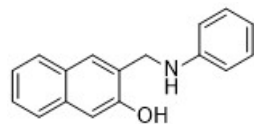
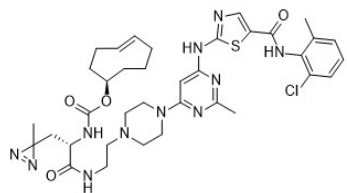
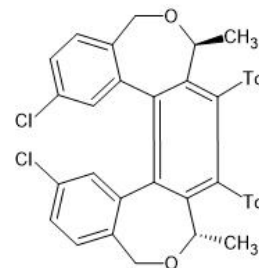
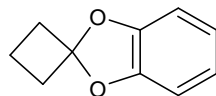
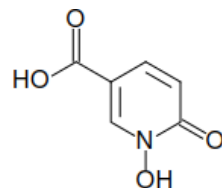
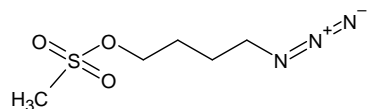
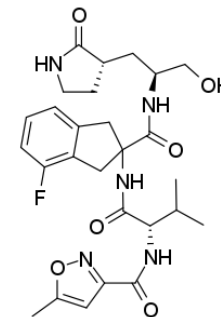
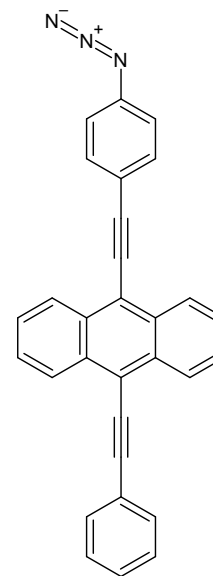
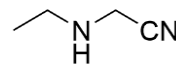


Ionization methods used @ IOCB

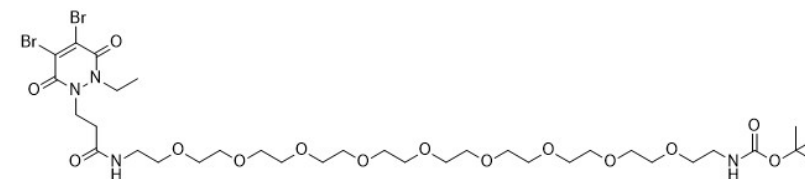
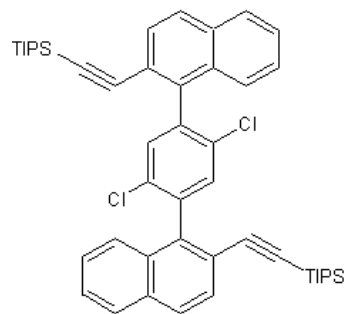
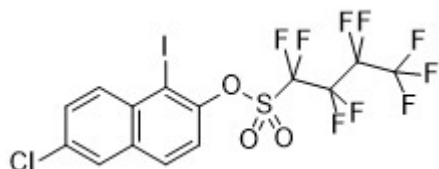
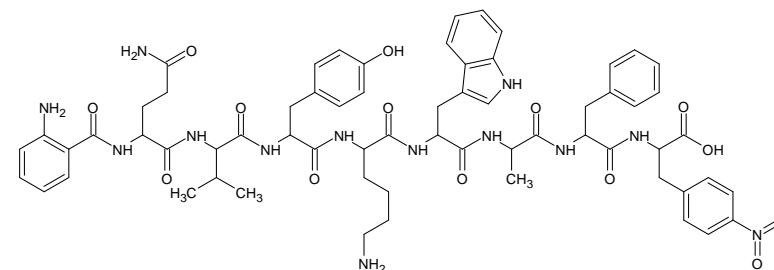
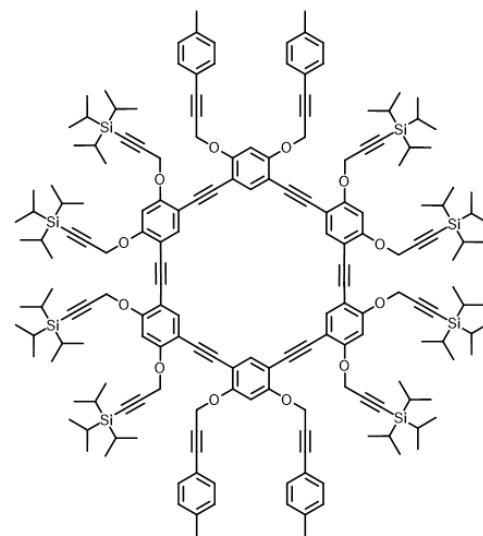
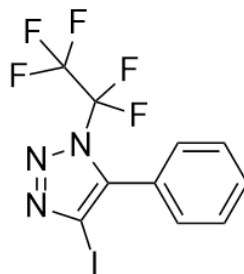
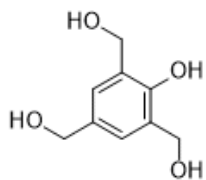
What ionization technique to choose?



proteins



oligonucleotides



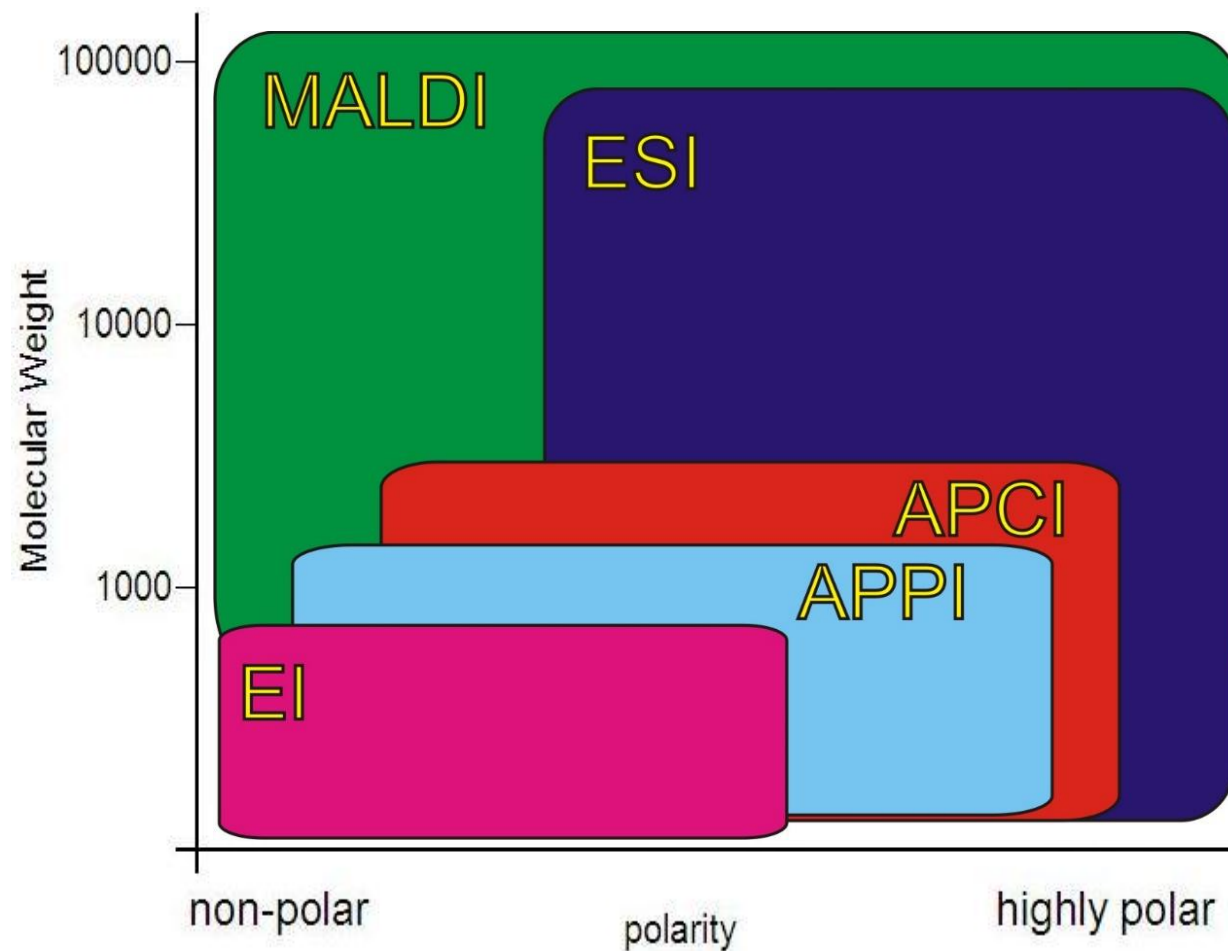
What ionization technique to choose?

Universal ionization technique does not exist

Choice depends on structure and polarity compound

Almost all compounds can be ionized by more than one technique.

Depends on molecular mass, polarity, ionization energy, solubility, ...



Ion source / ionization technique

Ion source – to produce charged molecule in the gas phase (positively or negatively charged) to be able to measure molecules

Basic differentiation:

By energy

- Hard ionization – high energy, a lot of fragments in the spectra, (typically EI)
- Soft ionization – molecular adduct, a few (or no) fragments in the spectra
(ESI, CI, MALDI,.....)

By pressure

- Vacuum (EI, CI, MALDI,...)
- Atmospheric pressure (ESI, APCI,...)

Mass of ion in MS

Mass spectrometry is using ions to determine mass of molecule. Because of the occurrence of isotopes and mass defect in elements, there are different type of masses.

Nominal mass: is the sum of the nominal masses of the constituent atoms, e.g. : Caffeine

$$M = (8 \times 12) + (10 \times 1) + (4 \times 14) + (2 \times 16) = 194 \text{ Da}$$

Monoisotopic mass: The monoisotopic mass is the sum of the masses of the atoms in a molecule

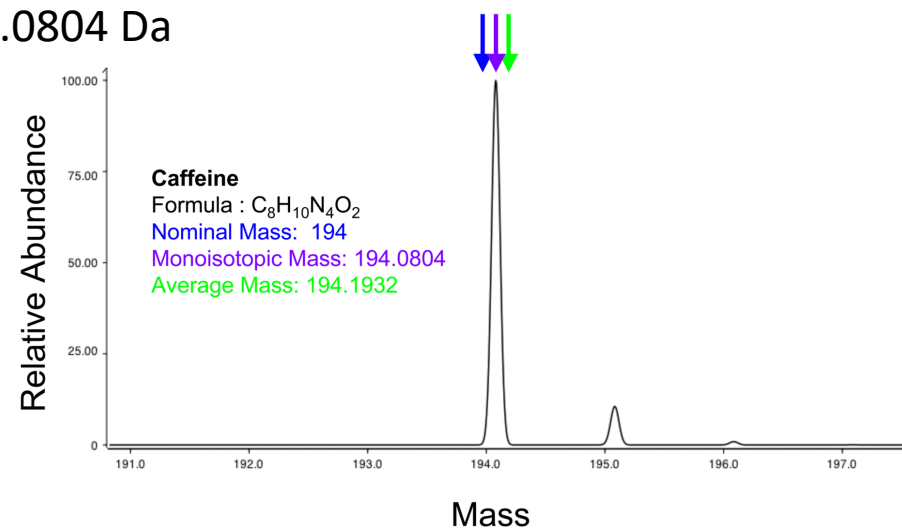
using the unbound, ground-state, rest mass of the principal (most abundant) isotope for each element.

problem: heavy atoms – the most abundant isotope is not necessary the lightest one (e.g. iron, 54 lightest one, 57 most abundant), so it must be specified to which isotopes is monoisotopic mass related

$$\text{Caffeine: } M = (8 \times 12.0000) + (10 \times 1.0078) + (2 \times 15.9949) + (4 \times 14.0031) = 194.0804 \text{ Da}$$

Average mass: obtained by summing the average atomic masses of the constituent elements

$$\text{Caffeine: } M = 194.1932 \text{ Da}$$



Resolution in MS – difference between two closest peaks

There are several methods to define resolution in MS but two are used predominantly.

