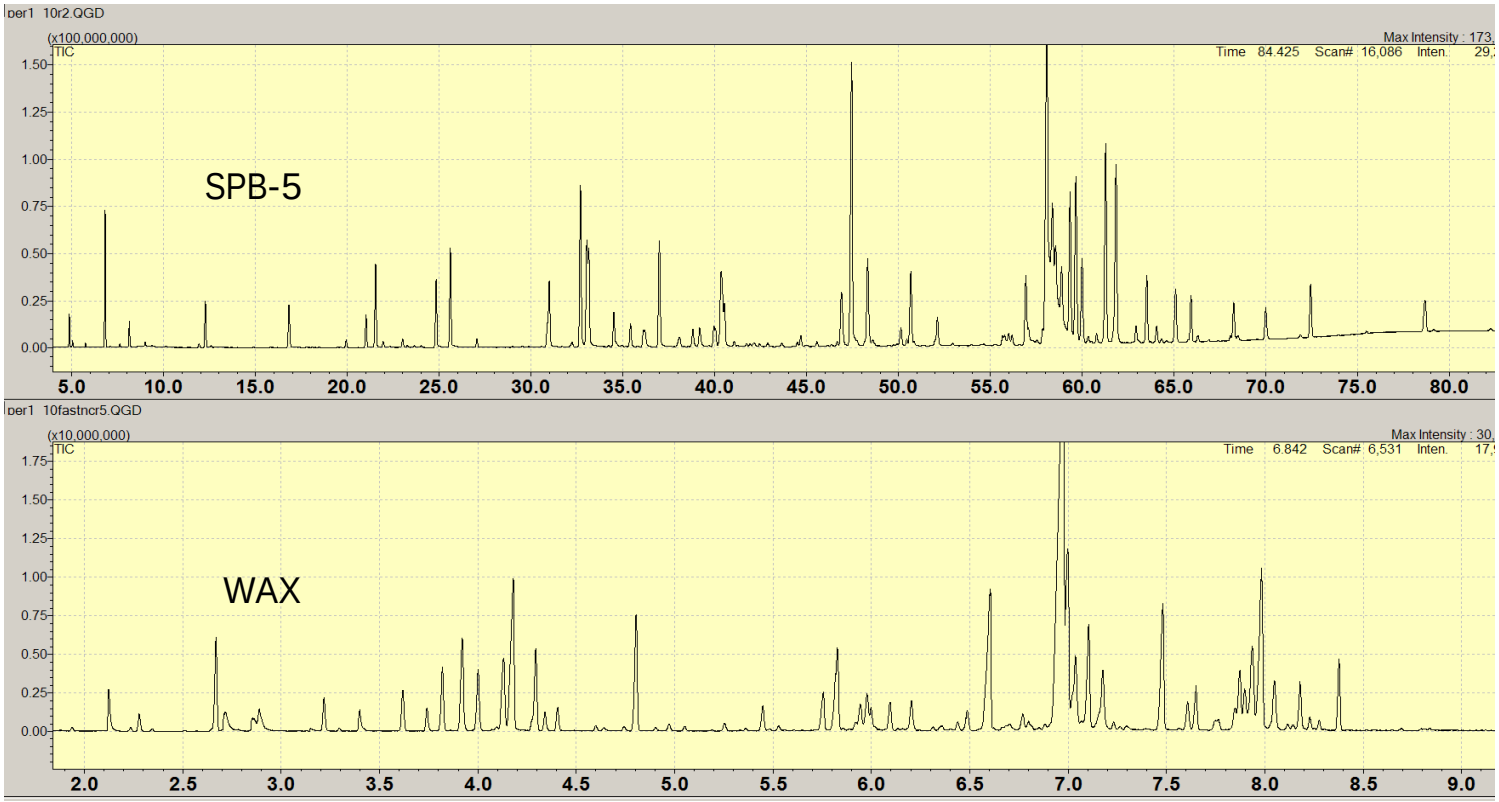
Résultat : 2 chromato / run

essais



Travaux publiés :

H. Leijs et al : J. Agric. Food Chem. 2005,53,6487

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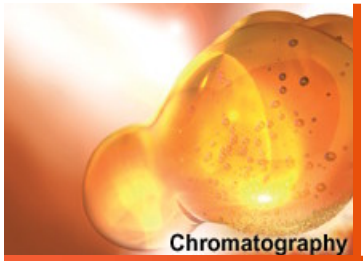


2ème approche : Fast GCMS

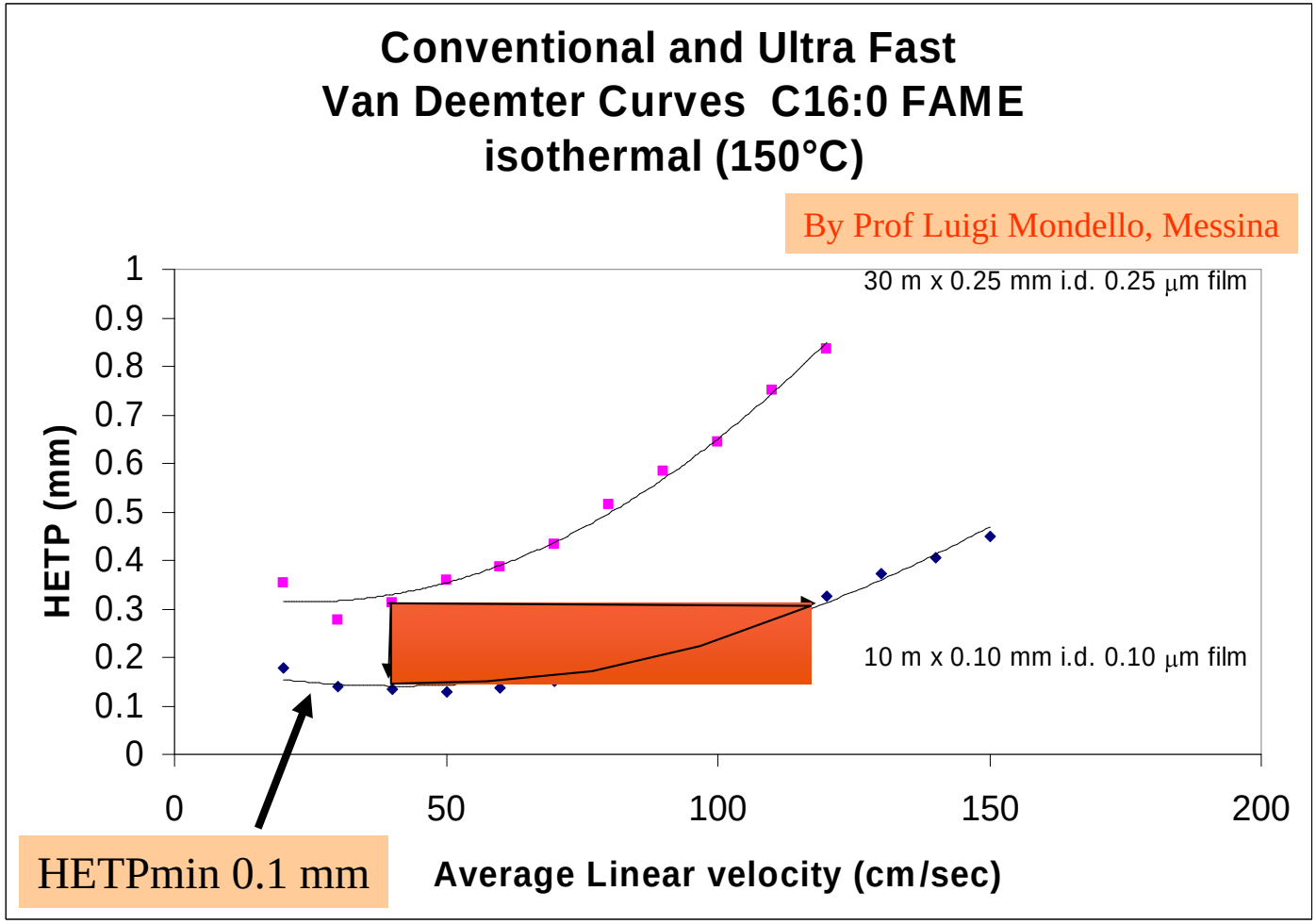
essais

Intérêts :

- Réponse rapide en contrôle Qualité et production
- Gain de sensibilité
- Pour certains composés, une meilleure précision résultant de la baisse d'interactions dans la phase stationnaire.

Notions en fast GC & GCMS

essais



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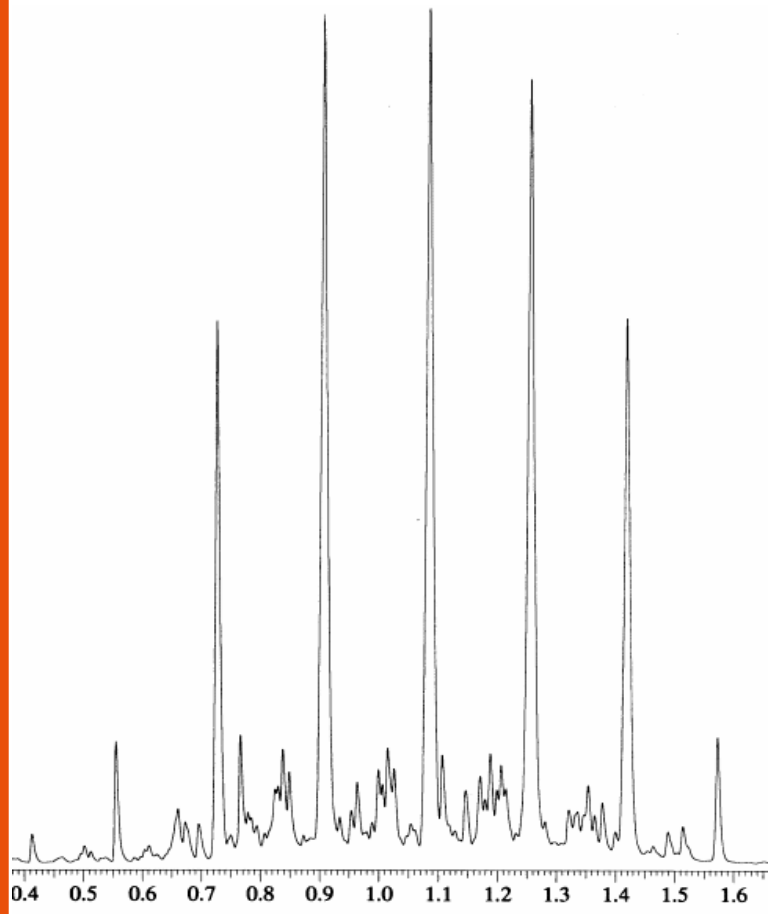
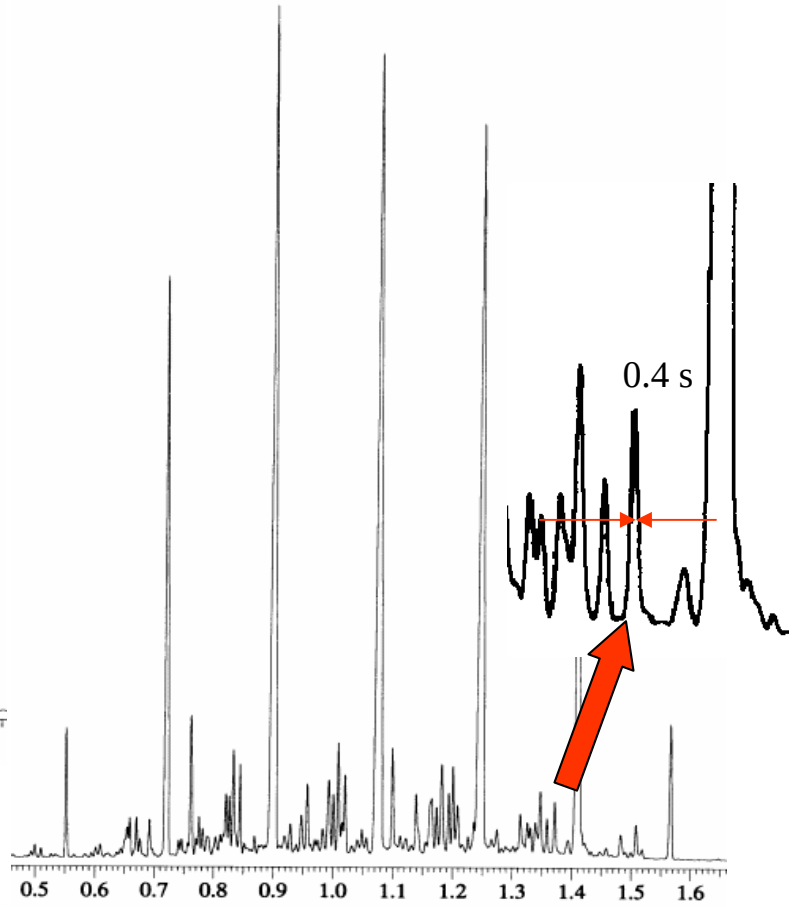
Contrainte : détecteur MS rapide



