

Characterization of lipids

*Lipid extraction
GC/MS analysis*



Clémence PENHOAT

*NuMeCan Institute
EXPRES team, INSERM U1241*



02nd of february 2021 – Technical meeting NPhDA

Introduction

Why ?

**Technological interest :**

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

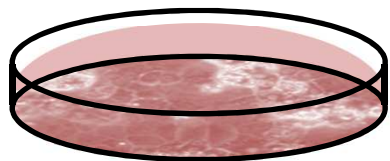
Clinical interest :

- Markers of consumption, deficiency, risk, metabolic diseases

**Research interest :**

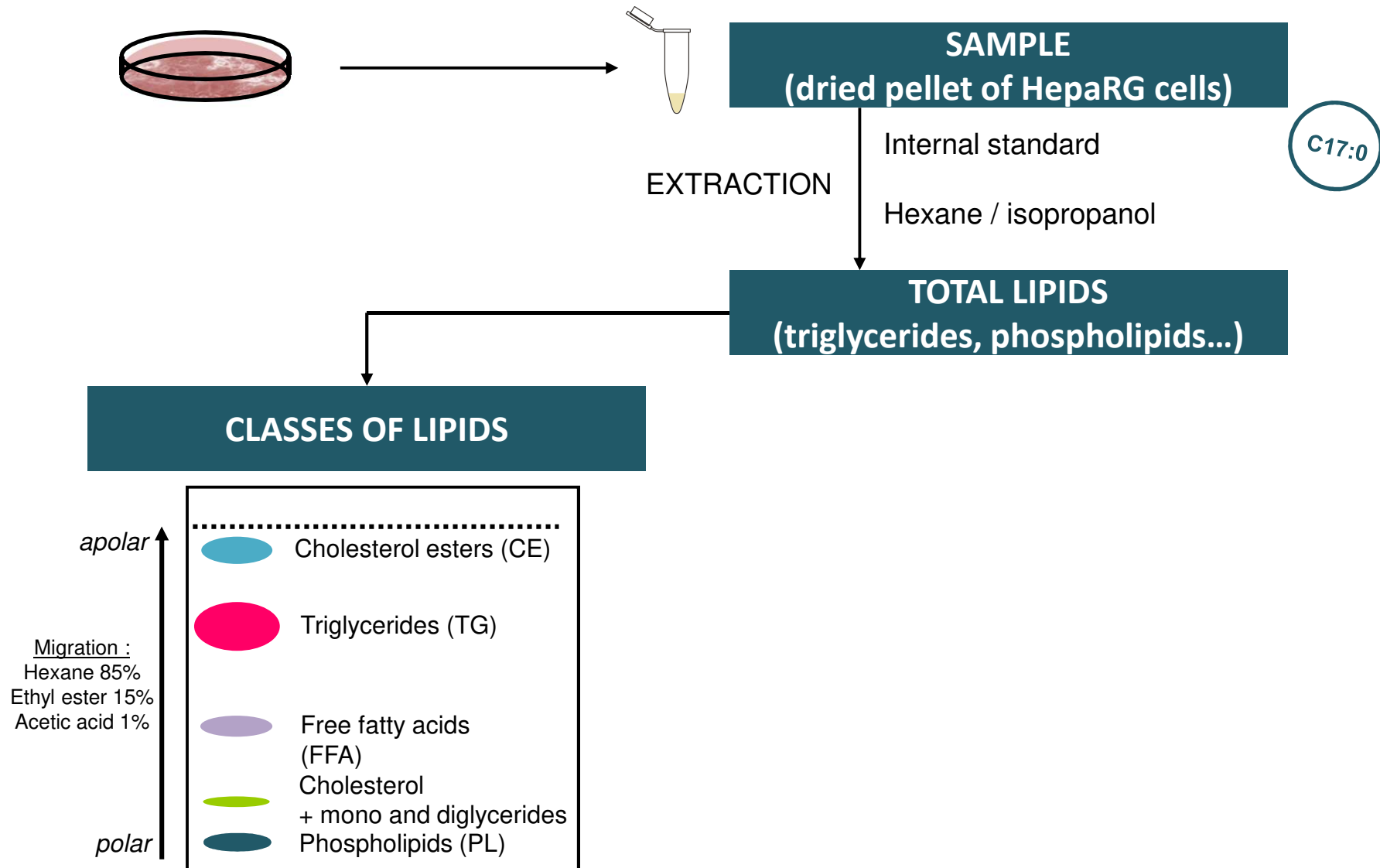
- Structures, metabolic pathways

Example : characterize the lipids present in HepaRG cells

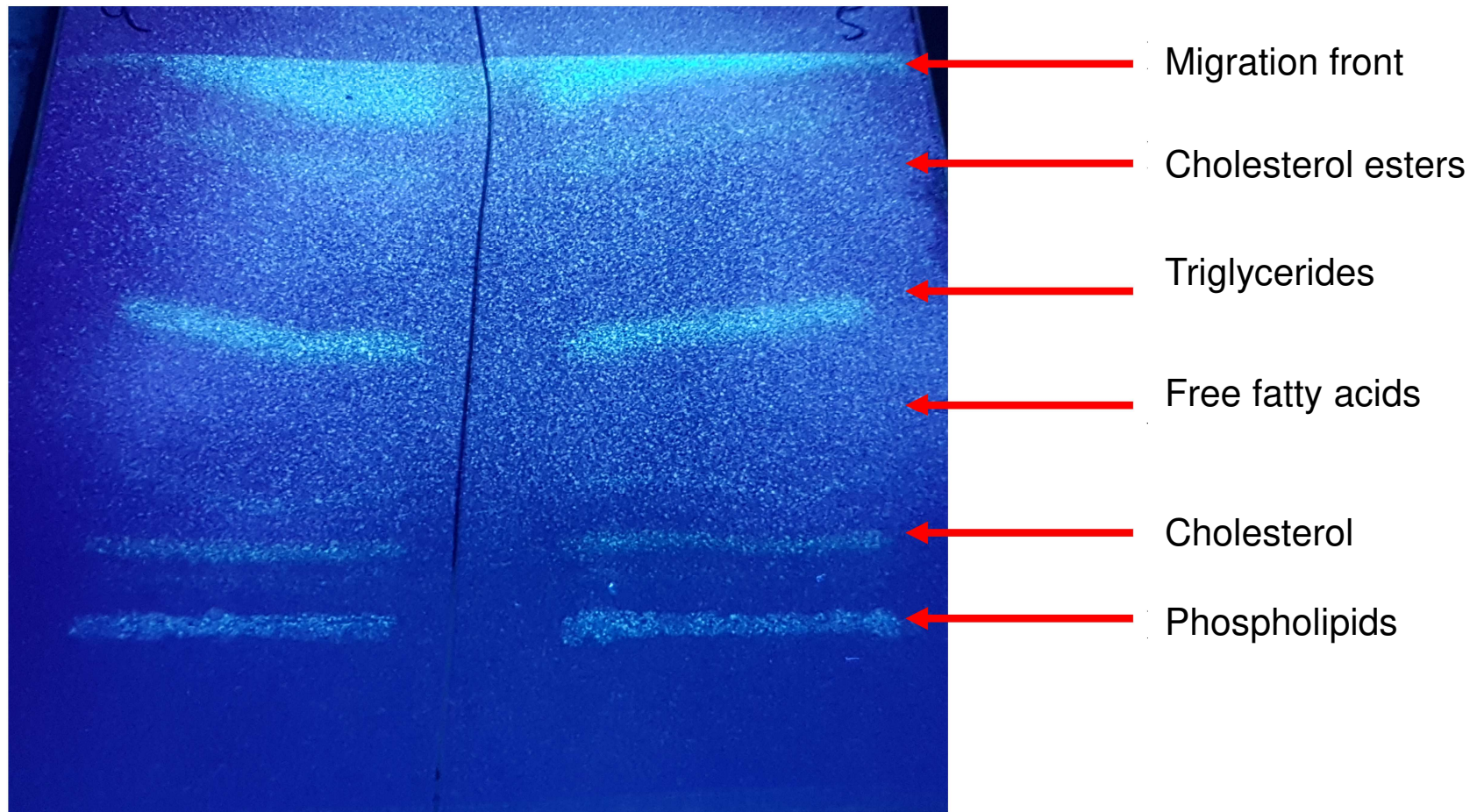


Lipid
characterization

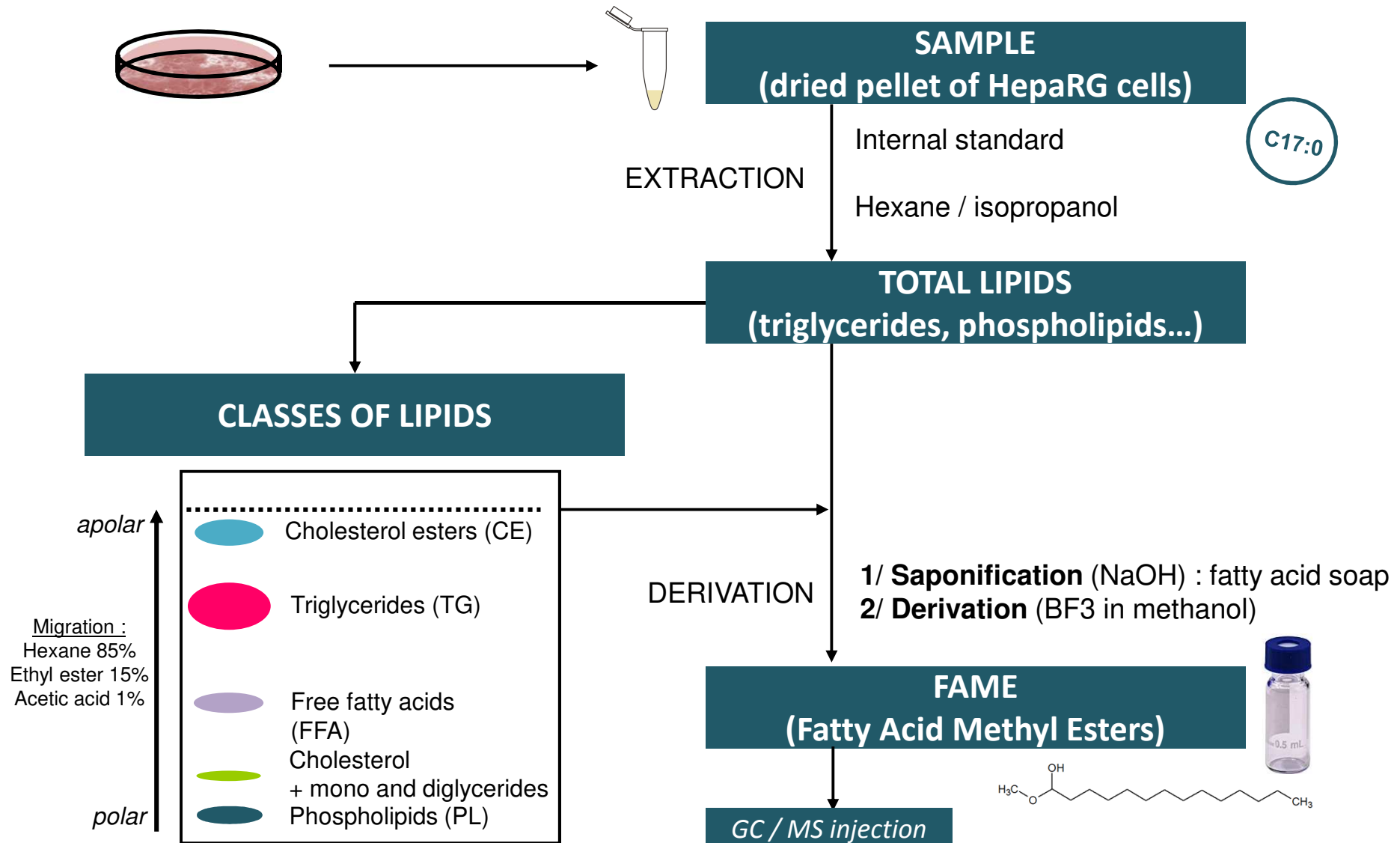
Preparation of the sample



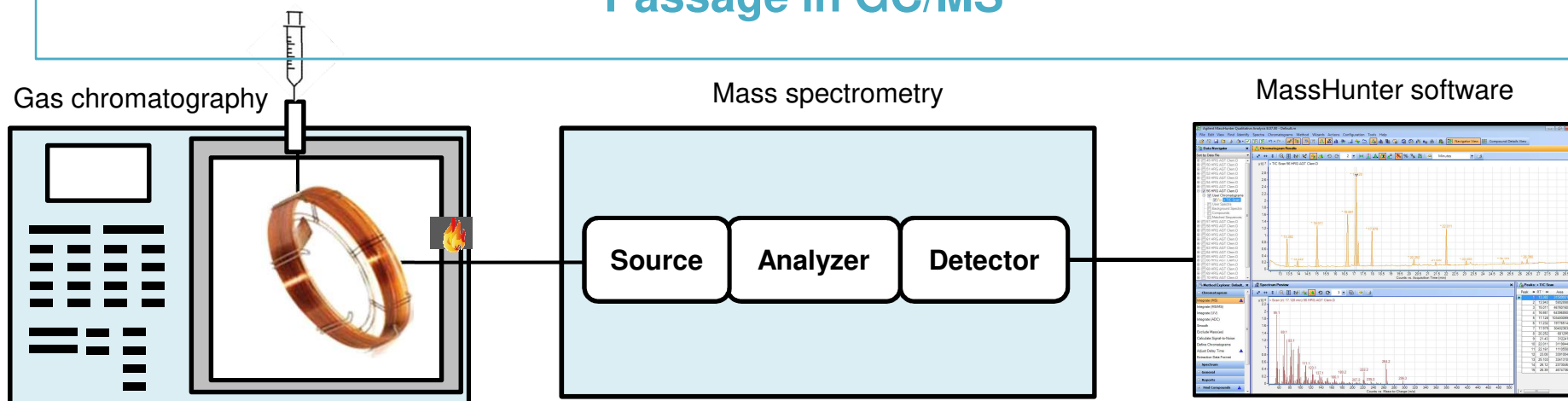