10% valley: the closest spacing of two peaks of equal intensity with the valley (lowest value of signal) between them less than a specified fraction of the peak height.

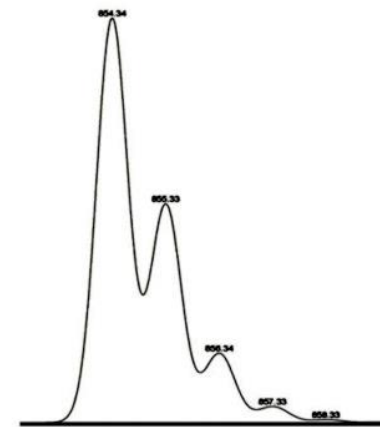
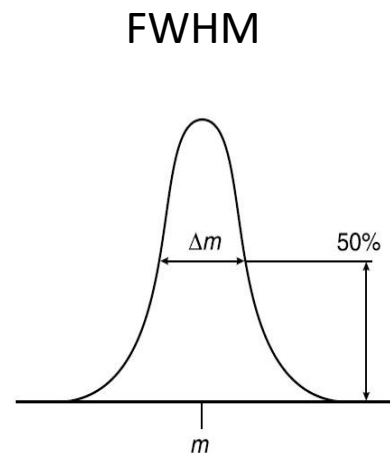
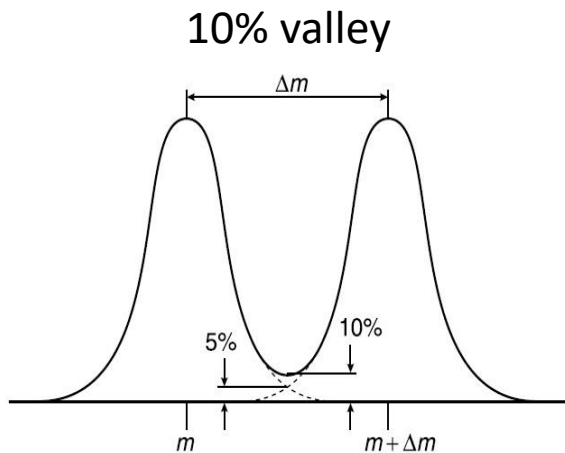
FWHM: the width of the peak measured at a specified fraction of the peak height – 50% is called Full Width at Half Maximum (FWHM)

$$R = \frac{m}{\Delta m}$$

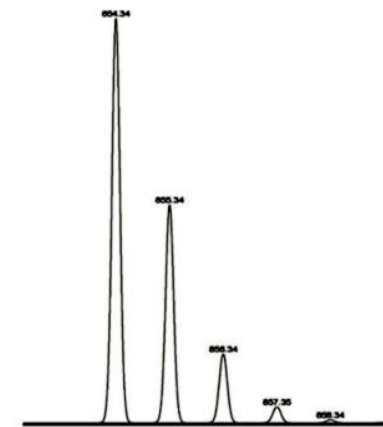
Low resolution $R < 10\,000$

High resolution $R = 10\,000 - 100\,000$

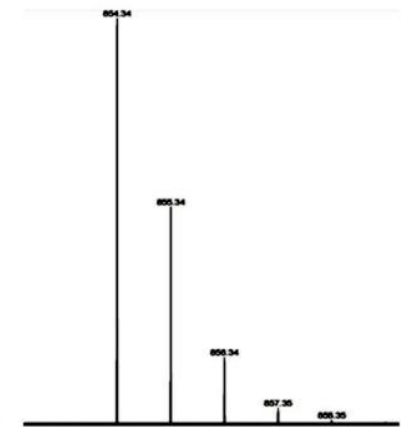
Ultra-high resolution $R > 100\,000$



Low resolution



↔



high resolution

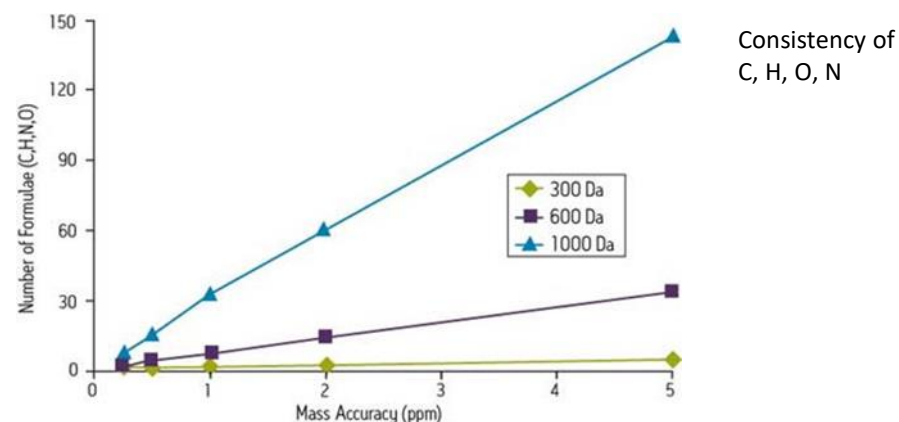
Mass accuracy

The mass of the molecules could be measured with different accuracy.

Mass accuracy: the ratio of the m/z measurement error to the true m/z.

Mass accuracy is usually measured in ppm or milli mass units.

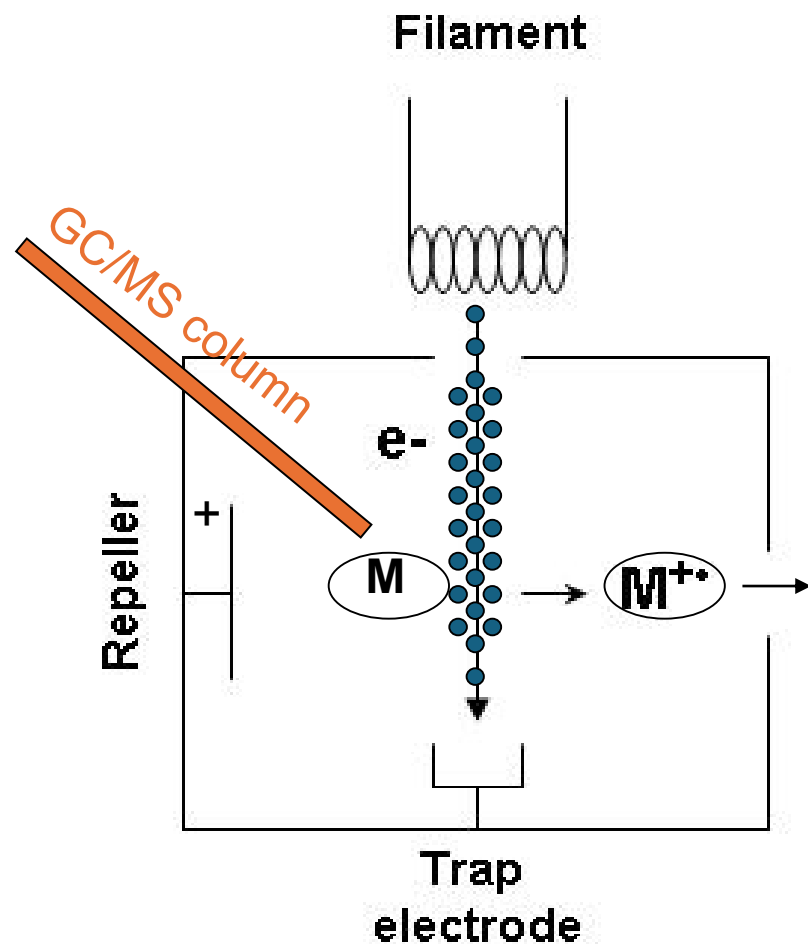
$$E_{ppm} = 10^6 \frac{M_{measured} - M_{calculated}}{M_{calculated}}$$



Low resolution measurement – quick scan on less sophisticated machine, good for quick orientation
I have/ don't have my assumed molecule in the sample

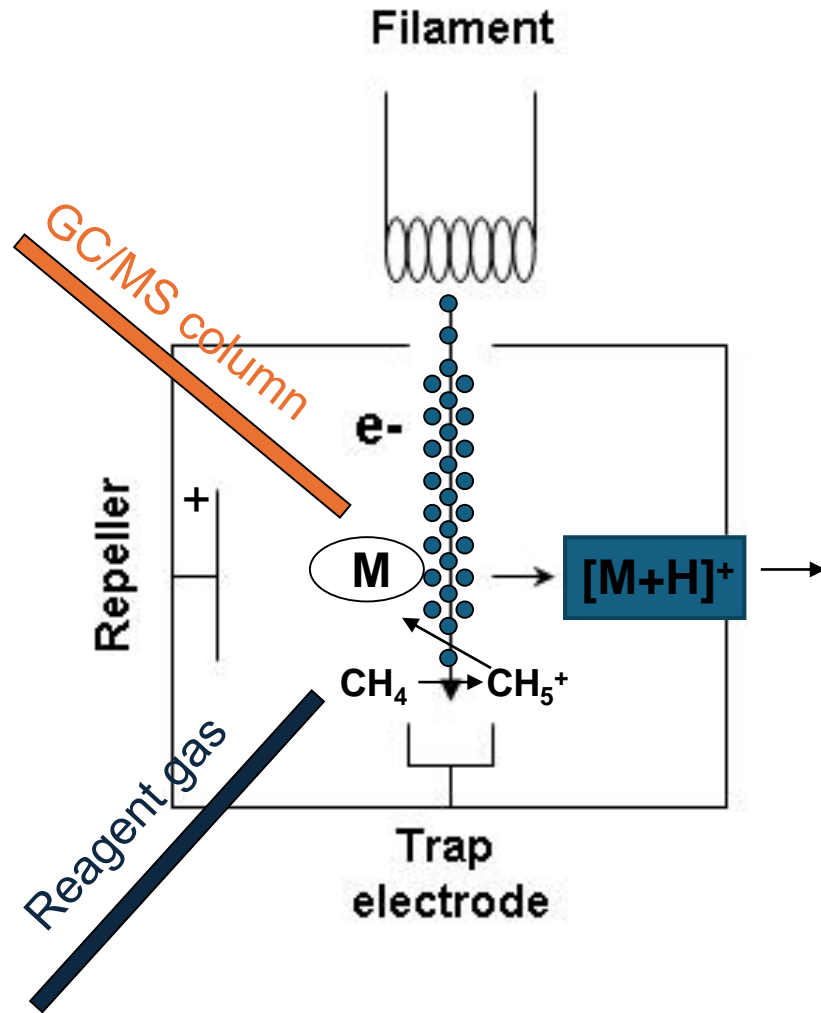
High-resolution measurement – to confirm (with high accuracy) that my assumed molecule is in the sample
– to suggest some structure possibilities of unknown compounds

