Applications au contrôle des produits naturels de la chromatographie gazeuse bidimensionnelle

Bidimentionnal gas chromatography applications for natural products control

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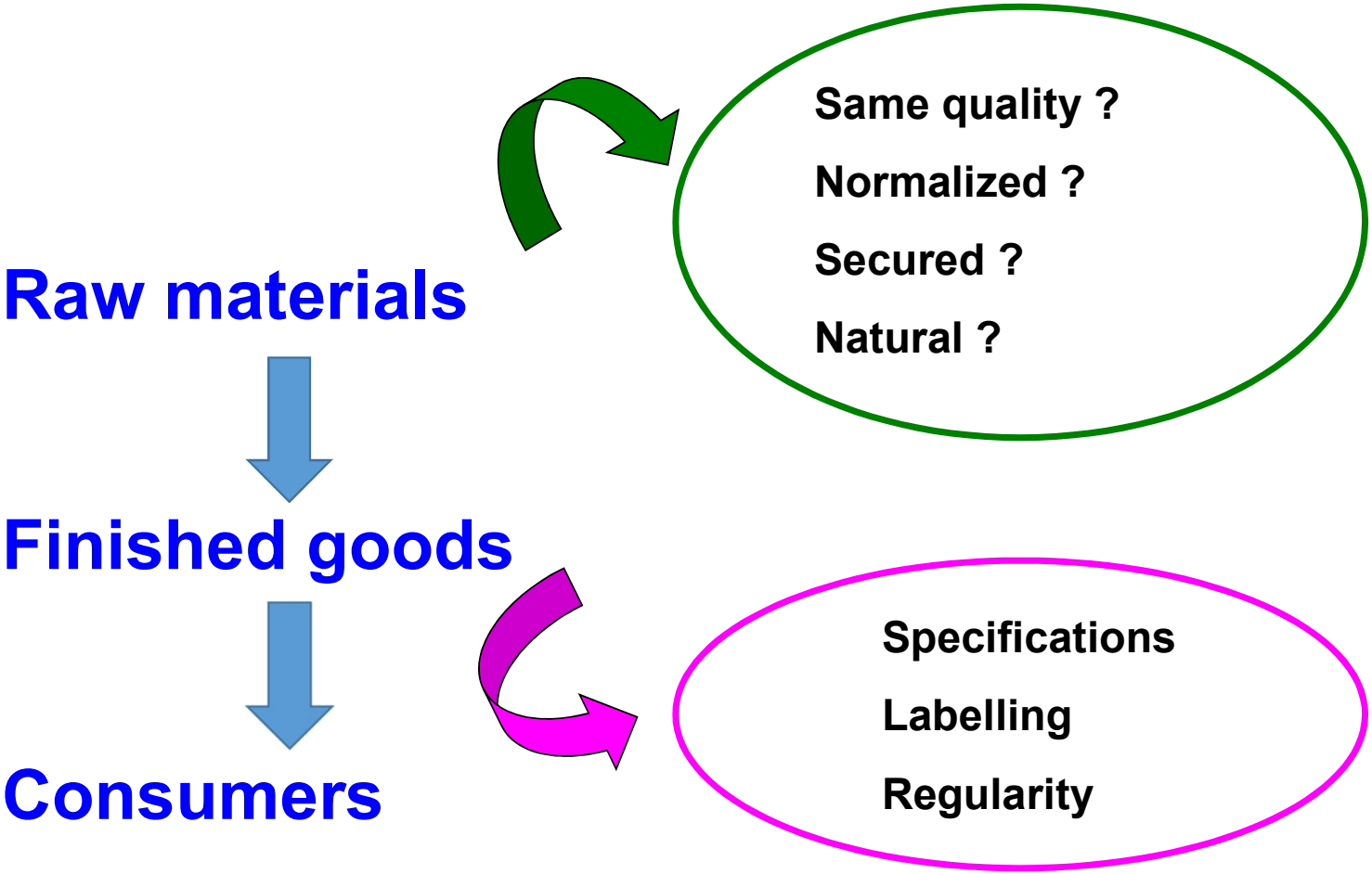
Why analysis is important ?



Food product: Fruit juice example

look for what needs to be there:

Sugars → Chrom. Ionic, HPLC, Chrom. Gaz

Color → UV-Vis, HPLC

Flavours → Chrom. Gaz

Minerals → EA / AA, ICP, ICP-MS

Vitamines → HPLC

look for what should not be there:

Pesticides → GC-NPD/ECG/MS, LC-MS

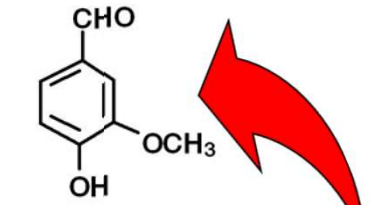
Heavy Metals → ICP-MS

Nature identical → ?

Using classical analysis, you are not able to differentiate Natural from Synthetic flavouring molecule !

Exactly same formula , Identical nature !

We need other tools !



Vanillin

1 kg synthetic 66 euros

1 kg (50 kg beans) 25,000 euros

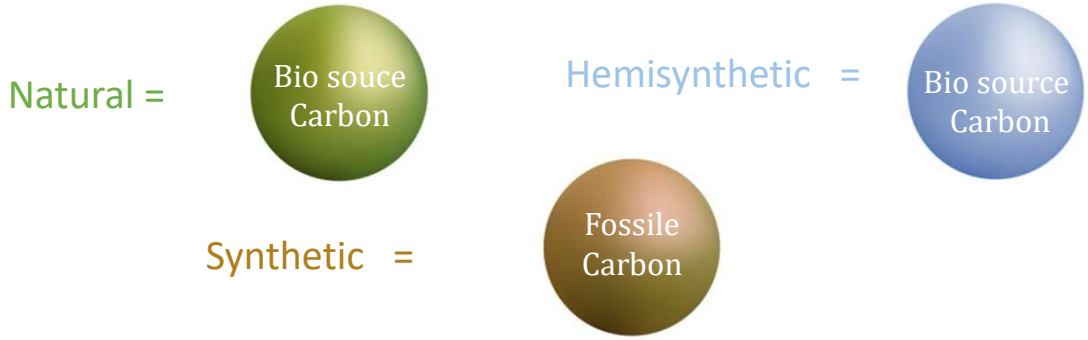
INTRODUCTION

Authenticity definition

Essential oil authentication = Naturalness and purity control

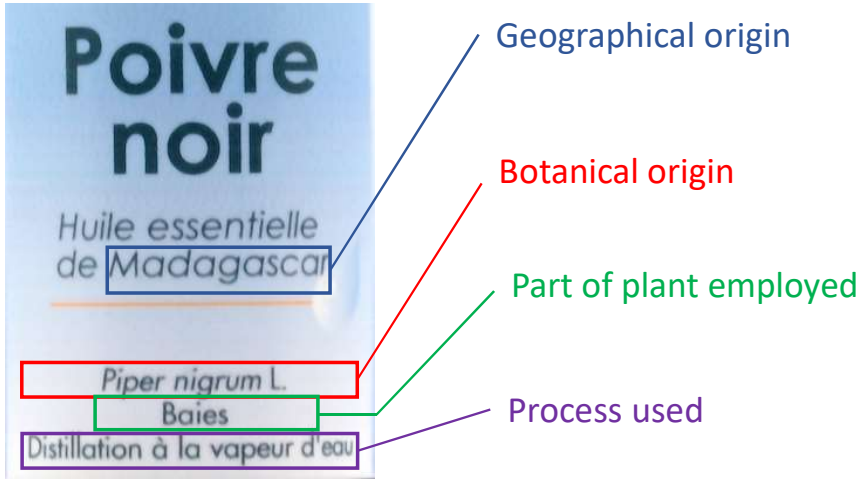
Naturalness

- A product is 100 % natural when composed of 100% bio source carbon
- At the opposite, a synthetic material is composed of 100 % fossile carbon (petroleum)
- Particular case: **hemisynthesis**, composed of 100% bio source carbon, but chemically modified



Pureté

- An essential oil is difined by Geographical origin, botanic, part of vegetal used, process employed .
- An essential oil is pure when no substance addition, even natural, different from original vegetal material.

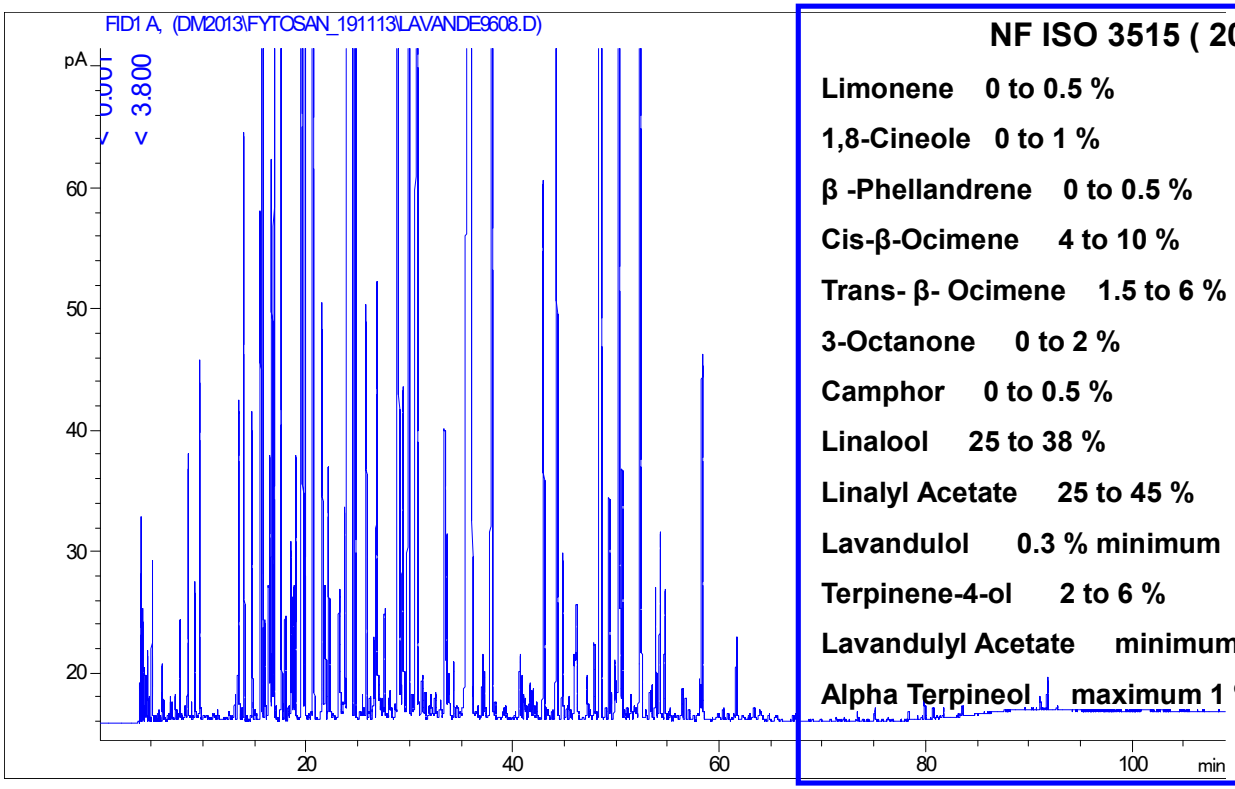


“Food fraud was recently defined in a report funded by the National Center for Food Protection and Defense (University of Minnesota) as a collective term that encompasses the deliberate substitution, addition, tampering or misrepresentation of food, food ingredients or food packaging, or false or misleading statements made about a product for economic gain”

Estimation in France: Frauds penalise 30.000 employments

With AFNOR norm, only 50 to 70% of an essential oil (EO) composition is discribed

What are other peaks? Why an EO is never known at 100 %?



