Characterization of lipids

Lipid extraction
GC/MS analysis

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02nd of february 2021 – Technical meeting NPhDA

Introduction

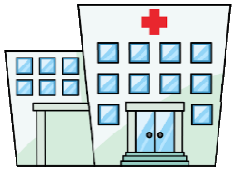
Why ?

Technological interest :

- Choice of raw materials
- Food fraud detection
- Nutritionnal and health claims

Clinical interest :

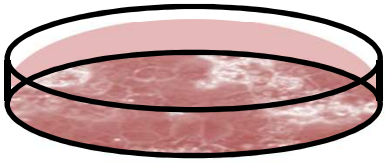
- Markers of consumption, deficiency, risk, metabolic diseases



Research interest :

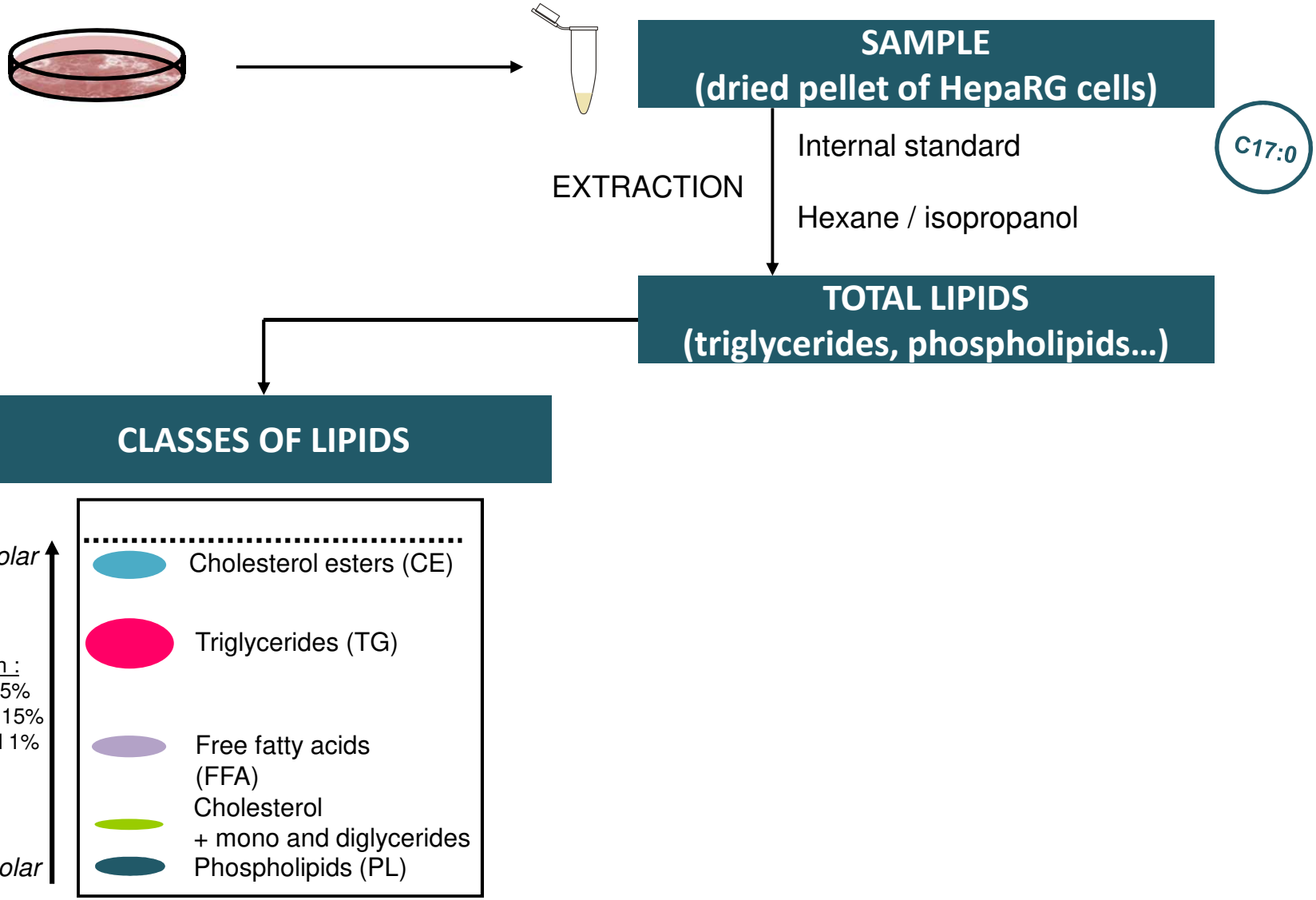
- Structures, metabolic pathways

Example : characterize the lipids present in HepaRG cells



Lipid characterization

Preparation of the sample



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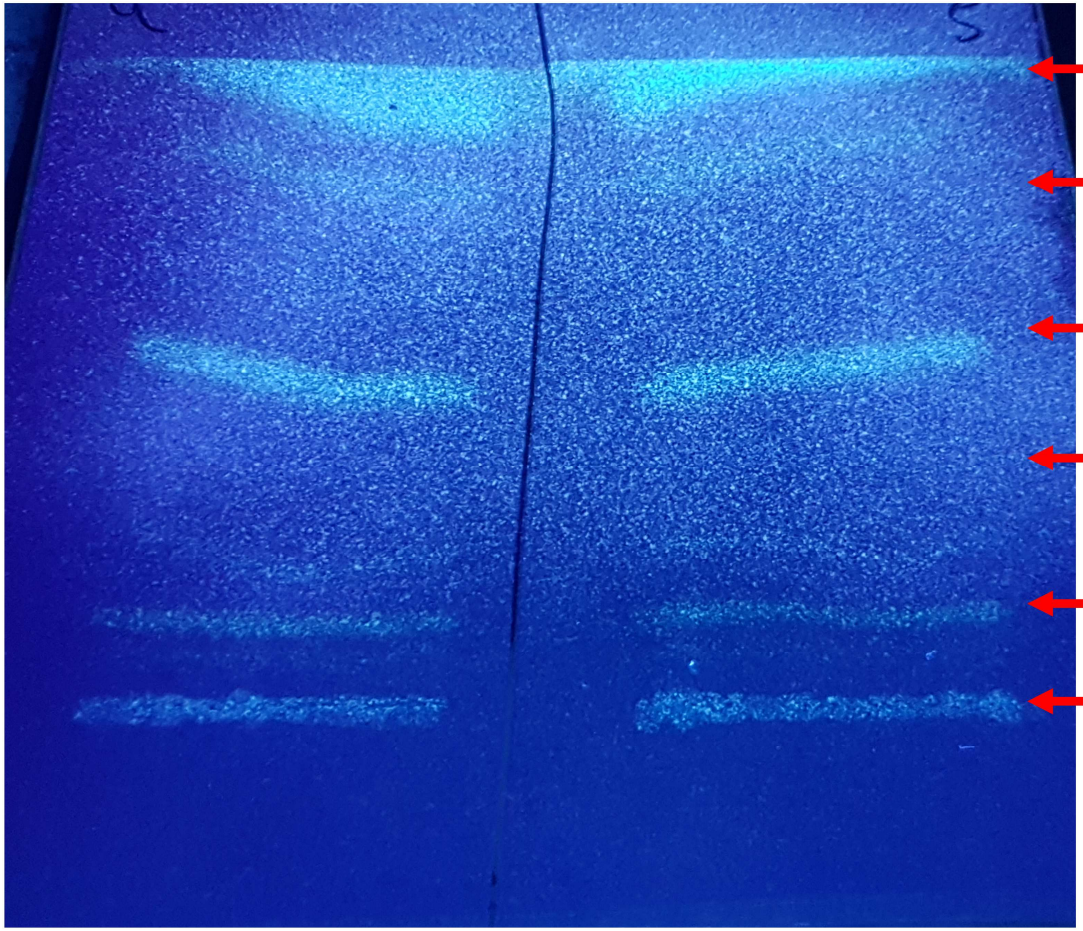

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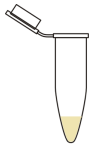


SAMPLE
(dried pellet of HepaRG cells)

Internal standard

C17:0

EXTRACTION

Hexane / isopropanol

TOTAL LIPIDS
(triglycerides, phospholipids…)

CLASSES OF LIPIDS

apolar

Cholesterol esters (CE)

Triglycerides (TG)

Free fatty acids (FFA)

Cholesterol + mono and diglycerides

Phospholipids (PL)

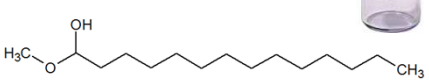
polar

Migration :
Hexane 85%
Ethyl ester 15%
Acetic acid 1%

DERIVATION

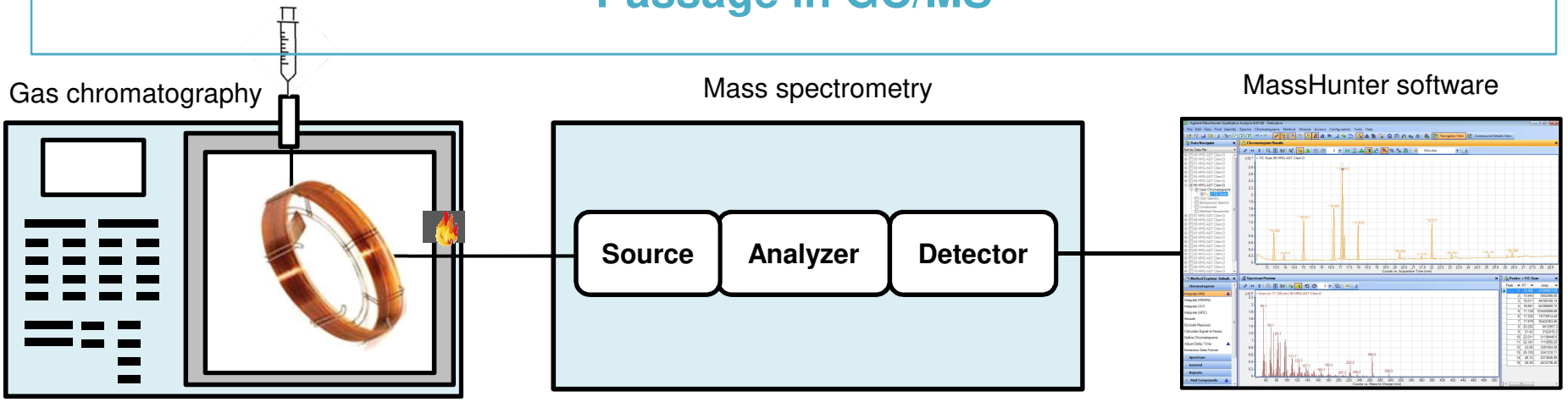
1/ Saponification (NaOH) : fatty acid soap
2/ Derivation (BF3 in methanol)