kerosène



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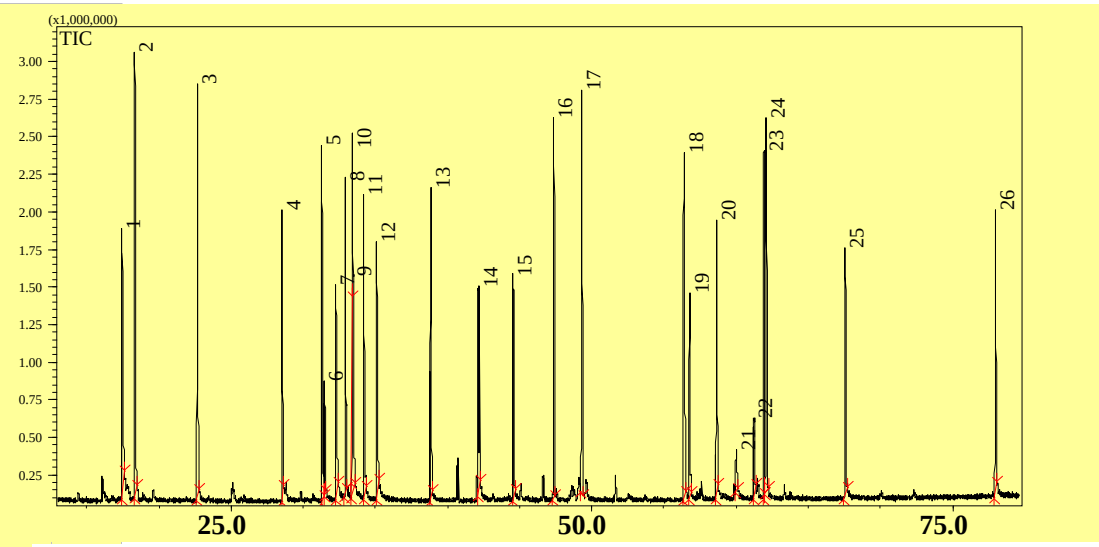
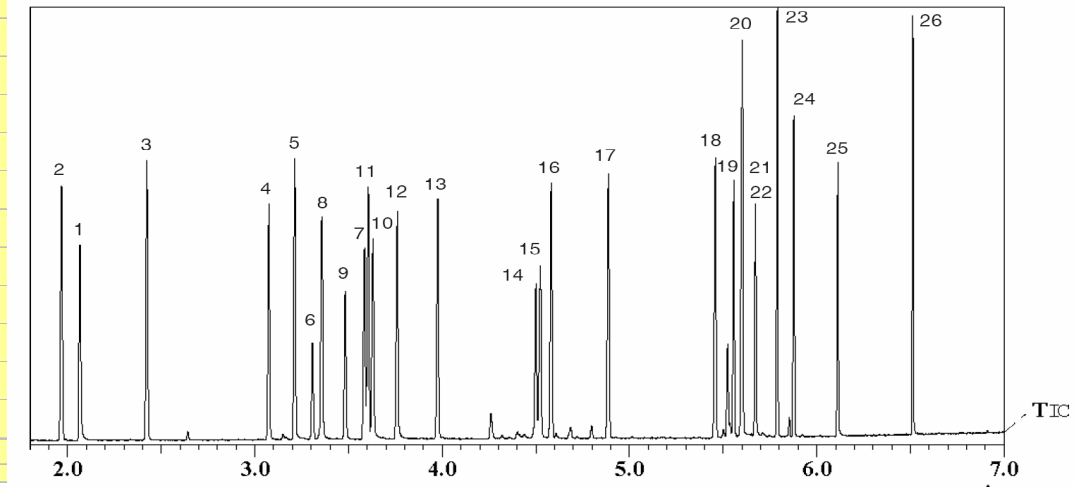
Comparatif GC conventionnelle et Fast

essais

1. Benzyl Alcohol	
2. Limonene	
3. Linalol	
4. Methyl HeptinCarbonate	
5. Citronellol	
6. Citral (Neral)	
7. Cinnamic Aldehyde	
8. Geraniol	
9. Citral (Geranial)	
10.Anisic Alcohol	
11.Hydroxy Citronellal	
12.Cinnamic Alcohol	
13.Eugenol	
14.Coumarin	
15.Iso-Eugenol	
16.Methyl Gamma Ionone	
17.Lilial	
18.Amyl Cinnamic Aldehyde	
19.Lyral	
20.Amyl Cinnamic Alcohol	
21.Farnesol 1	
22.Farnesol 2	
23.Hexyl Cinnamic Aldehyde	
24.Benzyl Benzoate	
25.Benzyl Salicylate	
26.Benzyl Cinnamate	





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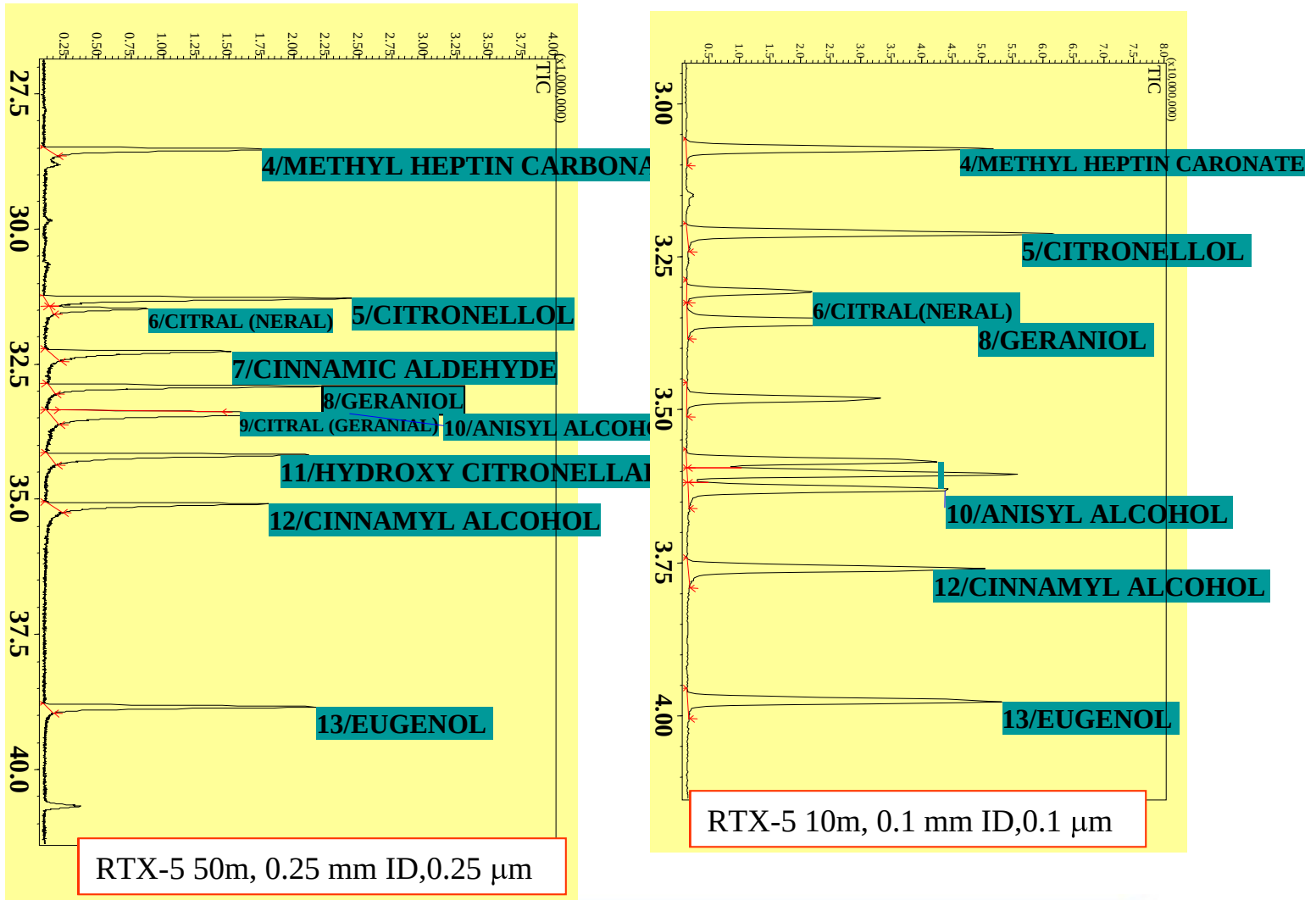
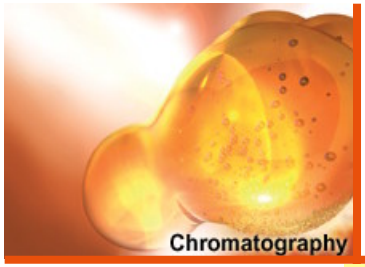
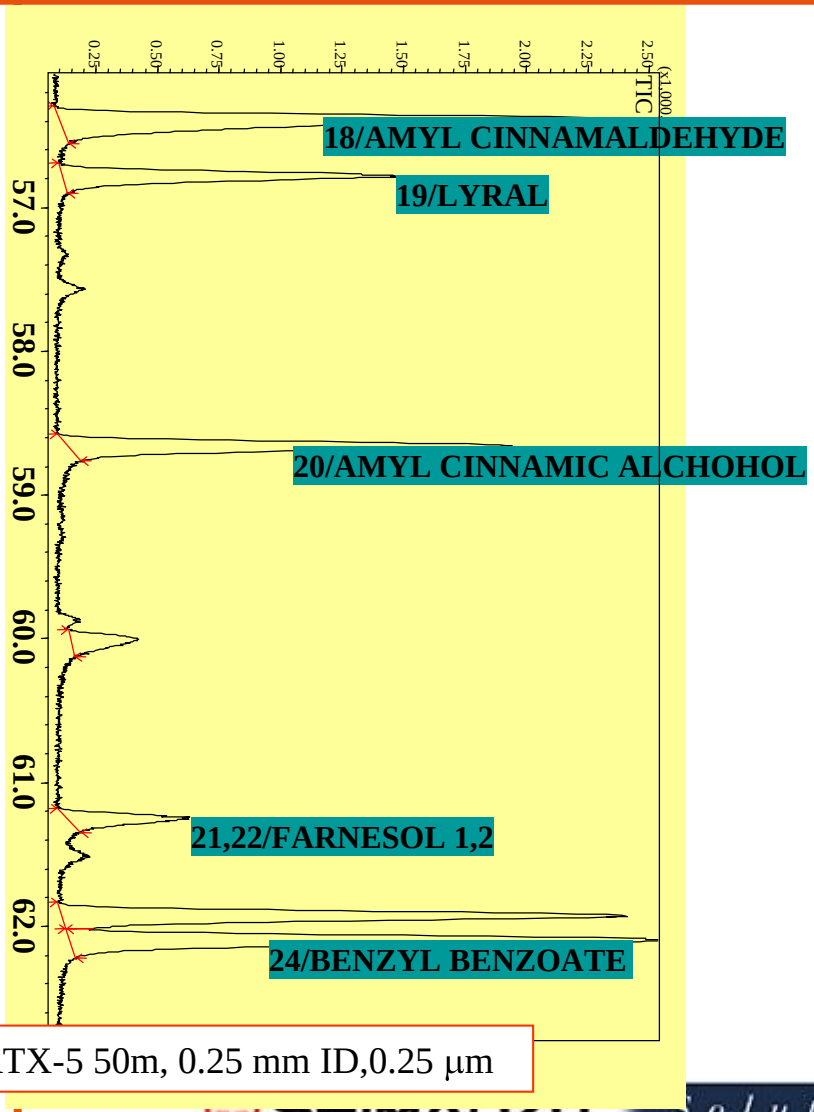
Chromatography