NF ISO 3515 (2004)

Limonene	0 to 0.5 %
1,8-Cineole	0 to 1 %
β -Phellandrene	0 to 0.5 %
Cis-β-Ocimene	4 to 10 %
Trans- β- Ocimene	1.5 to 6 %
3-Octanone	0 to 2 %
Camphor	0 to 0.5 %
Linalool	25 to 38 %
Linalyl Acetate	25 to 45 %
Lavandulol	0.3 % minimum
Terpinene-4-ol	2 to 6 %
Lavandulyl Acetate	minimum 2 %
Alpha Terpineol	maximum 1 %

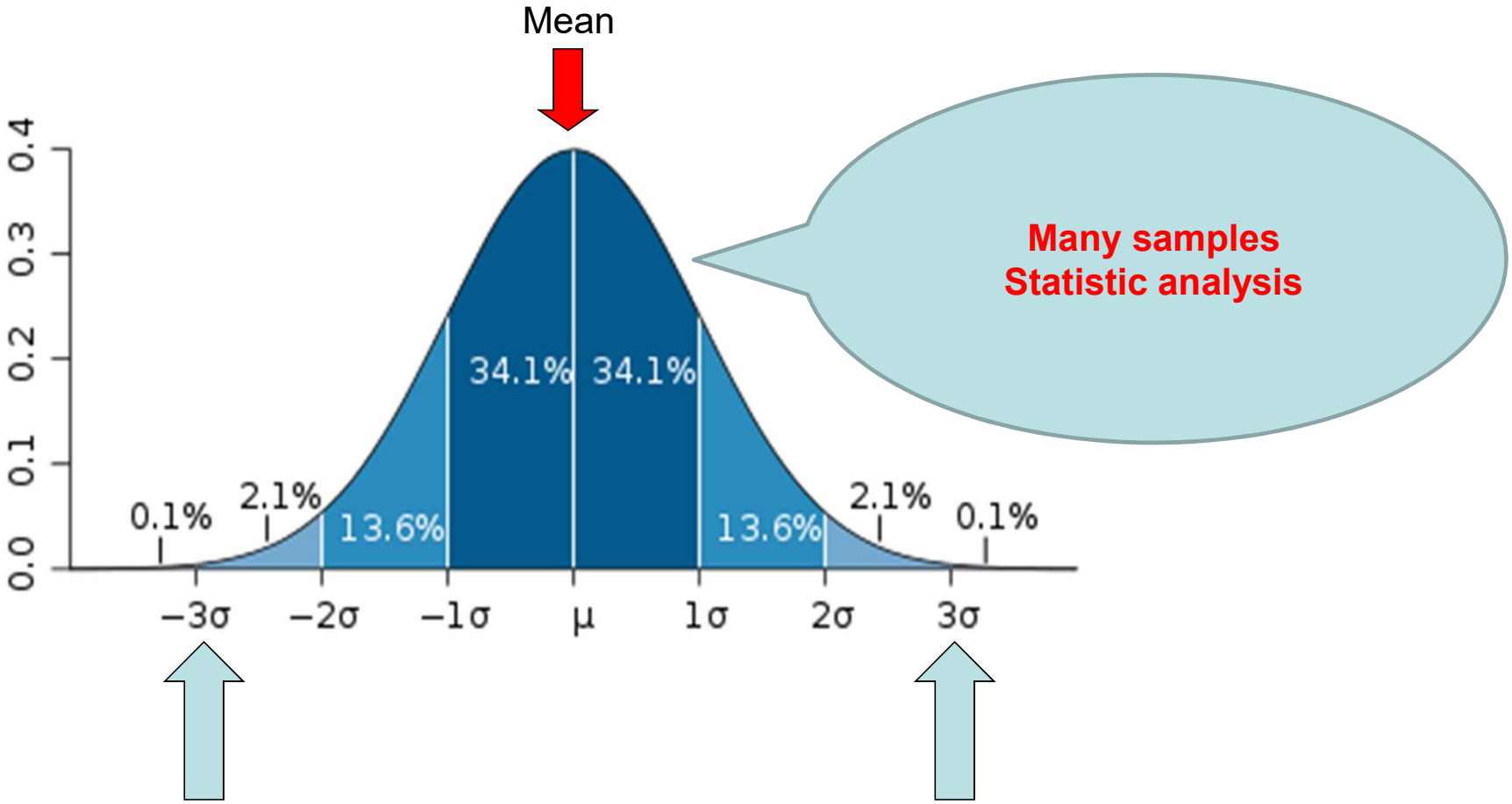
Typical chromatogarm of Lavender EO (>120 pics), Only 13 in norm !

You must remenber something : an essential oil can be compliant with norm and adulterated, an essential oil can be out of scope and natural !

R&D on essential oils knowledge

- Since more than 20 years, R&D for better knowledge of Aromatic and Medicinal plants composition : Isolation , identification
- Spectral and retention indices data banks(> 4000 molecules)
- Stable isotopes measurements and enantiomers profiles
- Authenticity data banks

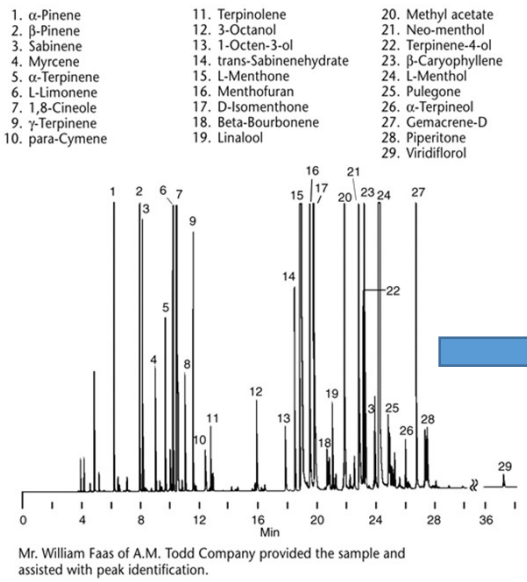
Natural products have one disadvantage : variability !



Mean with +/- 3* Standard deviation represents >99 % population

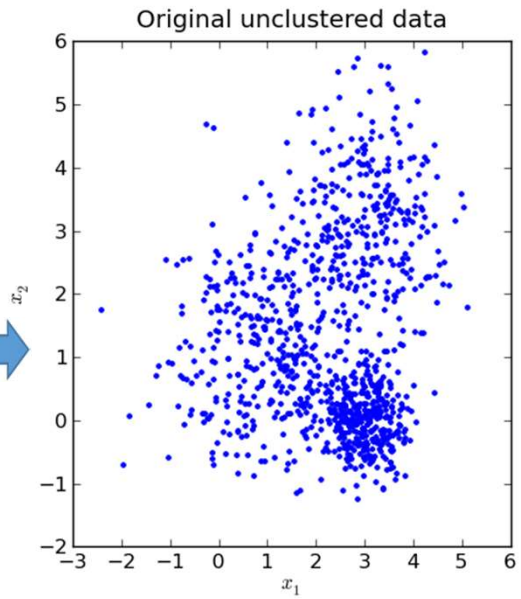
Natural distribution : Gauss Curve

Analytical stategy

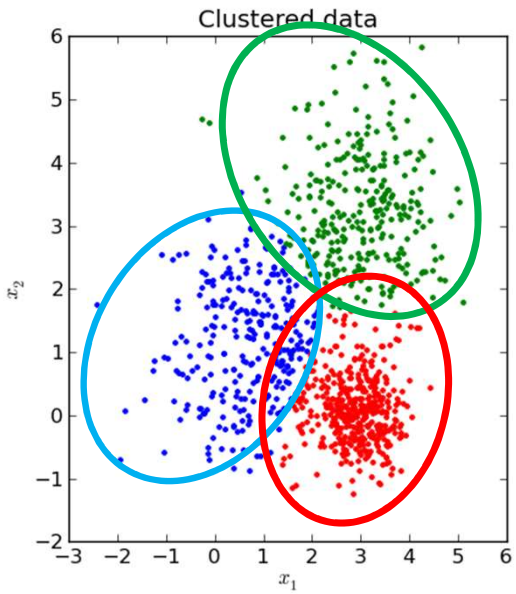


Sample chromatogram

Datas Banks



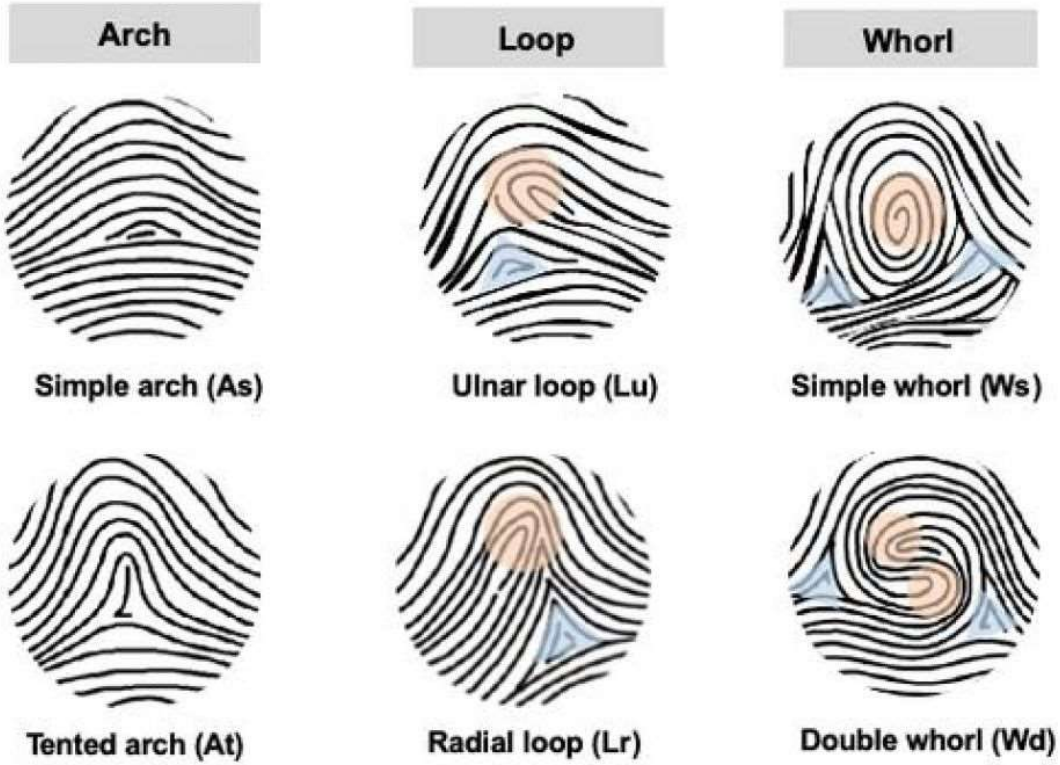
Unsupervised analysis



Supervised analysis

Metabonomic : Vegetal specificity (Metabolisme /Soil/ Enzymatic pathway) , secondary active metabolites biosynthesis is as trackable as fingerprints.

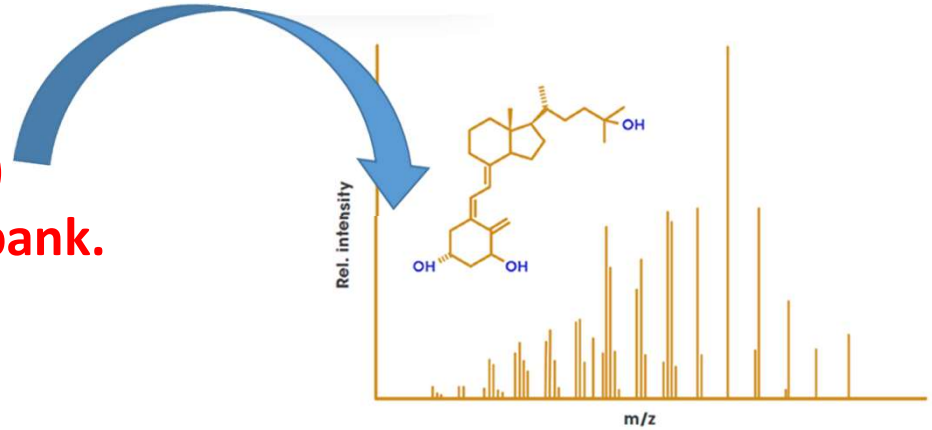
Guilty or innocent?



Mass spectra instument representation

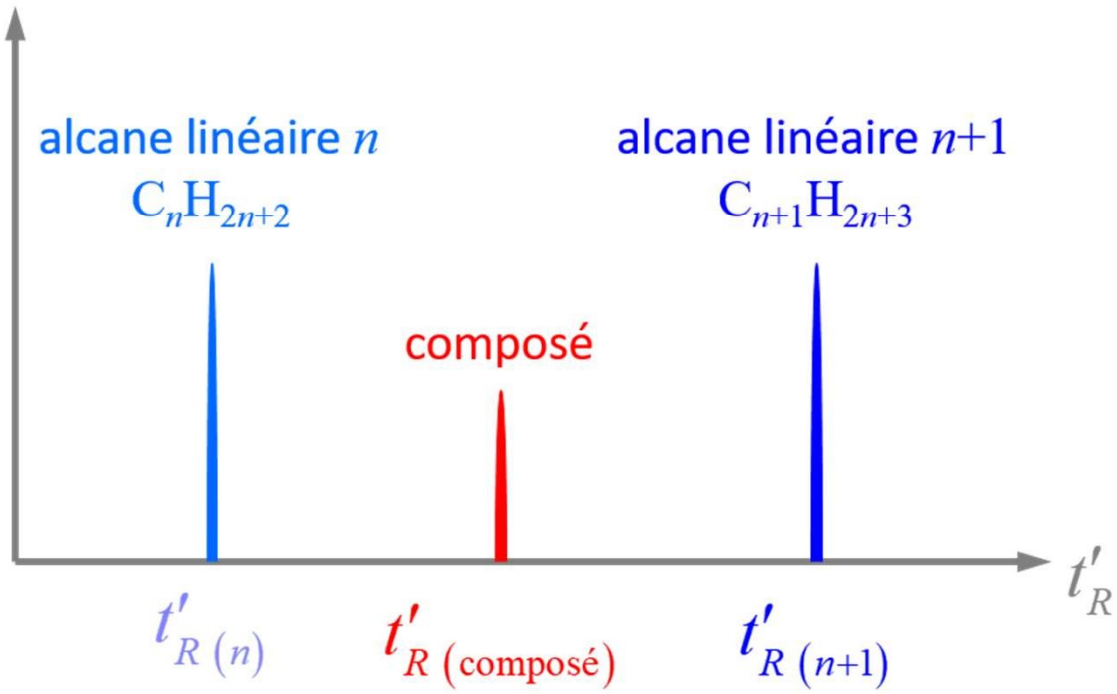
Ion Source	m/z mass filter	Detection Amplification	Signal processing
Gas phase Ion production	Mass separation Fonction of m/z SCAN or SIM mode	Ionic current conversion In electric power	Mass spectra representation

SCAN mode acquisition (scanning a mass range)
identification by comparing with spectras data bank.
SIM mode acquisition
Selection of specific ions, more sensitive mode.