FAME
(Fatty Acid Methyl Esters)

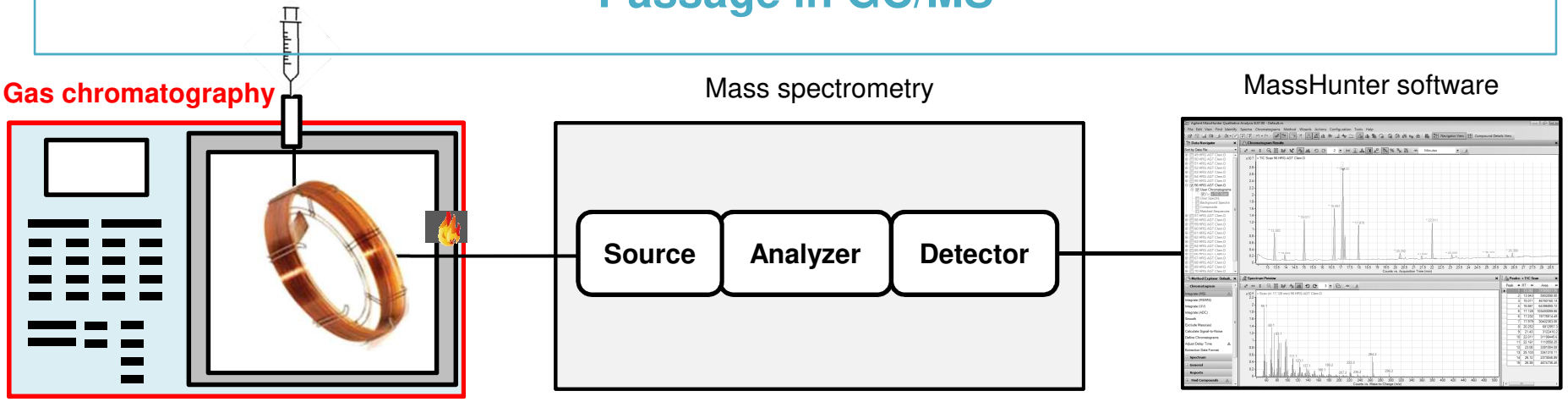


GC / MS injection

Passage in GC/MS



Passage in GC/MS

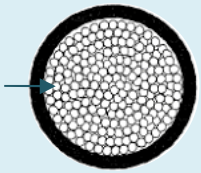


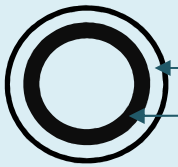
- Technique for **separating** a mixture of **volatile molecules**
- Separation by a **stationary phase** and a **mobile phase**
- Separation according to polarity

The column

Filled column

Stationary phase impregnated silica granules





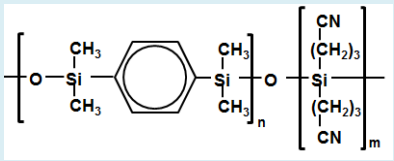
Capillary column

Fused silica

Stationary phase

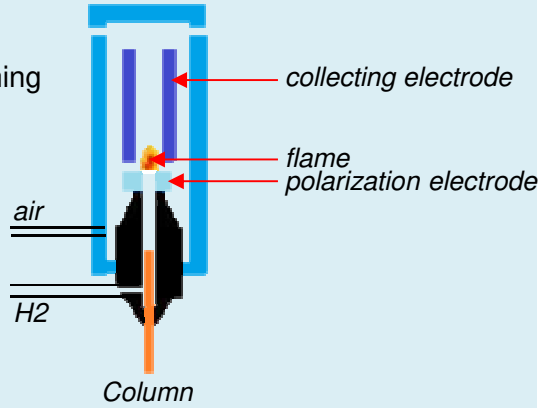
Stationary phases:

- Polydiméthylsiloxane
- Polyester
- Polyethylèneglycol
- Cyanopropysilphenylene-siloxane

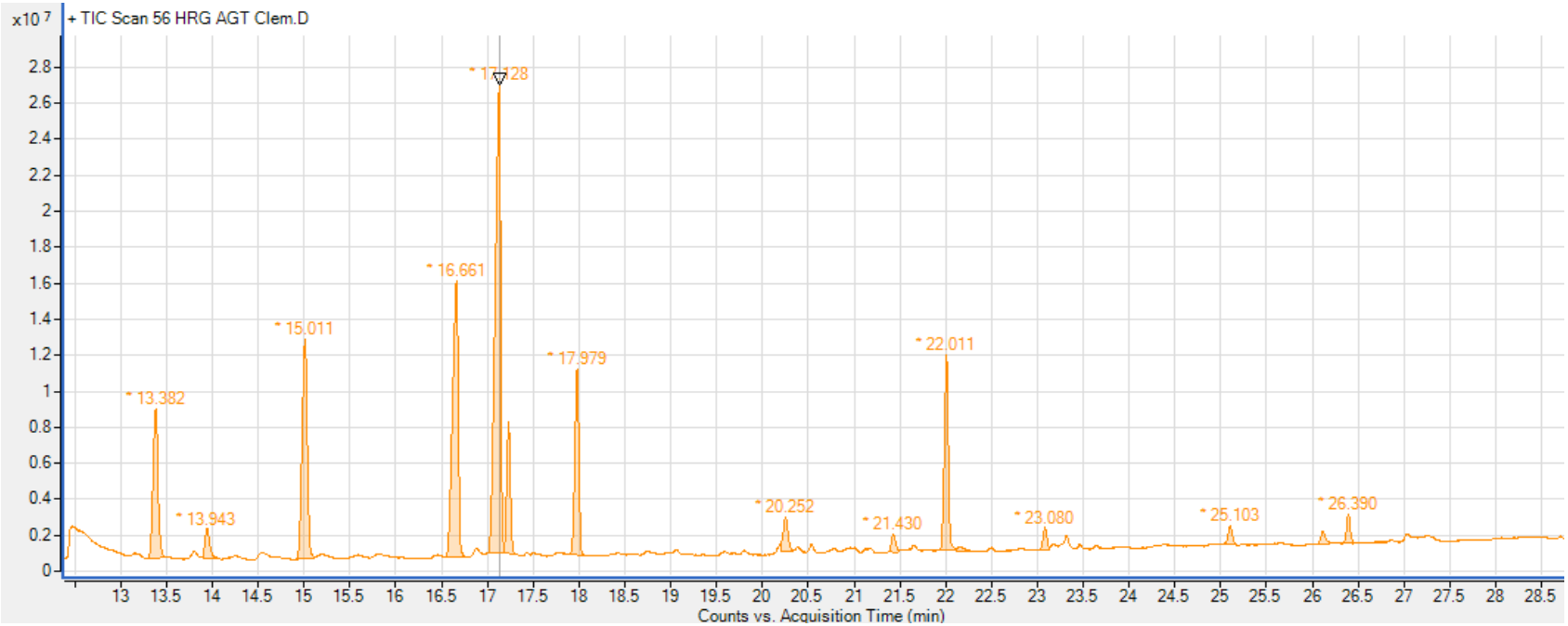


The FID (flamme ionization detector)