Preparation of the sample



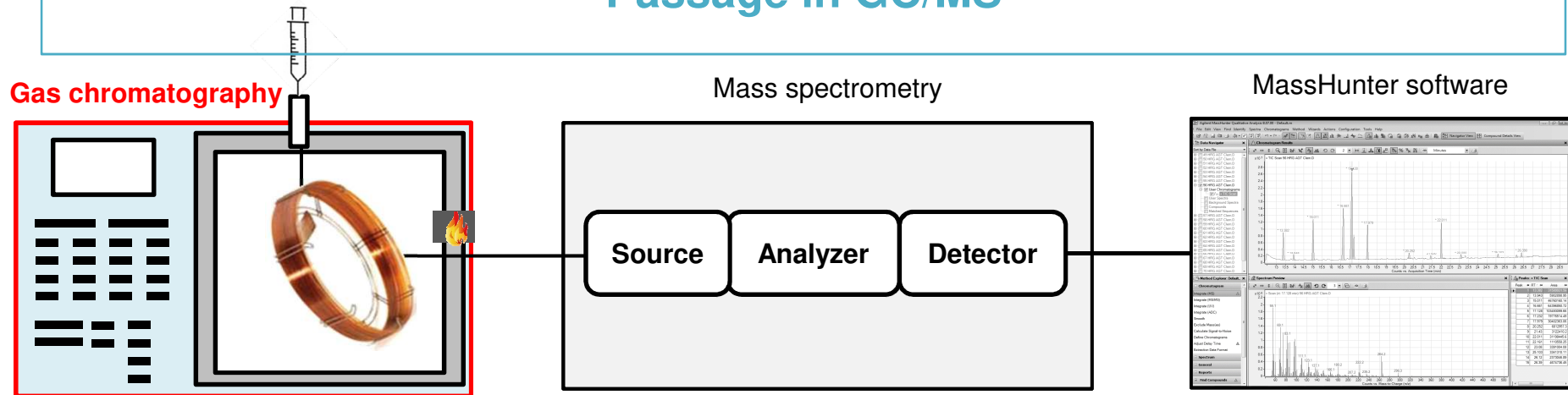
Preparation of the sample



Passage in GC/MS



Passage in GC/MS

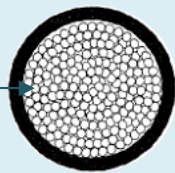


- Technique for separating a mixture of volatile molecules
- Separation by a stationary phase and a mobile phase
 - Separation according to polarity

The column

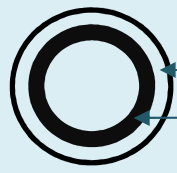
Filled column

Stationary phase
impregnated silica
granules



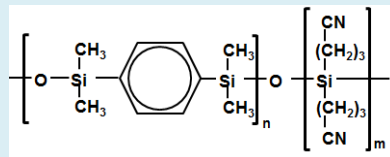
Capillary column

Fused silica
Stationary phase



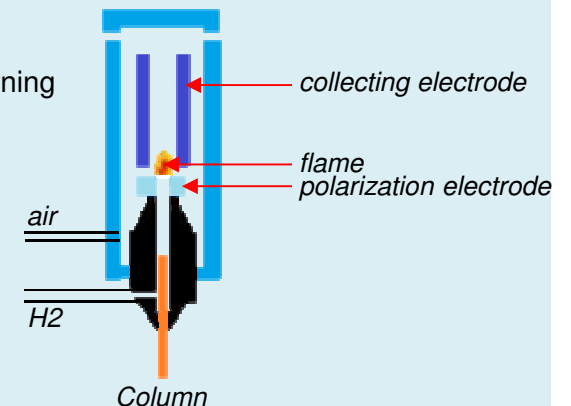
Stationary phases:

- Polydiméthylsiloxane
- Polyester
- Polyéthylèneglycol
- Cyanopropylphenylene-siloxane

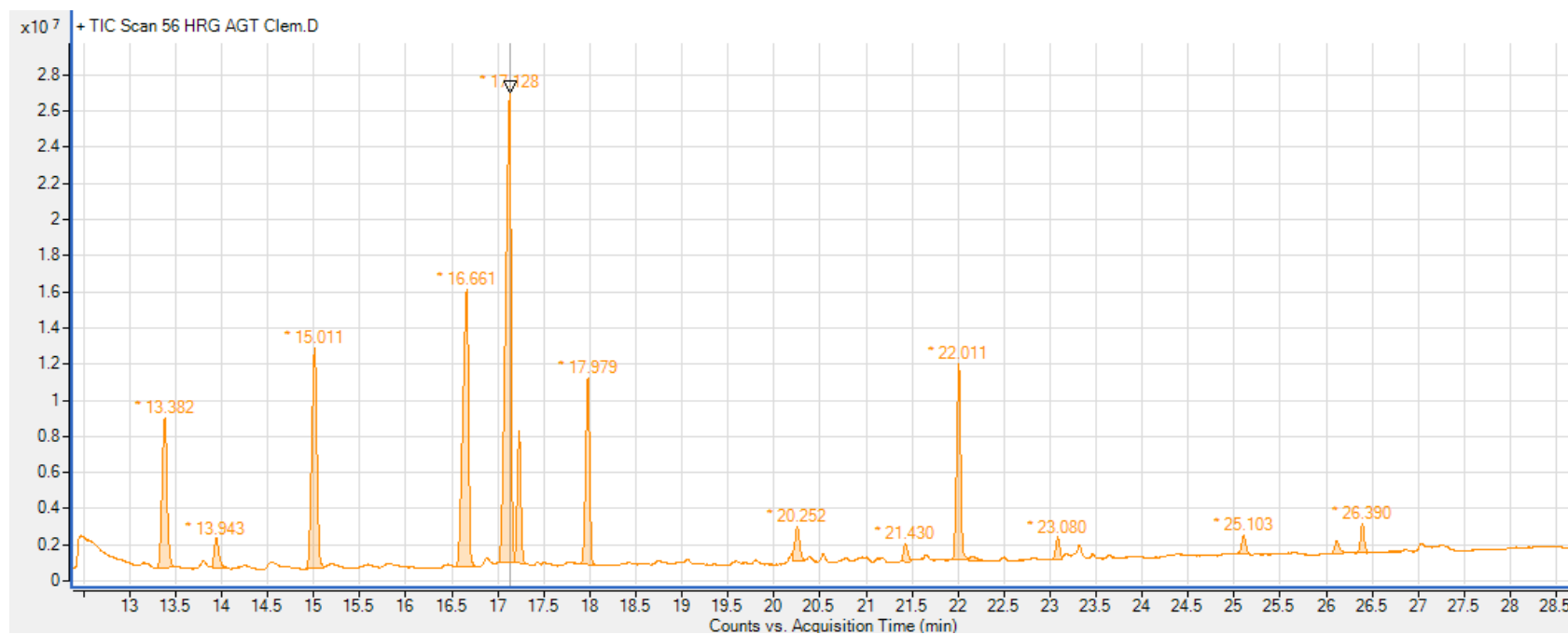


The FID (flamme ionization detector)