Gas chromatography parameter : retention time t_R

to compensate for variations (aging of column) , retention indices are used



indice de Kovàts du composé (hors isotherme)

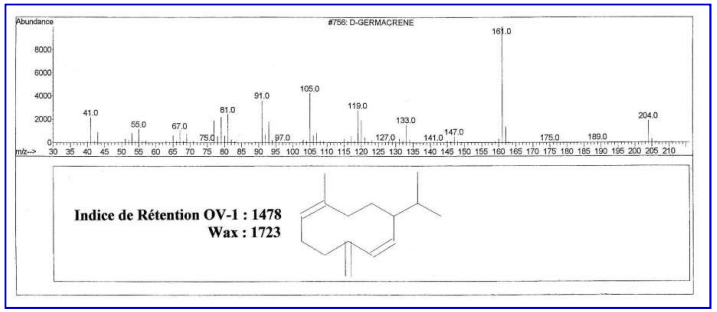
$$I = 100 \cdot \left[n + \frac{t'_{R\,(\text{composé})} - t'_{R\,(n)}}{t'_{R\,(n+1)} - t'_{R\,(n)}} \right]$$

Important R&D for better mass spectra identification:

retention indices and mass spectra data banks(4000 molecules)

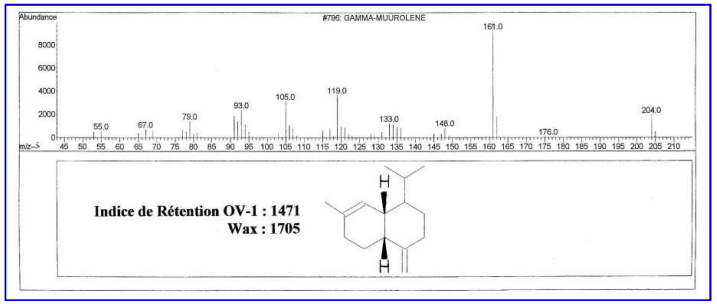
Germacrene-D

OV-1 1478
WAX 1723



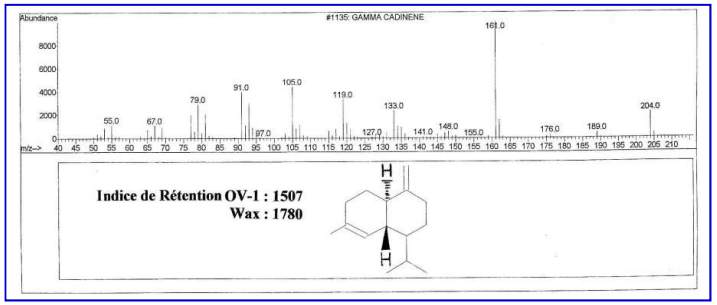
Gamma Muurolene

OV-1 1471
WAX 1705



Gamma-Cadinene

OV-1 1507
WAX 1709

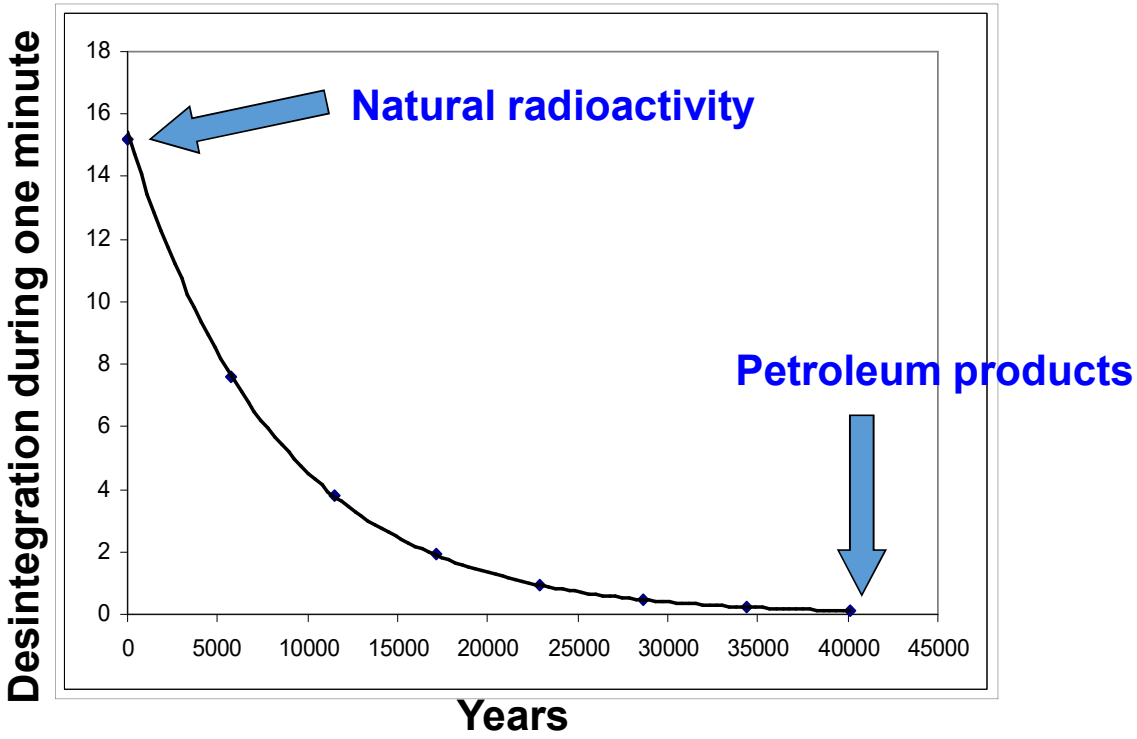


Many traps!
Identical spectras

Even with high tech instrument
Caution !!!!!!

Experience is important !

C^{14} desintegration curve : Half time period : 5730 years



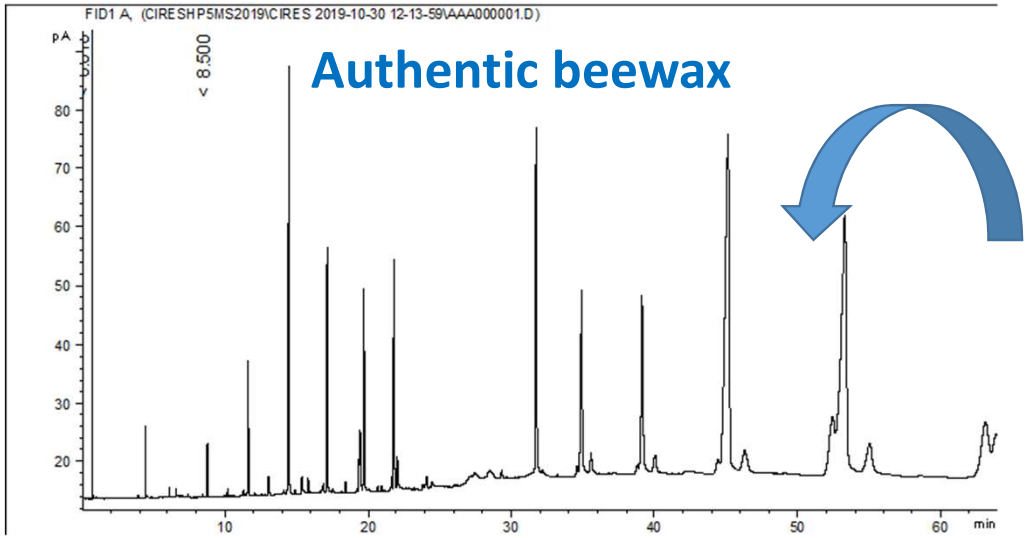
A molecule from natural source modified with organic synthesis also leads to a good C14 measure !

For example oxydation (chemistry catalyse) of alcohol leads to corresponding acid : Butanol-1 gives butyric acid

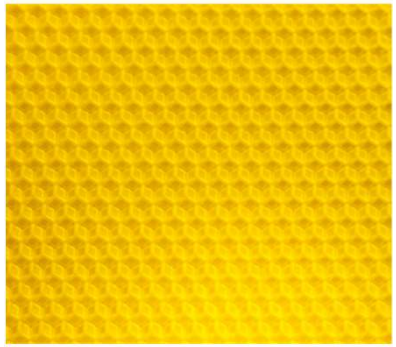
This method canno't be a complete proof !

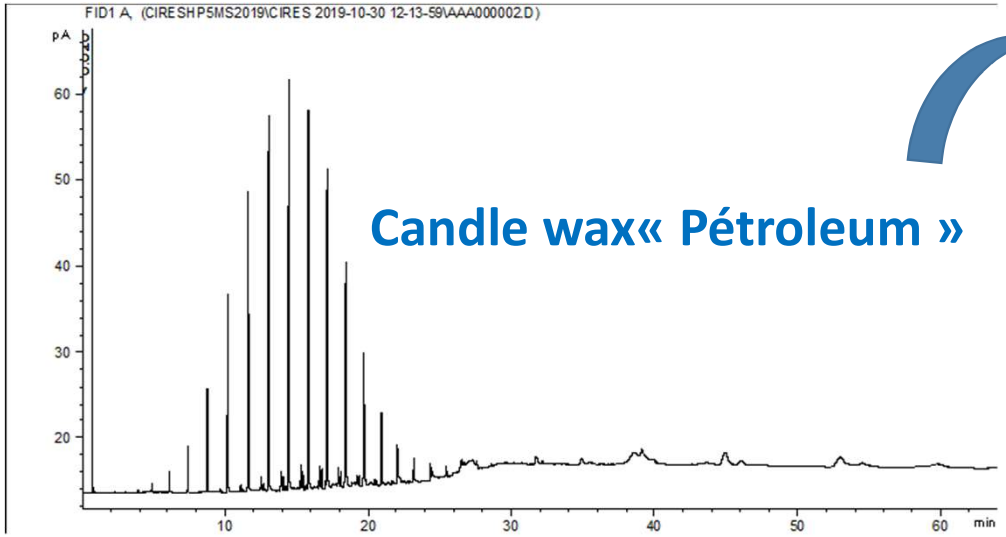
Forensic examination of a seizure by customs of embossed beeswax