Segment S1

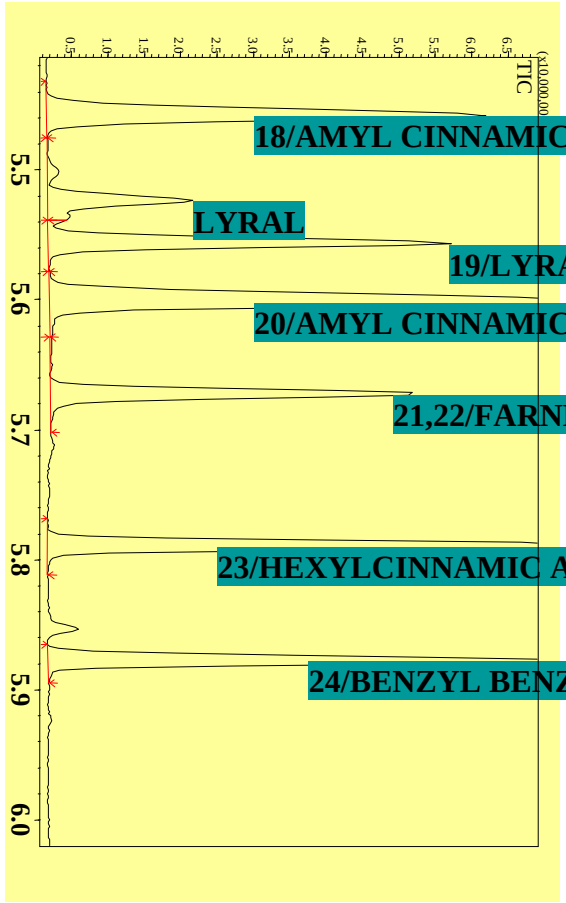




Segment S2

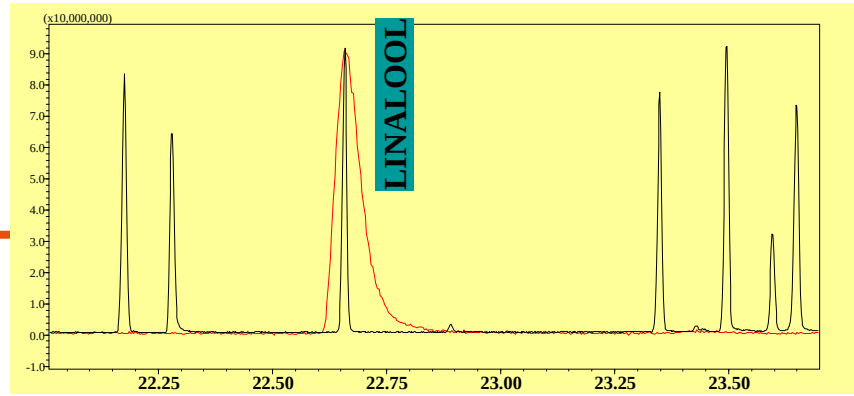


RTX-5 50m, 0.25 mm ID,0.25 μm

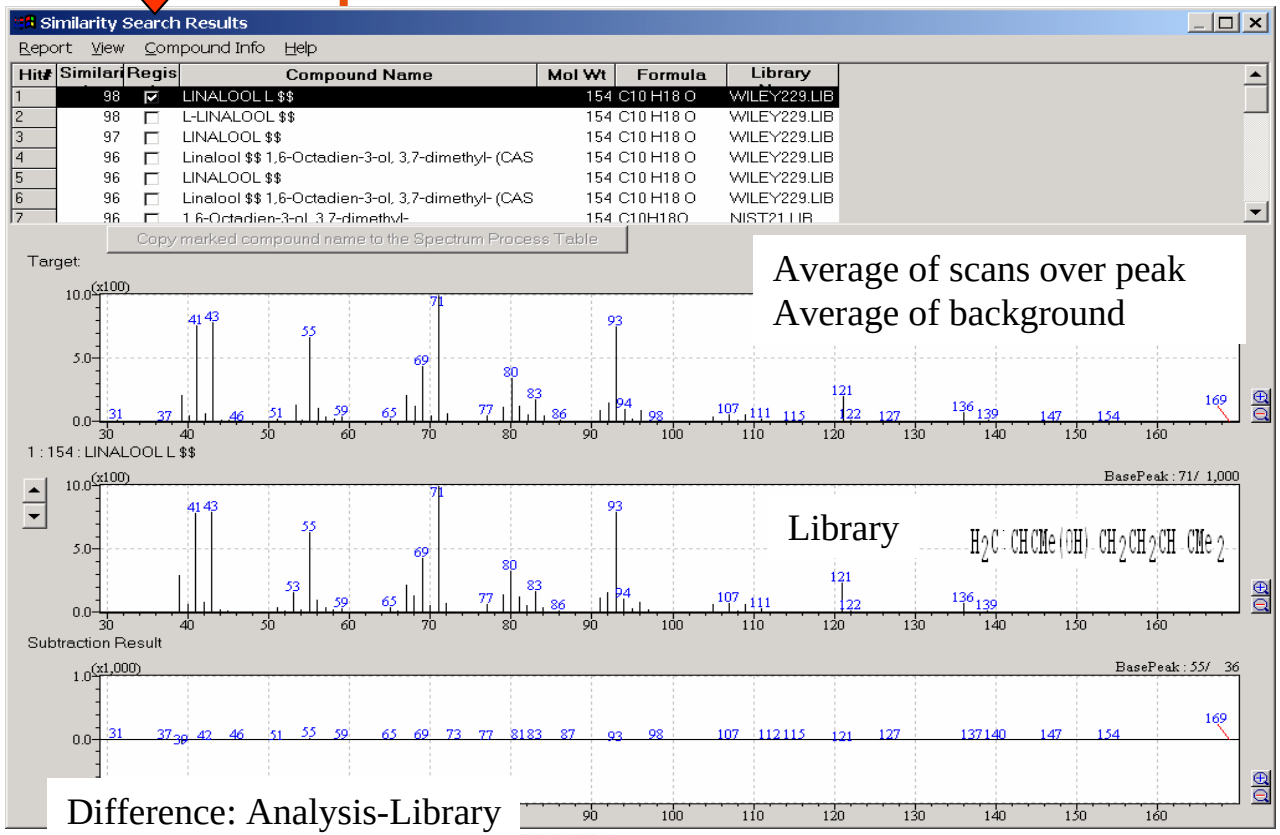


RTX-5 10m, 0.1 mm ID,0.1 μm

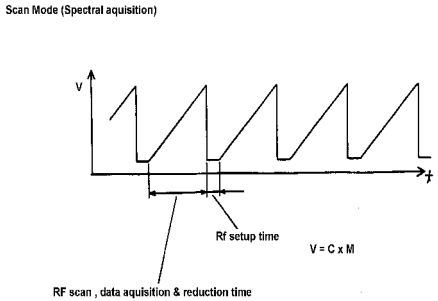
Qualité du spectre
En Fast GCMS



SI = 98



- Standard analysis
- Fast (shifted and normalized)



Fast GCMS Analysis needs:

1. High speed Quad scan and low Rf setup time
2. High No of spectra/sec FWHM 0.1-0.5 sec: sampling ≥ 20 Hz [2]

[2] J. V. Hinshaw, LCGC (2002)vol 15 p. 152

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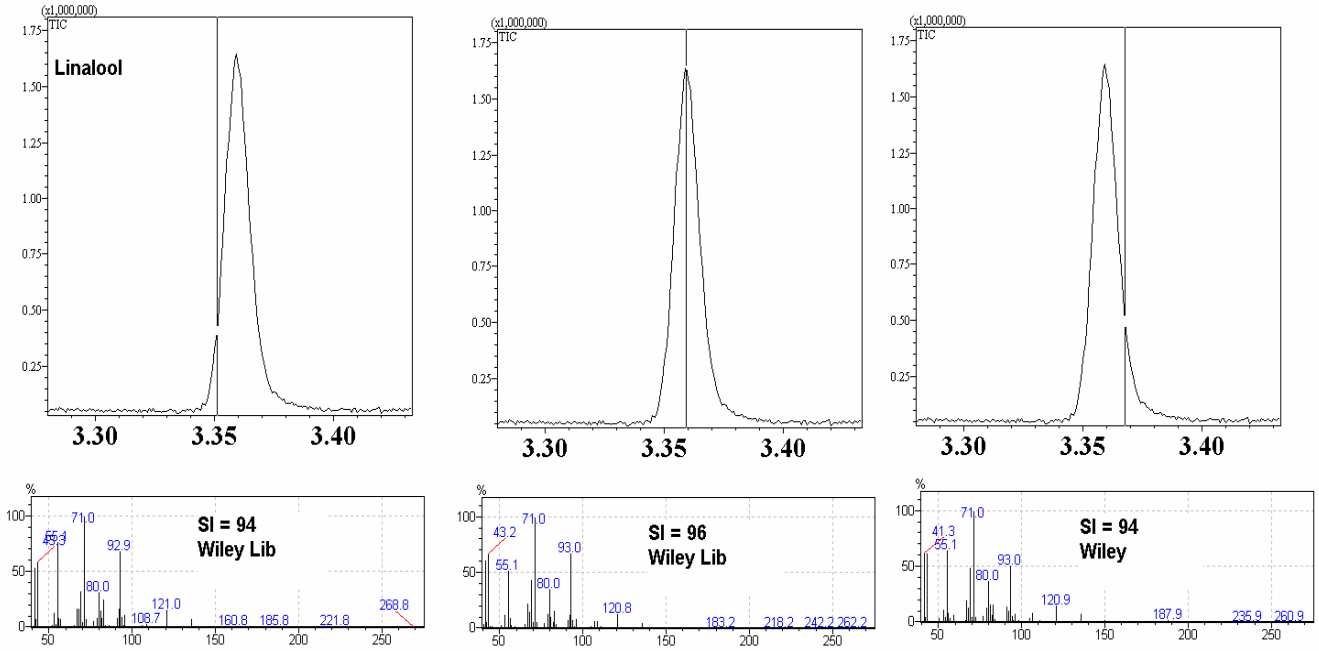
Qualité du spectre



Conditions : FWHM 0.6 - 20 Hz, 30-450 amu

Spectre identique à l'APEX, en début et fin de pic

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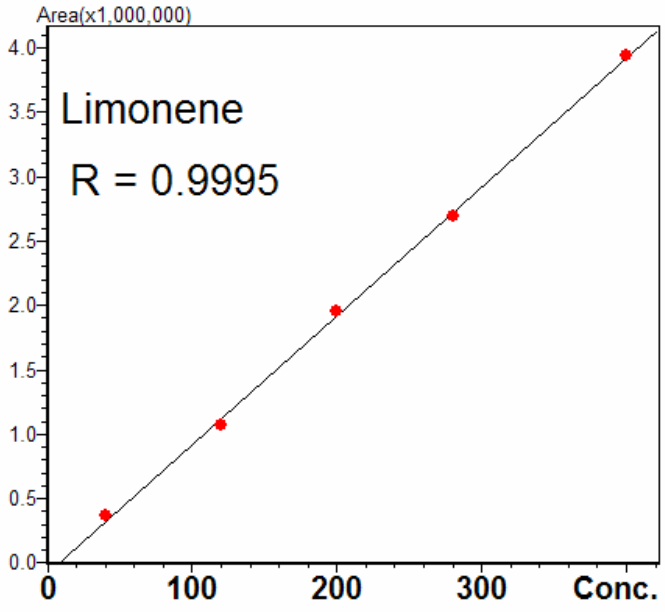
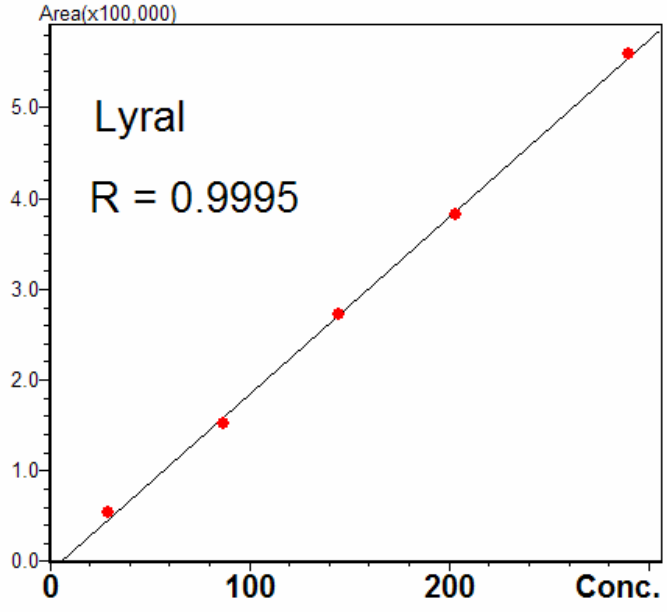
Calibration et linéarité en Fast GCMS

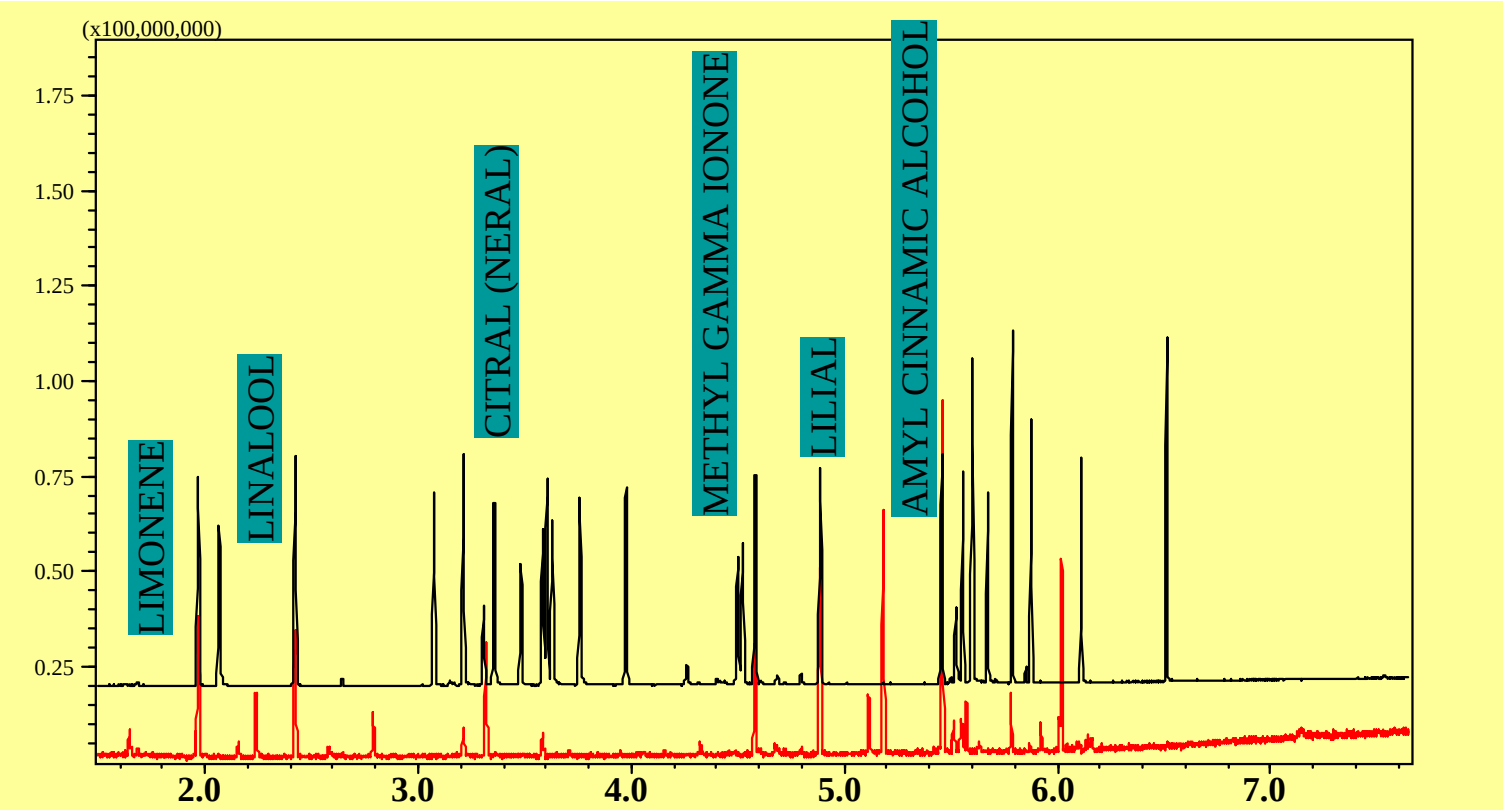




Linéarité de la courbe de calibration contrôlée entre 4 et 400 ppm : $R^2 = 0.99998$