- Great sensitivity (<1ng)
- Only detects what is burning
- Destructive

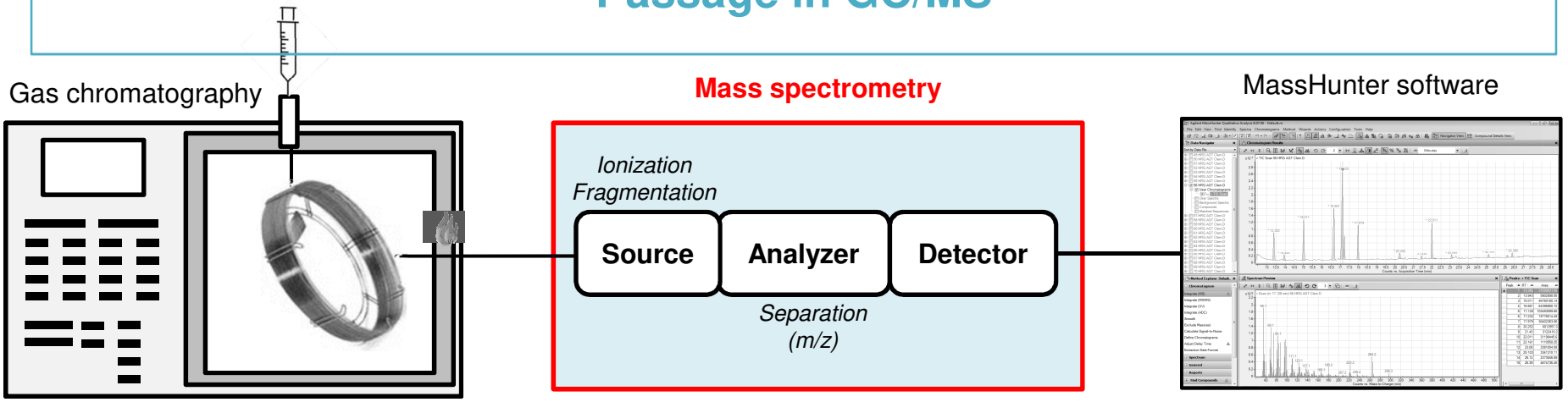


Passage in GC/MS

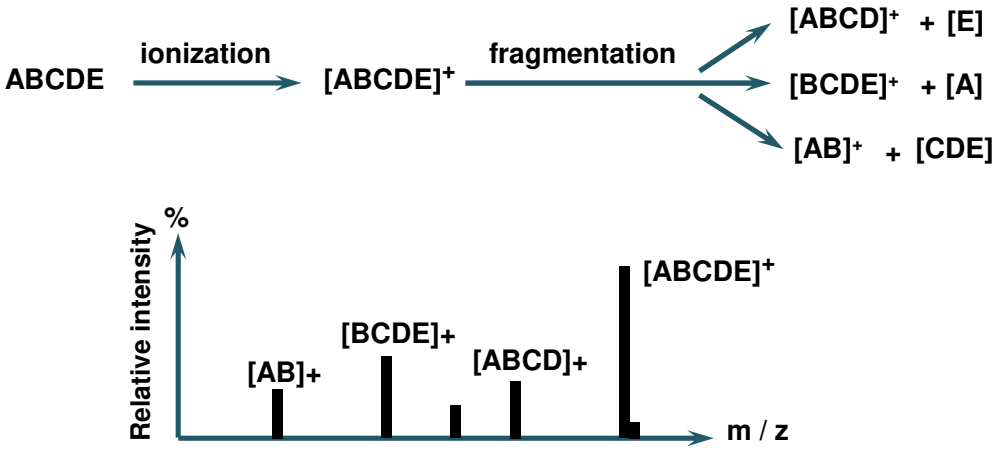


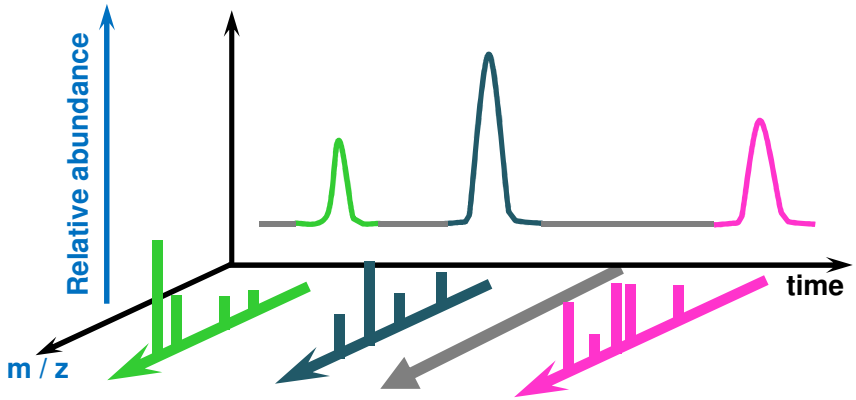
1 peak = 1 detected compound

Passage in GC/MS



- Method for **structural analysis** of organic compounds
- **Ionization** of a molecule, **fragmentation** and analyze the **fragments abundance** in terms of their mass to load ratio (**m/z**)





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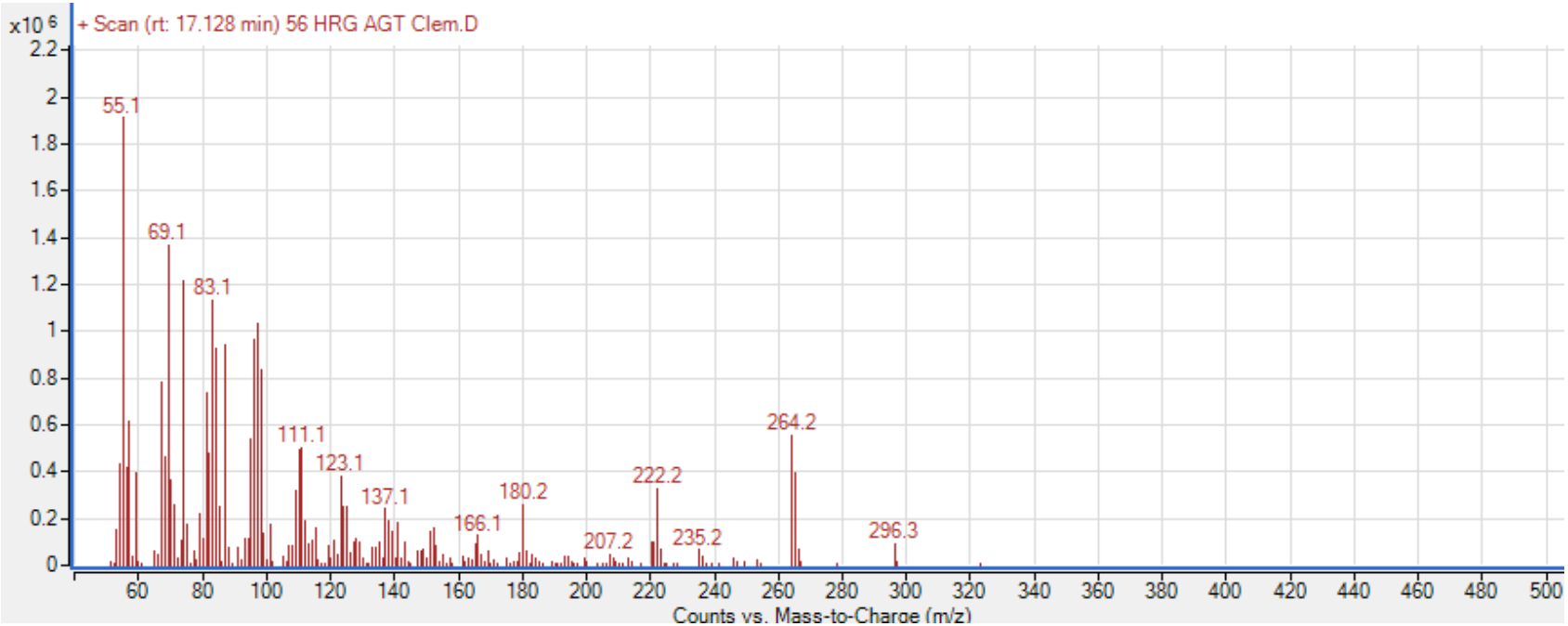
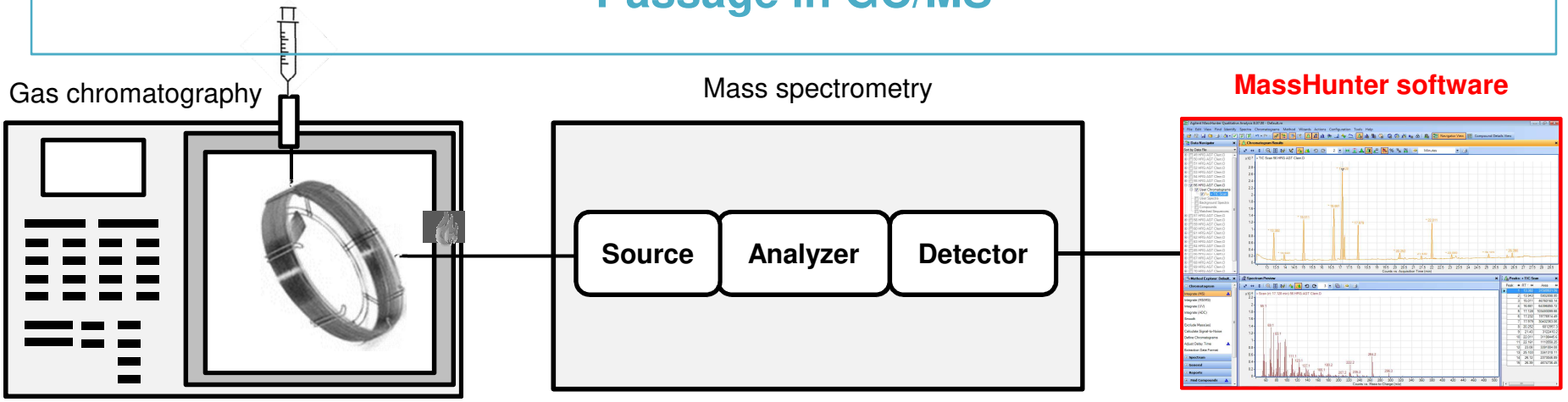