Electron impact ionization - EI



- An ionization method in which energetic electrons interact with gas-phase molecules to produce ions.
 - Electron emission by heating a wire filament
 - Good reproducibility – spectral library – easy interpretation
 - energy of the electrons 70 eV
- $M + e^- \rightarrow M^{+\bullet} + 2 e^-$
 - M is the analyte molecule being ionized
 - e^- - electron
 - $M^{+\bullet}$ - resulting ion
- Widely used for **volatile** organic molecules
- Often coupled with GC = GC/EI-MS

Chemical ionization - CI

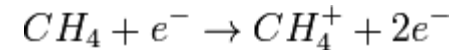


- Analyzed ions are produced through the collision of the analyte with ions of a reagent gas, that are present in the ion source
 - Methane**, ammonia, isobutane, acetonitrile,.....
- Soft ionization technique
 - $[M + H]^+$, $[M + \text{reagent gas}]^+$, fragments (depend on condition)

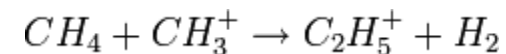
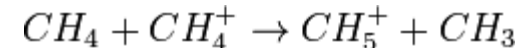
Example

- CH_4 as a reagent gas

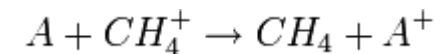
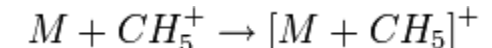
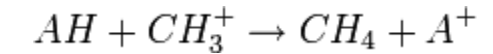
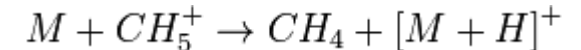
Primary ion formation



Secondary reagent ions



Product ion formation



- Coupled with GC = GC/CI-MS
- Used for volatile organic molecules

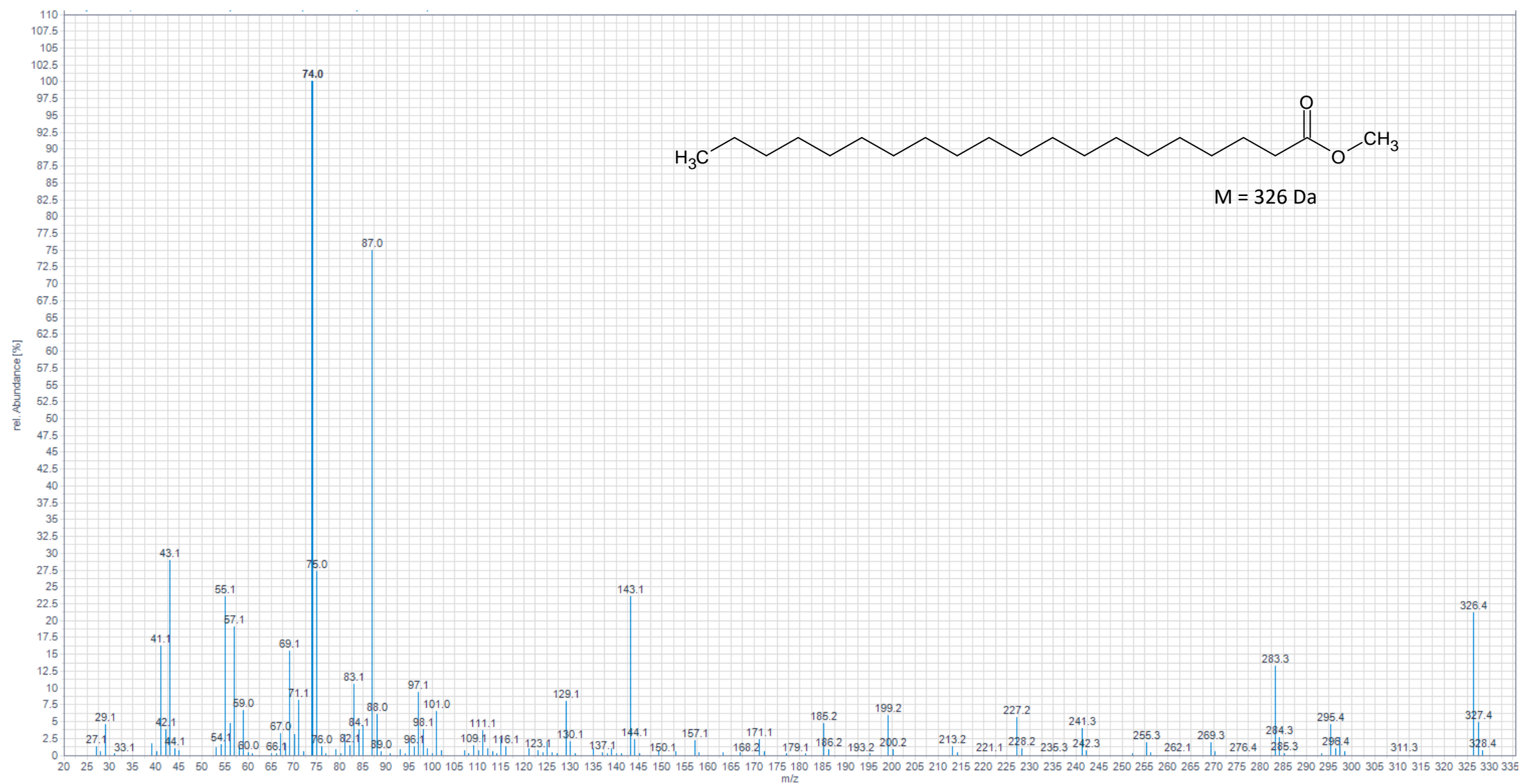
Machines at our laboratory

Agilent 5975C Inert MSD



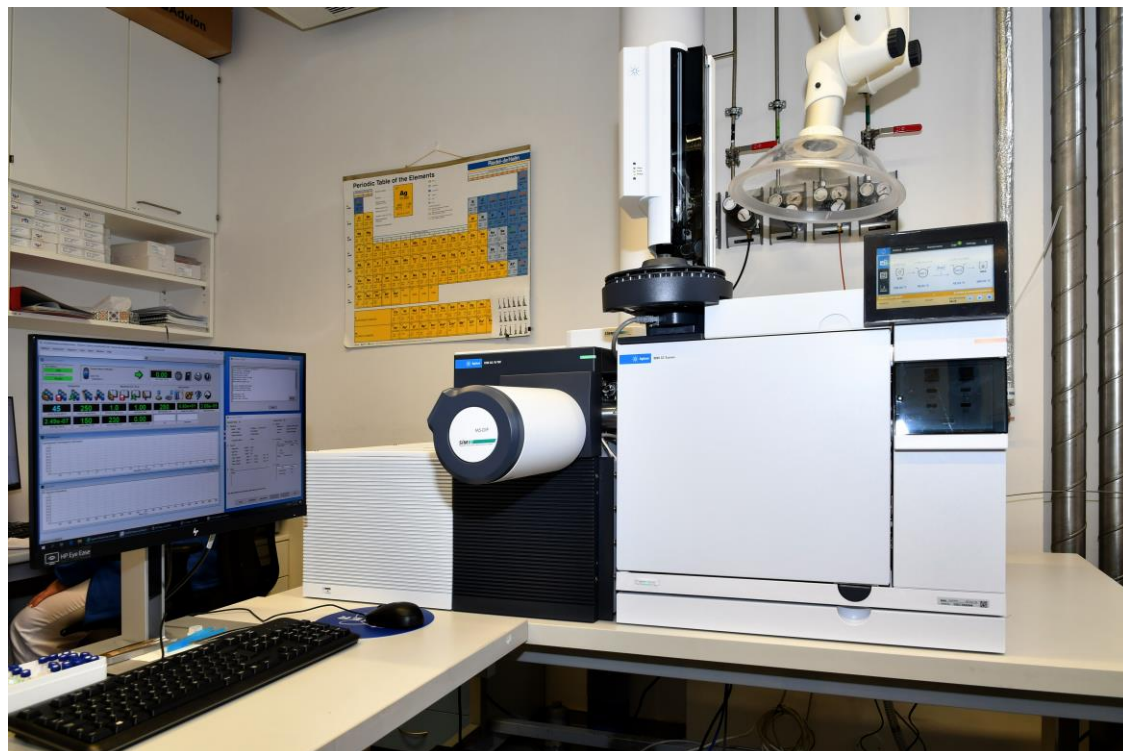
- low resolution
- nominal mass only
- coupled with GC
- mass range up to 1000 Da
- only EI ion source
- self-service

El spectrum



Machines at our laboratory

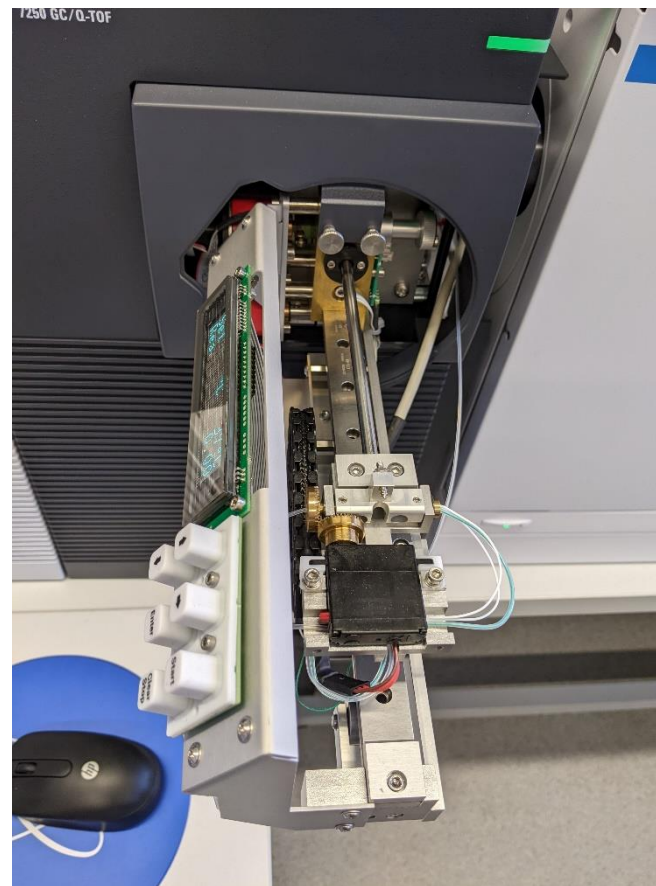
Agilent 7250 GC/Q-ToF



- high-resolution
- accurate mass
- coupled with GC
- Direct inlet probe (DIP) attached
- mass range up to 3000 Da
- EI and CI ion source