Exemple en Fast GCMS

Rouge : Parfum dilué dans l'Acétone 1000:1
Noir : Standard 200-400 ppm



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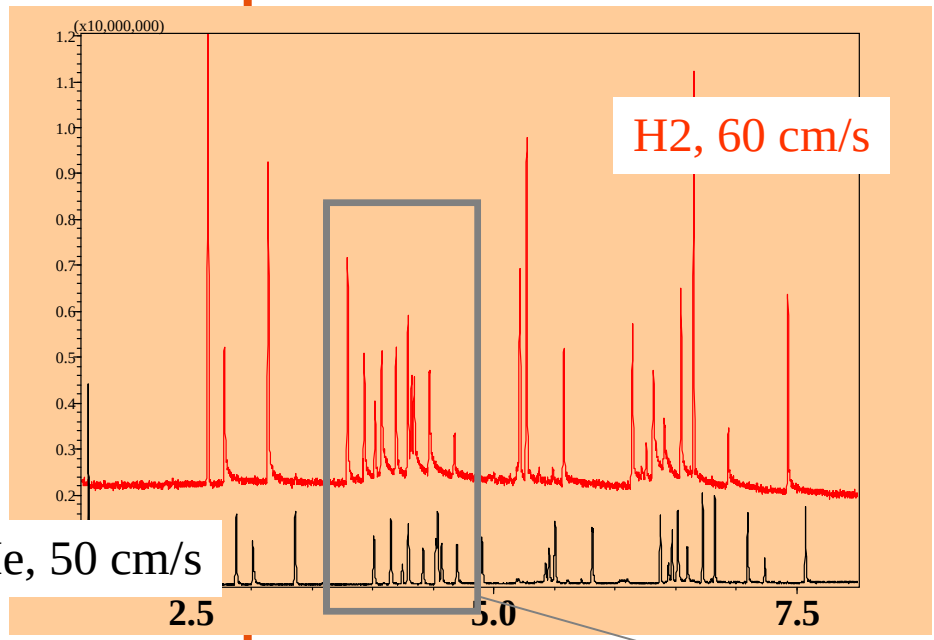
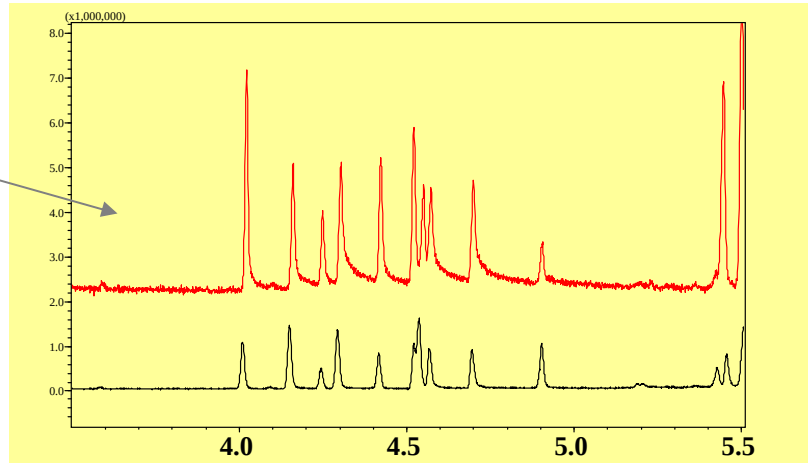
NB : Possibilité d'utiliser H2 en gaz vecteur sans risque de CI – débit des colonnes FAST plus faible.



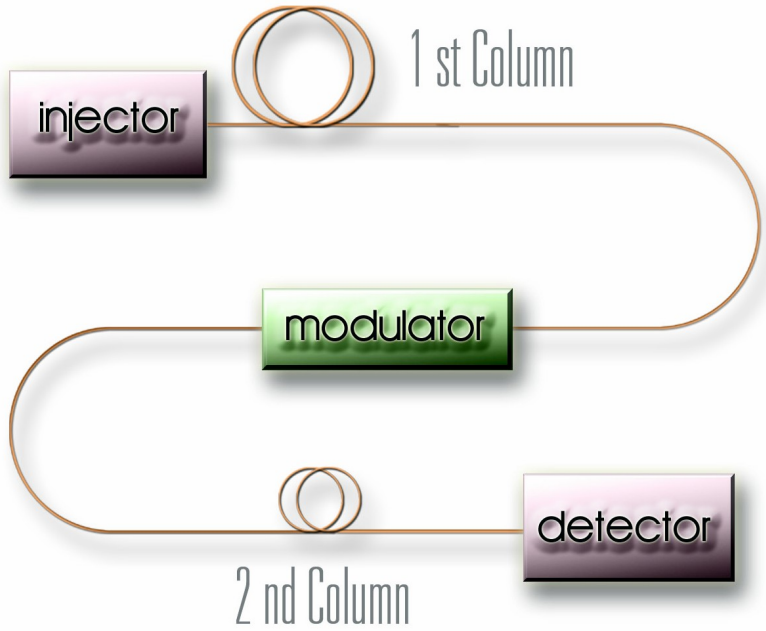


Chromatography

GCxGC-qMS

3ème approche : Comprehensive GCMS

- Principe :

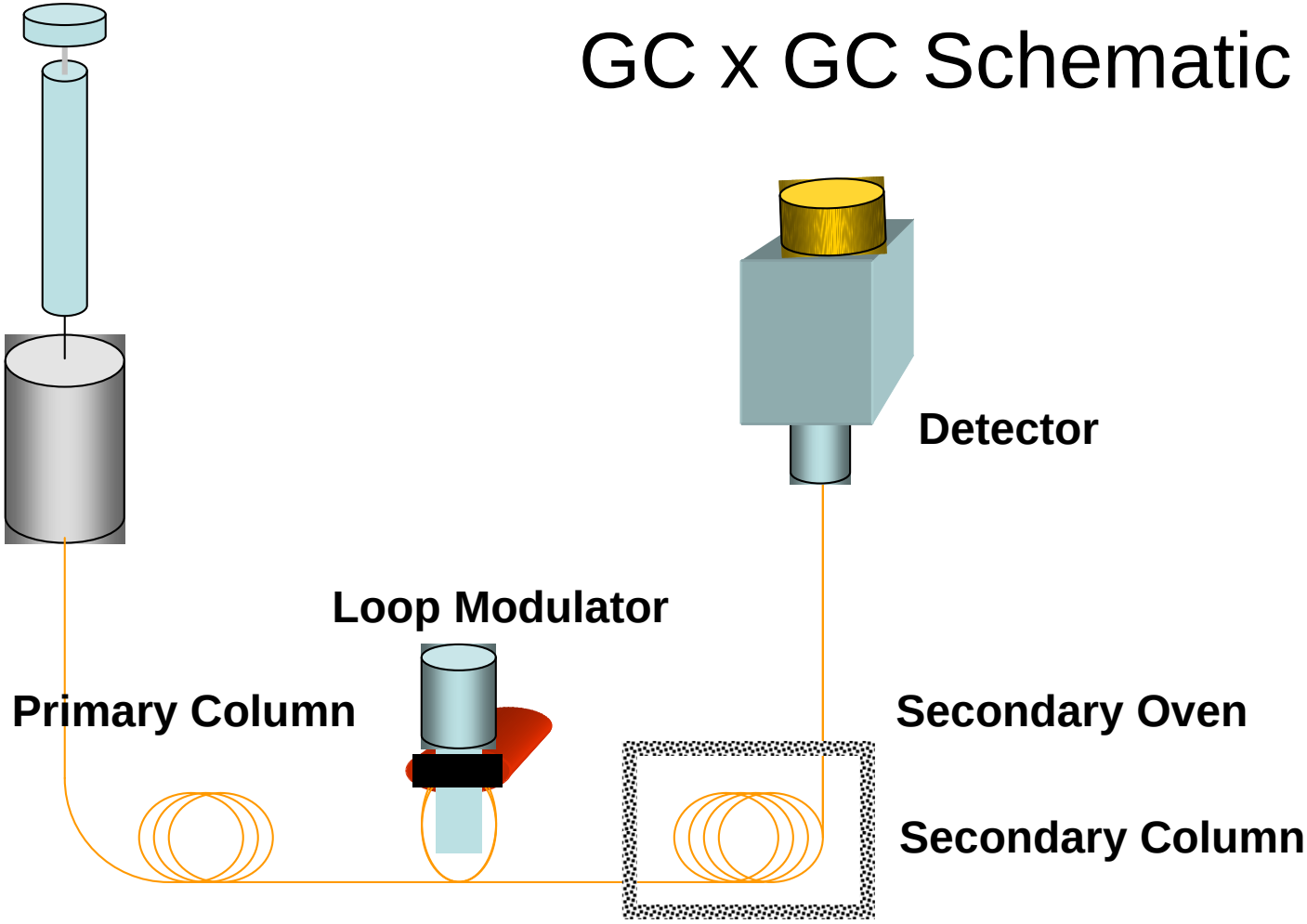


Chromatography

3ème approche : Comprehensive GCMS

GCxGC-qMS

GC x GC Schematic



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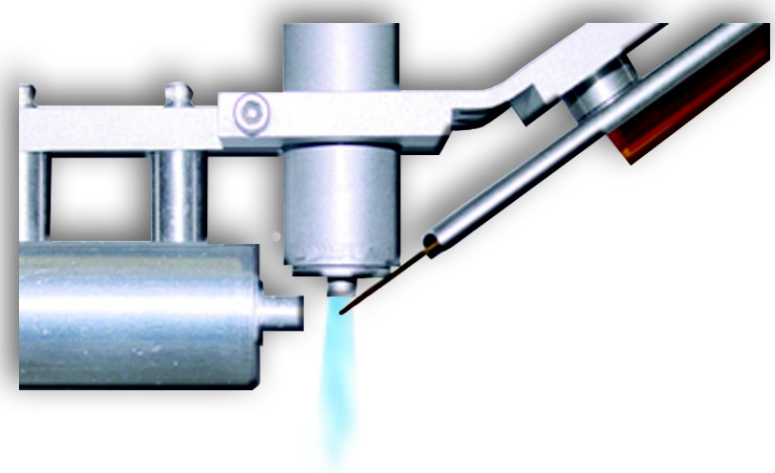
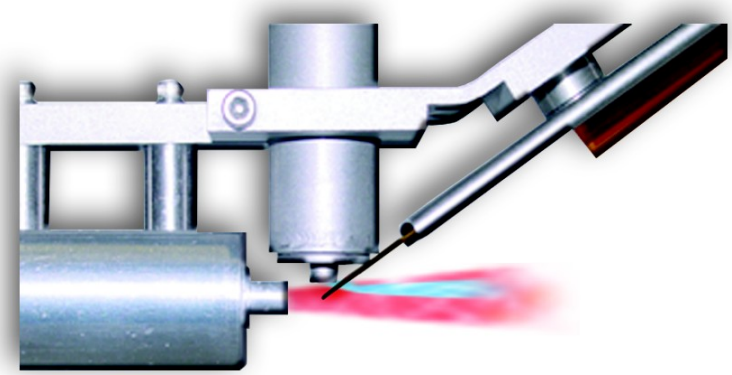
GCxGC-qMS