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

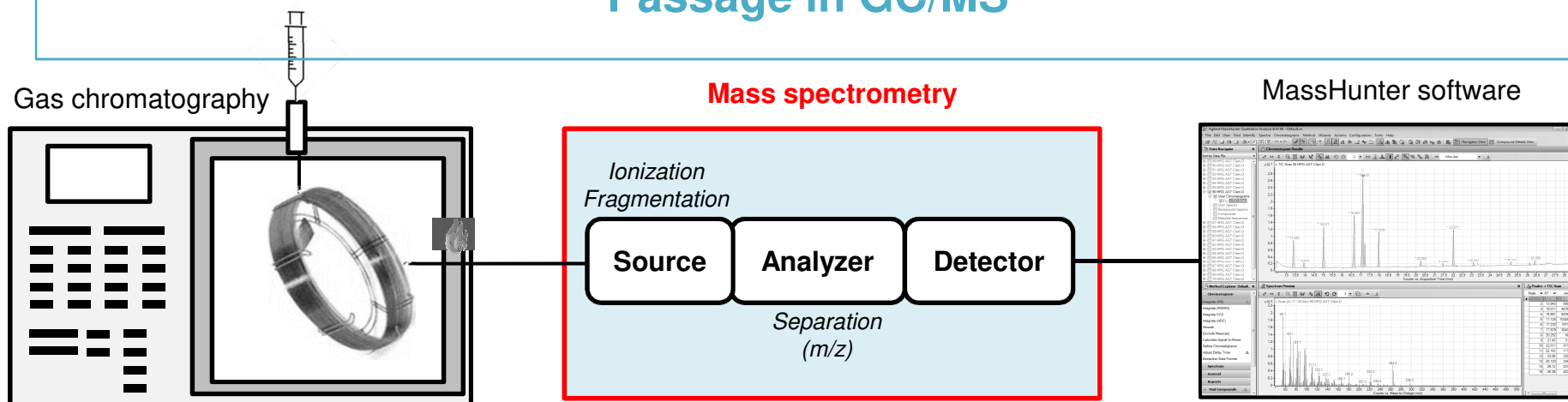


Passage in GC/MS

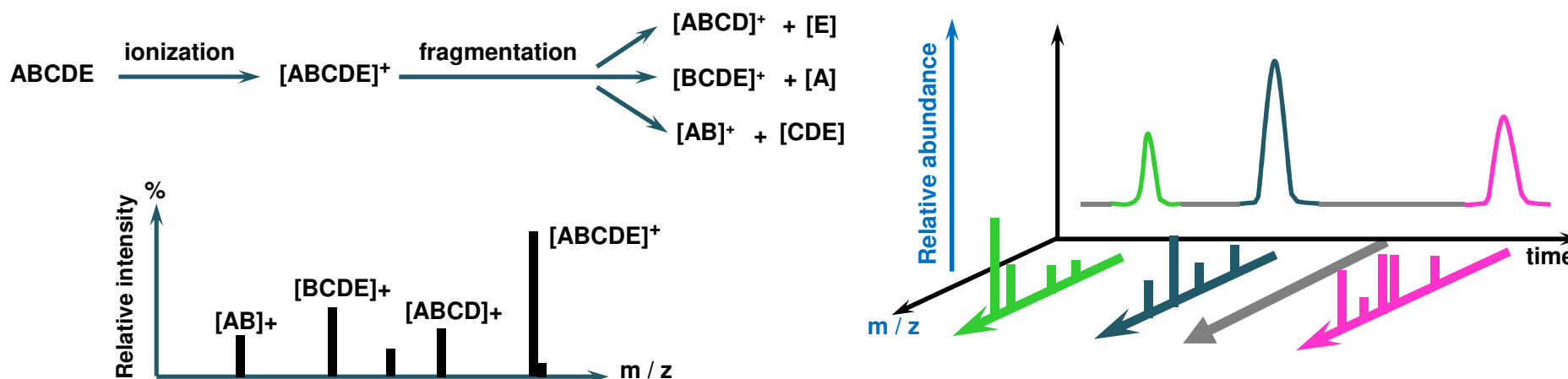


1 peak = 1 detected compound

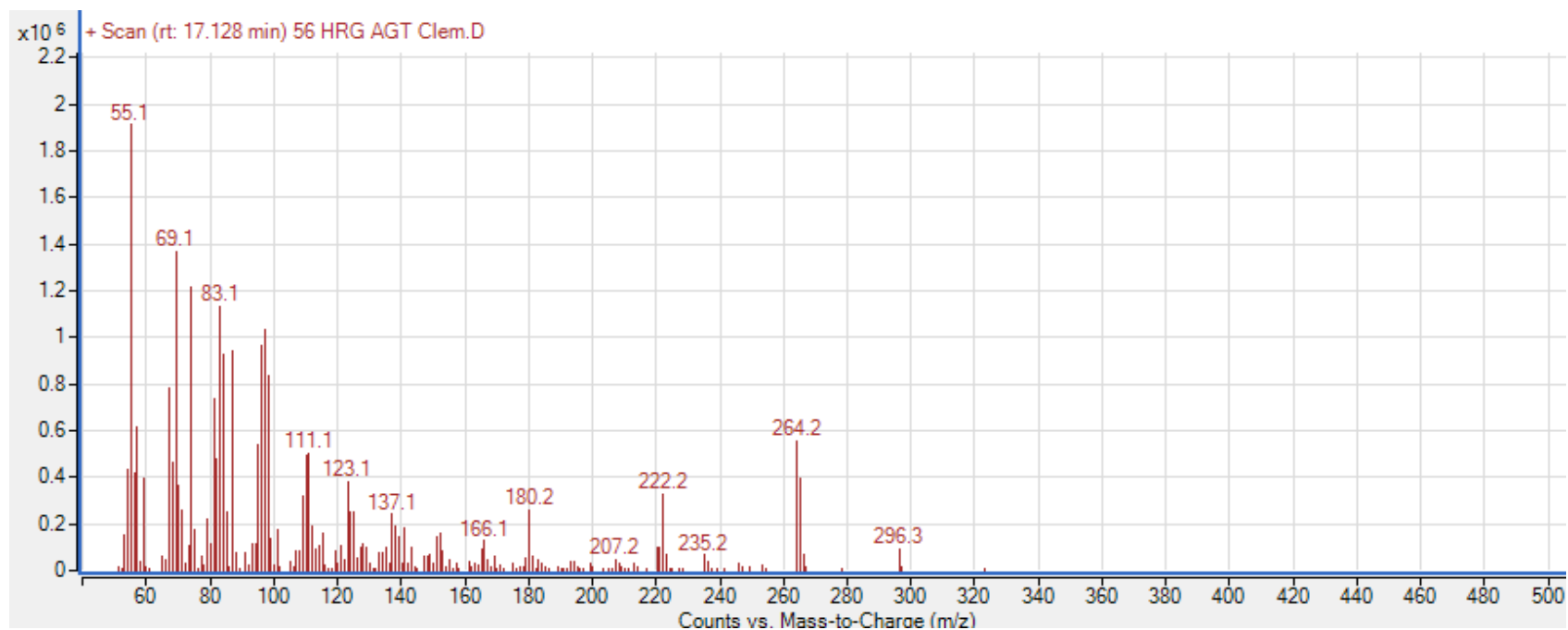
Passage in GC/MS



- 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**)