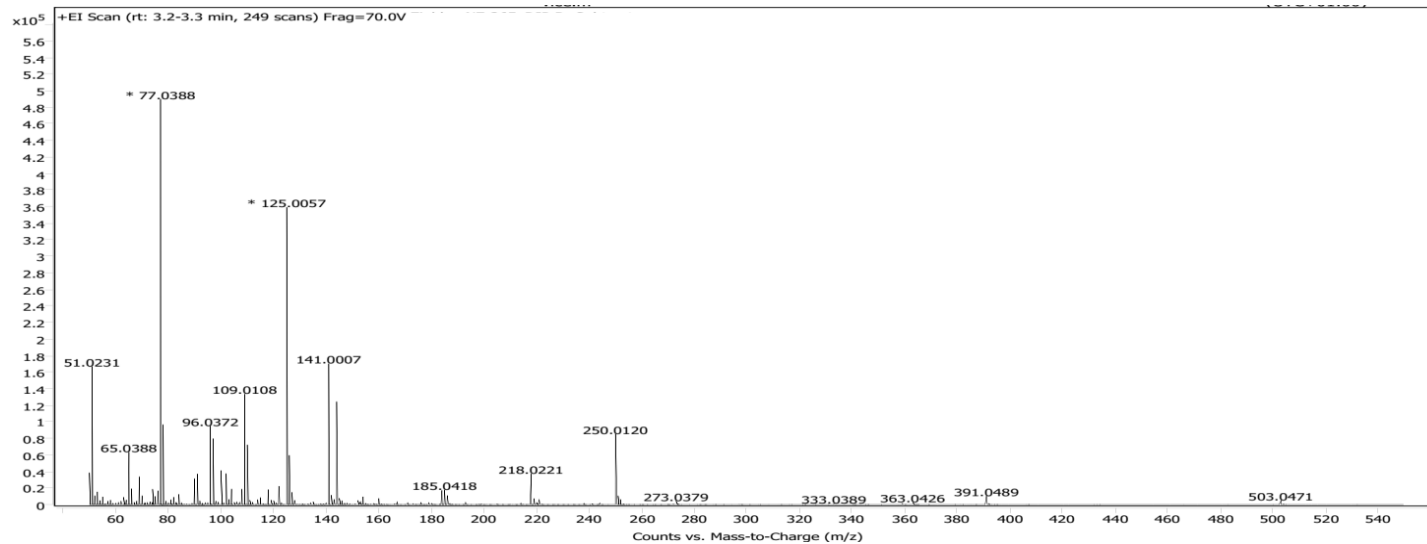
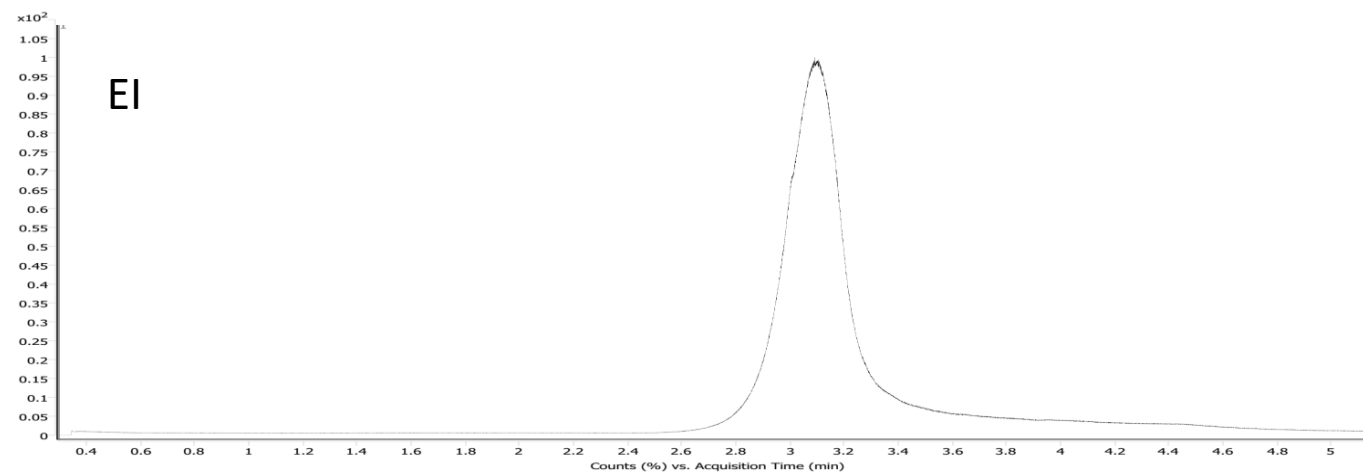
Machines at our laboratory