3ème approche : Comprehensive GCMS

1ère étape



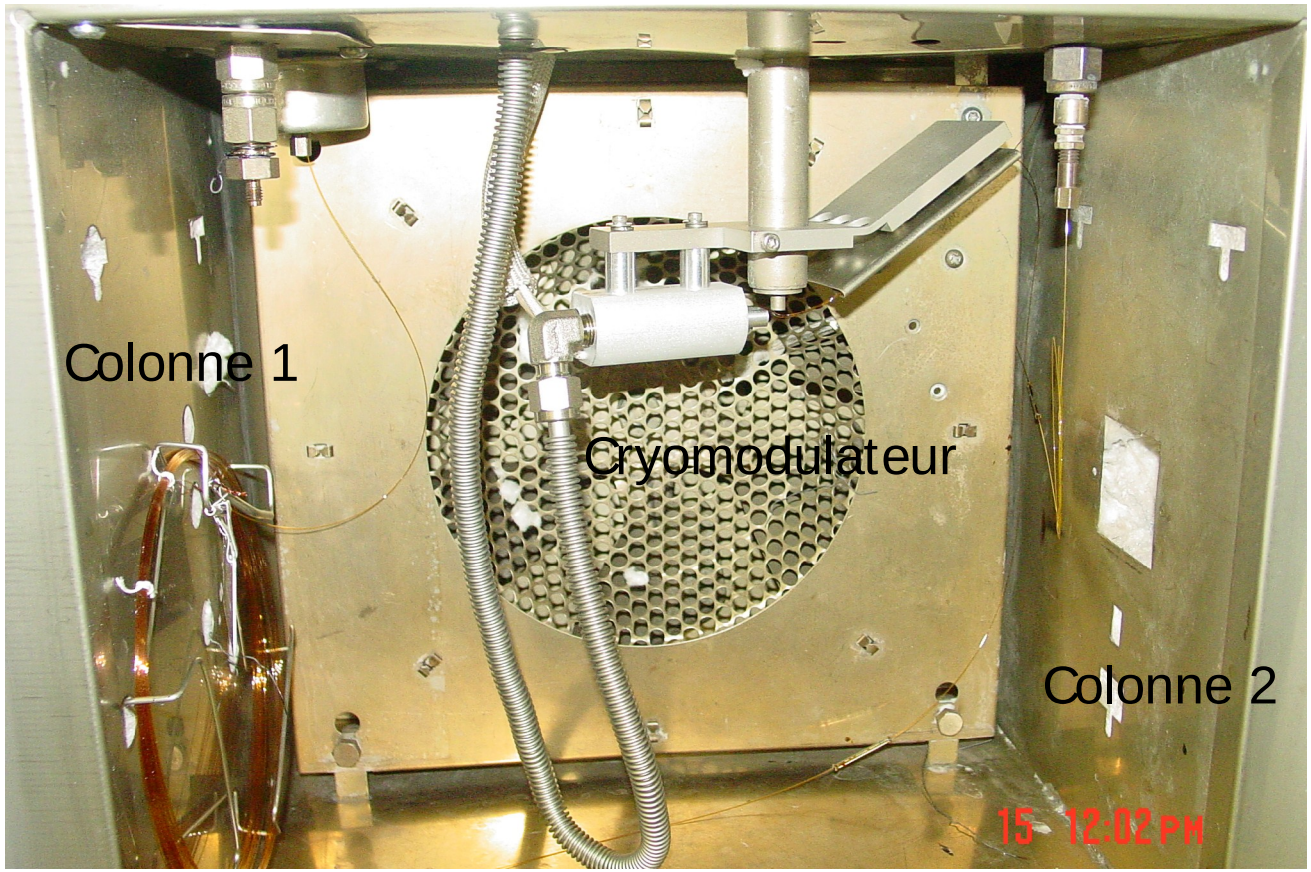
2ème étape



Chromatography

GCxGC-qMS

3ème approche : Comprehensive GCMS



Images fournies par Mr Ed LEDFORD – ZOEX Corporation

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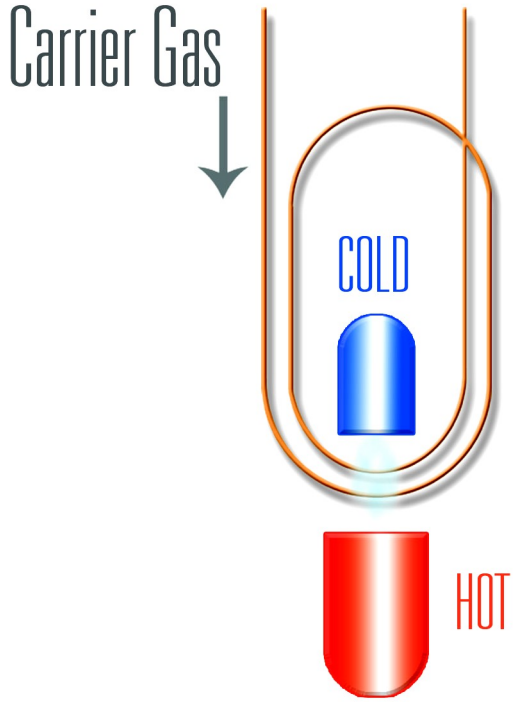
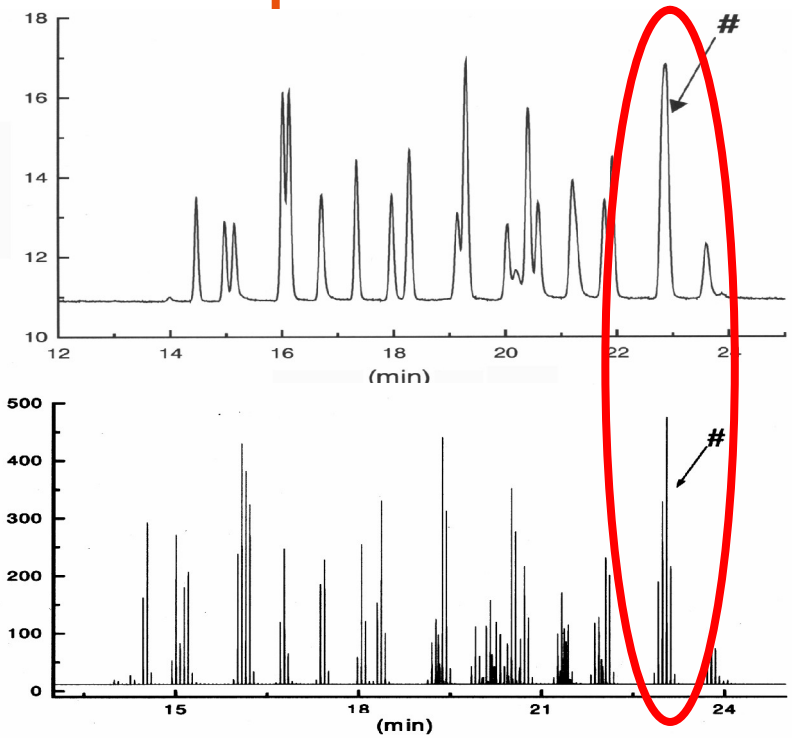
3ème approche : Comprehensive GCMS