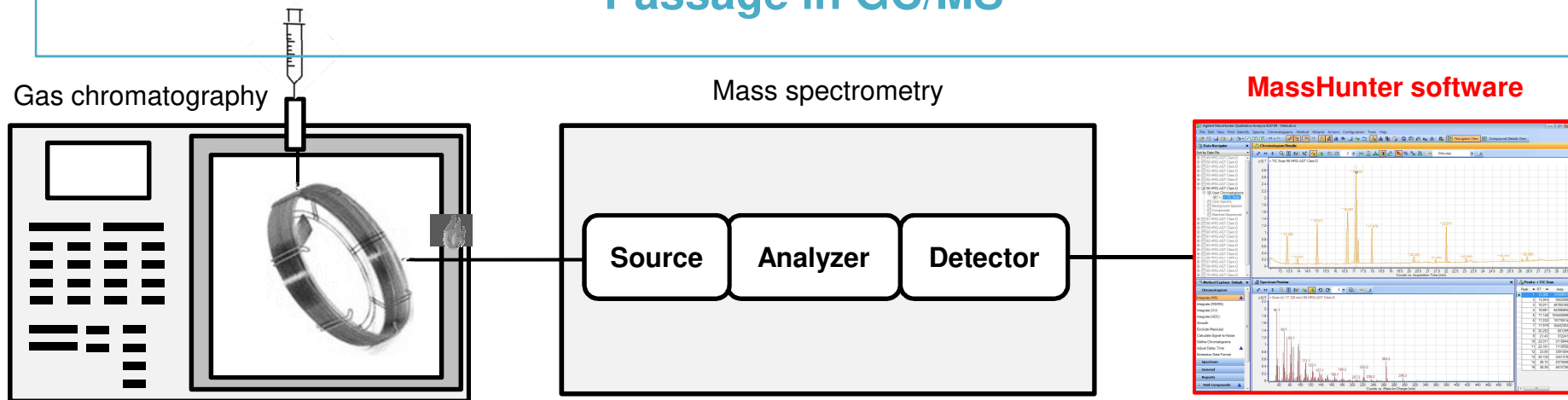


Passage in GC/MS



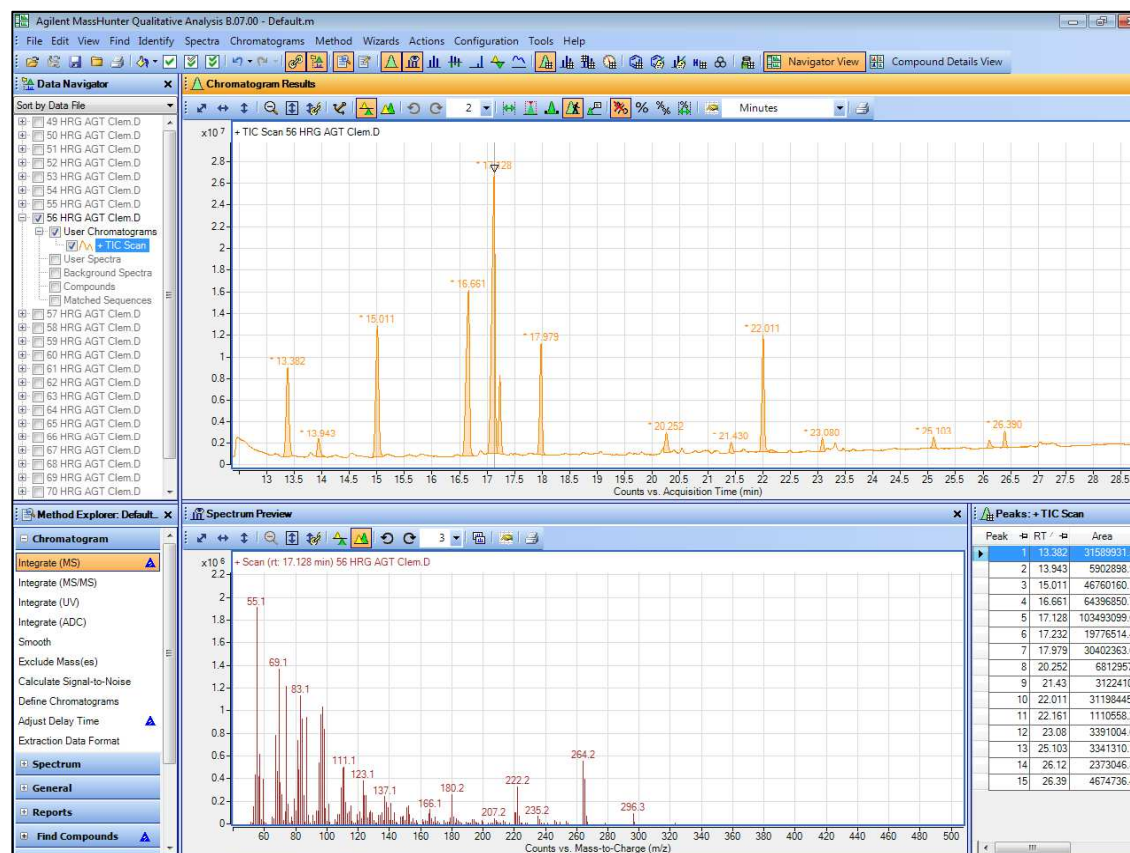
Molecule characteristic

Passage in GC/MS



Identification of fatty acids

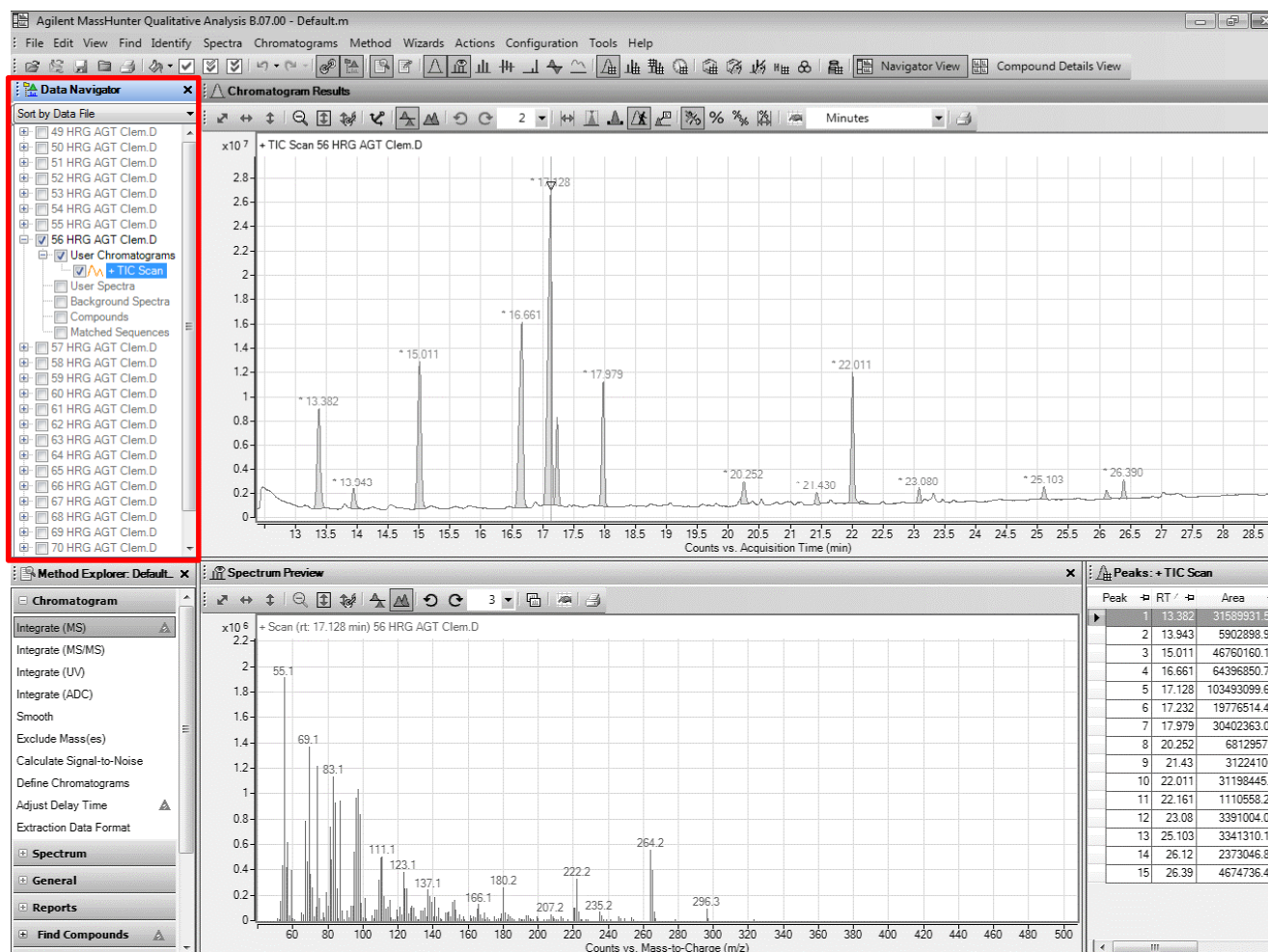
1. Software interface : MassHunter



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

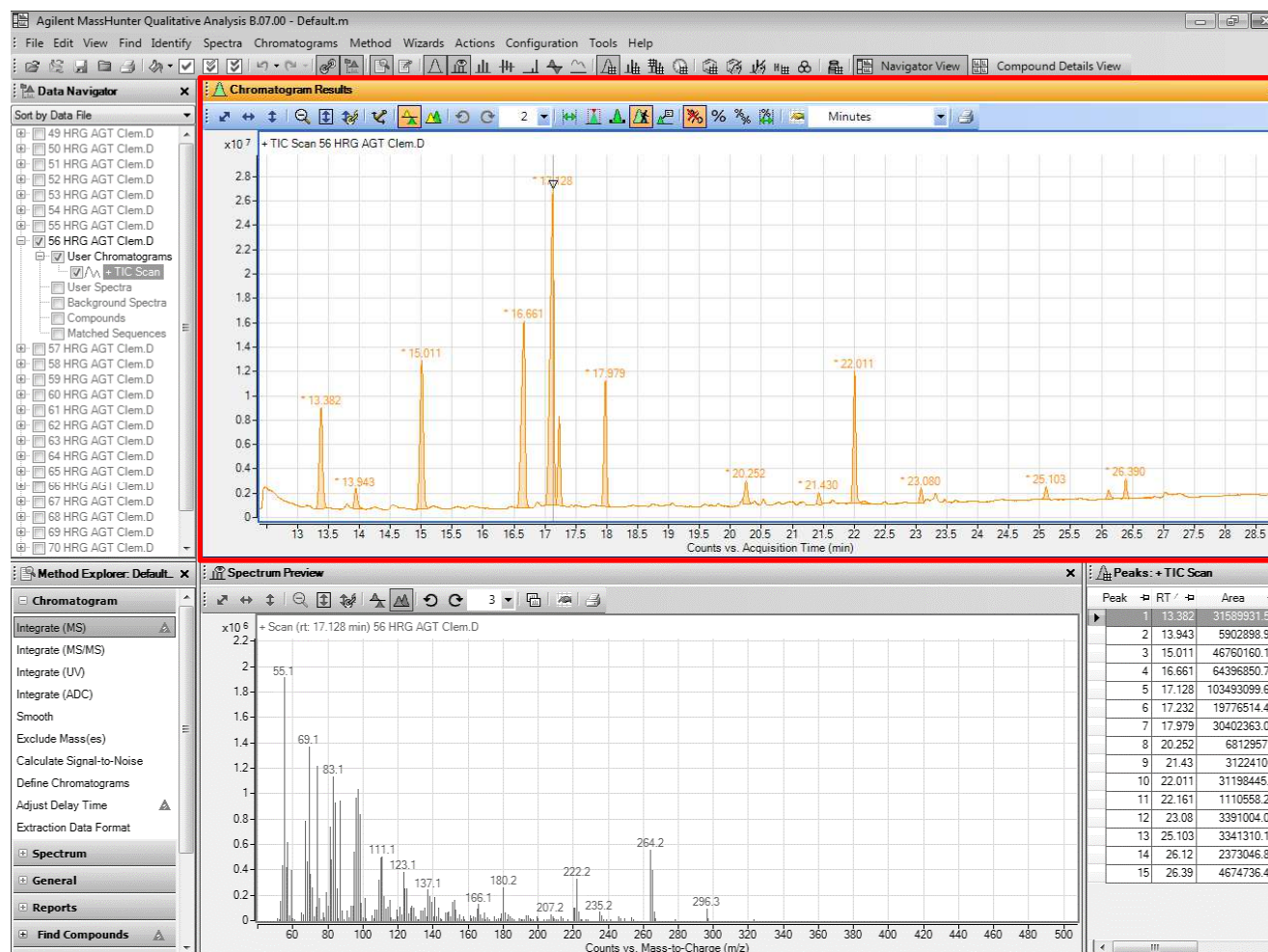