Analyses par GC-FID

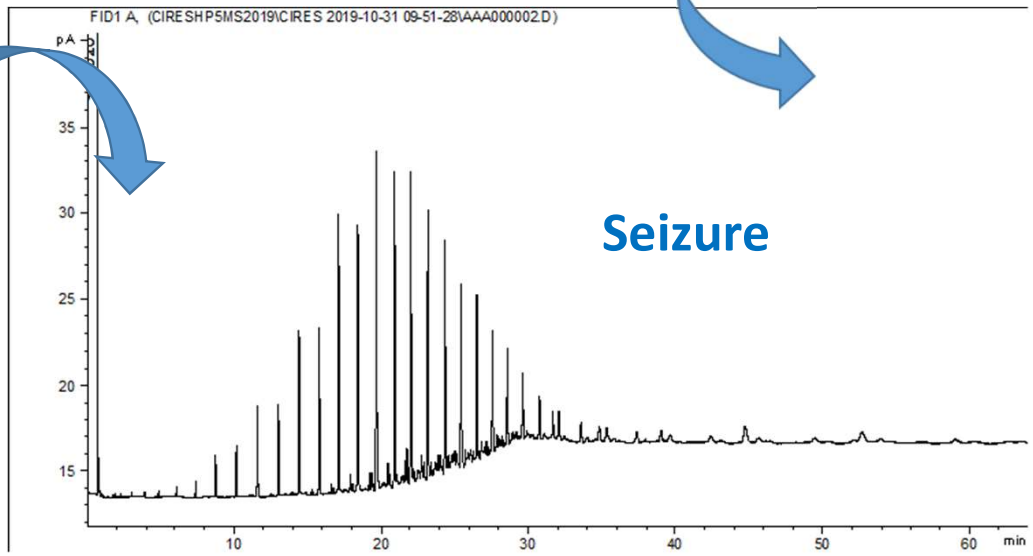


Authentic beewax



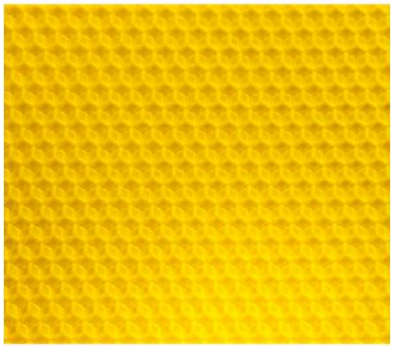


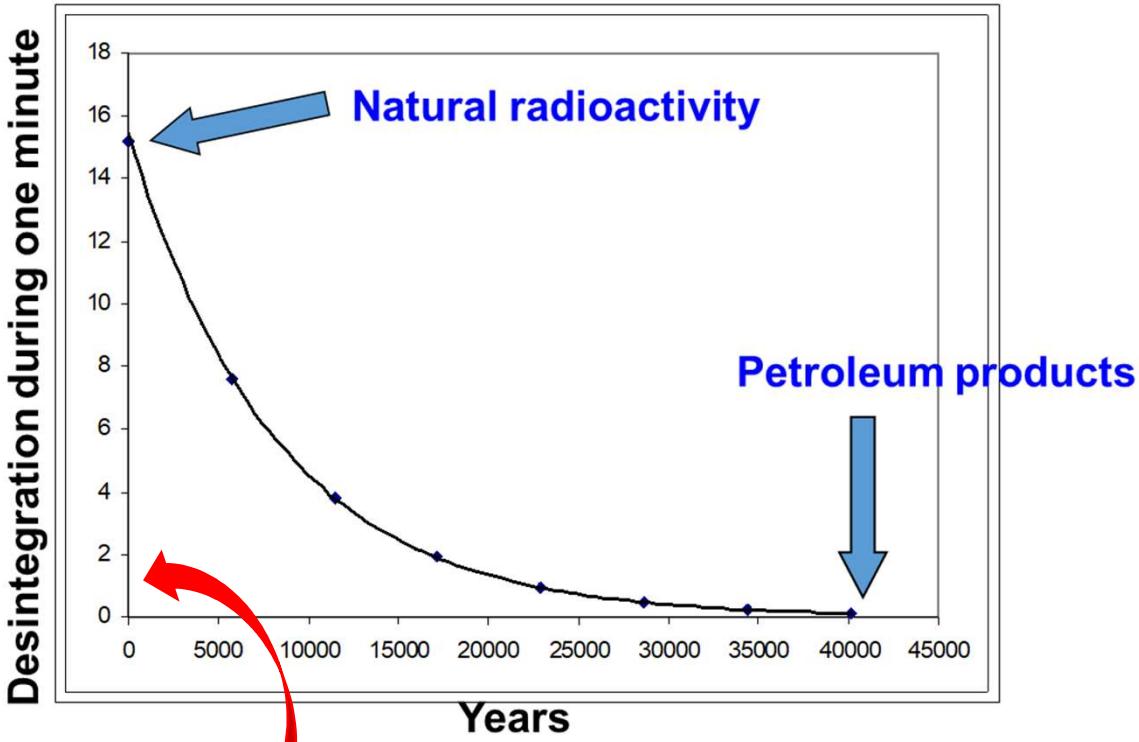
Candle wax« Pétroleum »



Seizure

Forensic examination of a seizure by customs of embossed beeswax

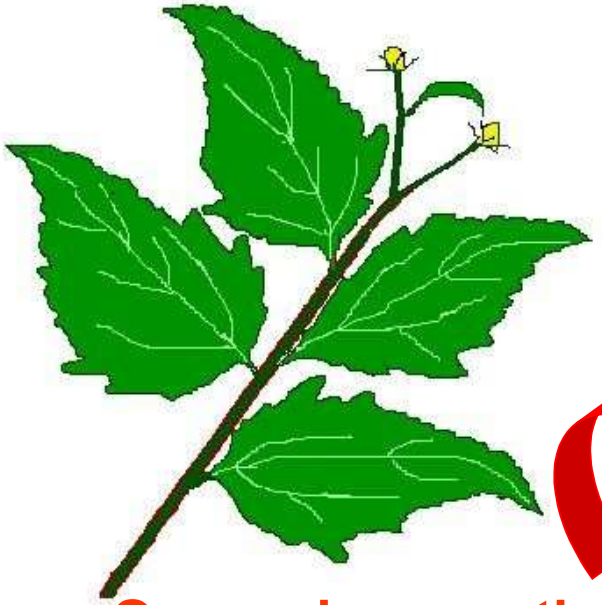


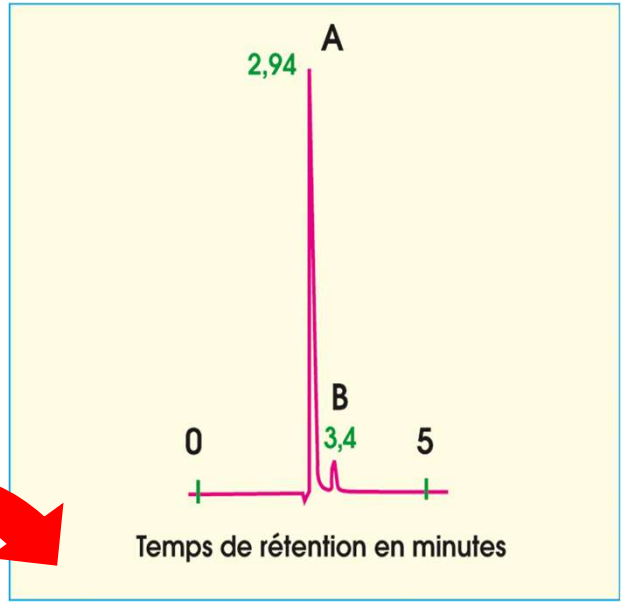


1.6 dpm (+/- 0,02)/ g is measured

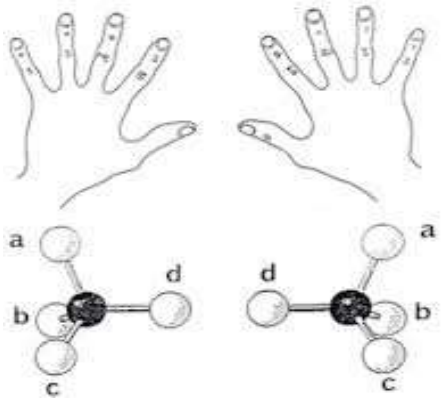
12% natural origin / 88 % petroleum origin

Enzyme catalyst leads to stereoselective synthesis



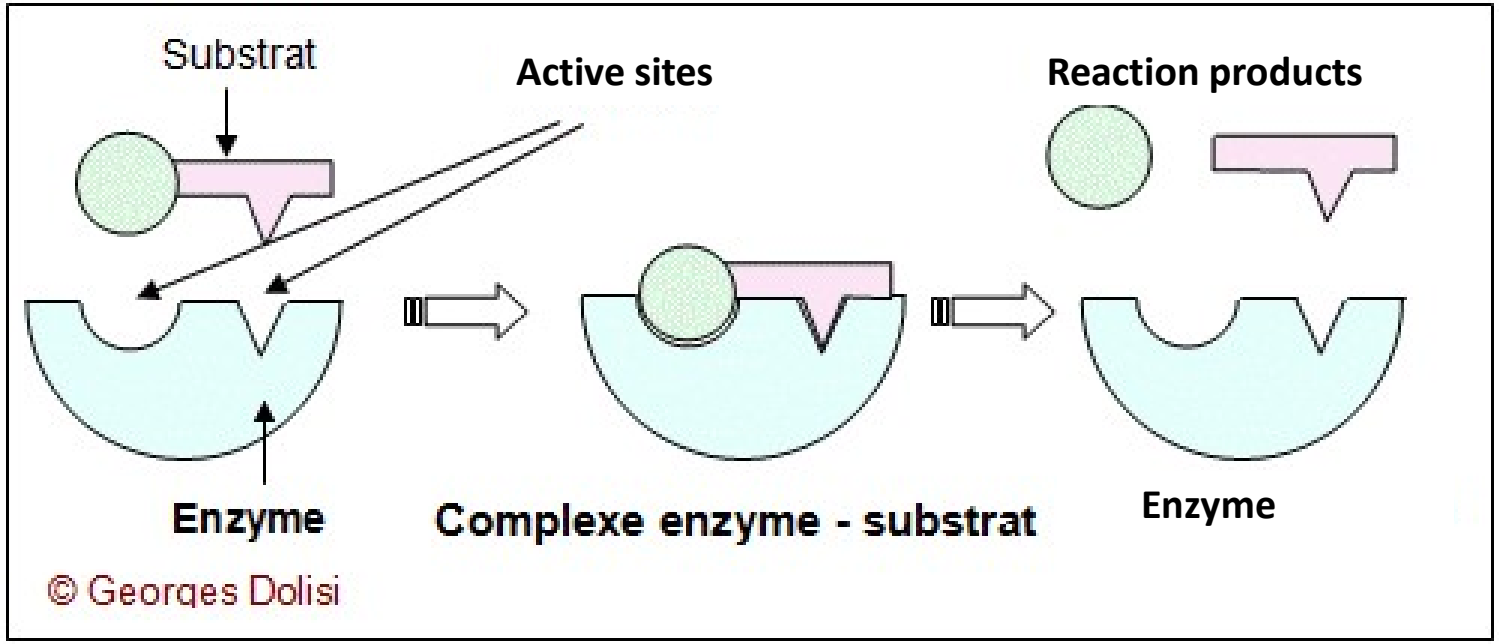


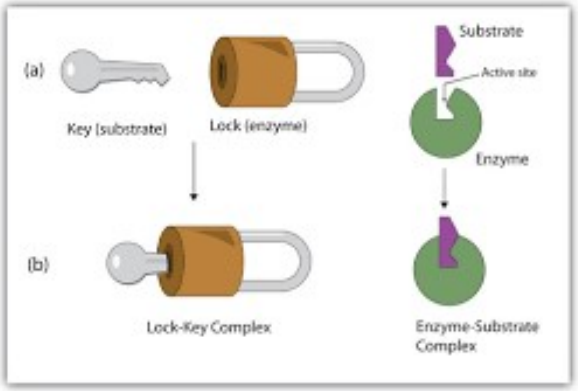
One major enantiomer in lot of cases.



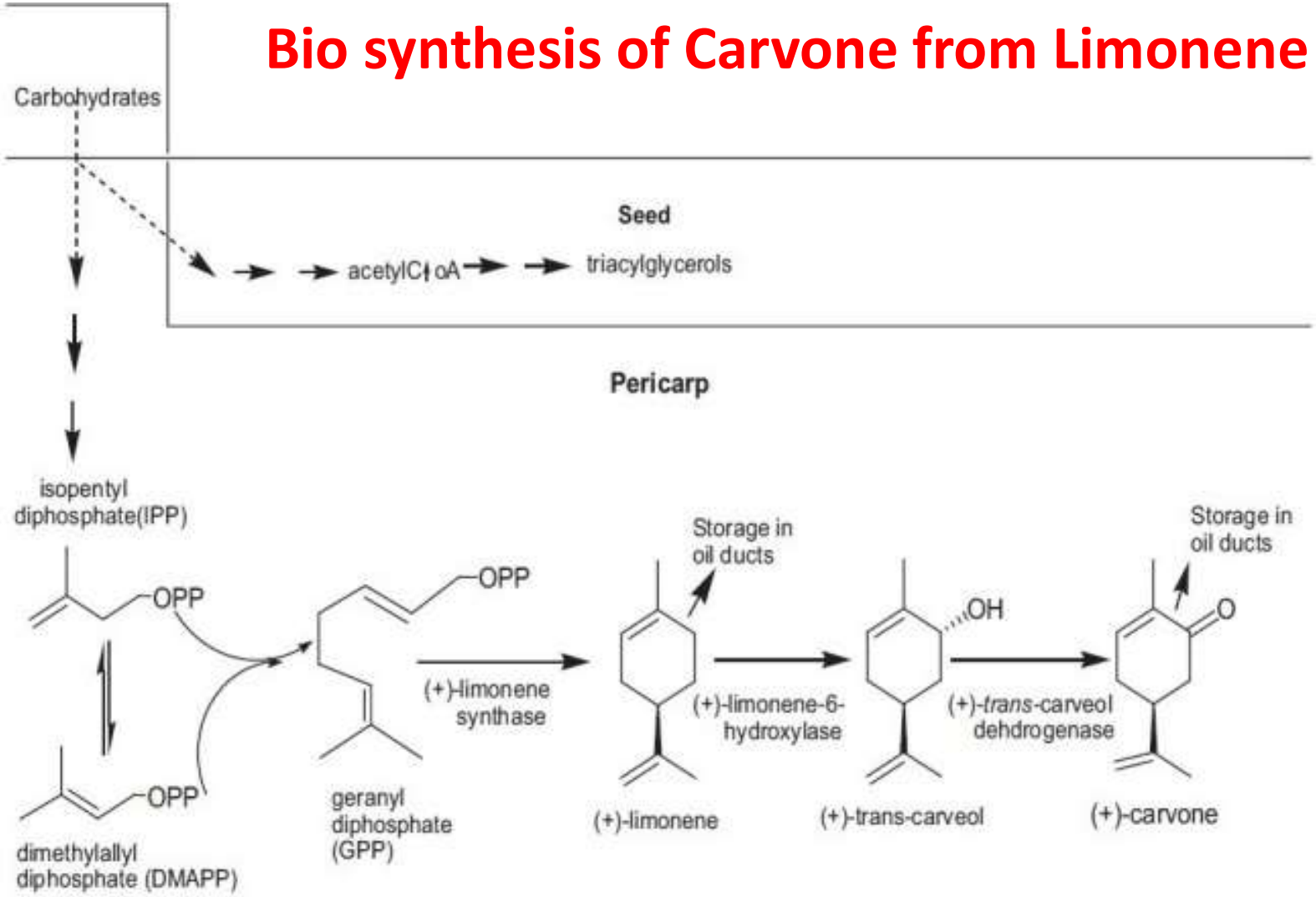
Enzymes give specific molecule geometry (enantioselectivity)