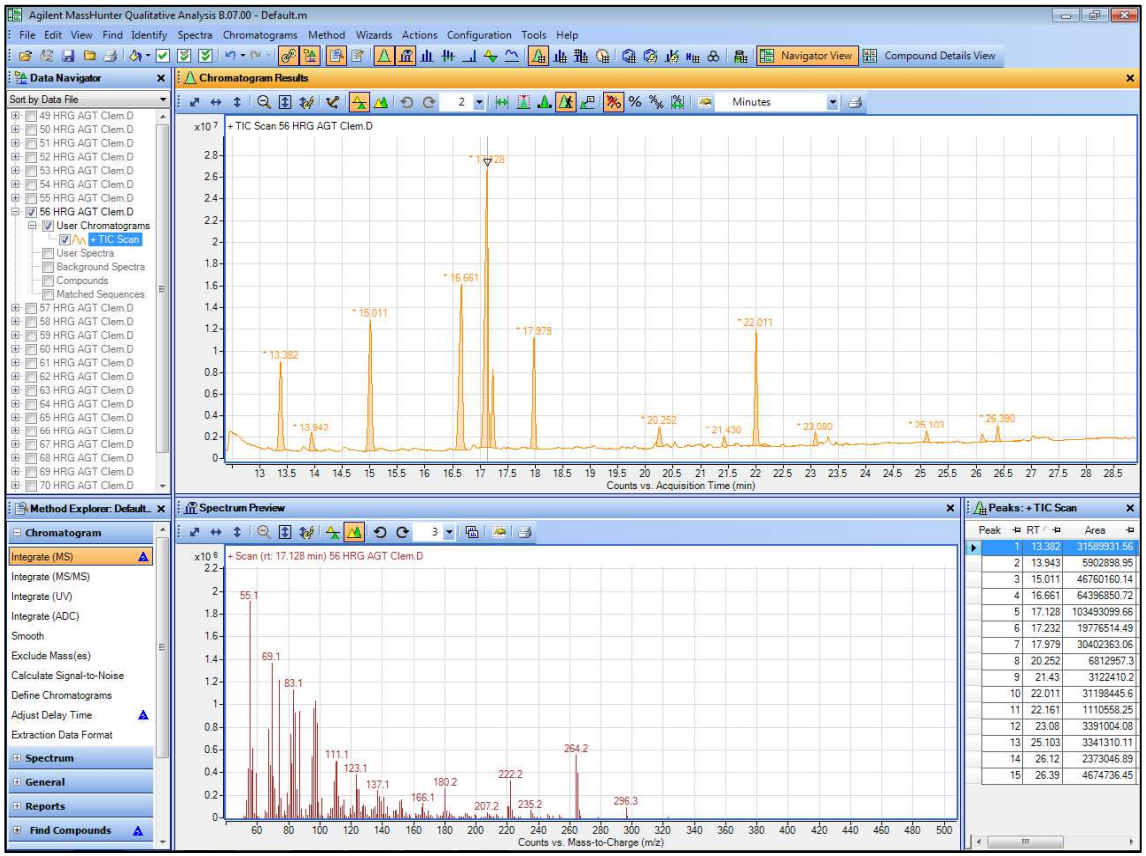
Molecule characteristic

Passage in GC/MS



Identification of fatty acids

1. Software interface : MassHunter



Chromatogram of lipids extracted from HepaRG cells, treated with 150µM C18:1

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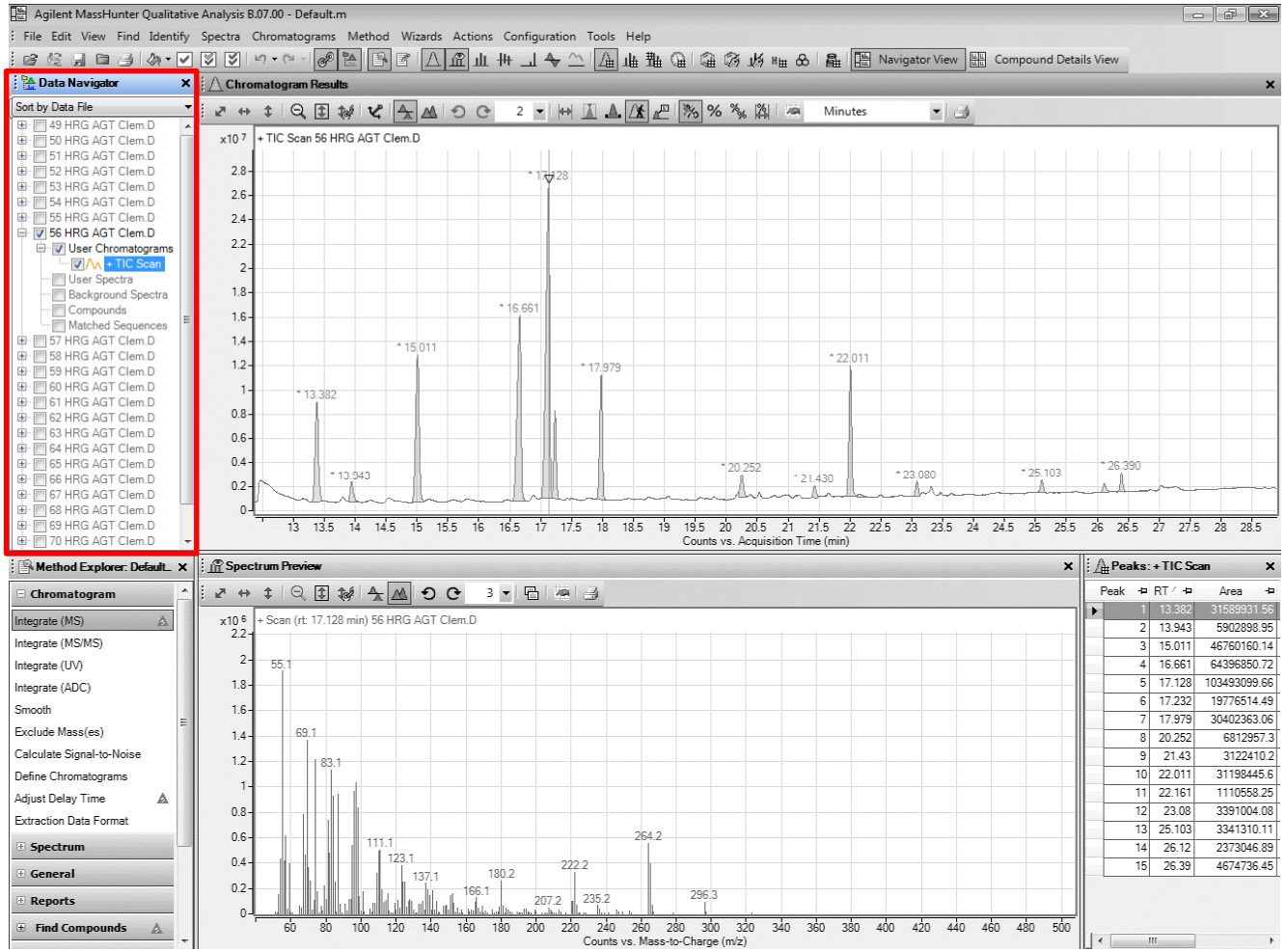

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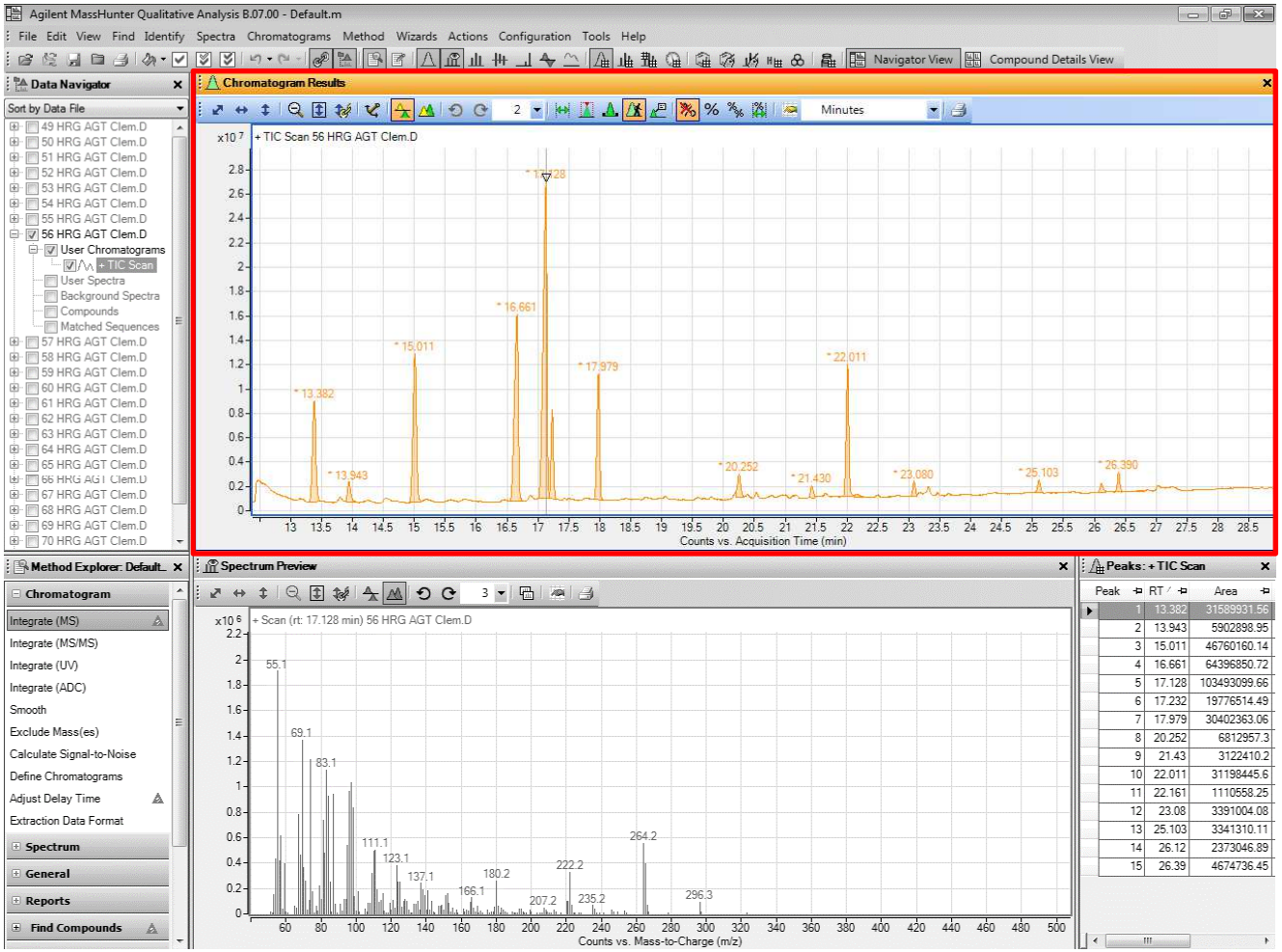

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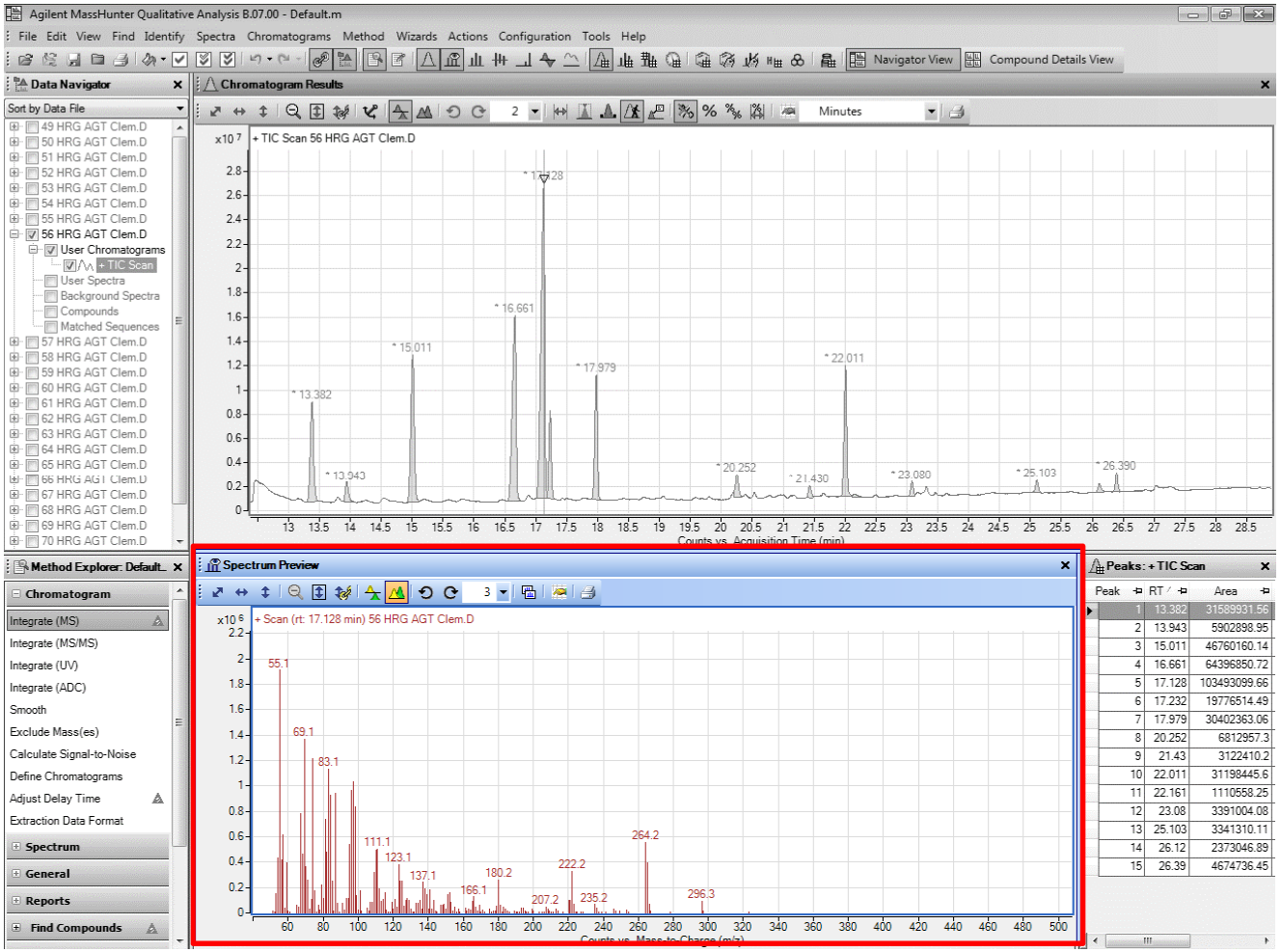