Identification of fatty acids

1. Software interface : MassHunter



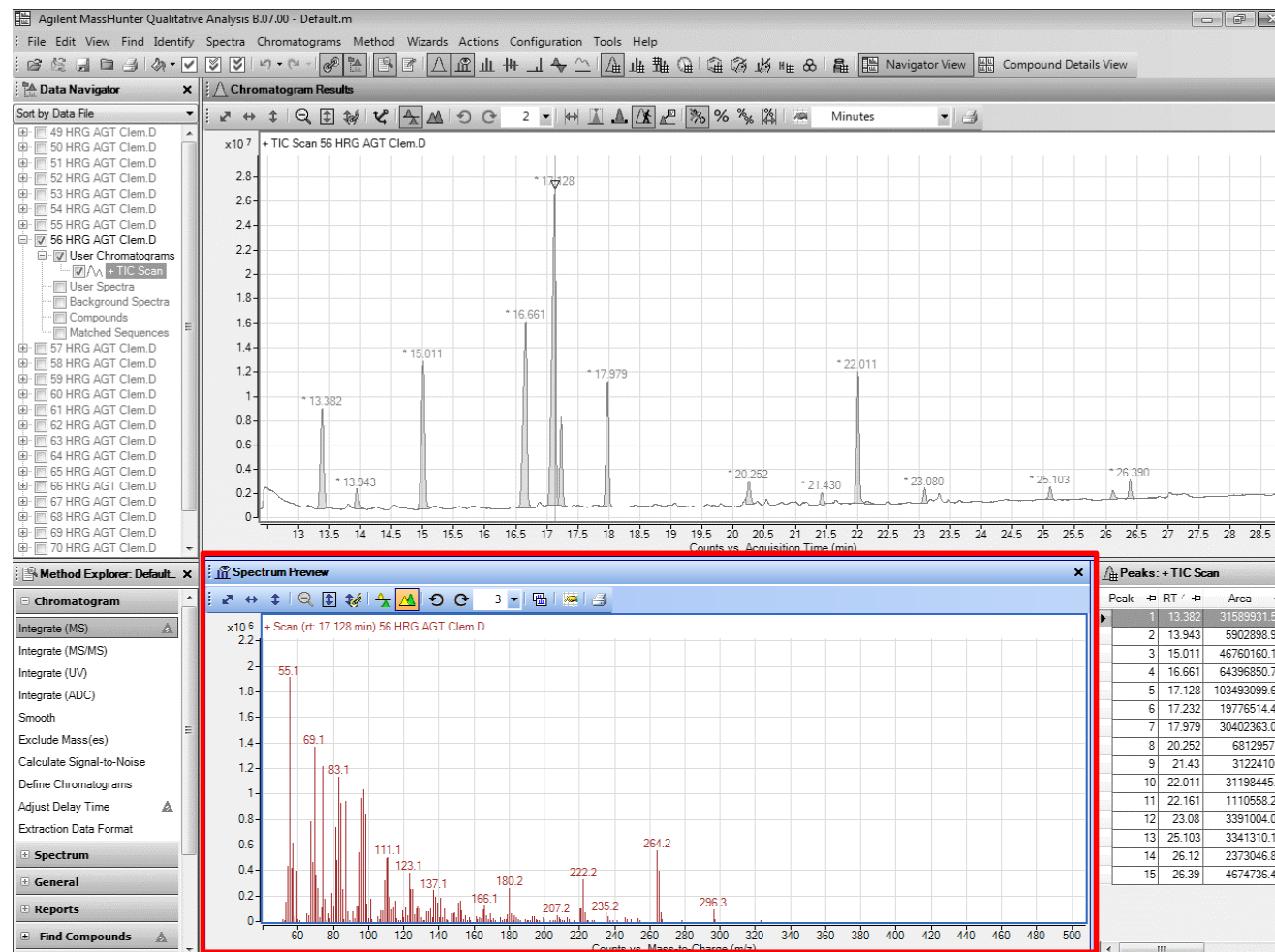
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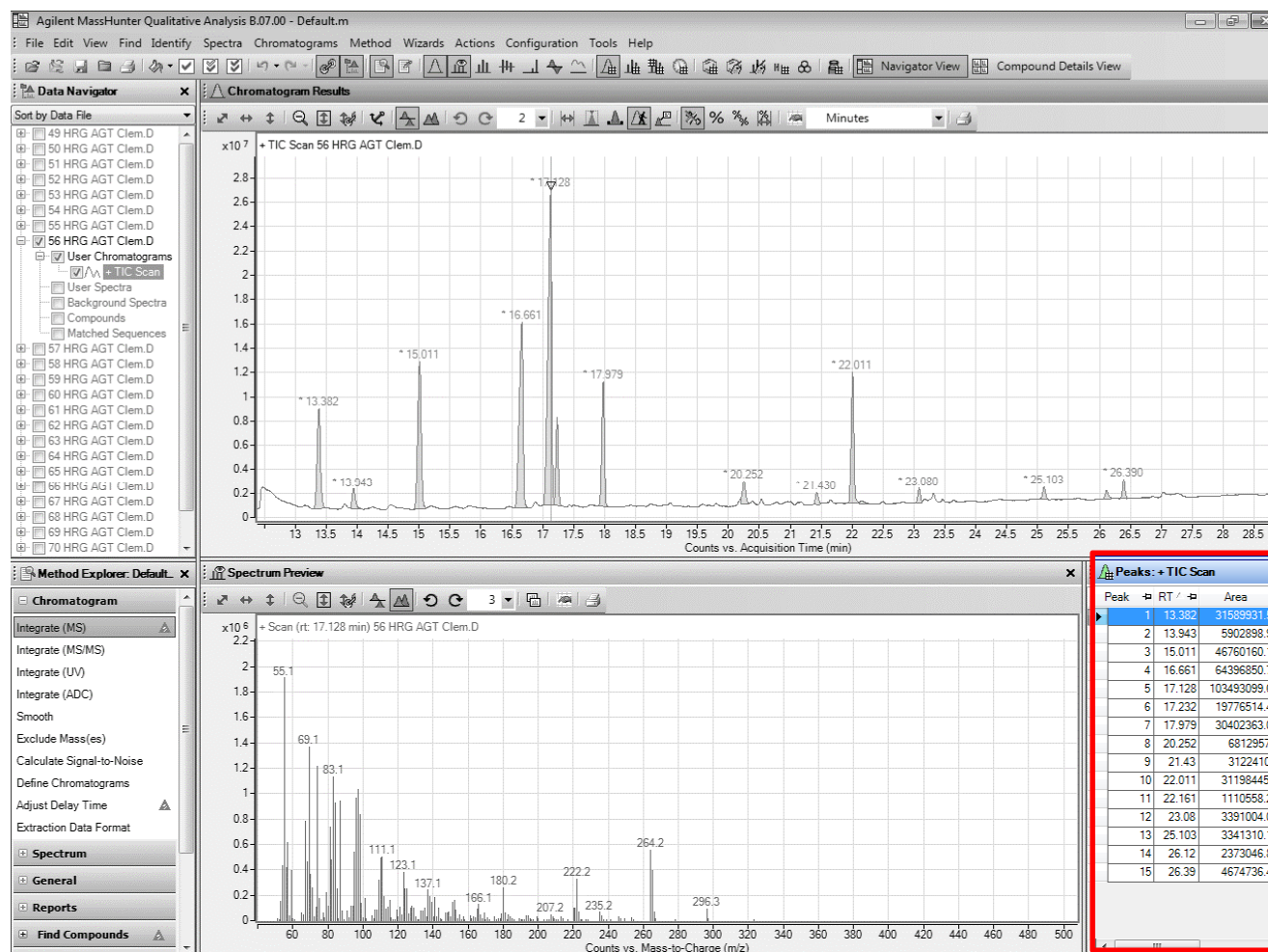
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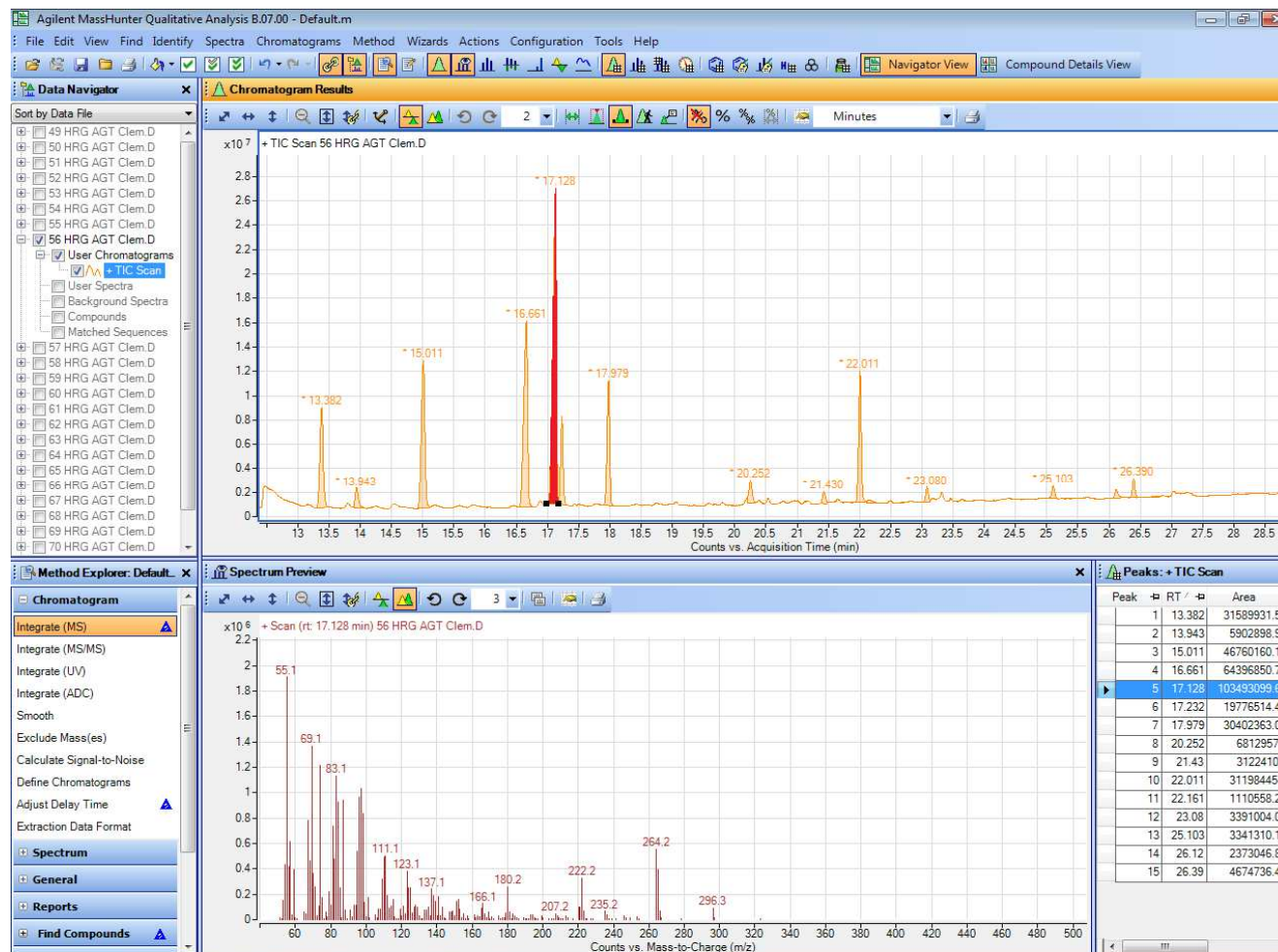
Identification of fatty acids