Agilent 7250 GC/Q-ToF - DIP

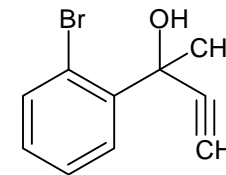


Machines at our laboratory

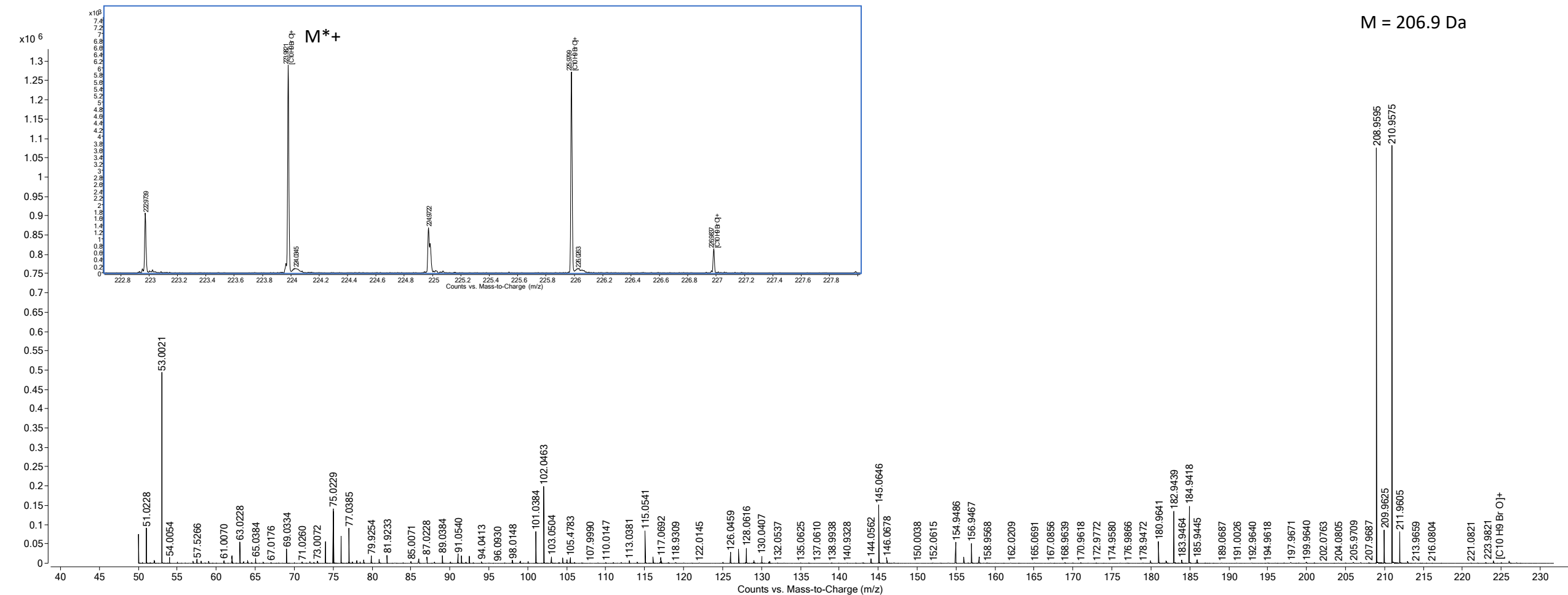
Agilent 7250 GC/Q-ToF - DIP



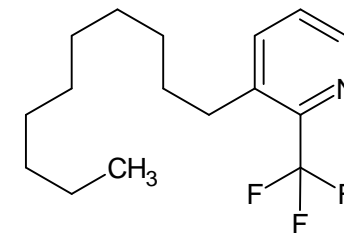
El spectrum



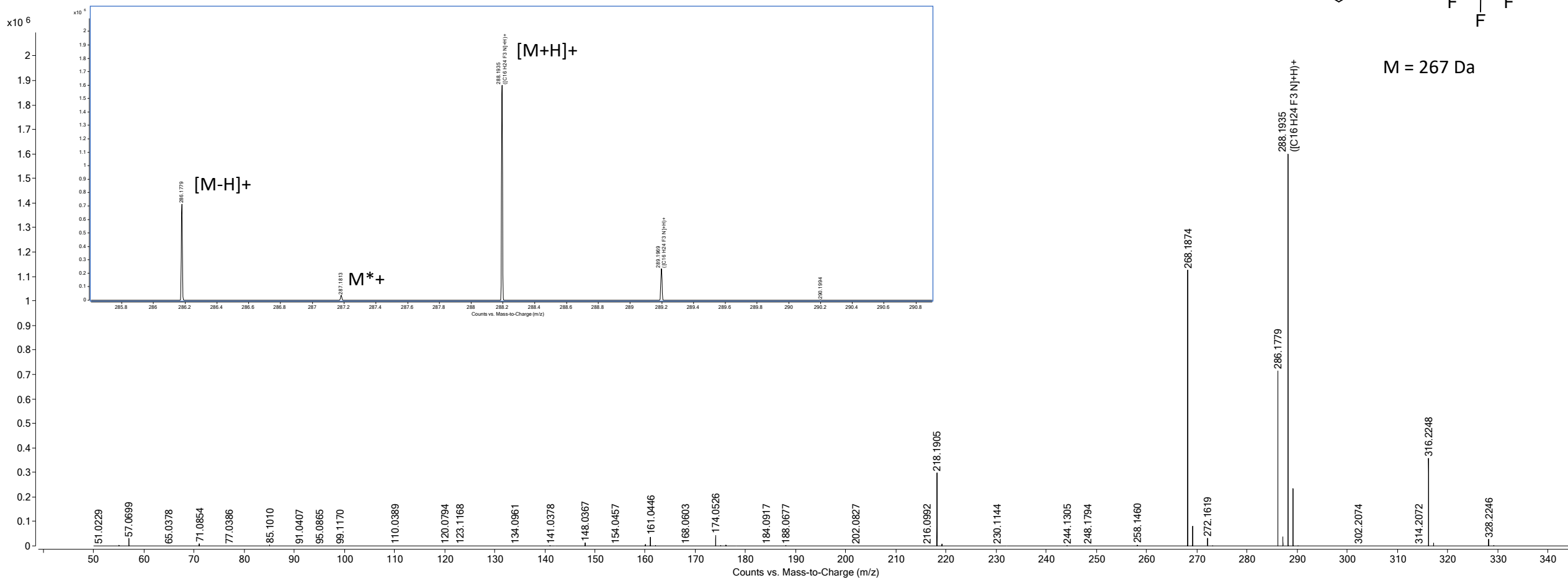
M = 206.9 Da



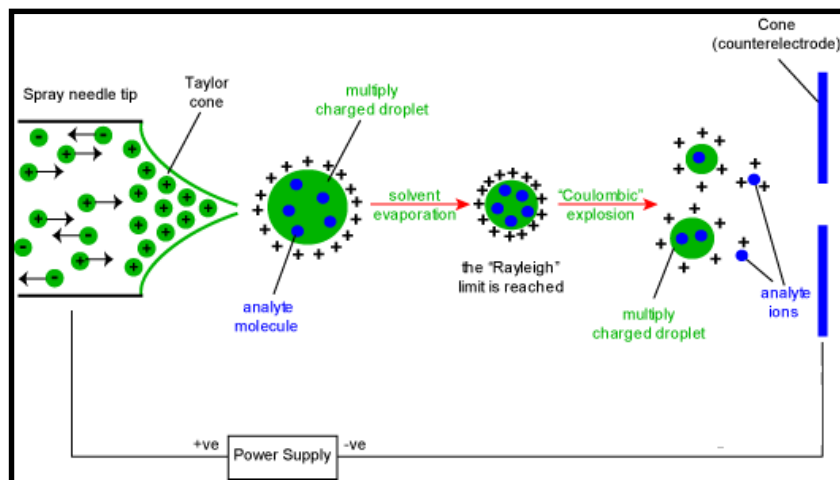
CI spectrum



M = 267 Da



Electrospray ion source - ESI



- the liquid containing the analyte(s) is dispersed by electrospray into an aerosol
 - Charged droplets
 - Solvent evaporation, Coulombic explosion
- soft ionization technique
 - $[M + H]^+$, $[M + Na]^+$, $[M + K]^+$, ...molecular adducts of solvent
 - Multiply charged ions, dimers, multimers
 - Quite complex spectrum
- mobile phase – polar solvent (as a donor of H^+)
- ionization in both positive and negative mod
- coupling with HPLC systems

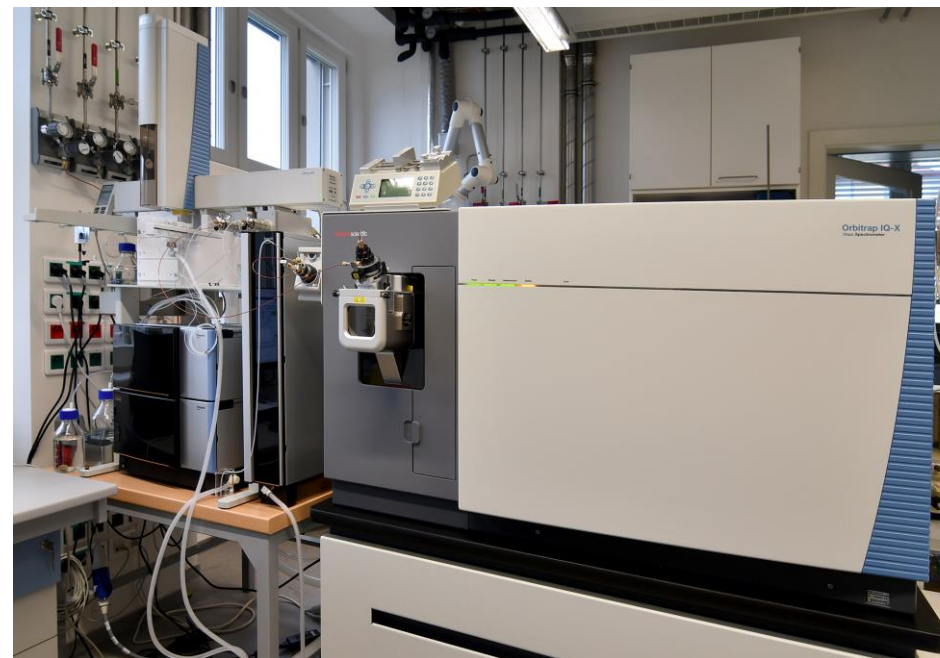
Machines at our laboratory

LTQ Orbitrap XL



- HR machine
- API source
- installed in 2008

Orbitrap IQ-X Tribrid



- HR machine
- API source

Machines at our laboratory

LCQ Advantage



- LR machine
- coupled with HPLC system

Sciex QTRAP 6500+



- LR machine
- used for quantification
- targeted metabolomics studies
- coupled with HPLC system

ESI+

13b

M = 1382 Da

[M+H]⁺

[M+Na]⁺

[M+K]⁺

Relative Abundance

m/z

1383.72662
C₆₆H₁₀₇O₂₅ Na⁺ = 1383.72804
0.41697 ppm

1384.73180

1385.73478

1386.73725

1387.74015

1383.7

1407.7

1406.7

1423.7

1422.7

1421.7

1405.7

722.3

722.8

730.3

714.9

714.4

723.3

731.3

731.8

723.8

584.3

584.8

565.7

565.3

563.7

487.2

481.9

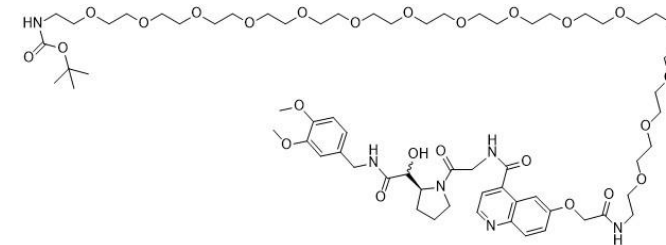
432.2

416.2

352.2

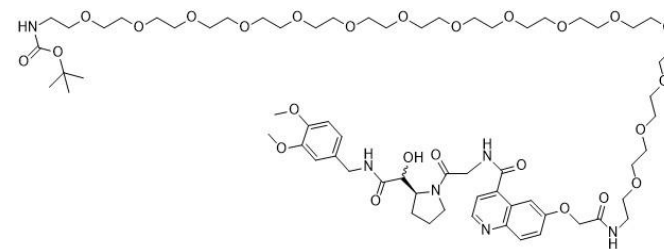
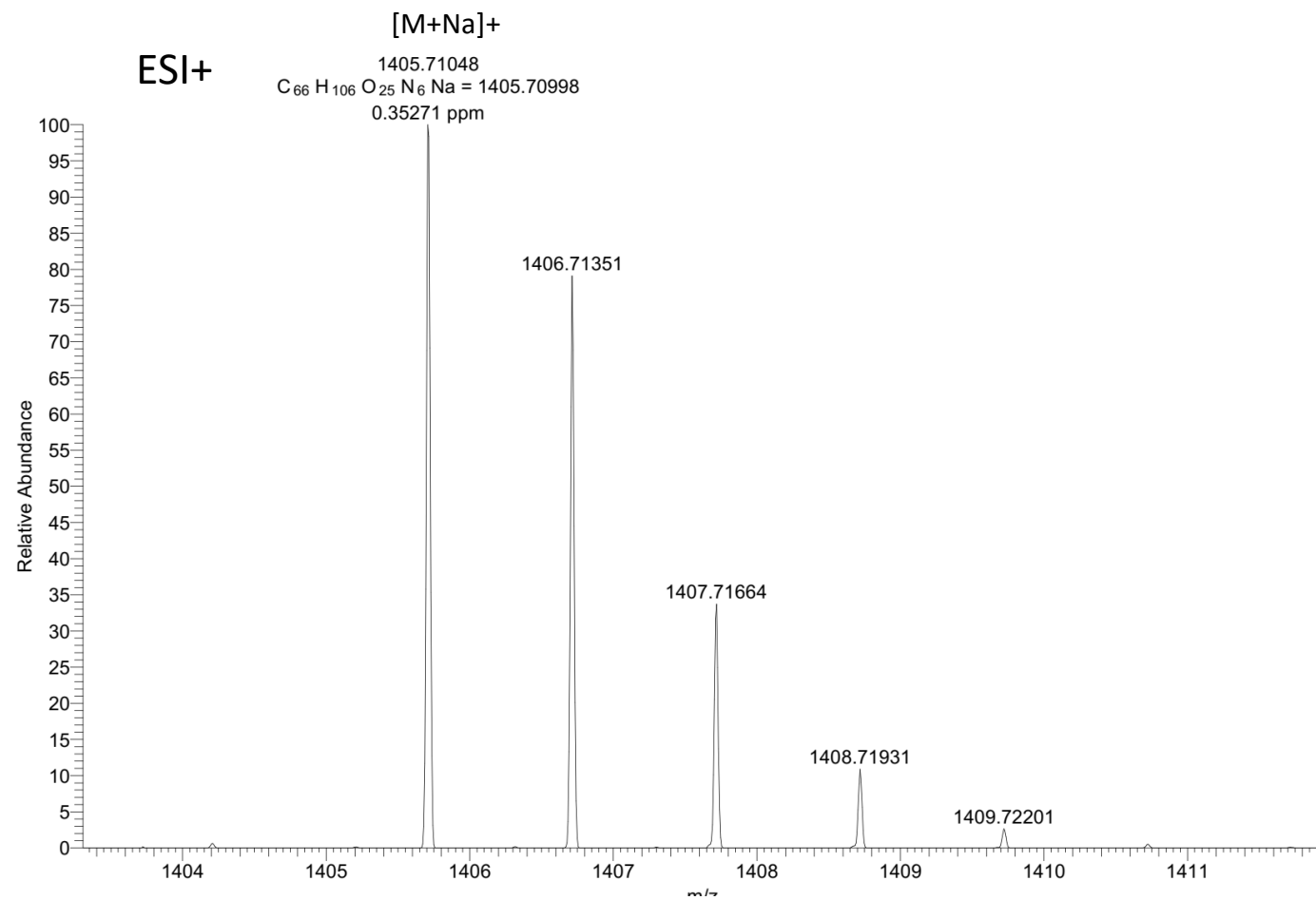
902.5