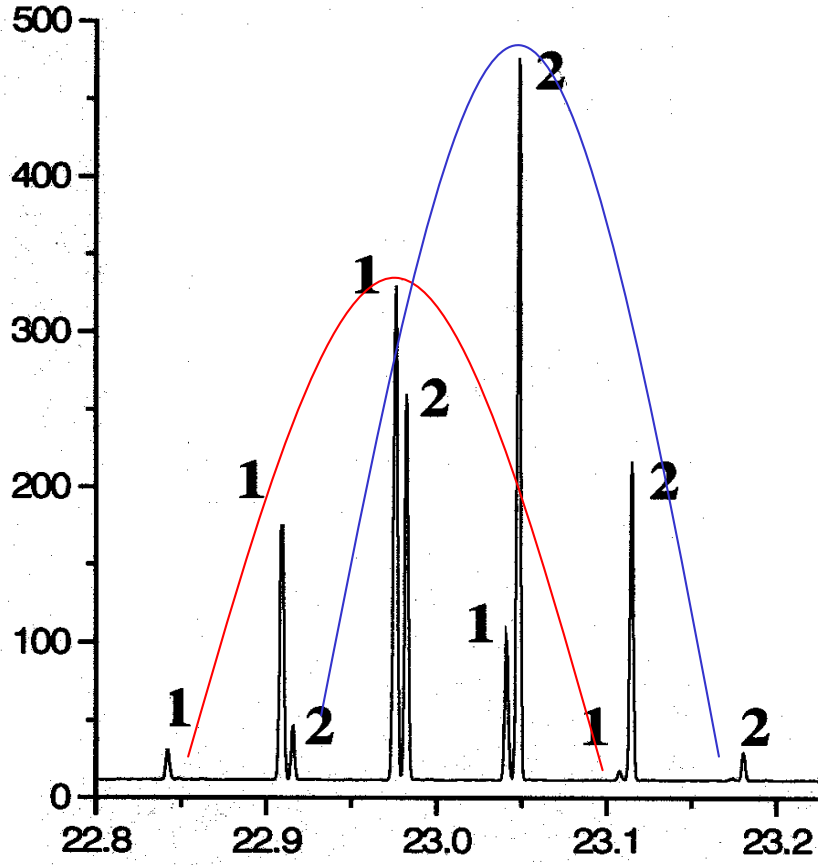
The Loop Modulator



Les deux boucles permettent de refocaliser les composés

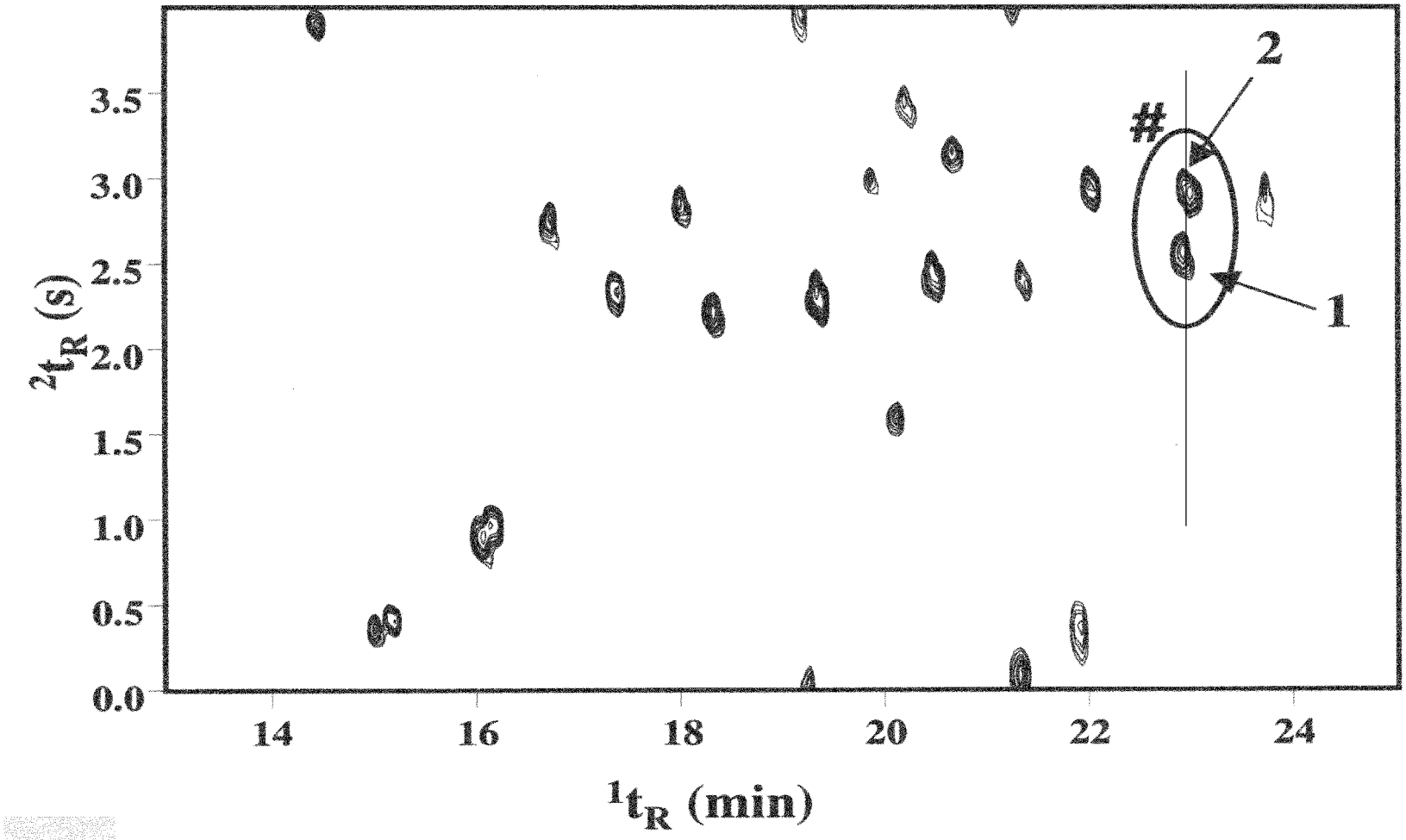
3ème approche : Comprehensive GCMS





Chromatography

3ème approche : Comprehensive GCMS

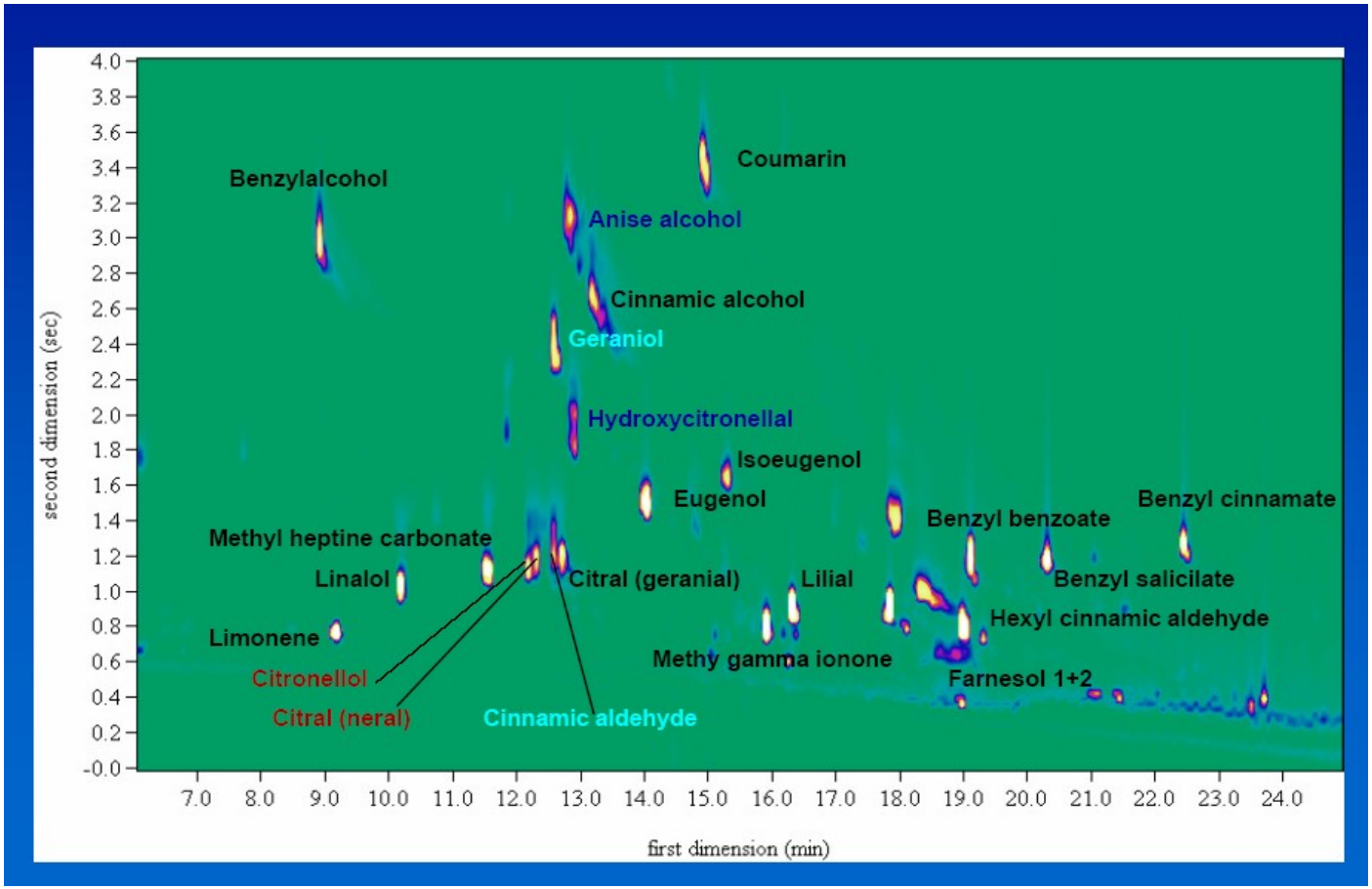


Chromatography

GCxGC-qMS

3ème approche : Comprehensive GCMS

GCxGC-qMS on Allergens



- Mohamed Adahchour et al

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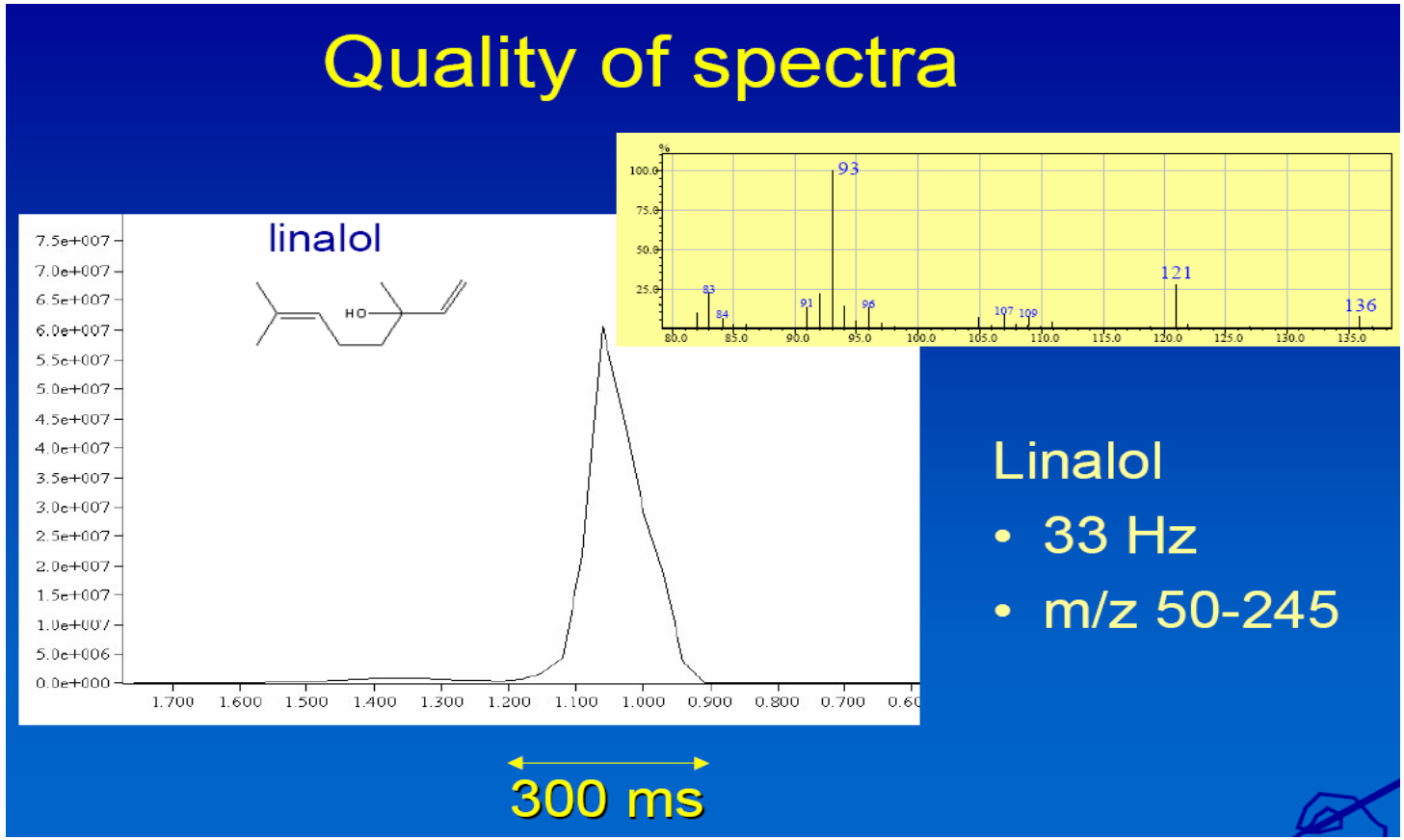
Chromatography

GCxGC-qMS

3ème approche : Comprehensive GCMS



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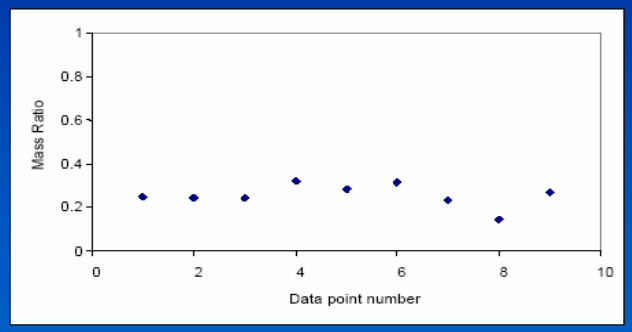
3ème approche : Comprehensive GCMS

GCxGC-qMS

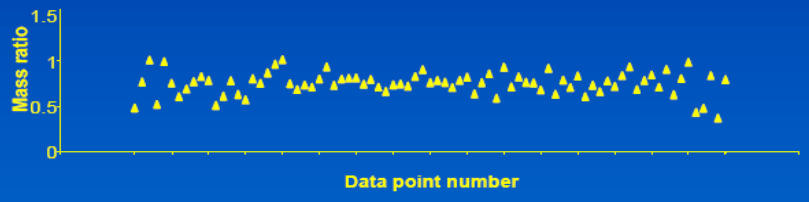
No skewing

mass ratio plot (m/z 136/121)

Shimadzu qMS



TOF MS



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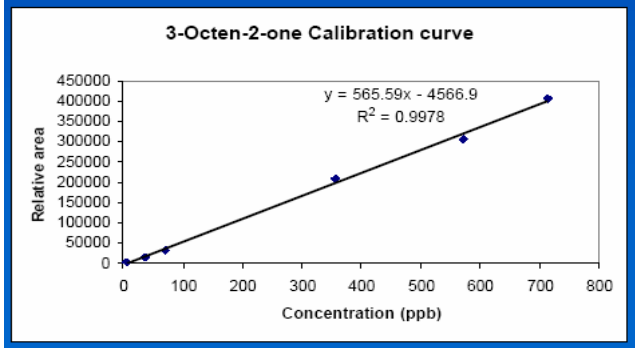
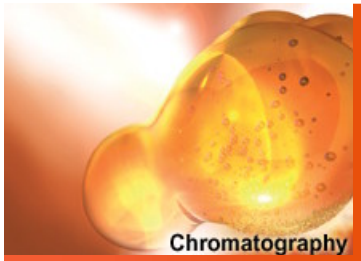
GCxGC-qMS

Quantitative data

Compound	Selective mass	R^2	Detection limits (pg)
3-Octen-2-one	111	0.9978	4
Trans-2,4-Heptadienal	81	0.9992	11
Ethyl-2-methylbutyrate	102	0.9957	1
Nonanal	98	0.9981	4
trans-2-Nonenal	83	0.9951	19



- Mohamed Adahchour et al



3ème approche : Comprehensive GCMS

GCxGC-qMS

1. Comprehensive two-dimensional gas chromatography in combination with rapid scanning quadrupole mass spectrometry in perfume analysis

Luigi Mondello et al:

Journal of Chromatography A, 1067 (2005) 235–243

2.Comprehensive Two-Dimensional Gas Chromatography coupled to rapid-scanning Quadrupole Mass Spectrometer (GC×GC–qMS): Principles and Applications

Mohamed Adahchour, Menno Brandt, Hans-Ulrich Baier, René J.J. Vreuls and Udo A.Th. Brinkman:

Journal of Chromatography A
Volume 1067, Issues 1-2 , 4 March 2005, Pages 245-254
Mass Spectrometry: Innovation and Application. Part IV

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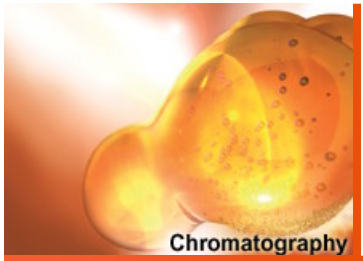

3ème approche : Comprehensive GCMS

GCxGC-qMS

FLAVOUR AND FRAGRANCE JOURNAL
Flavour Fragr. J. 2004; **19**: 91–98
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ffj.1334

Quantitation of suspected allergens in fragrances (Part I): evaluation of comprehensive two-dimensional gas chromatography for quality control