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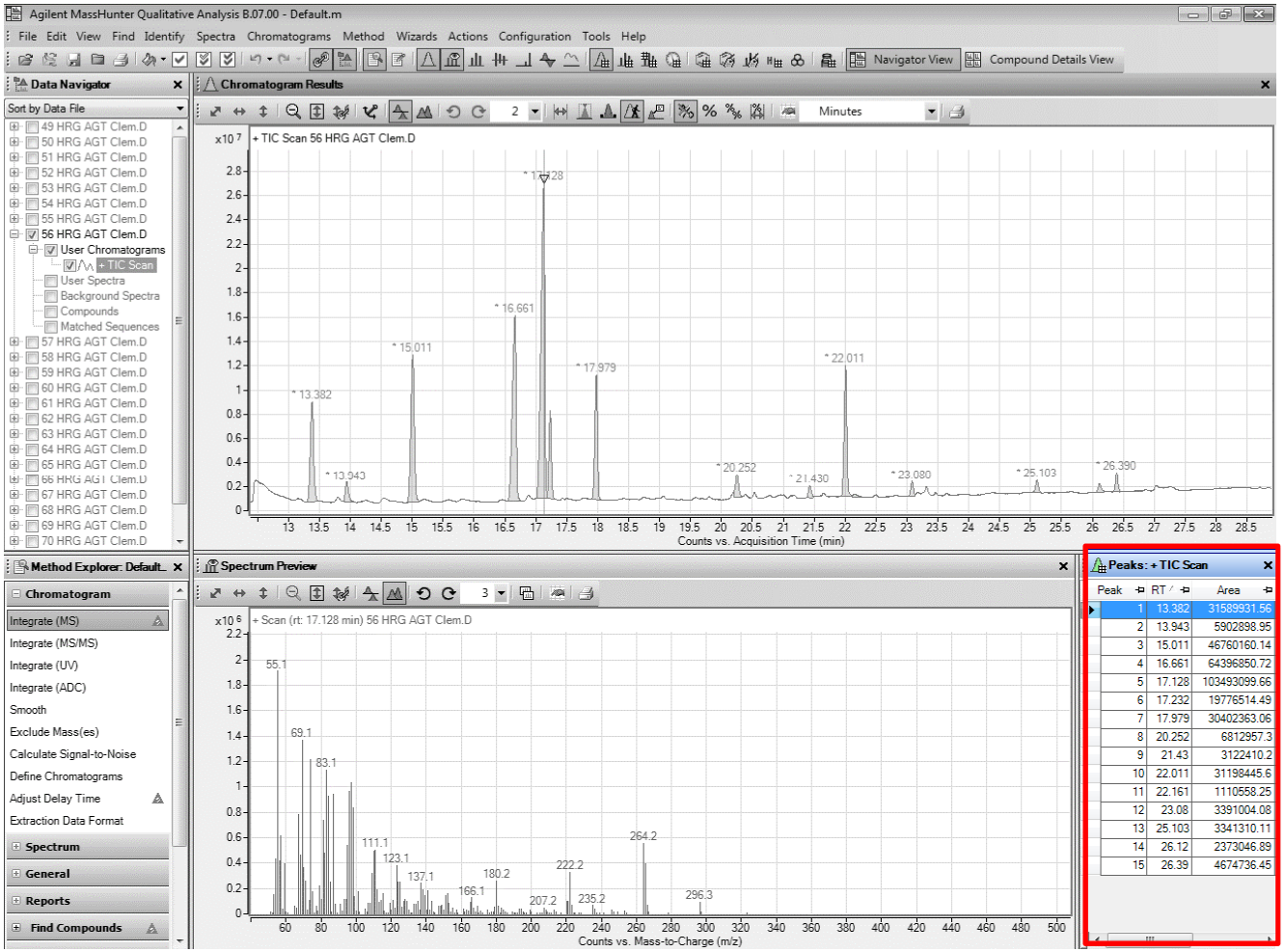
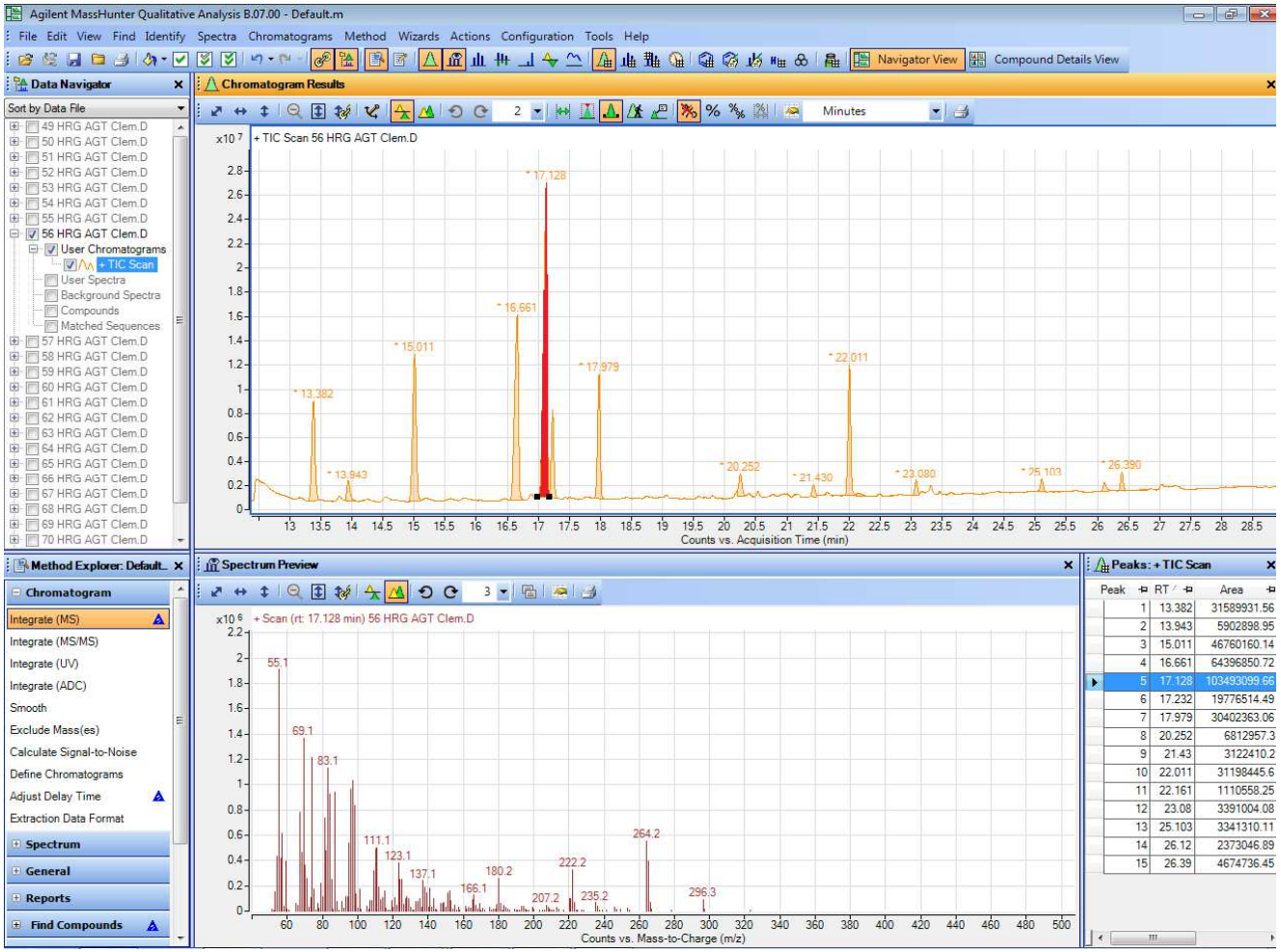


Identification of fatty acids

2. Peak identification



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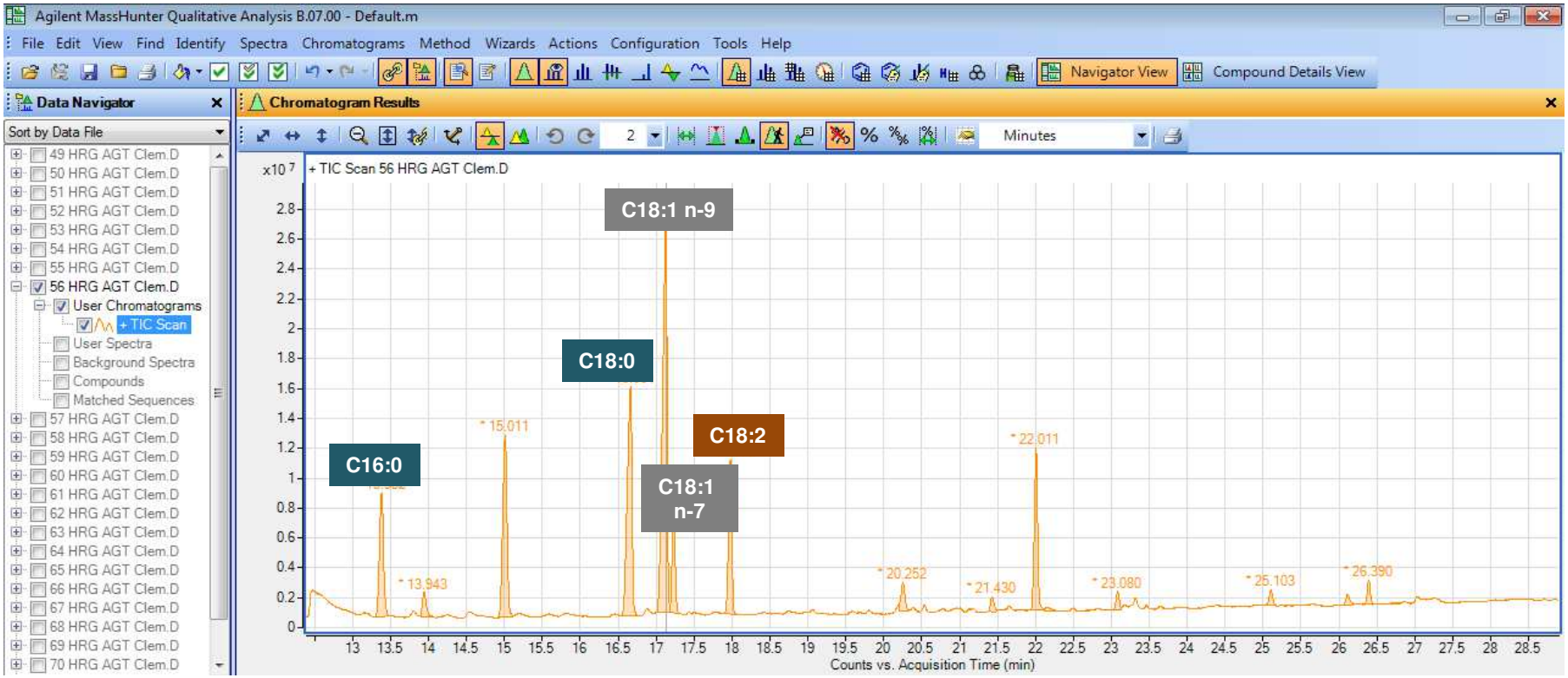