901.5



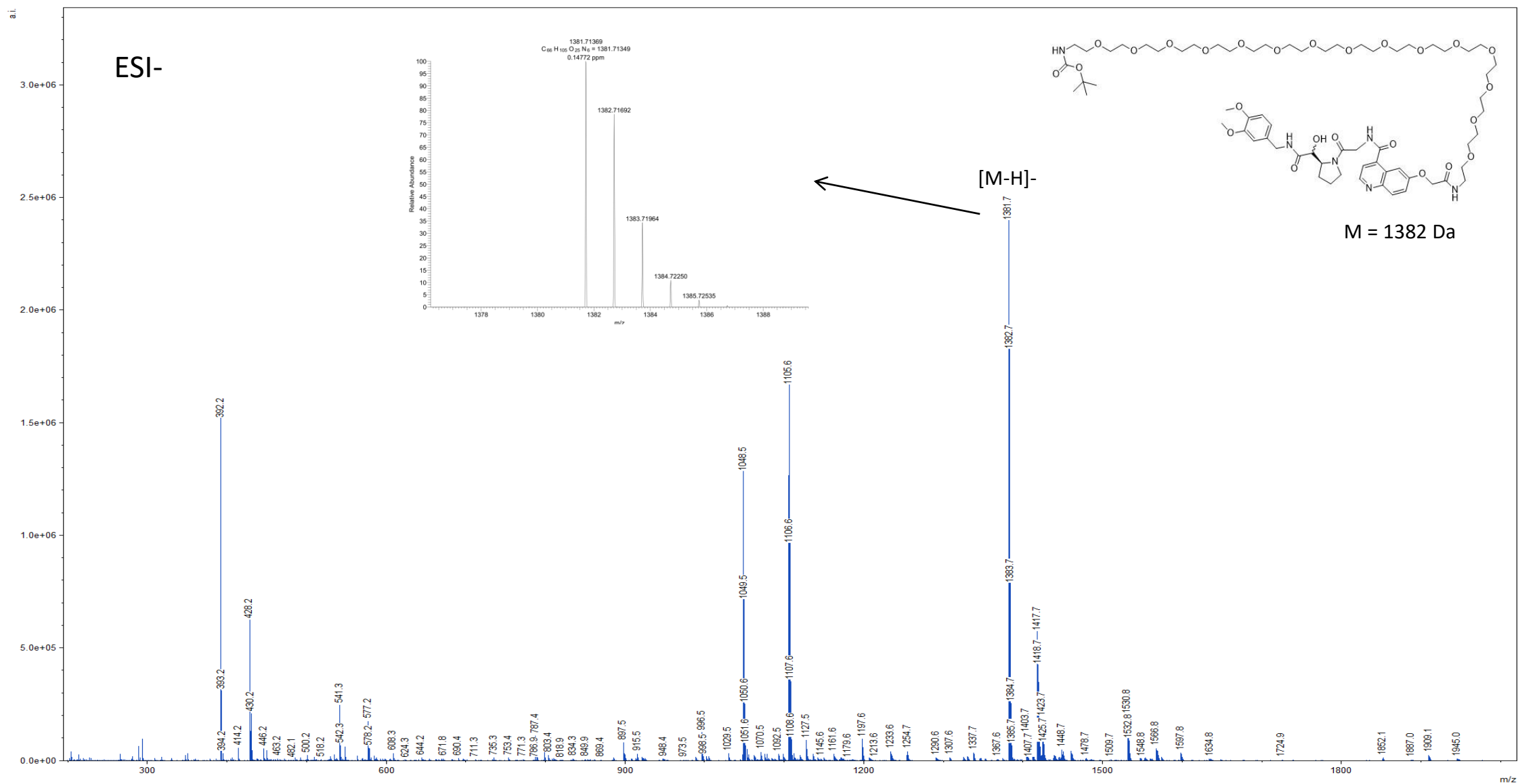
M = 1382 Da

ESI spectrum

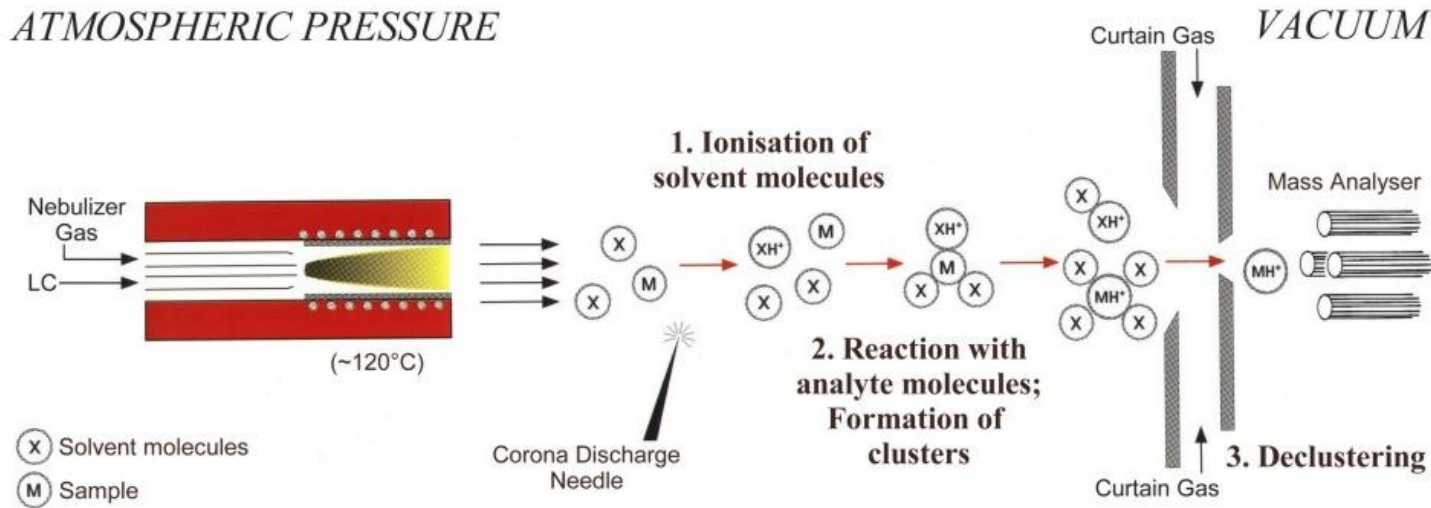


M = 1382 Da

ESI spectrum



Atmospheric Pressure Chemical Ionization - APCI

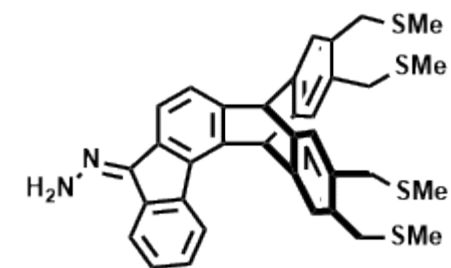


- the mobile phase containing analyte is heated to high temperature (above 400 °C), sprayed with high flow rates of nitrogen
- molecules of solvent and gas are ionized by corona discharge
- analyte are ionized by ionized gas molecules and solvent

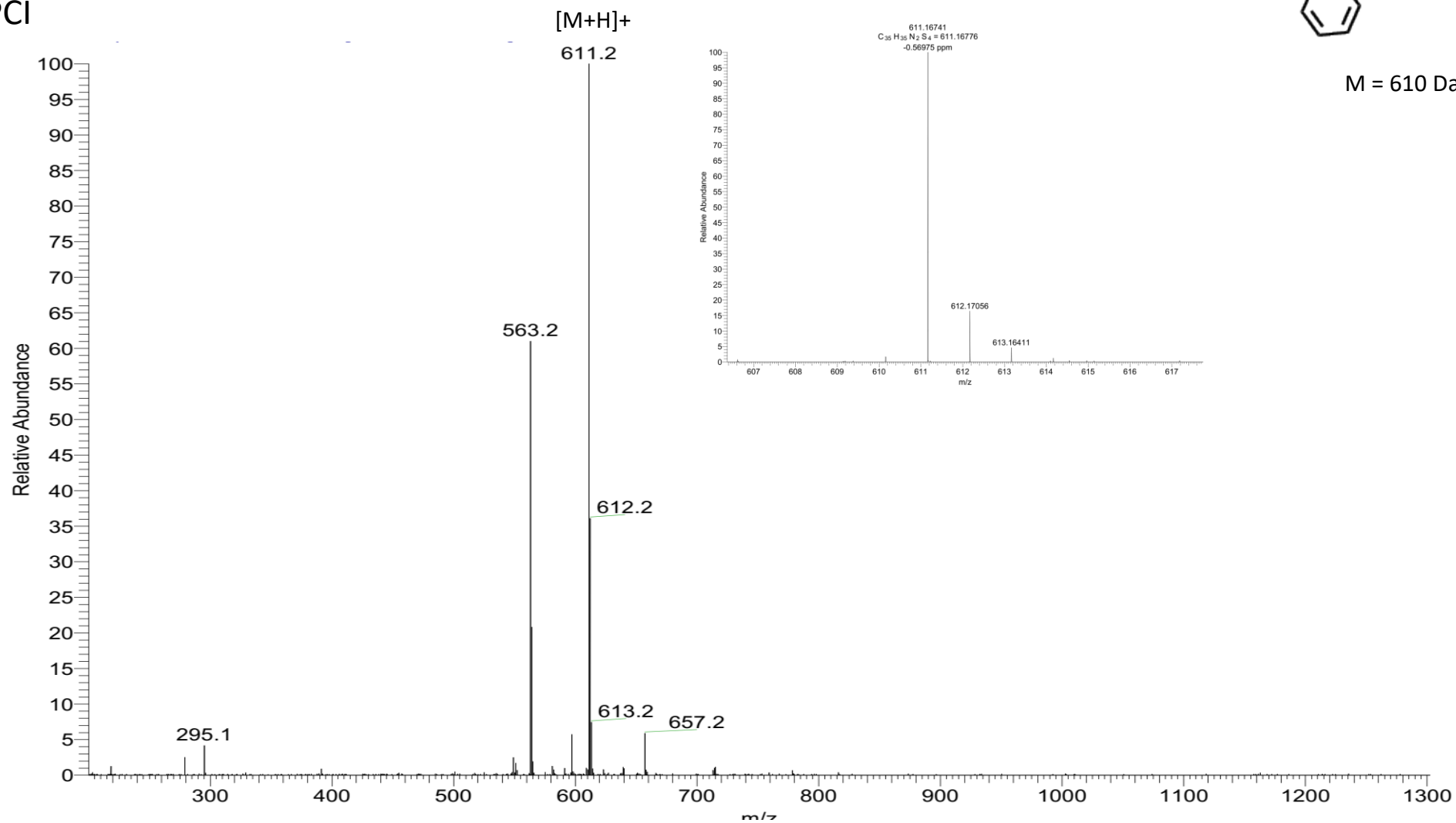
Atmospheric Pressure Chemical Ionization - APCI

- device is similar to ESI source
- mechanism of ionization similar to CI
- APCI is a less "soft" ionization technique than ESI
- generates more fragment ions
- advantage of APCI - possible to use a nonpolar mobile phase
- higher appearance M^{*+} ion than in ESI
- higher appearance adduct with solvent than in ESI
- less Na, K adduct