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Accepted 25 March 2003

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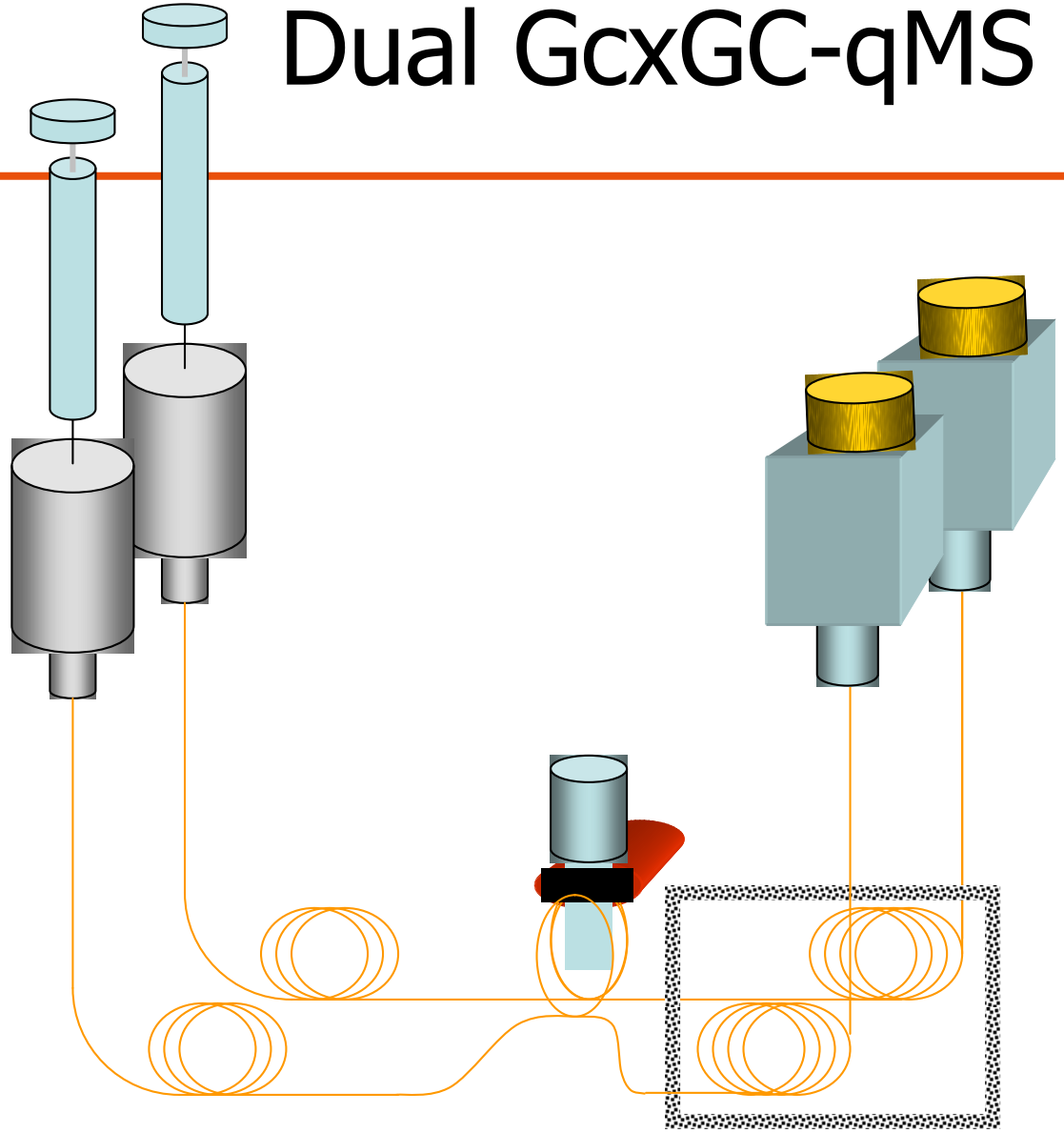
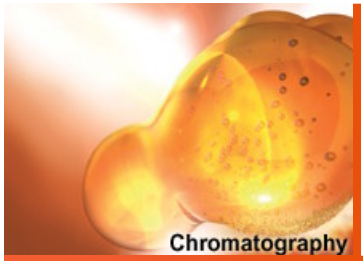
GCxGC-qMS

Dual GcxGC-qMS





4ème approche : Crèmes et produits finis cosmétiques

Produits Finis

- Matrices complexes et variées :

  Savons, crèmes, shampoing, ...

  Techniques envisagées à base de thermodésorption. → deux appareils sur le marché :

  CIS (GERSTEL)

  OPTIC (ATAS)

Chromatography

4ème approche : Crèmes et produits finis cosmétiques

Produits Finis

CIS-4

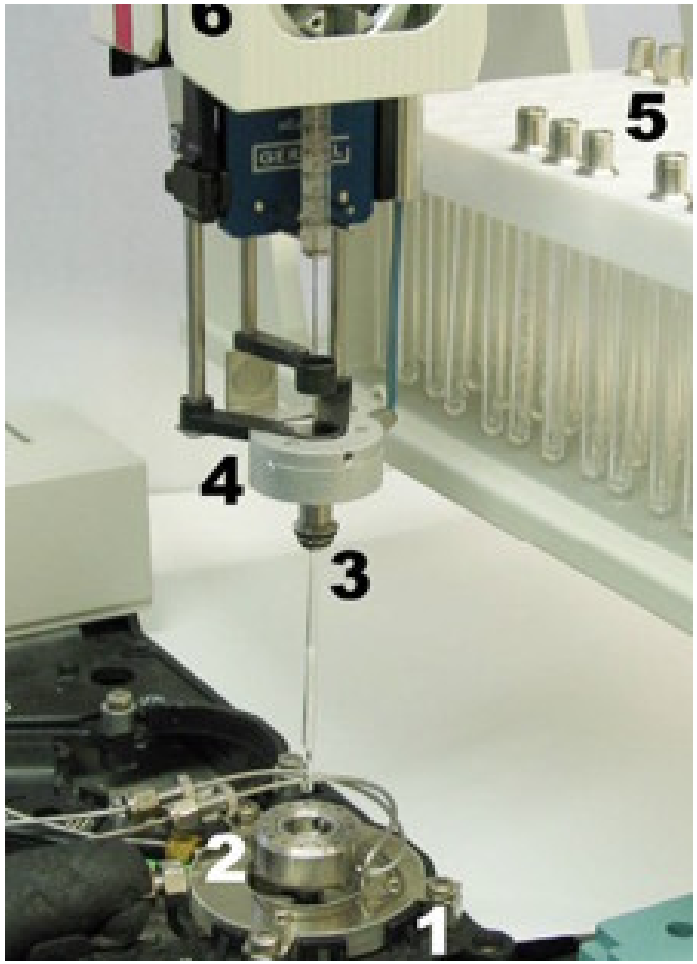
Automated Liner EXchange

SHIMADZU Solutions for Science

Chromatography

Produits Finis

Automated Liner Exhange



- Changement automatique du liner à chaque analyse.
- évite la contamination des colonnes
- Pas de ligne de transfert
- Injection de matrices chargées

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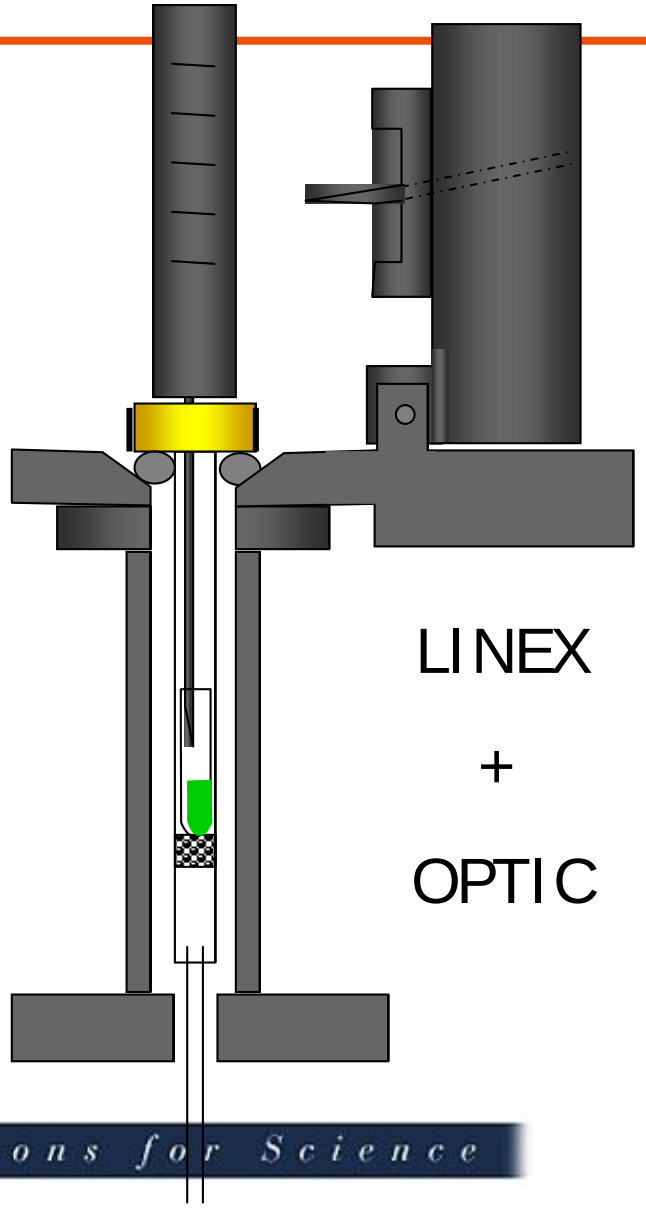
Chromatography

Produits Finis

LINEX (Liner Exchange)



LINEX

+

OPTIC

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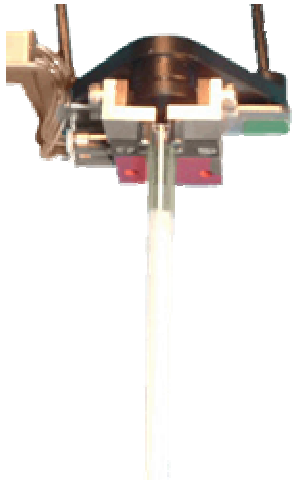
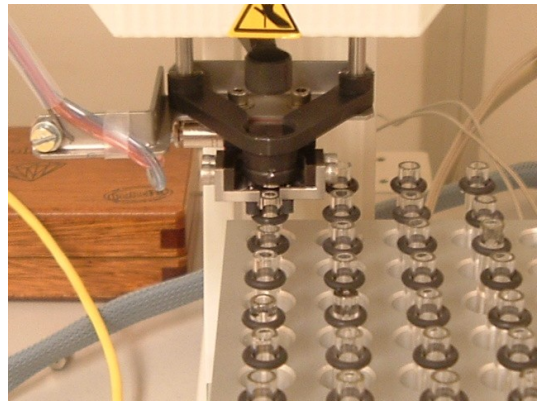
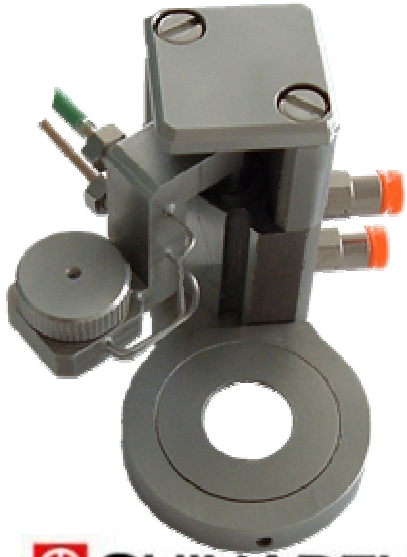
Chromatography

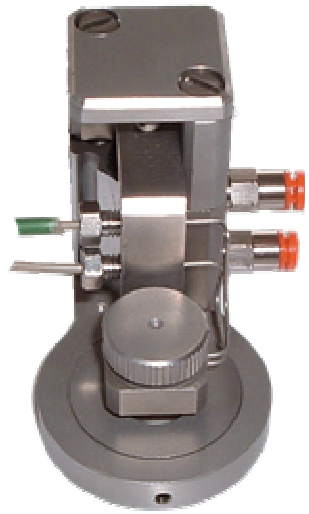
LINEX

Produits Finis









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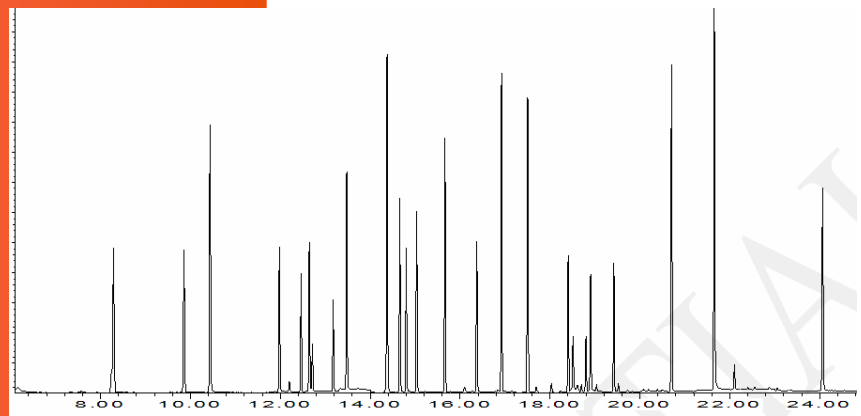
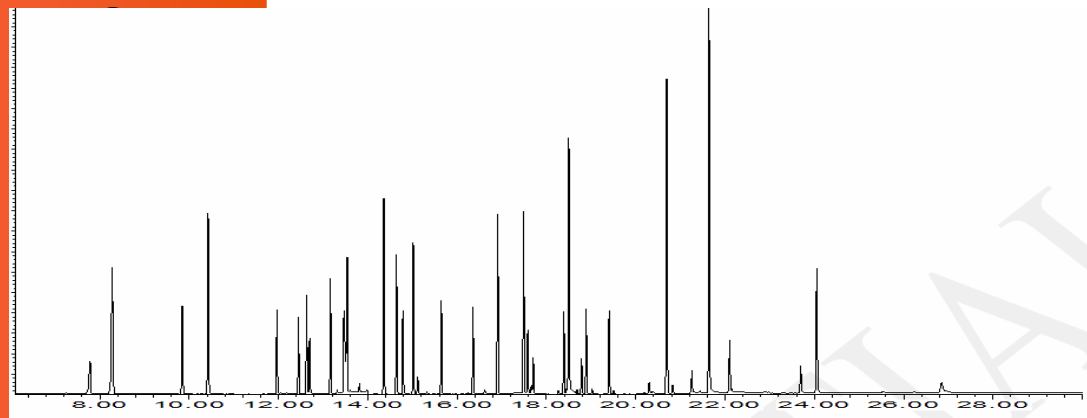
4ème approche : Crèmes et produits finis cosmétiques