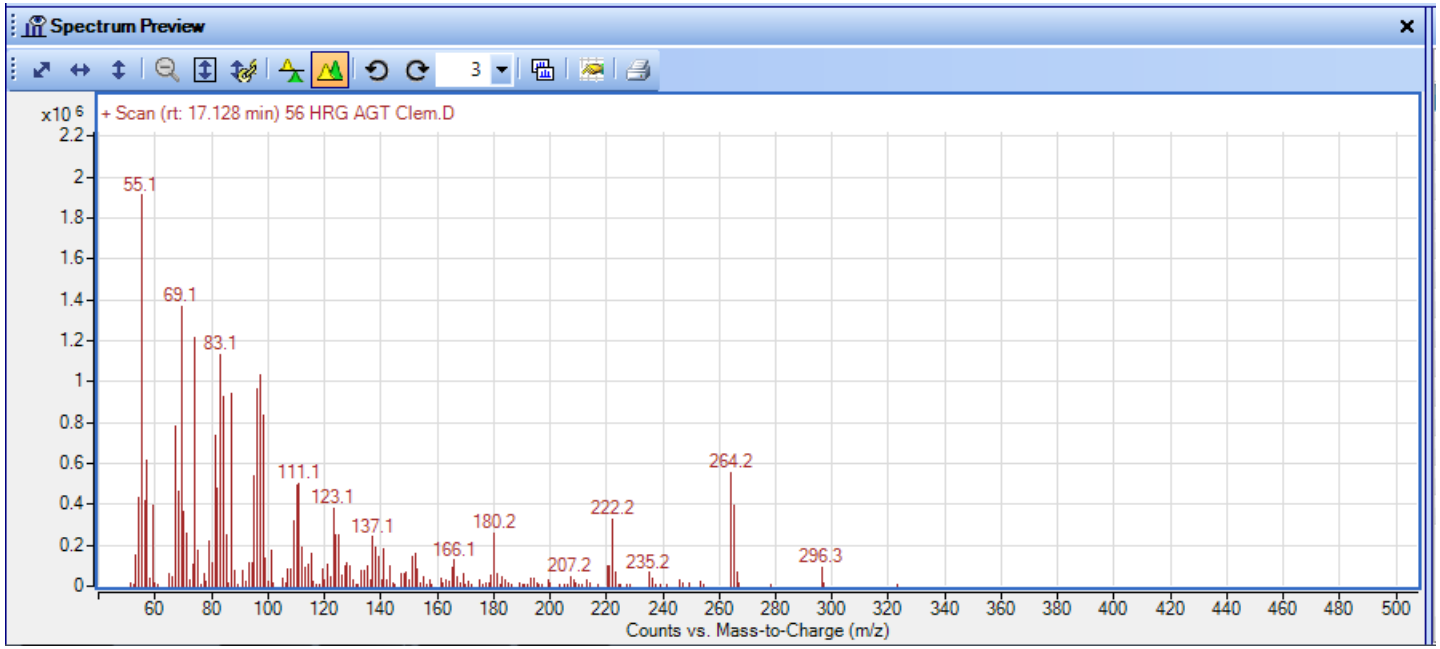
The peak order depends of:

Chain lengh
Number of double bonds
Positon of double bonds
Double bonds geometry

Identification of fatty acids

2. Peak identification: characteristic ions

Fatty acid category	Ions (m/z)
Saturated	74, 87, 101, 115, 129, 143…
Mono-unsaturated	74, 55, 69, 83, 97…
Diene	67, 81, 95, 109, 123, 137…
Triene	79, 91, 107, 121, 135… n-6 : 150 n-3 : 108
Poly-unsaturated	79, 91, 105, 119, 133… n-3 : 108



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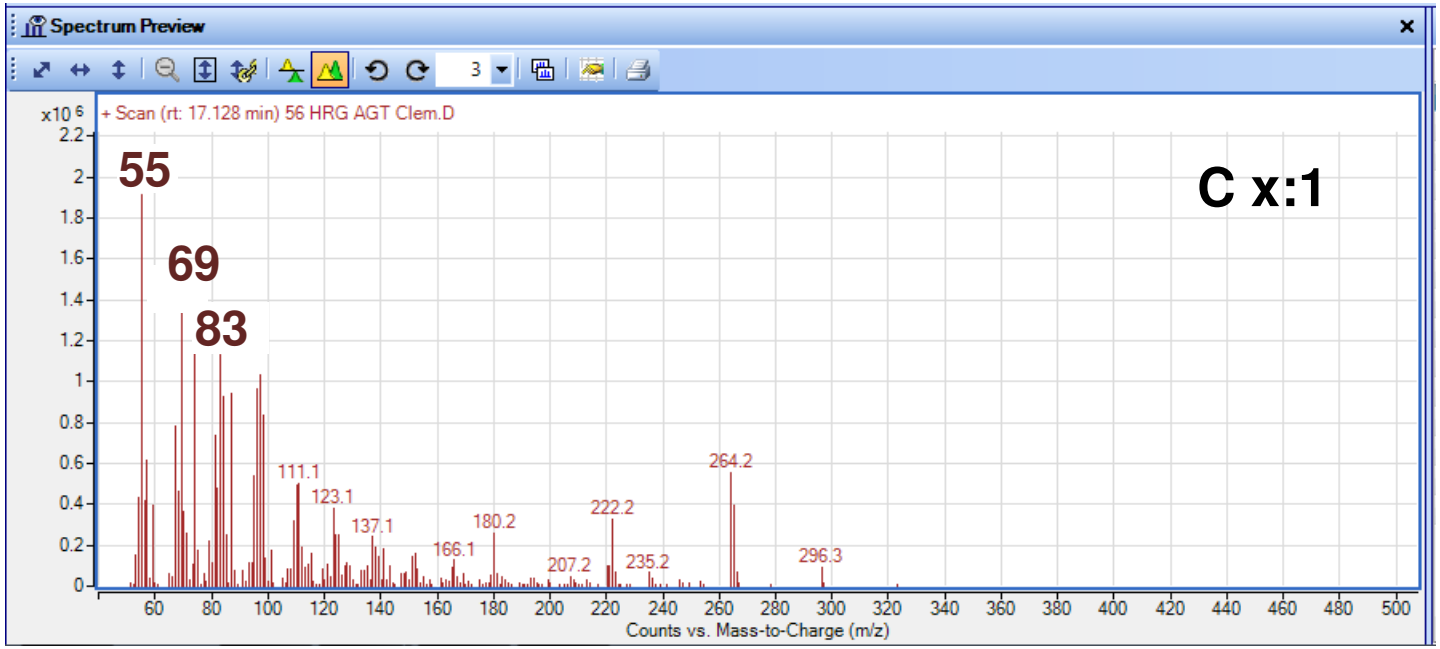
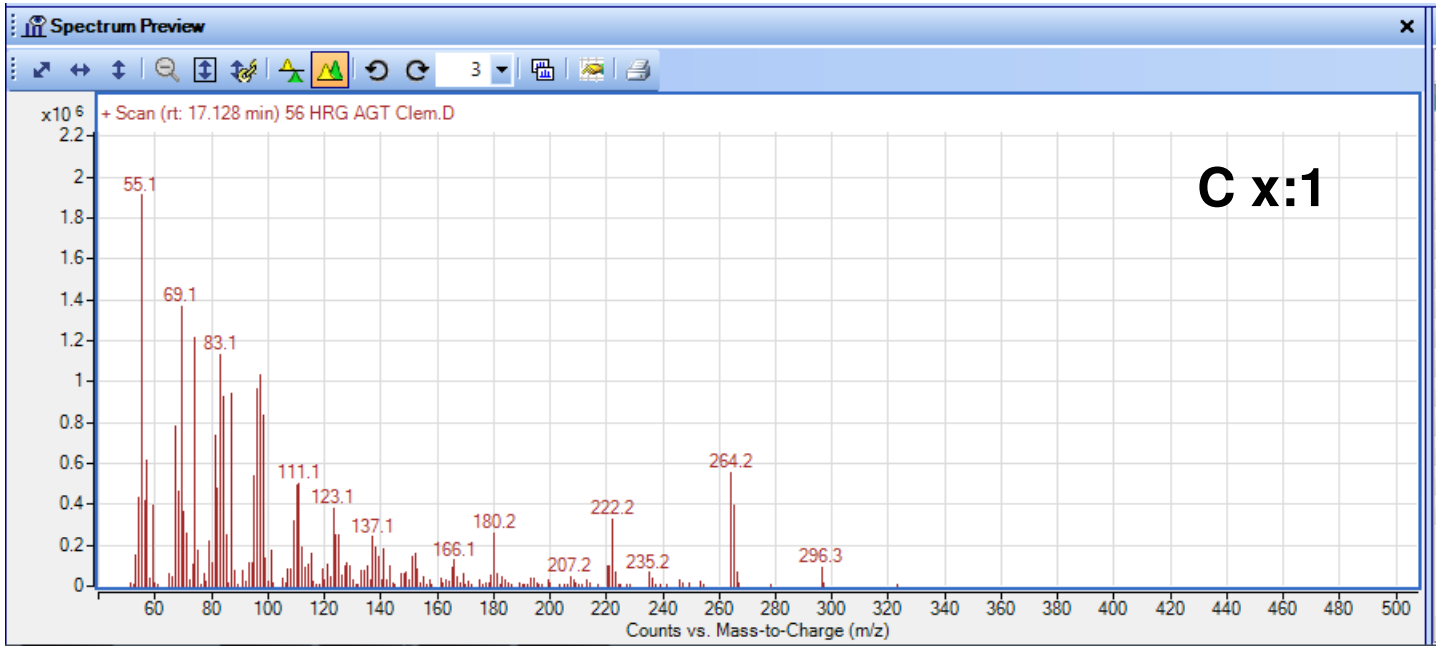