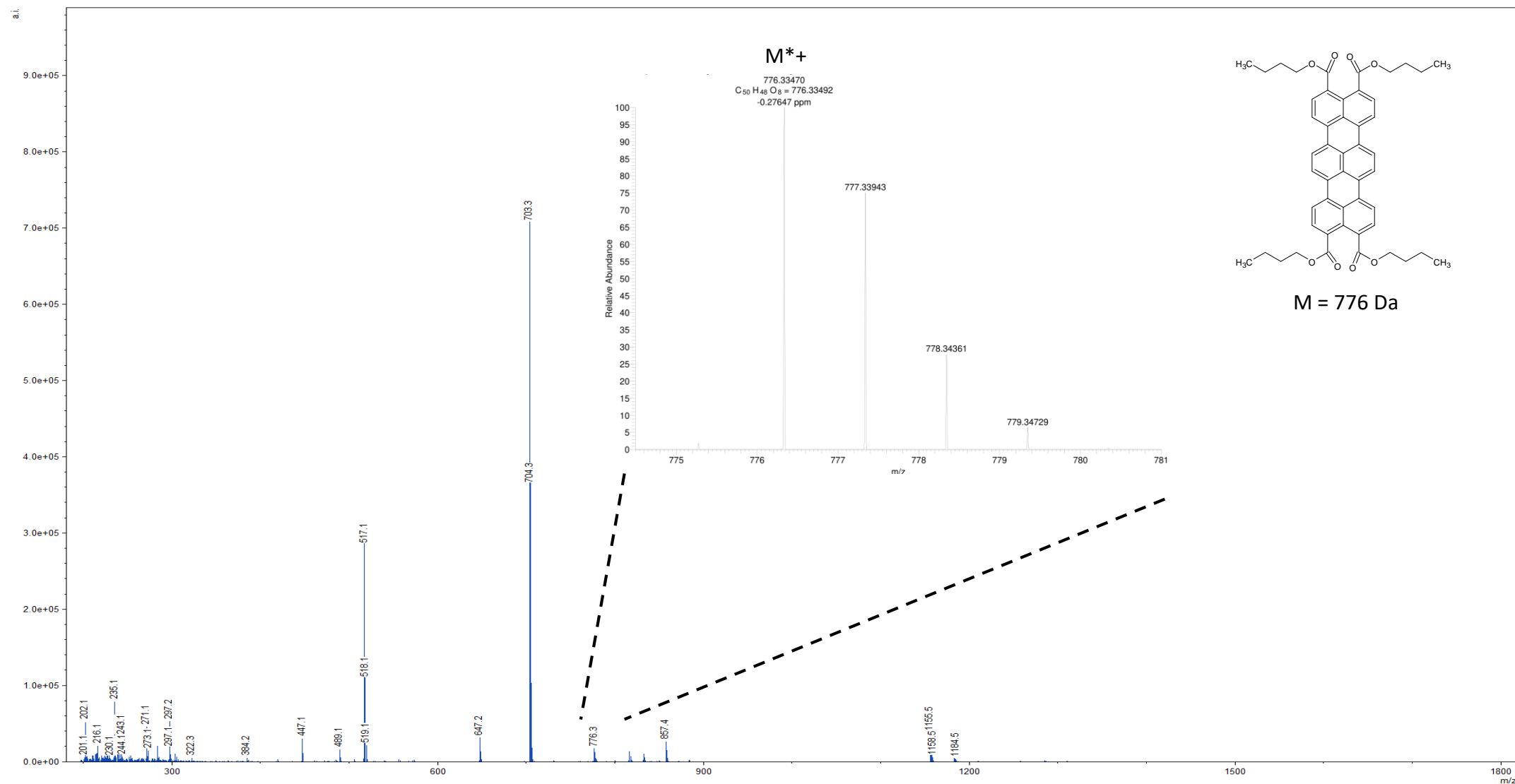
APCI spectrum



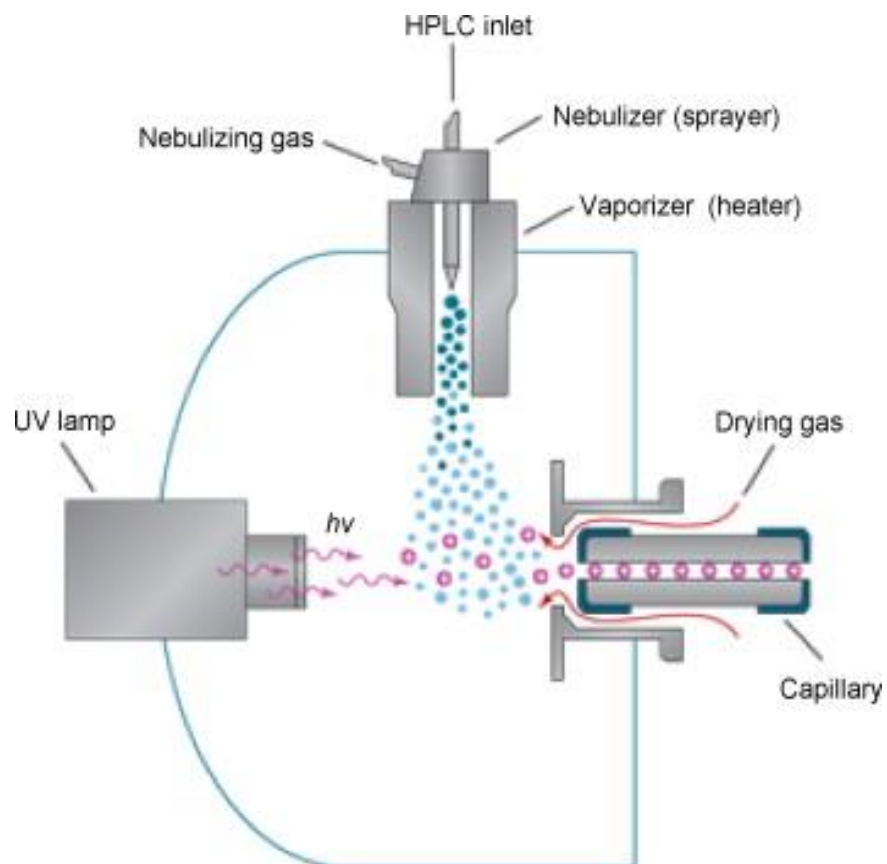
M = 610 Da



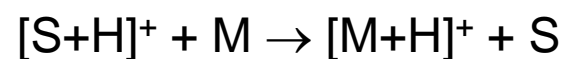
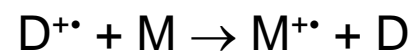
APCI spectrum



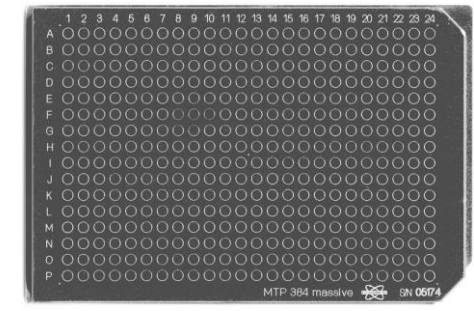
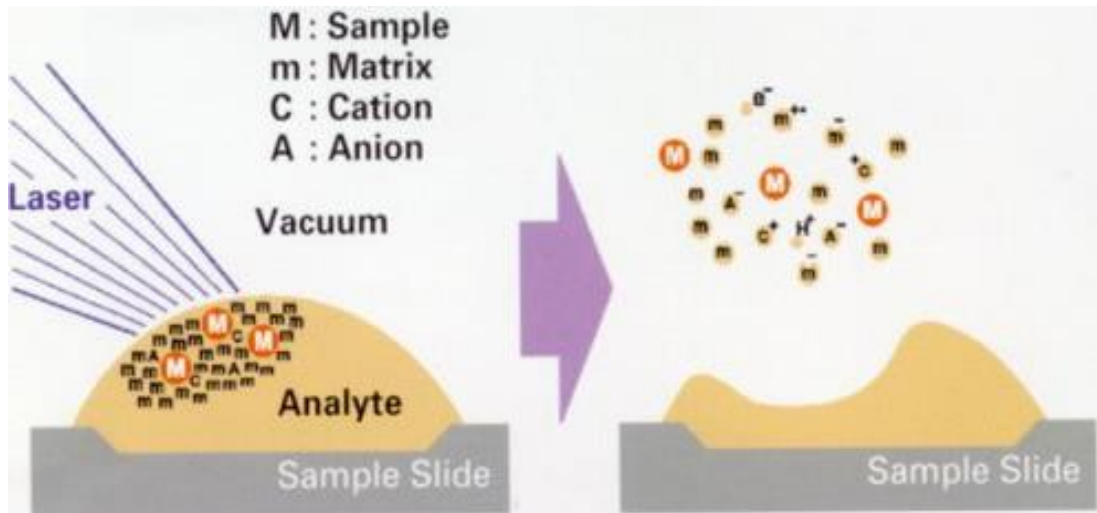
Atmospheric Pressure Photoionization - APPI



- source of energy UV lamp
- mainly for nonpolar compounds
- direct ionization – absorption of UV by sample
- indirect ionization – by dopant (toluene, acetone, ...)



Matrix-Assisted Laser Desorption/Ionization - MALDI

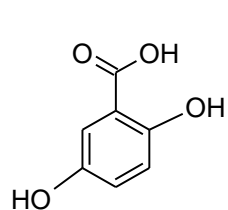


sample plate

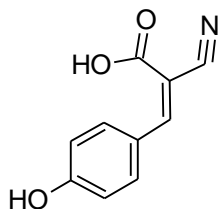
- soft ionization method
- matrix and analyte are mixed on the target plate
- the UV laser (355 nm or 337 nm) shoots the mixture
- the energy is transferred to the matrix, which is vaporized, carrying analyte into the vapor phase and charging it
- the mechanism of ionization has still been discussed

Matrix-Assisted Laser Desorption/Ionization - MALDI

- MALDI is useful method for broad spectrum of molecule (small and large)
- mostly singly charged analytes $[M+H]^+$ or $[M-H]^-$
- adduct of salts Na, K (if present in the matrix Li adduct)
- could be dimmers, trimmers $[2M+H]^+$ or double charged molecule $[M+2H]^{2+}$ as well
- Matrices – small organic molecules (2,5-dihydroxybenzoic acid, sinapic acid, a-cyano-4-hydroxycinnamic acid, ...)