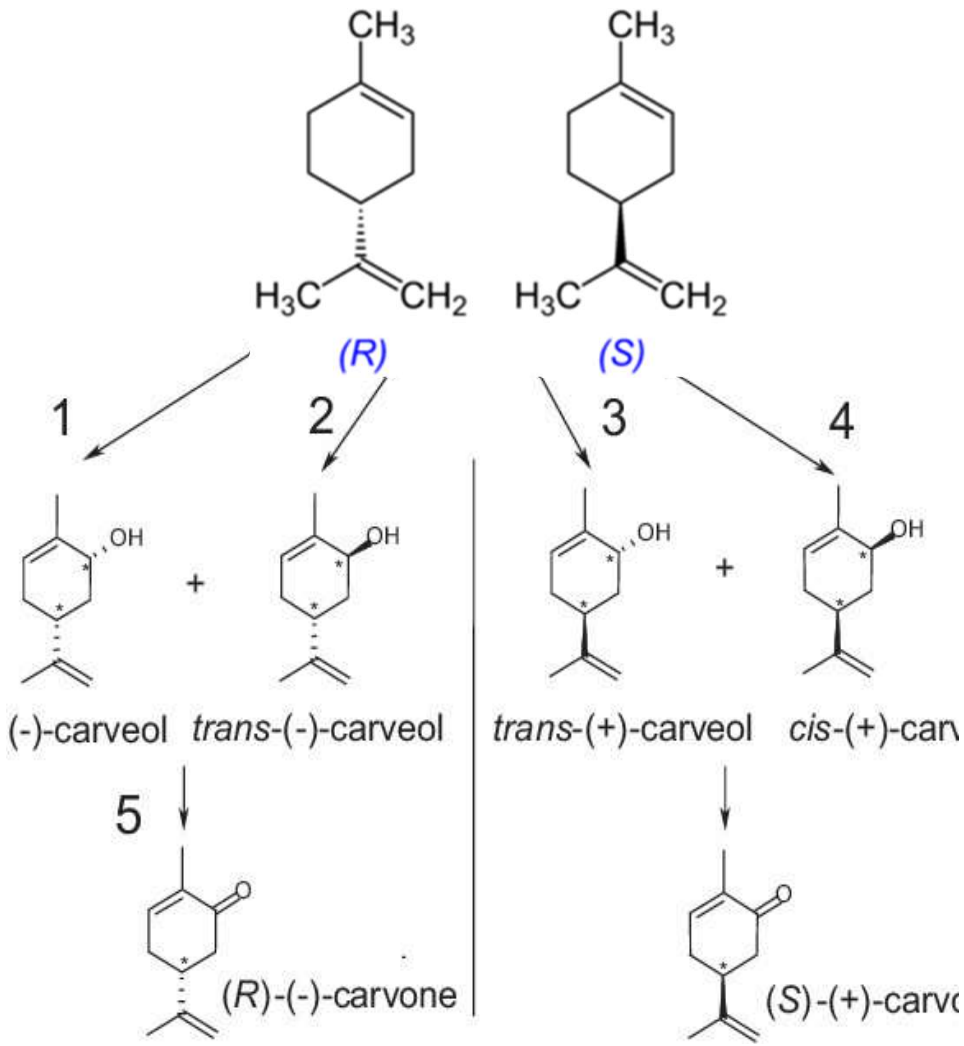
« Same principe than lock and key »





Bio synthesis of Carvone from Limonene





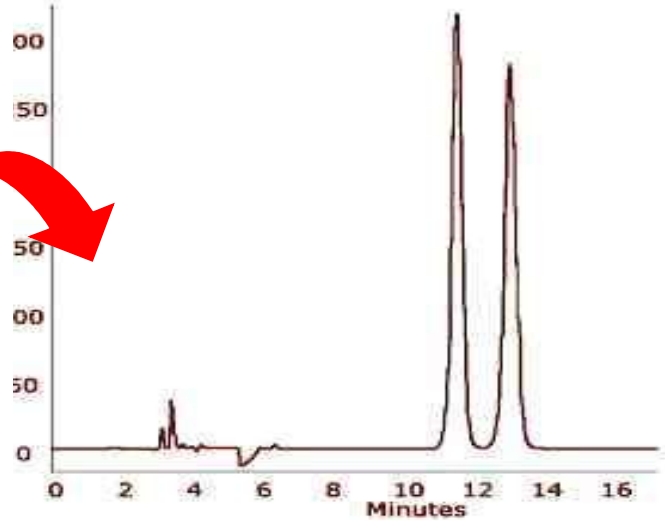
Carvone Mint chlorophyll smell

Carvone Carvi cumin smell

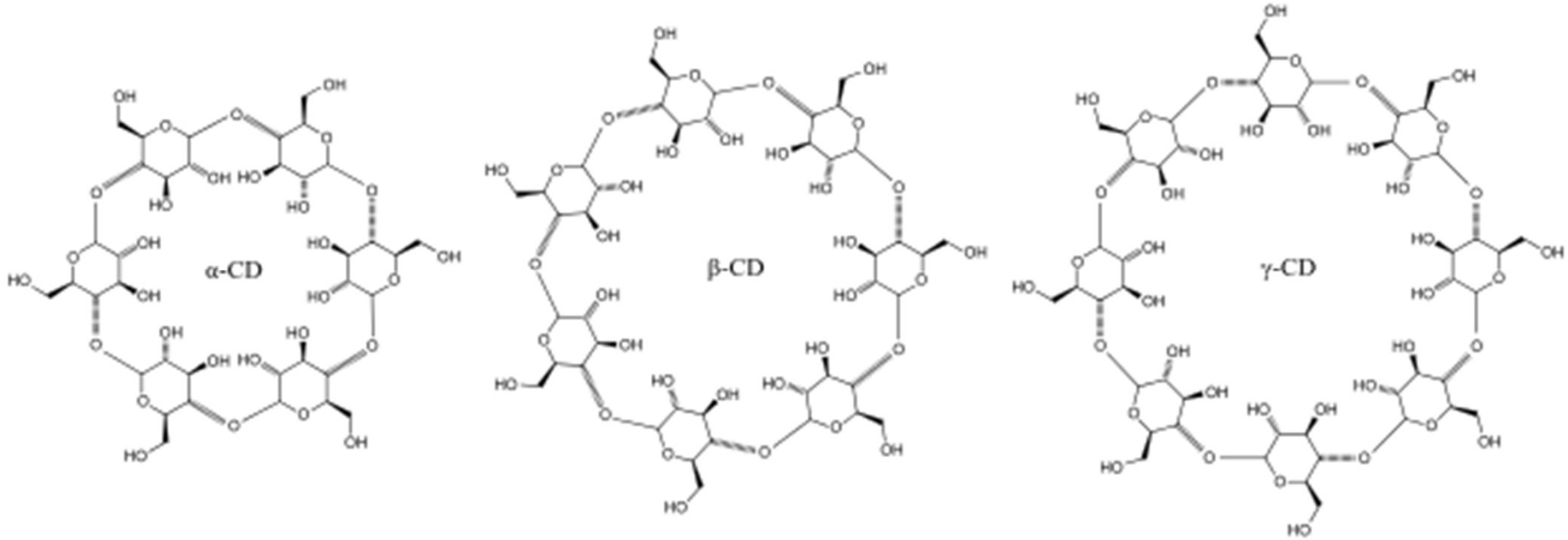
Organic chemistry leads to racemic compound

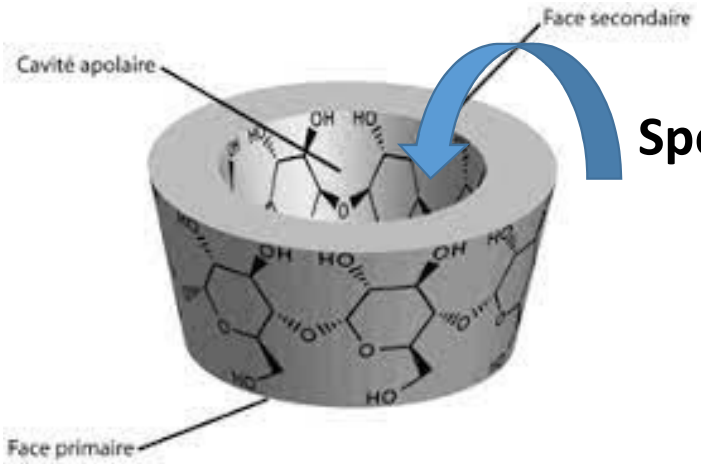
Two enantiomers at the same concentration

Racemic mixture



Chiral selectors : cyclodextrines stationnary phases





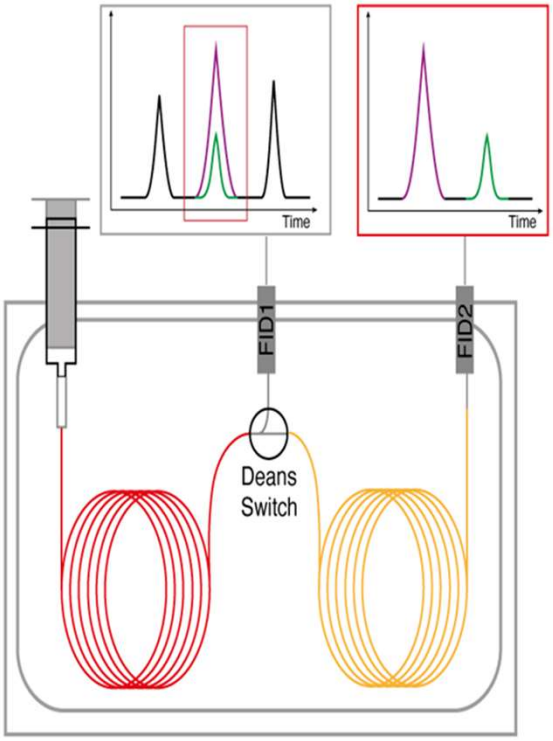
Specific interaction with one enantiomer in cavity

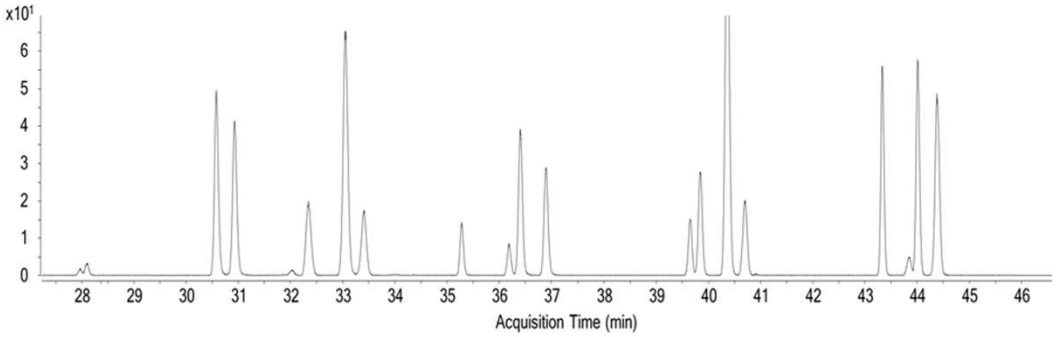
Chromatographic resolution

GC-2D en mode Heart-Cutting

ANALYSES ENANTIOSELECTIVES

Développement de méthodes GC multidimensionnelles
Approche « Métabonomique »