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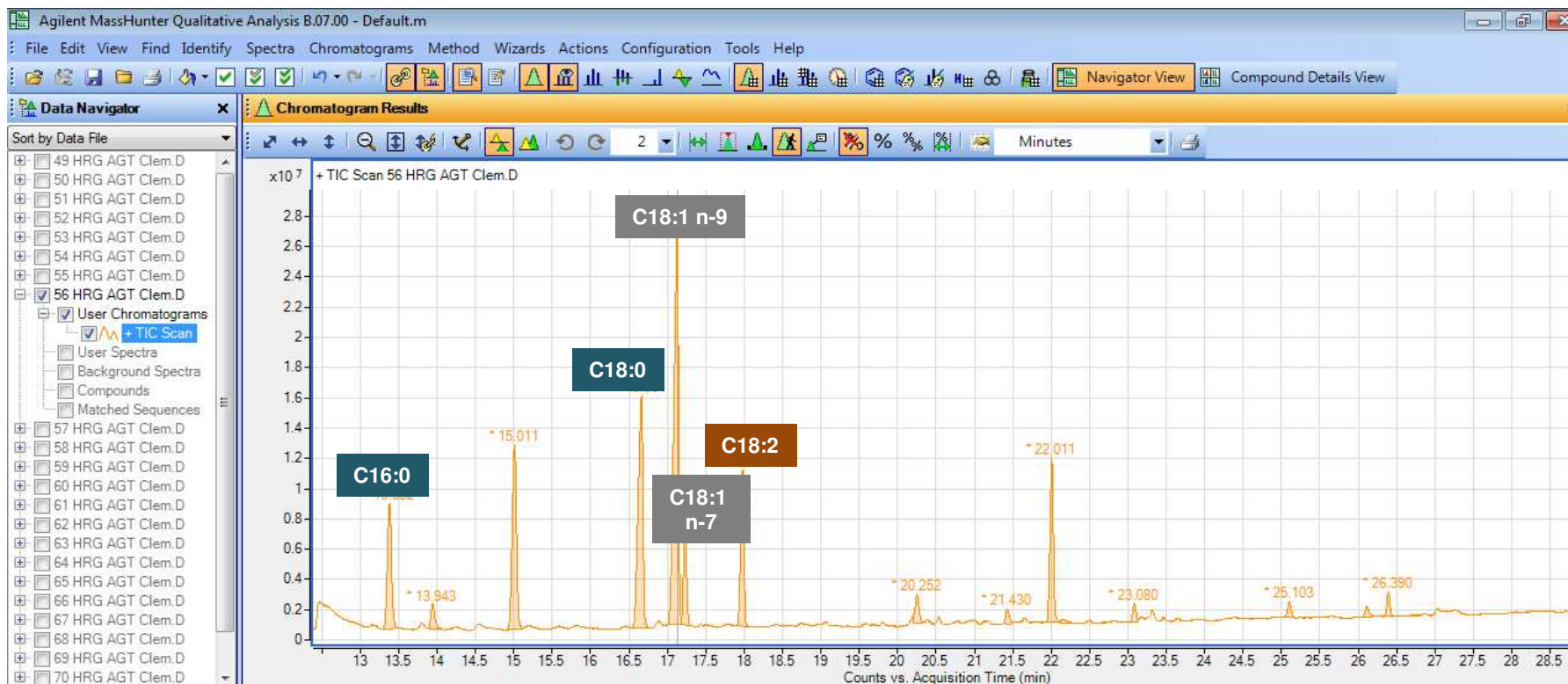
Identification of fatty acids

2. Peak identification



Identification of fatty acids

2. Peak identification



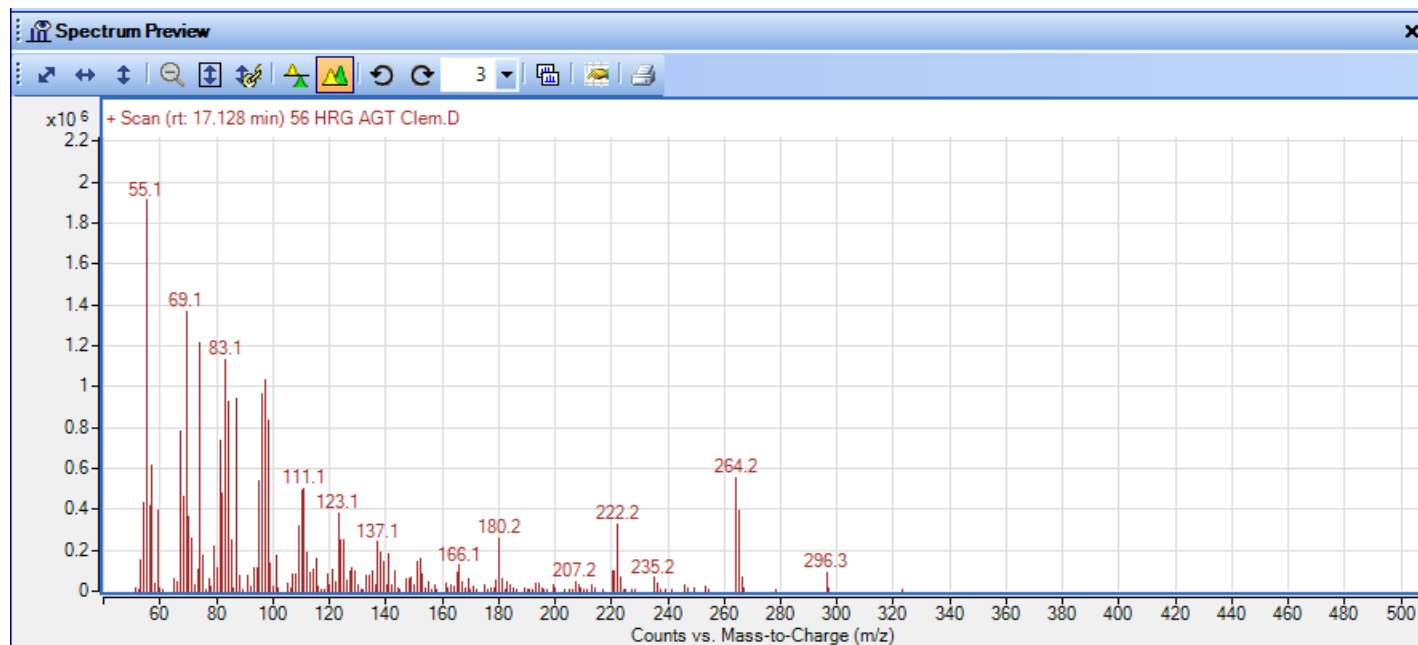
The peak order depends of:

Chain length
Number of double bonds
Position of double bonds
Double bonds geometry