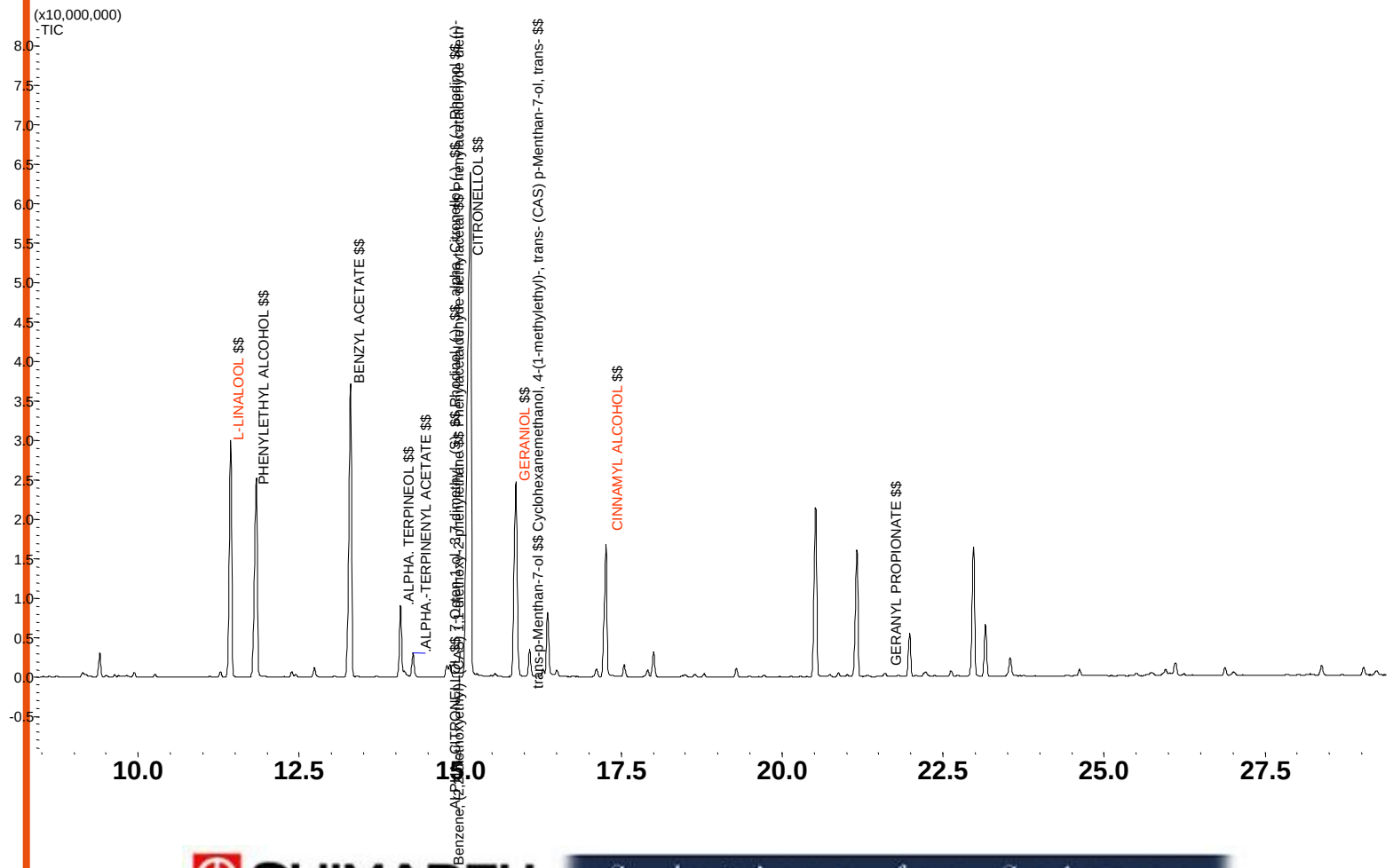


Allergen Standard 10 ppm



Emulsion spiked with 100 ppm

4ème approche : Crèmes et produits finis cosmétiques



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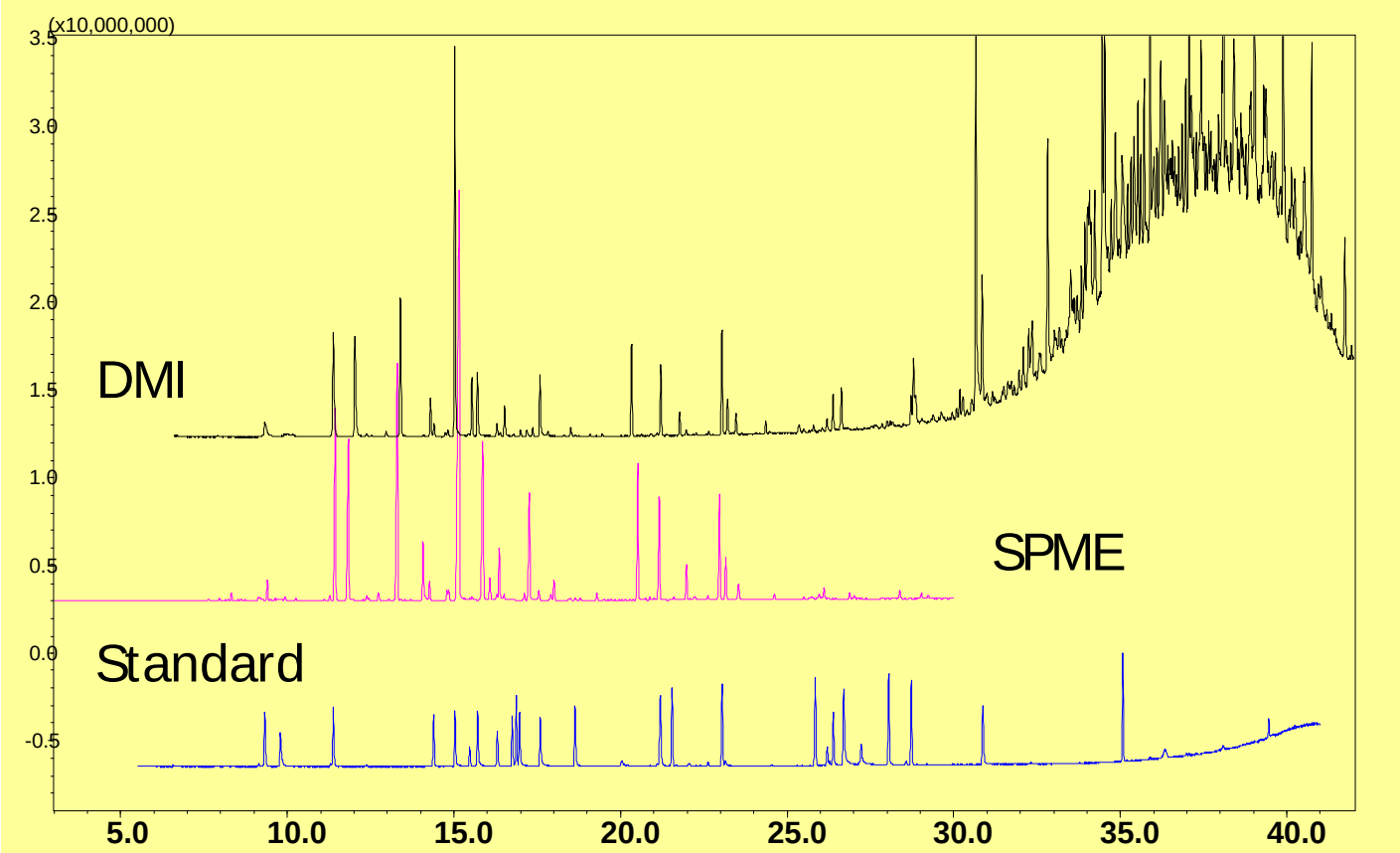
Chromatography

Exemple de crème

Crèmes

- Extrait dans l'acétone pour l'analyse en DMI
- Crème déposée directement dans le flacon HS pour la SPME



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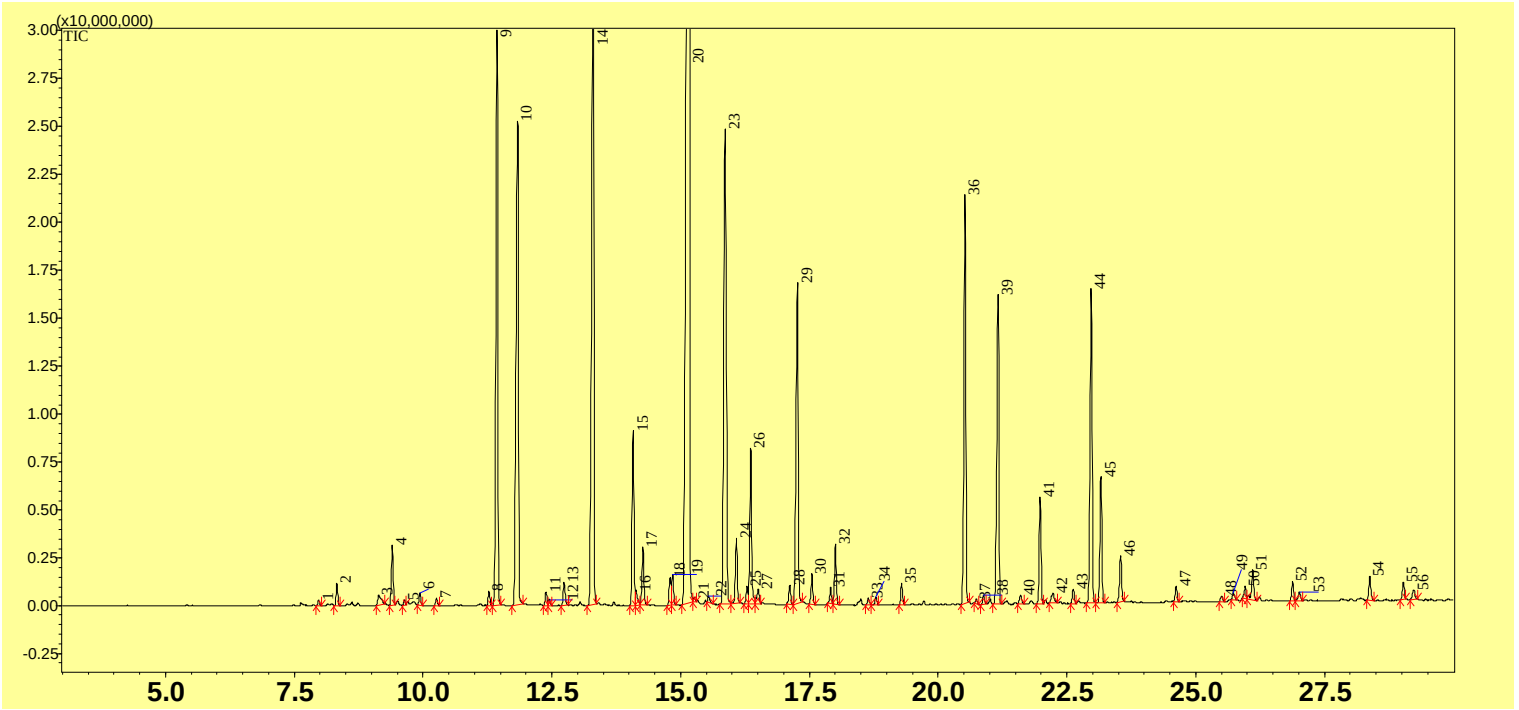
SPME

Crèmes

SPME with hand creme (100 mg dans 10 ml)

- - undiluted into headspace vial and heated at 60°C
- Adsorption 20 min with DVB/PDMS



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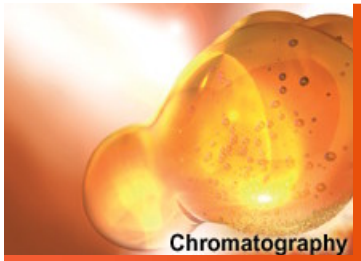

résumé

Résumé

Contrôle des matières premières	Coélution Faux positifs / faux négatifs	GCMS-SCAN **(OK)** GCMS-SIM **(Pb Coelution)** GCMS-Dual column **(OK)** Comprehensive GCMS **(OK)** GCxGC-TOF **(Trop cher)**
Contrôle des compositions	Coélution Faux positifs / faux négatifs	GCMS-SCAN GCMS-SIM GCMS-Dual column Comprehensive GCMS GCxGC-TOF
Contrôle des crèmes, shampoing, émulsions, ...	Coélution Faux positifs / faux négatifs Pb matrice	ALEX-GCMS **(OK)** LINEX-GCMS **(OK)** SPME **(OK)**