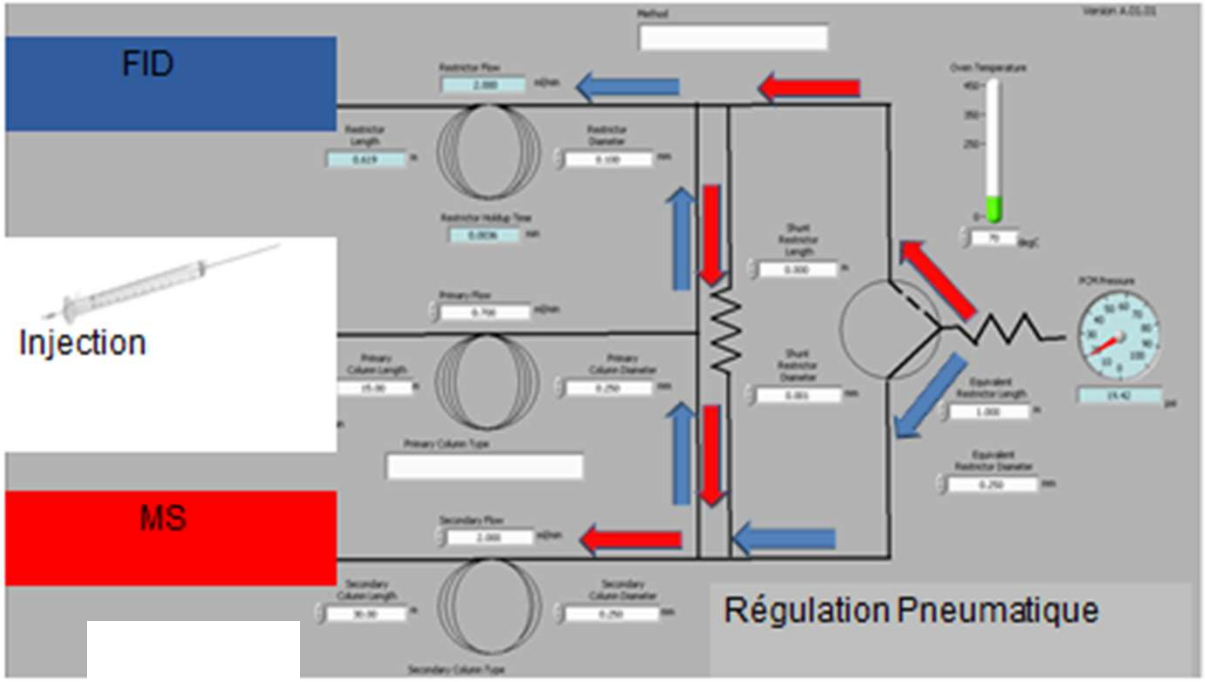


- **Détection d'ajouts de synthèse (Mélange racémique)**
- **Empreintes complétes des métabolites secondaires actifs (molécules/énantiomères)**
- **Effet varietal ou géographique (terroir)**
- **Mise en évidence de coupe entre huiles essentielles (Différences de profils énantiomériques)**

Heart-cutting GC-2D better enantiomers analysis with specific transfer of investigated molecules

INSTITUT DES SCIENCES ANALYTIQUES

Principe du Heart-Cutting avec un Dean-Switch

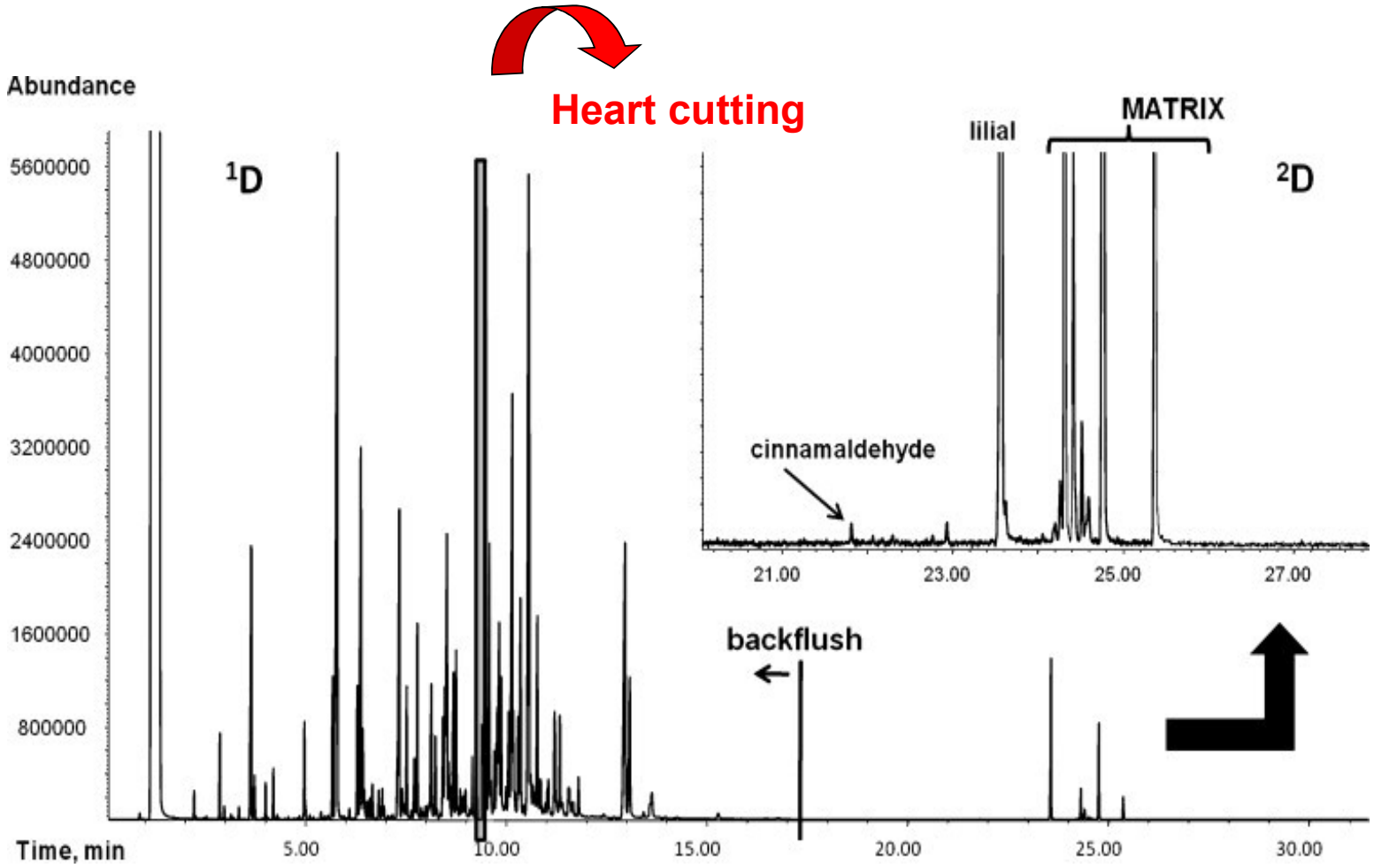


Vers FID

Vers Spectromètre de masse

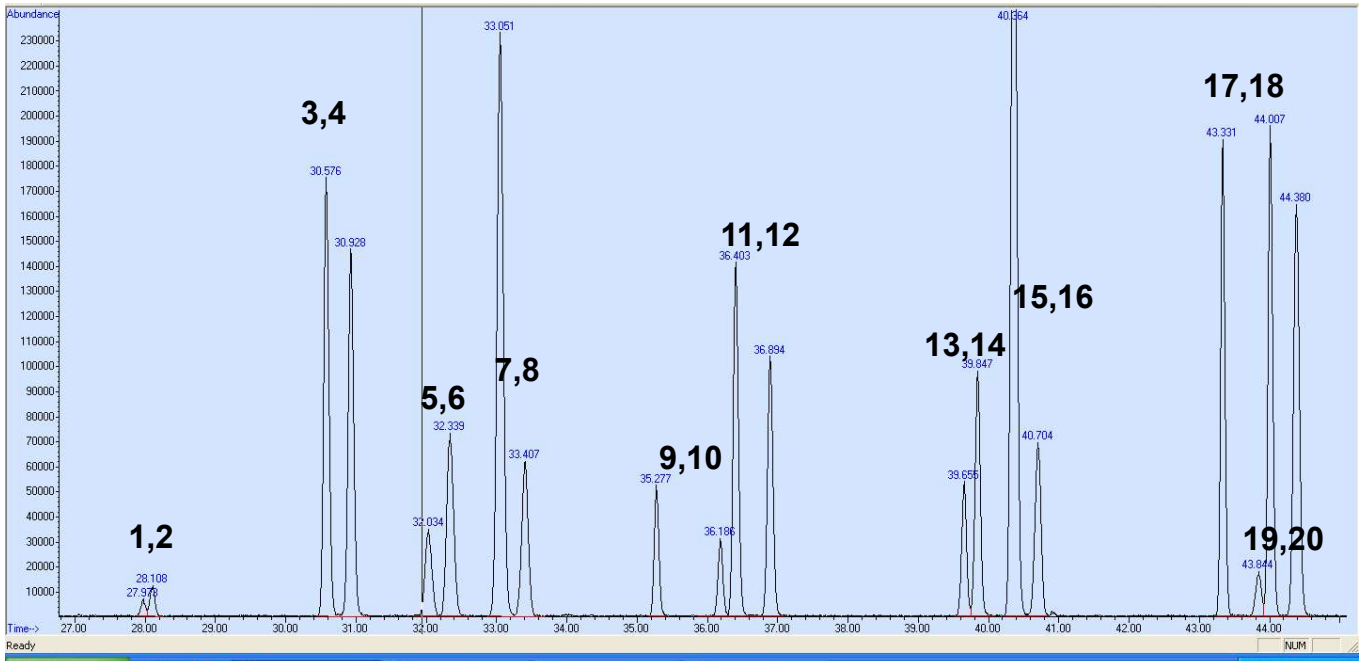
CAF SAA 2017

Application : Allergens substances in perfume



Plant metabolism fingerprint of secondary active metabolites enantiomers

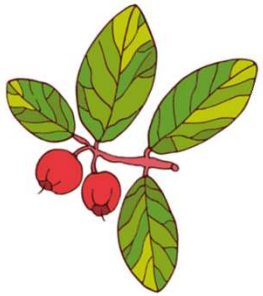
Enantio sélective resolution of mono terpenes using Dean-Switch



Alpha Thuyène 1(+), 2(-) ; Alpha Pinène 3(-),4(+); Alpha Fenchène 5(+),6(-);
Camphène 7(-),8(+); Sabinène 9(+),10(-), Bêta Pinène 11(+),12(-); Alpha Phellandrène 13(-),14(+);
Delta-3-Carène 15(+),16(-), Limonène 17(-),18(+); Bêta Phellandrène 19(-), 20(+)

Wintergreen essential oil study (*Gaultheria*)

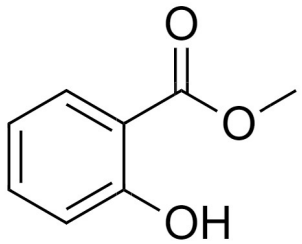
Authenticity problem



Gaultheria procumbens et *fragrantissima leaves*

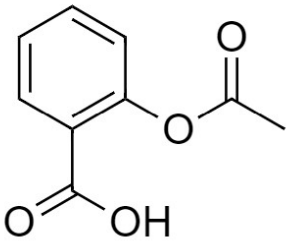
Wintergreen essential oil extraction from leaves

NATURAL

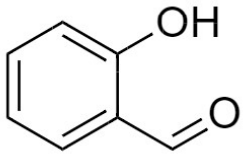


Methyle Salicylate

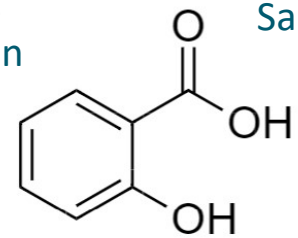
>99% oil composition



Aspirin



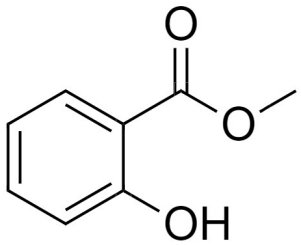
Salicylaldehyde



Salicylic acid

Methyle Salicylate synthesis

SYNTHESIS

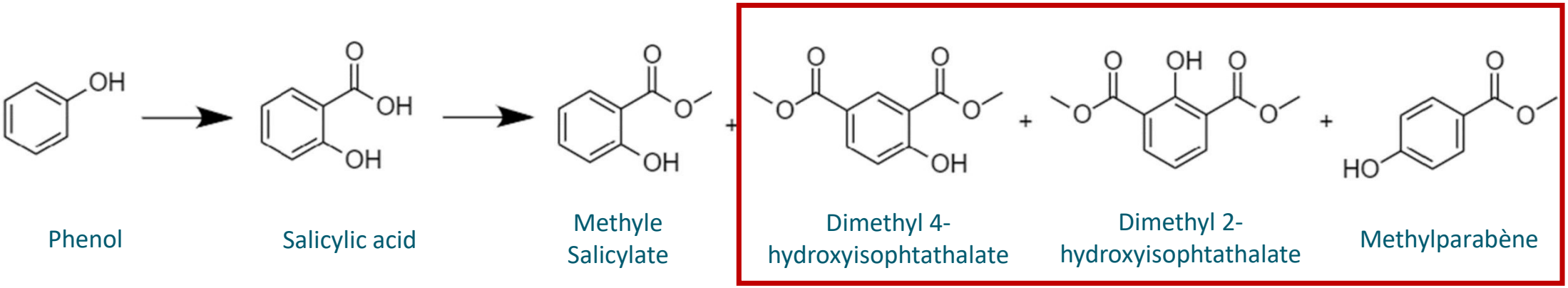


Methyl Salicylate

Easy synthesis from cheap materials

Wintergreen essential oil investigation(*Gaultheria*)