Identification of fatty acids

2. Peak identification: characteristic ions

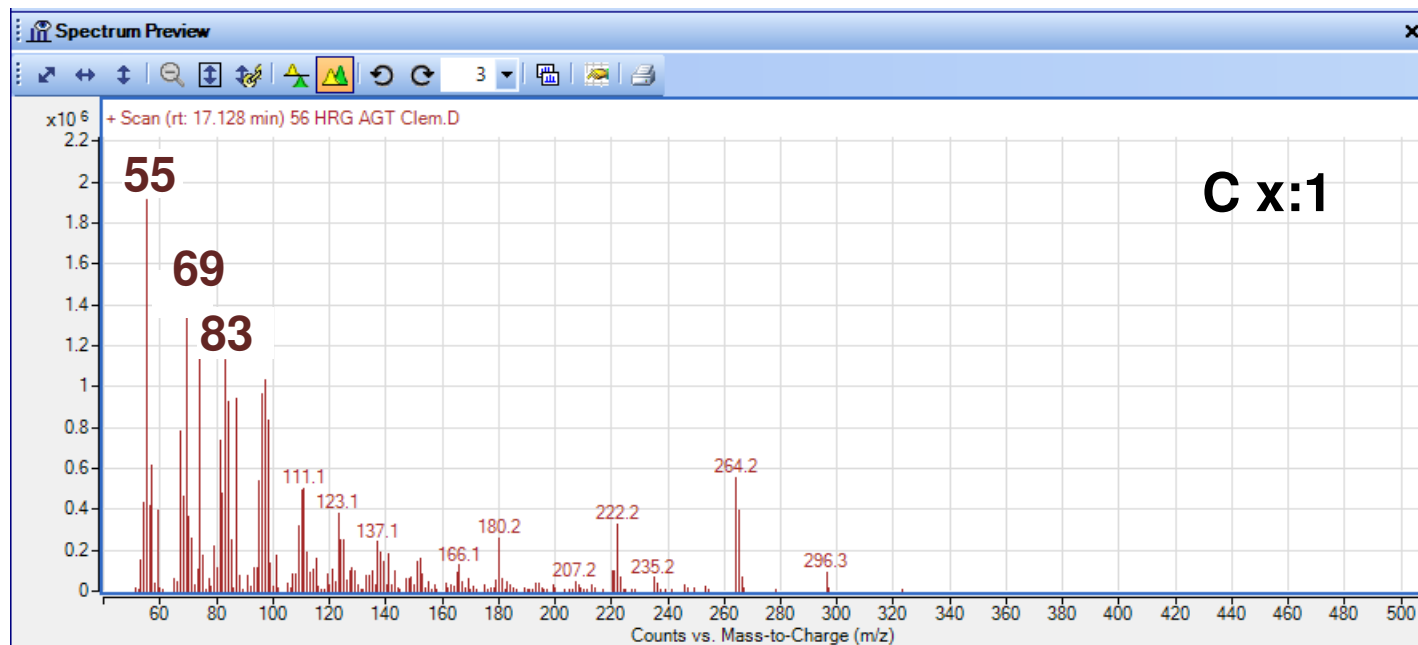
Fatty acid category	Ions (m/z)
Saturated	74, 87, 101, 115, 129, 143...
Mono-unsaturated	74, 55, 69, 83, 97...
Diene	67, 81, 95, 109, 123, 137...
Triene	79, 91, 107, 121, 135... n-6 : 150 n-3 : 108
Poly-unsaturated	79, 91, 105, 119, 133... n-3 : 108



Identification of fatty acids

2. Peak identification: characteristic ions

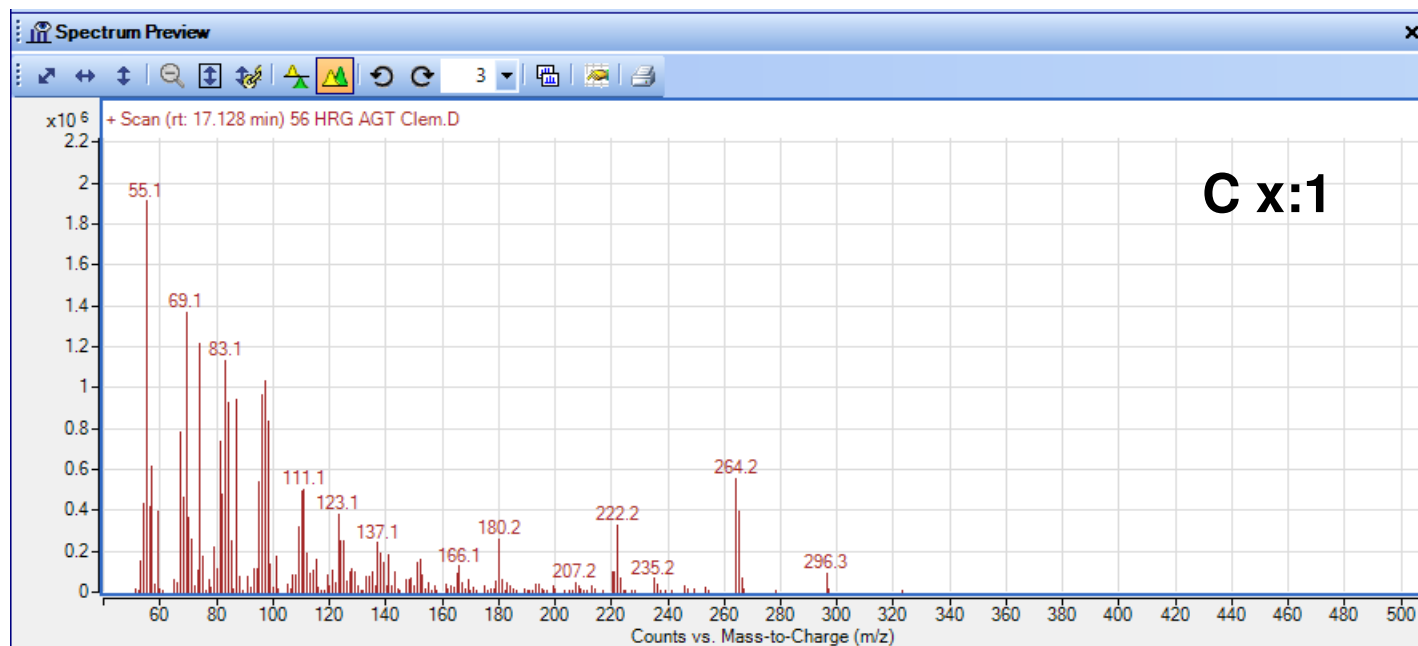
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Identification of fatty acids

2. Peak identification: exact mass of FAME

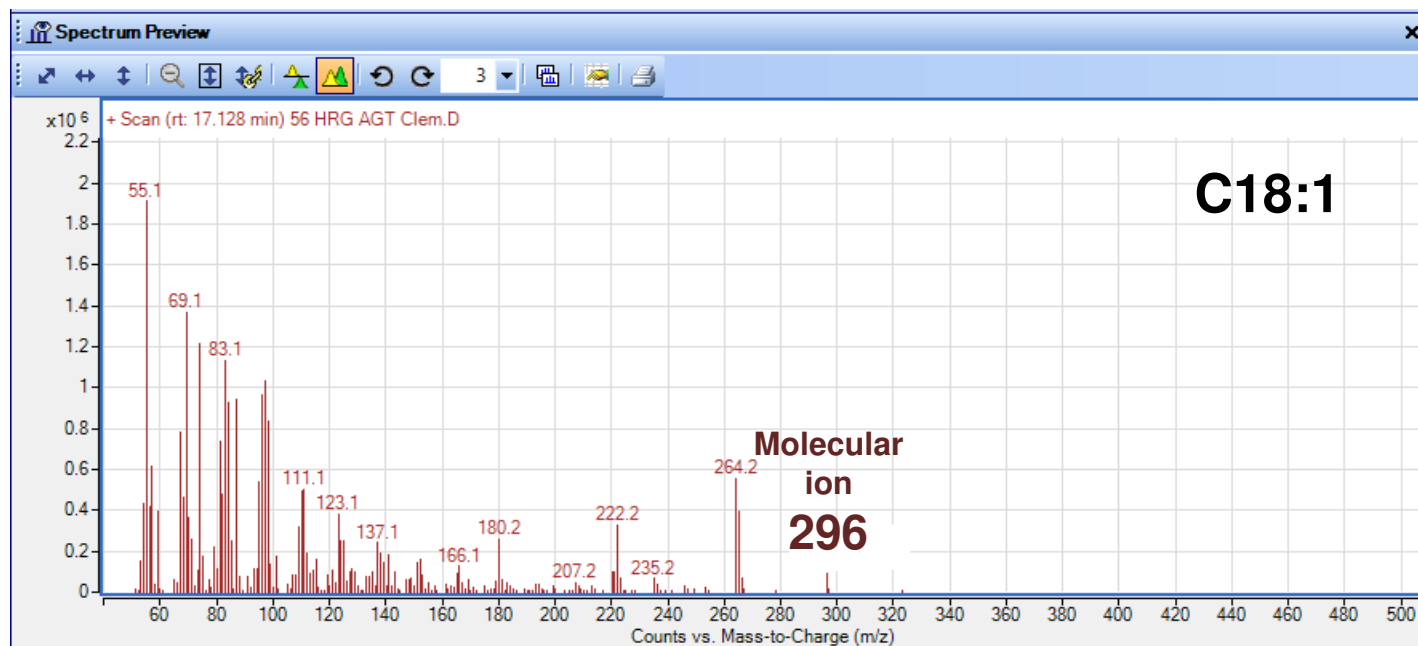
x	C x:0	C x:1	C x:2	C x:3	C x:4
16	270	268	266	264	262
18	298	296	294	292	290
20	326	324	322	320	318
22	354	352	350	348	346
24	382	380	378	376	374



Identification of fatty acids

2. Peak identification: exact mass of FAME

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Identification of fatty acids

2. Peak identification

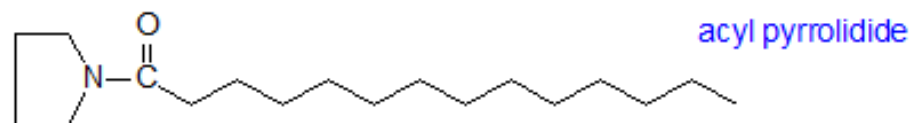
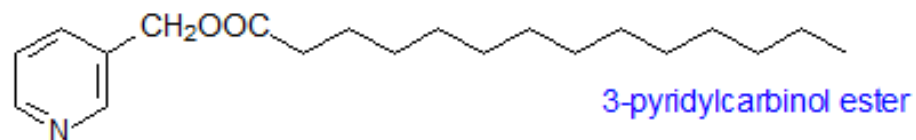


To identify the position of the double bonds:

Need to stabilize the double bonds



addition of a cycle containing nitrogen



Identification of fatty acids



3. Verification with NIST database

NIST MS Search 2.2 - [Ident, Presearch Default - InLib = -107, 100 spectra]