Identification of fatty acids

2. Peak identification: exact mass of FAME

x	C x:0	C x:1	C x:2	C x:3	C x:4
16	270	268	266	264	262
18	298	296	294	292	290
20	326	324	322	320	318
22	354	352	350	348	346
24	382	380	378	376	374



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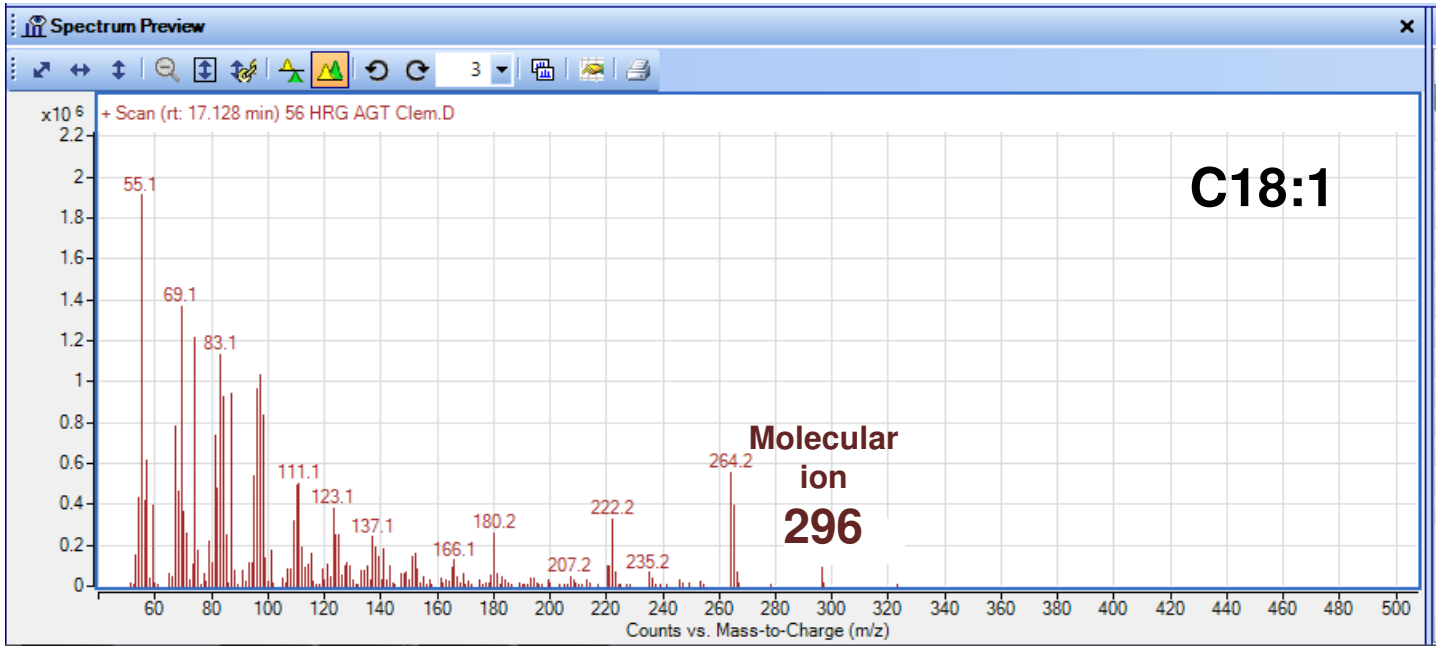


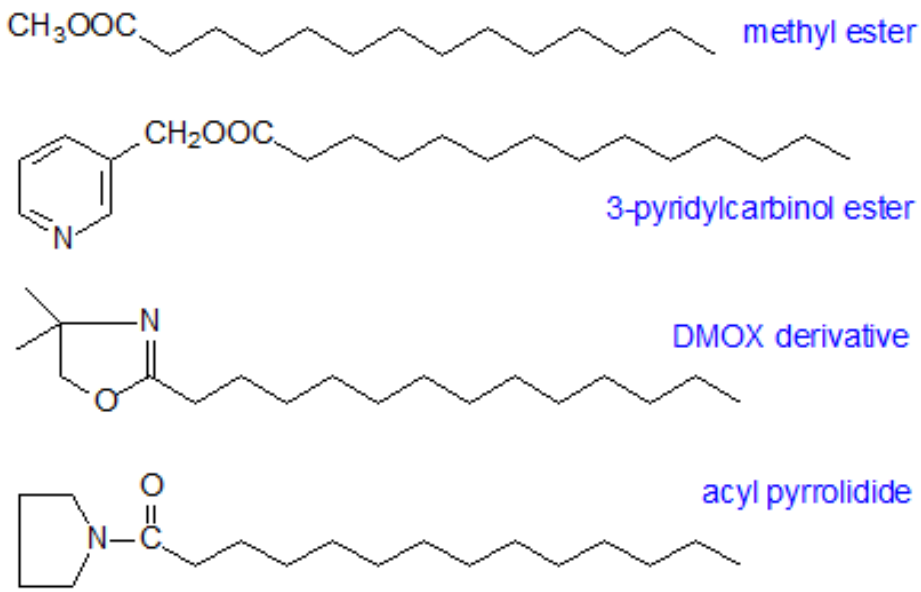
Identification of fatty acids

2. Peak identification

To identify the position of the double bonds:

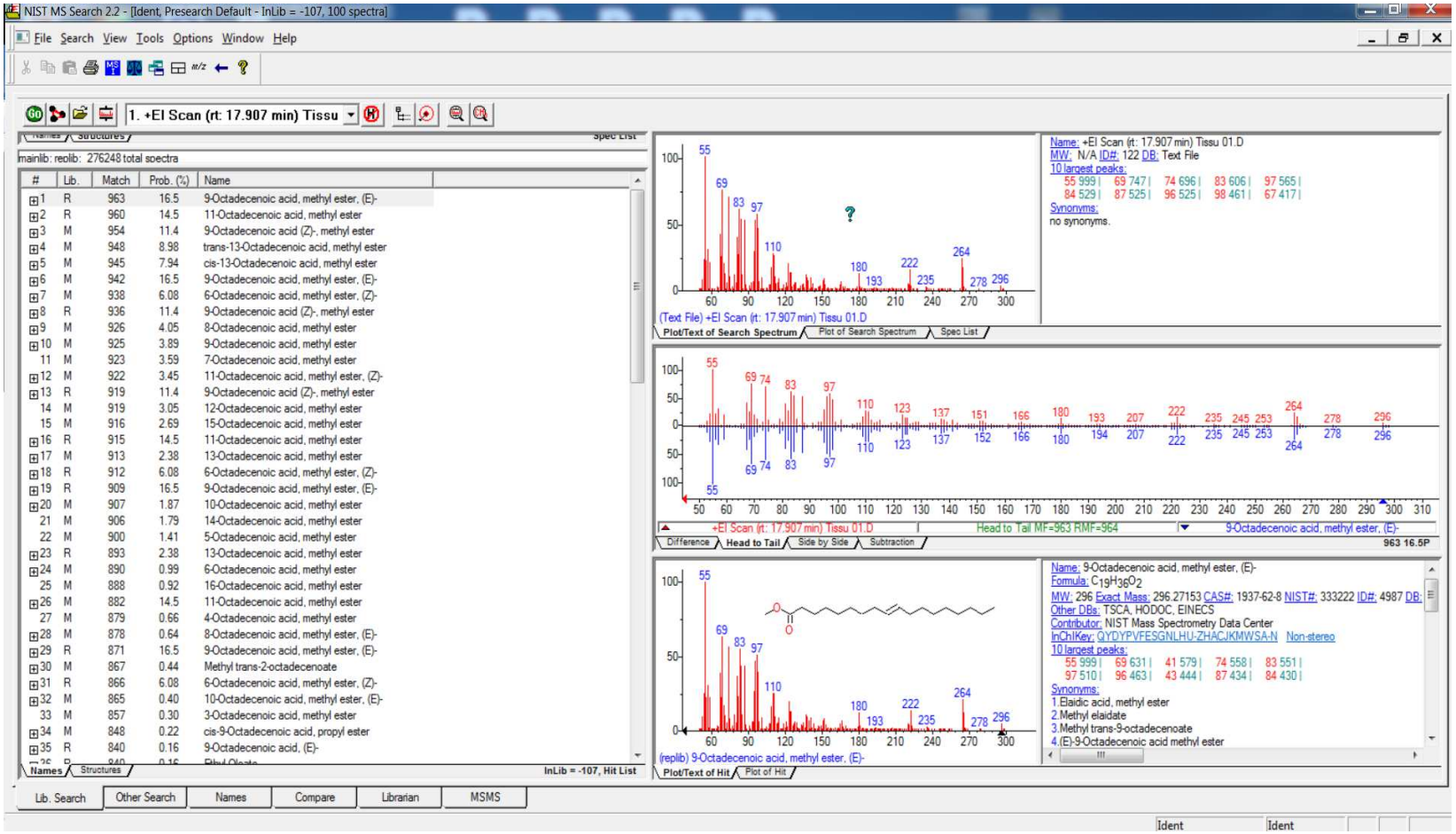
Need to stabilize the double bonds

→ addition of a cycle containing nitrogen



Identification of fatty acids

3. Verification with NIST database



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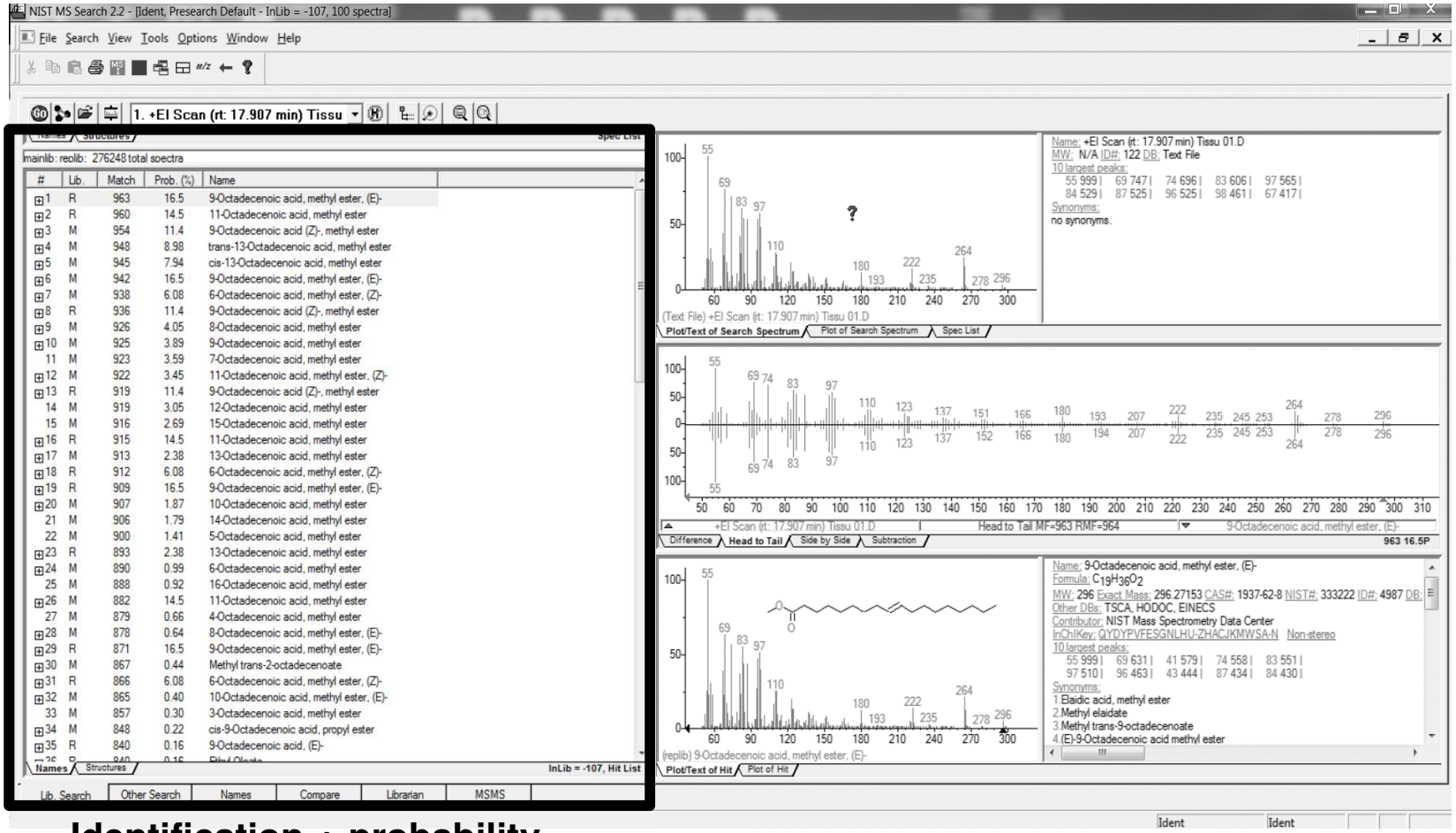

Identification + probability

Identification of fatty acids

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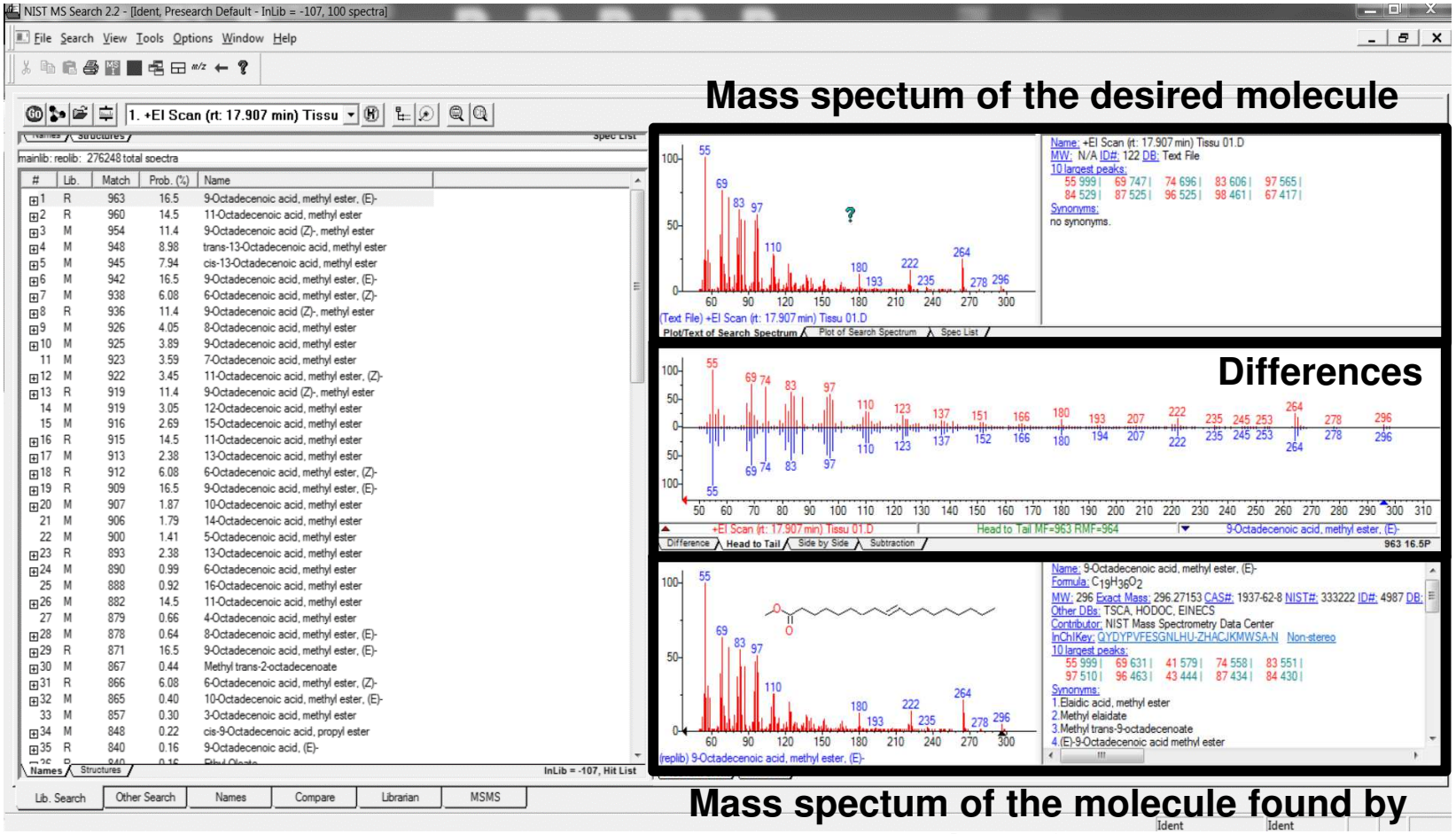

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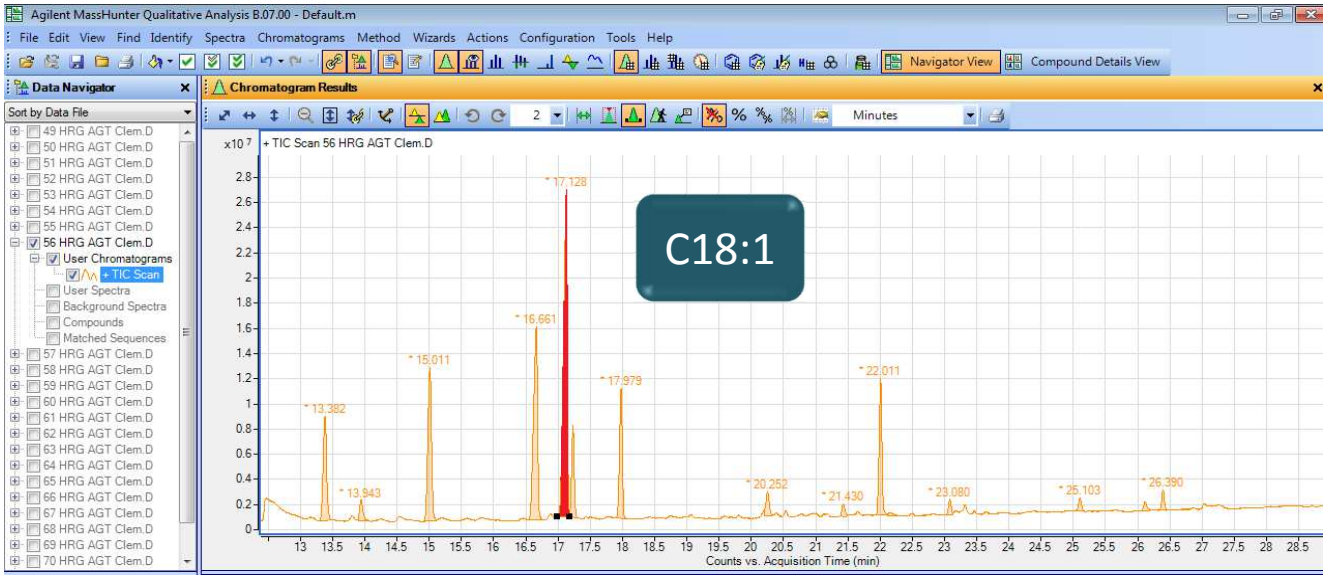

Σ areas = sum of the areas of the peaks identified (without the internal standard C17:0)

% of a fatty acid $\%(i) = \frac{A_i * 100}{\Sigma \text{ areas}}$

Total mass (mg/g) $= \frac{\Sigma \text{ Areas} * \text{mass (C17:0)}}{\text{Area (C17:0)}} \times 25 \times \frac{1}{\text{Sample mass (g)}}$

+ Desaturation index, %SFA, %MUFA, %PUFA, %n-3, triglyceride incorporation…

Other methods

Lipid extraction:

- Folch (chloroform/methanol – for tissues)
- Bligh and Dyer (methylal/methanol – for tissues)

Analysis of fatty acids:

- HPLC

LINKS


cyberlipids.gerli.com


lipidlibrary.aocs.org


lipidmaps.org

Thank you for your attention !

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