Impurities detection



Residual impurities production

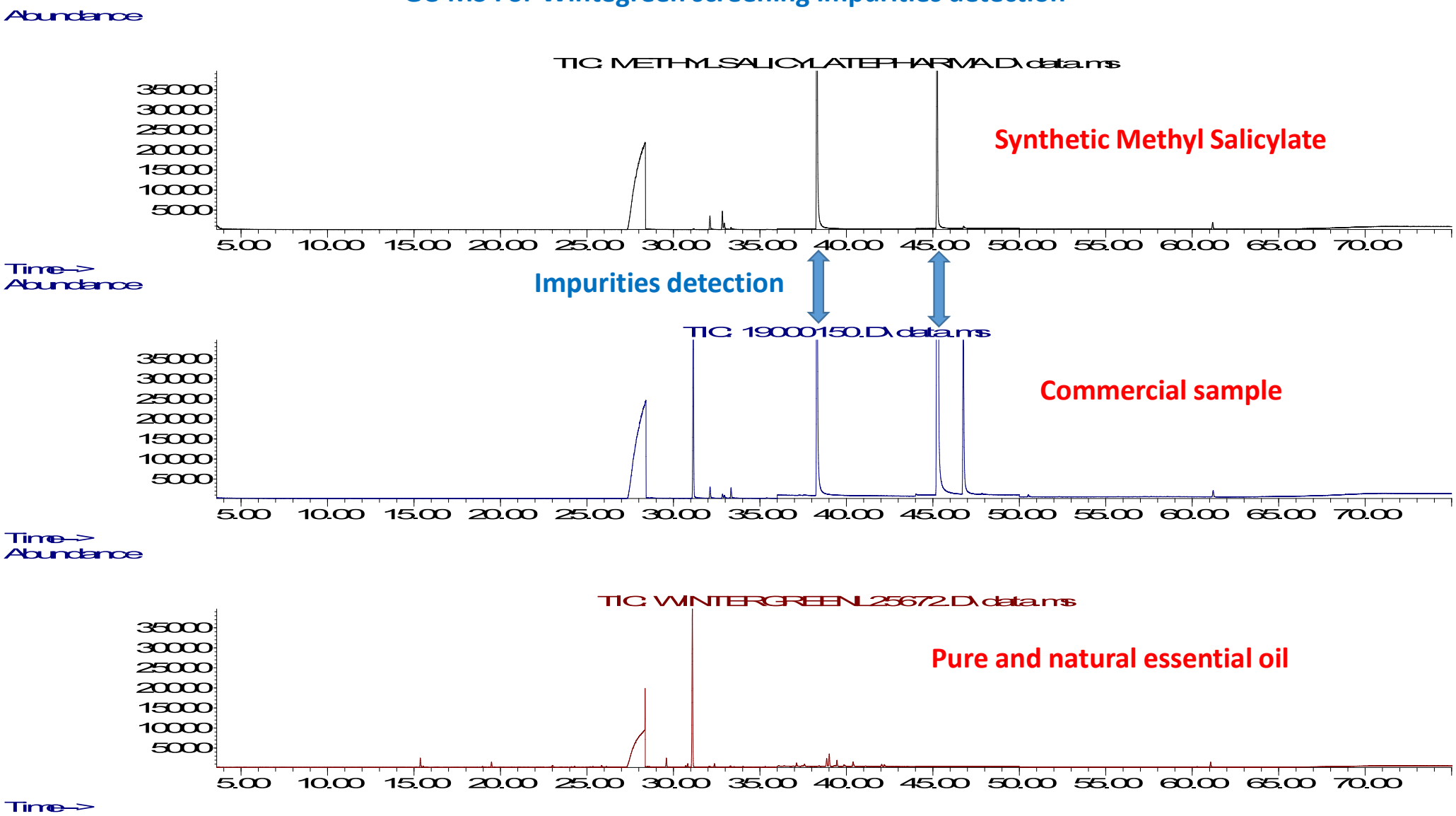
Indicators of synthetic Methyl Salicylate addition

GC-MS control of impurities presence over 64 Wintergreen essential oils samples

Presence of these molecules over 23 samples **(36%)**

Cuchet et al., **2019**. Authentication of the naturalness of wintergreen (*Gaultheria* genus) essential oils by gas chromatography, isotope ratio mass spectrometry and radiocarbon assessment. Ind. Crops Prod. 142, 111873.

GC-MS For Wintegreen screening impurities detection

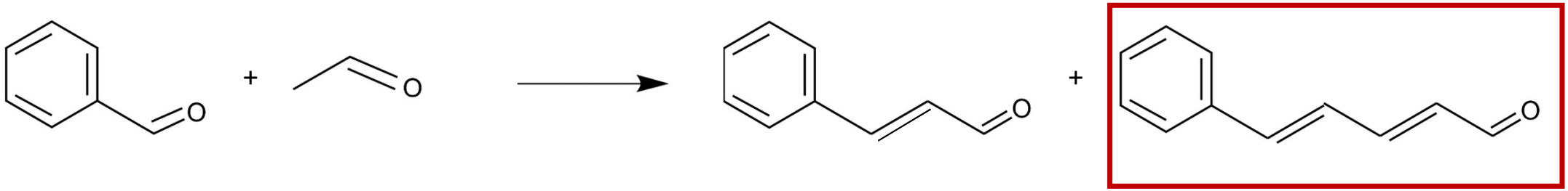


Cinnamon essential oil investigation (*Cinnamomum*)

Cinnamaldehyde hemi-synthesis

Preliminary study

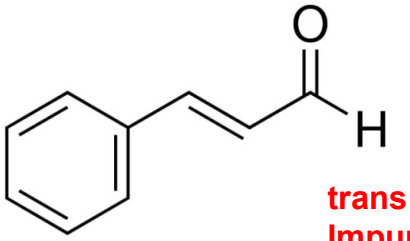
Residual synthetic impurity



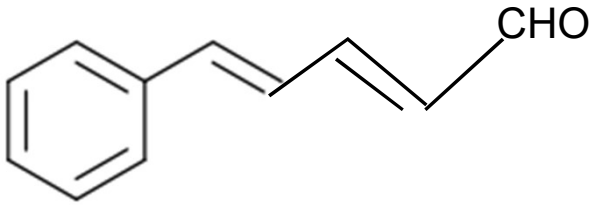
Impurity production
5-phenylpenta-2,4-diénal

GC-MS CONTOL OVER 55 Cinnamon essential oils
Identification in 43 samples……

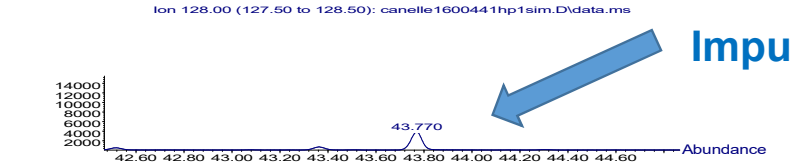
Cinnamon : *Cinnamonum zeylanicum*

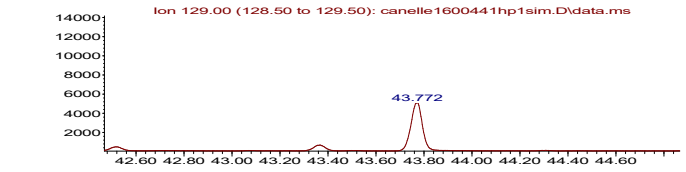


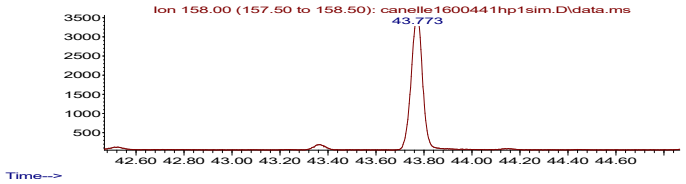
trans Cinnamaldehyde synthesis
Impurity production

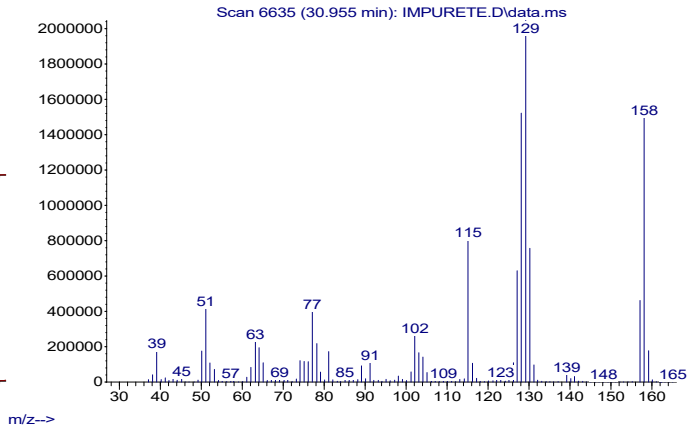


Impurity detection using SIM mode acquisition





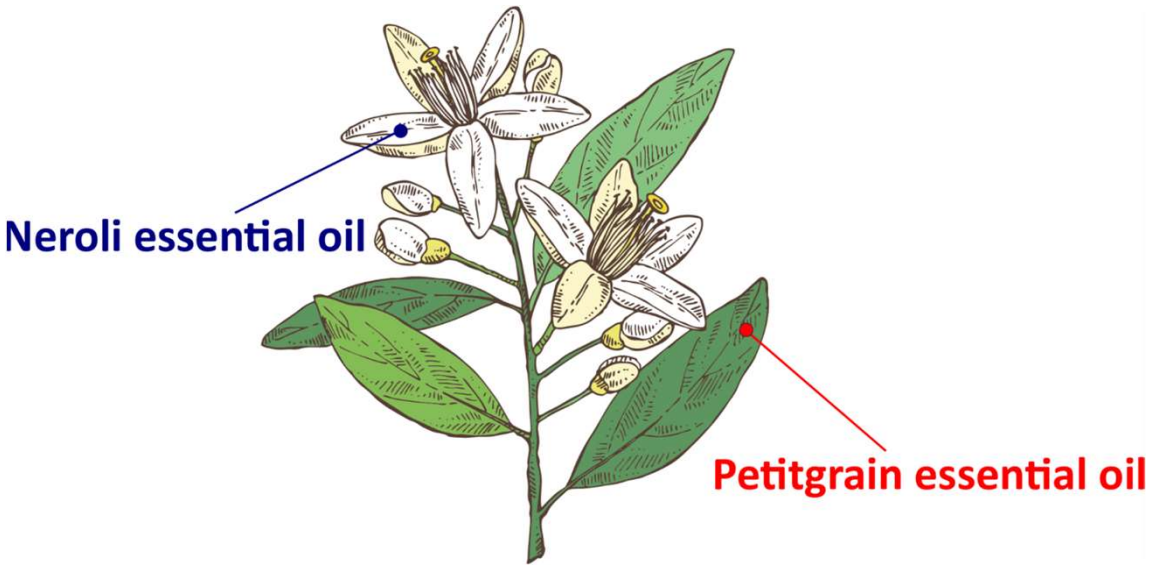




SIM mode acquisition: Single Ion Monitoring , more sensitive, ppb level detection

NEROLI essential oil (*Citrus aurantium L.*)

Purity and authenticity control



Similar composition,

Petitgrain addition in Néroli essential oil can't be detected with simple composition analysis(no specific molecular markers)