File Search View Tools Options Window Help

1. •EI Scan (rt: 17.907 min) Tissu

mainlib: reclib: 276248 total spectra

#	Lib.	Match	Prob. (%)	Name
1	R	963	16.5	9-Octadecenoic acid, methyl ester, (E)-
2	R	960	14.5	11-Octadecenoic acid, methyl ester
3	M	954	11.4	9-Octadecenoic acid (Z)-, methyl ester
4	M	948	8.98	trans-13-Octadecenoic acid, methyl ester
5	M	945	7.94	cis-13-Octadecenoic acid, methyl ester
6	M	942	16.5	9-Octadecenoic acid, methyl ester, (E)-
7	M	938	6.08	6-Octadecenoic acid, methyl ester, (Z)-
8	R	936	11.4	9-Octadecenoic acid (Z)-, methyl ester
9	M	926	4.05	8-Octadecenoic acid, methyl ester
10	M	925	3.89	9-Octadecenoic acid, methyl ester
11	M	923	3.59	7-Octadecenoic acid, methyl ester
12	M	922	3.45	11-Octadecenoic acid, methyl ester, (Z)-
13	R	919	11.4	9-Octadecenoic acid (Z)-, methyl ester
14	M	919	3.05	12-Octadecenoic acid, methyl ester
15	M	916	2.69	15-Octadecenoic acid, methyl ester
16	R	915	14.5	11-Octadecenoic acid, methyl ester
17	M	913	2.38	13-Octadecenoic acid, methyl ester
18	R	912	6.08	6-Octadecenoic acid, methyl ester, (Z)-
19	R	909	16.5	9-Octadecenoic acid, methyl ester, (E)-
20	M	907	1.87	10-Octadecenoic acid, methyl ester
21	M	906	1.79	14-Octadecenoic acid, methyl ester
22	M	900	1.41	5-Octadecenoic acid, methyl ester
23	R	893	2.38	13-Octadecenoic acid, methyl ester
24	M	890	0.99	6-Octadecenoic acid, methyl ester
25	M	888	0.92	16-Octadecenoic acid, methyl ester
26	M	882	14.5	11-Octadecenoic acid, methyl ester
27	M	879	0.66	4-Octadecenoic acid, methyl ester
28	M	878	0.64	8-Octadecenoic acid, methyl ester, (E)-
29	R	871	16.5	9-Octadecenoic acid, methyl ester, (E)-
30	M	867	0.44	Methyl trans-2-octadecenoate
31	R	866	6.08	6-Octadecenoic acid, methyl ester, (Z)-
32	M	865	0.40	10-Octadecenoic acid, methyl ester, (E)-
33	M	857	0.30	3-Octadecenoic acid, methyl ester
34	M	848	0.22	cis-9-Octadecenoic acid, propyl ester
35	R	840	0.16	9-Octadecenoic acid, (E)-

Names Structures InLib = -107, Hit List

Lib. Search Other Search Names Compare Librarian MSMS

Plot/Text of Search Spectrum Plot of Search Spectrum Spec List

Name: •EI Scan (rt: 17.907 min) Tissu 01.D
MW: N/A ID# 122 DB: Text File
10 largest peaks:
55 999 | 69 747 | 74 696 | 83 606 | 97 565 |
84 529 | 87 525 | 96 525 | 98 461 | 67 417 |
Synonyms:
no synonyms.

(Text File) •EI Scan (rt: 17.907 min) Tissu 01.D

Plot/Text of Search Spectrum Plot of Search Spectrum Spec List

Name: 9-Octadecenoic acid, methyl ester, (E)-
Formula: C₁₉H₃₆O₂
MW: 296 Exact Mass: 296.27153 CAS#: 1937-62-8 NIST#: 333222 ID#: 4987 DB:
Other DBs: TSCA, HODOC, EINECS
Contributor: NIST Mass Spectrometry Data Center
InChIkey: QYDYPVFESGNIHU-ZHACJHMWSA-N Non-stereo
10 largest peaks:
55 999 | 69 631 | 41 579 | 74 558 | 83 551 |
97 510 | 96 463 | 43 444 | 87 434 | 84 430 |
Synonyms:
1. Elaidic acid, methyl ester
2. Methyl elaidate
3. Methyl trans-9-octadecenoate
4. (E)-9-Octadecenoic acid methyl ester

Chemical structure of 9-Octadecenoic acid, methyl ester, (E)- is shown.

Identification of fatty acids



3. Verification with NIST database

NIST MS Search 2.2 - [Ident, Presearch Default - InLib = -107, 100 spectra]

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84 529 87 525 96 525 98 461 67 417
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(replib) 9-Octadecenoic acid, methyl ester, (E)-

Plot/Text of Hit Plot of Hit

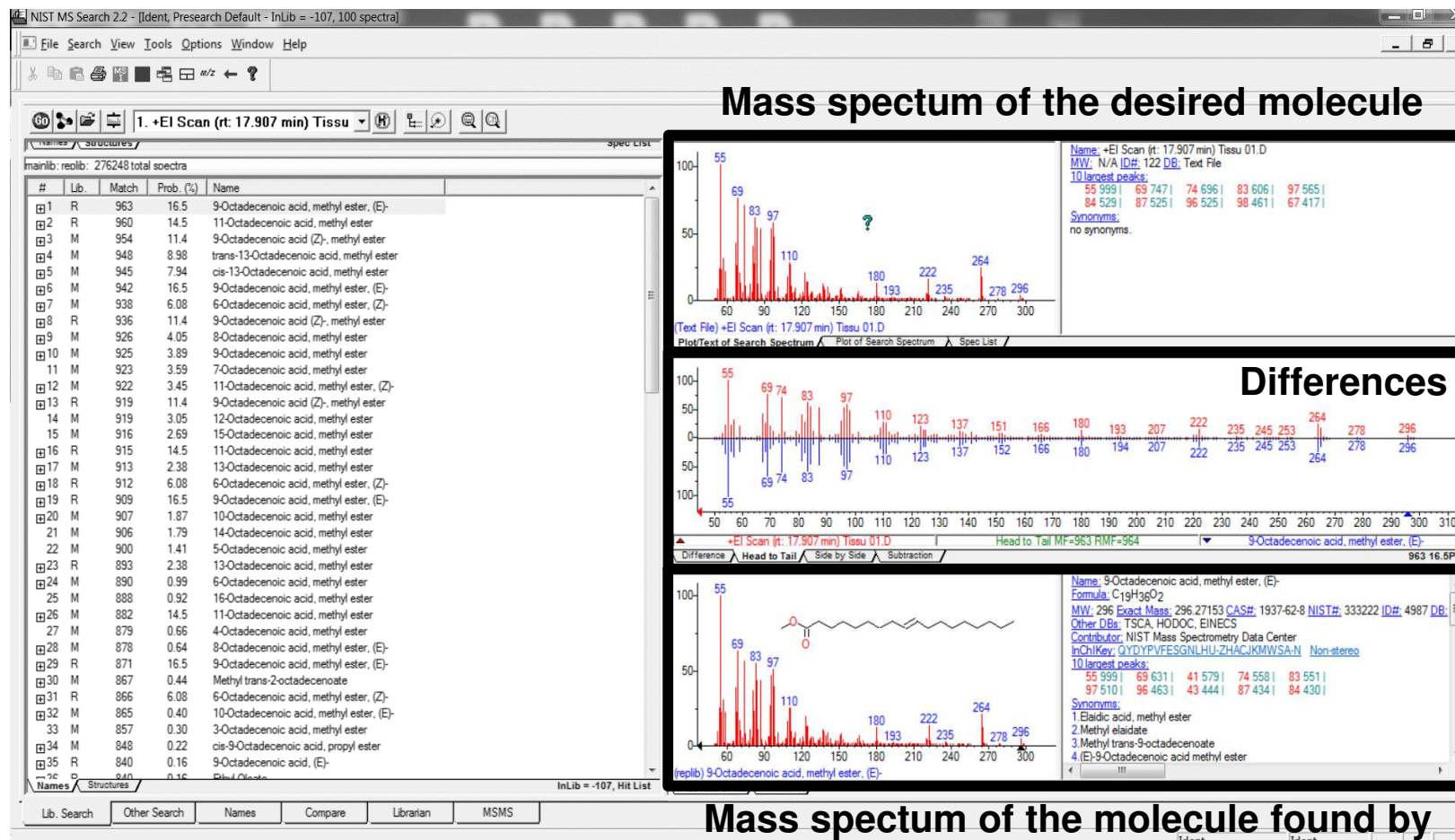
Ident Ident

Identification + probability

Identification of fatty acids

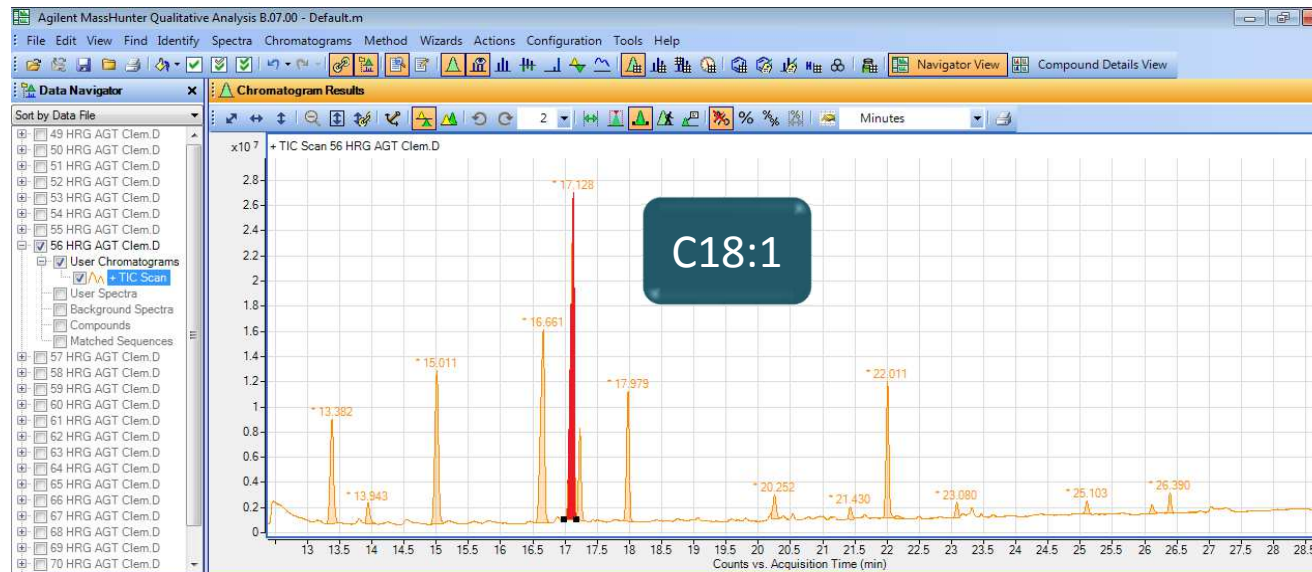


3. Verification with NIST database



Identification of fatty acids

2. Peak identification



$\Sigma \text{ areas}$ = sum of the areas of the peaks identified (without the internal standard C17:0)

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

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

+ 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 !



l'institut Agro
agriculture • alimentation • environnement