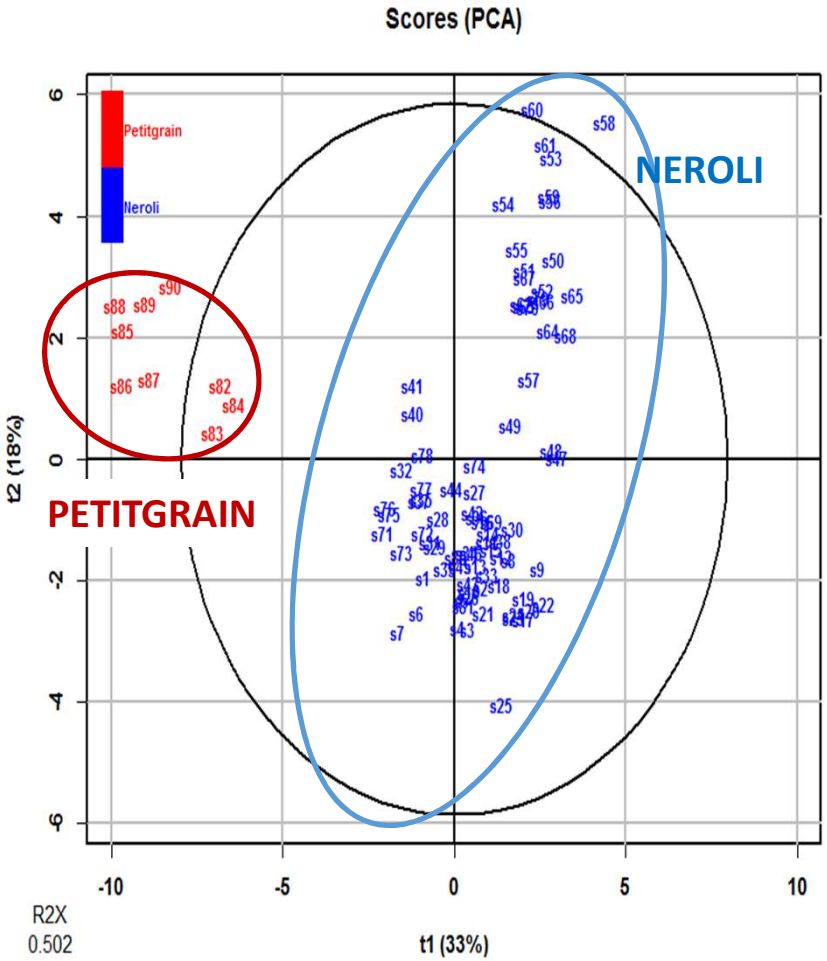
NEROLI essential oil investigation(*Citrus aurantium L.*)

Botanic Discrimination

Study : 90 samples of Neroli and Petitgrain

Several chiral molecules :
Enantiomers analysis over 14 chiral molecules

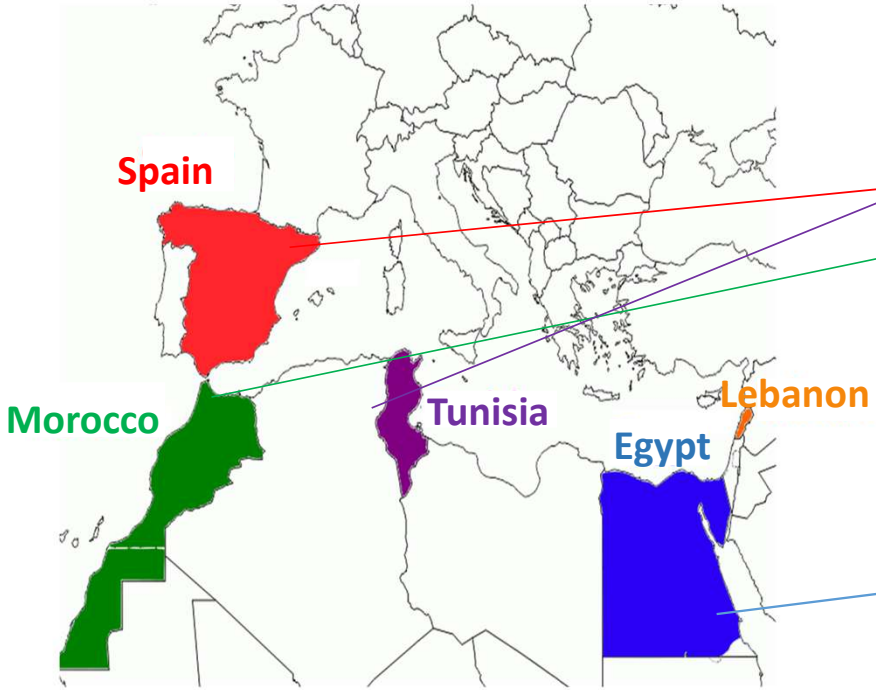
Stable isotopes investigation :
Isotopic analysis $\delta^{13}C$ et $\delta^{2}H$ over 10 molecules

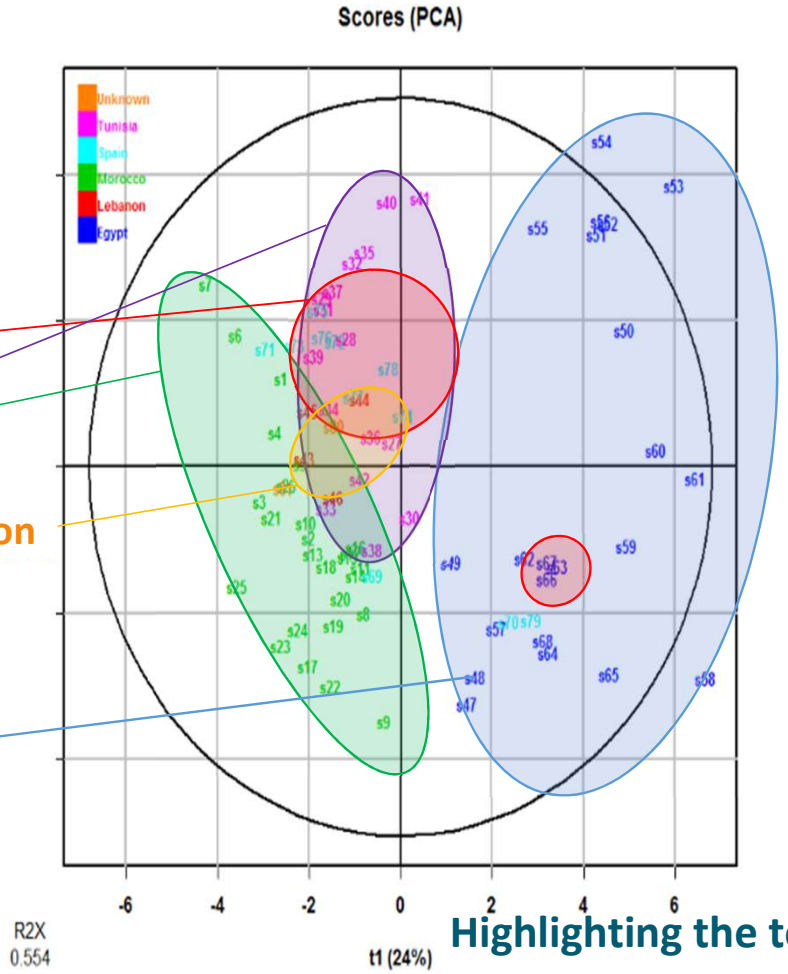


Cuchet et al., 2021. Determination of enantiomeric and stable isotope ratio fingerprints of active secondary metabolites in neroli (Citrus aurantium L.) essential oils for geographical and purity authentication. J,Chrom,B

NEROLI essential oil investigation (*Citrus aurantium L.*)

Geographic discrimination





Different prices in fonction of geographical origin

Highlighting the terroirs

Cuchet et al., **2021**. Determination of enantiomeric and stable isotope ratio fingerprints of active secondary metabolites in neroli (Citrus aurantium L.) essential oils for geographical and purity authentication. J.Chrom.B , 2021

Conclusion :
The development of methodologies based on the mechanisms of synthesis and photosynthesis of plants' bioactive secondary metabolites validate the natural character and therefore the authenticity of raw materials:
1-Stable isotopes ratios measurements
2-Enantiomers distributions
3-Radiocarbon ^{14}C

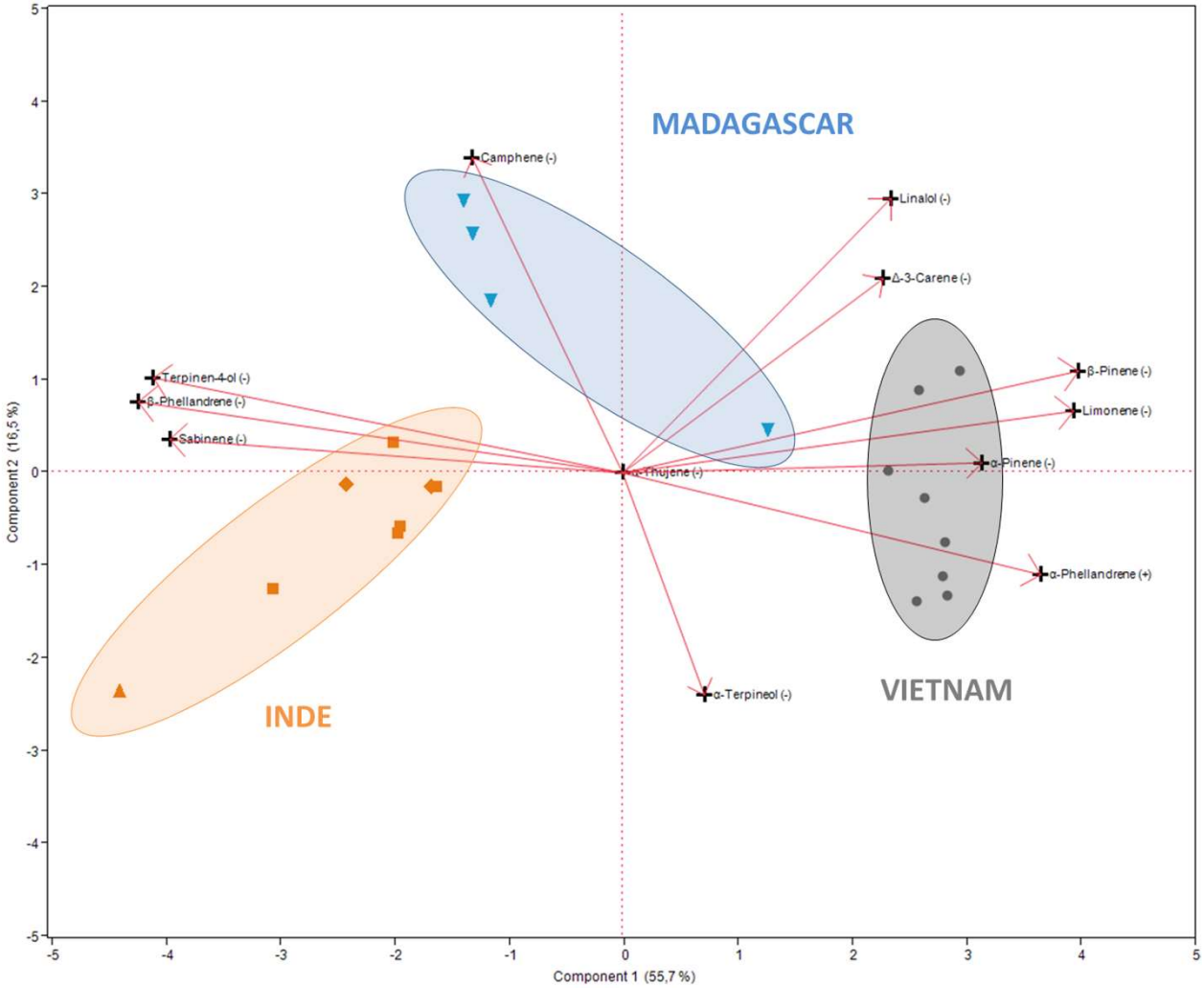
The search and detection for impurities revealing the use of so-called molecules. «nature-identical» is a methodology to combat fraud effectively.

Perspectives:
Develop methodologies to validate the terroir of a production (physicochemical fingerprint of a variety , climate, soil) : high added values of agricultural productions.

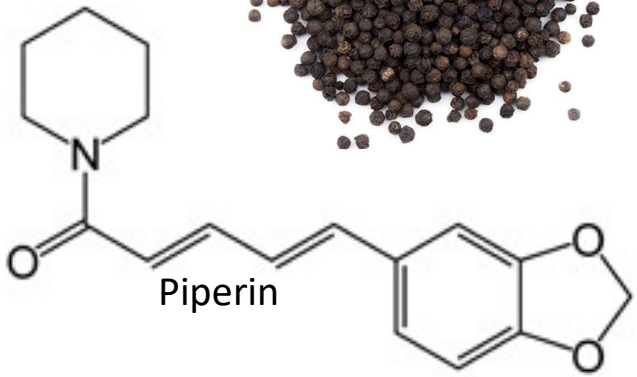
Kampot pepper : IGP

Pepper (*Piper nigrum*): Main production areas

Black pepper geographic origin discrimination using Mono terpenes enantiomers profiles

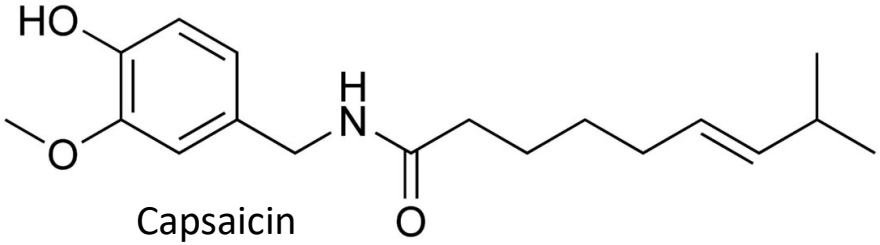


Composition : GC-FID constituants %
Enantiomers analysis : mono terpènes
Stable isotopes analysis : mono terpènes and Pipérine
Piperine % and other alcaloïds
Stable isotopes $^{15}N/^{14}N$ possible on Pipérine



Piperin

Similar study possible on Chilli pepper (*Capsicum* species) !



Capsaicin