2,5-DHB



CCA

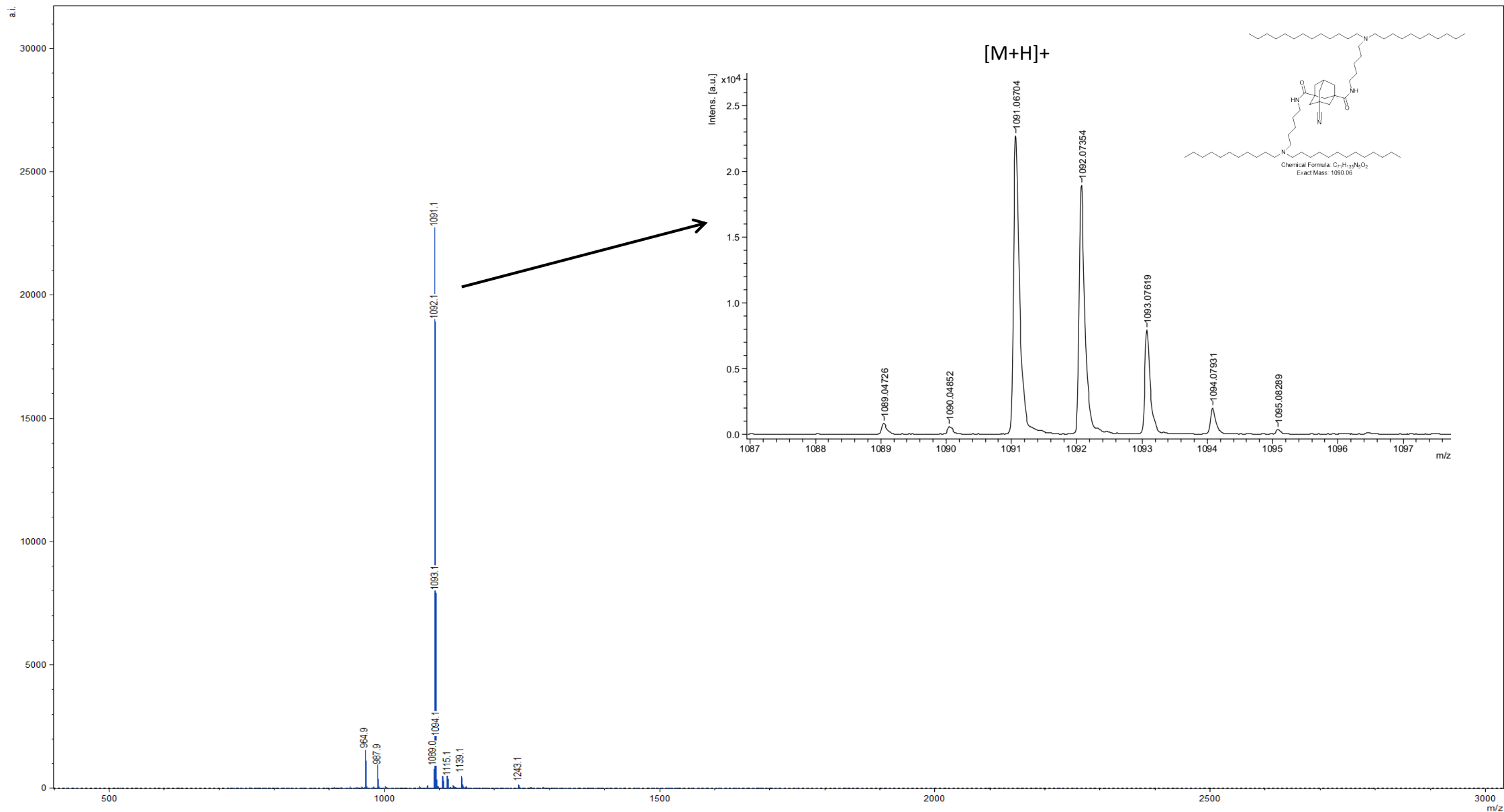


UltrafleXtreme
from Bruker comp.

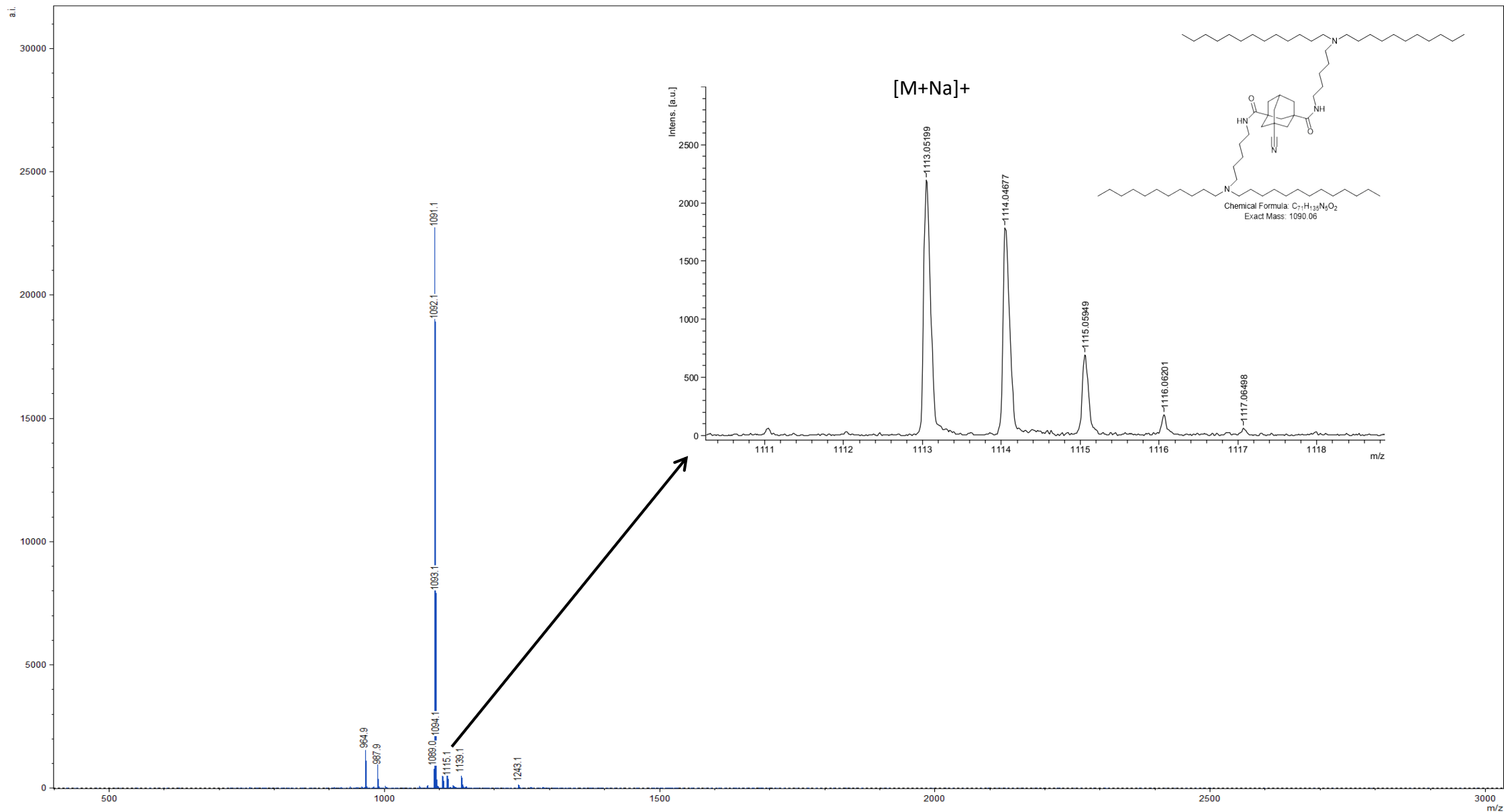
Requirements for matrices

- vacuum stability, be able to embed analytes (by co-crystallization), solubility in solvents compatible with analyte, absorb the laser wavelength, cause co-desorption of the analyte upon laser irradiation

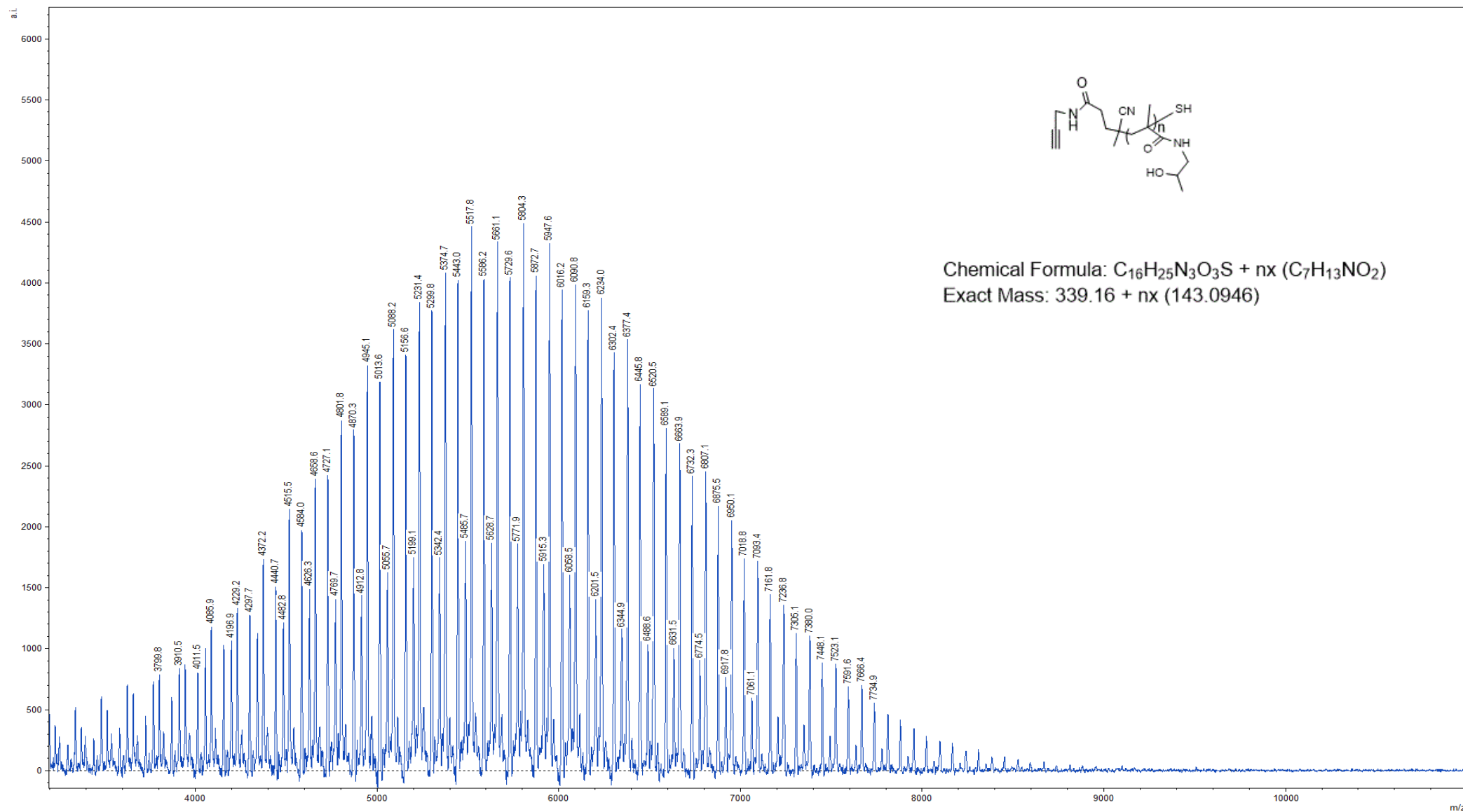
MALDI spectrum



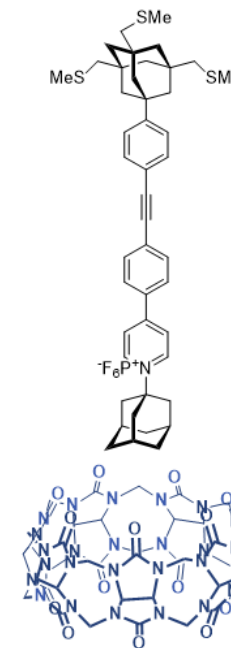
MALDI spectrum



MALDI spectrum



The figure displays the mass spectrum of compound 10, which is a phthalocyanine derivative. The main spectrum (bottom) shows relative intensity (0 to 60,000) versus m/z (0 to 5000). The base peak is at m/z 704.4. Other significant peaks are labeled at m/z 407.0, 409.1, 456.4, 501.0, 502.0, 545.0, 588.1, 589.1, 672.3, 705.4, 706.4, 707.4, 736.4, 752.3, 753.3, 755.4, 756.4, 757.4, 758.4, 759.4, 760.4, 761.4, 762.4, 763.4, 764.4, 765.4, 766.4, 767.4, 768.4, 769.4, 770.4, 771.4, 772.4, 773.4, 774.4, 775.4, 776.4, 777.4, 778.4, 779.4, 780.4, 781.4, 782.4, 783.4, 784.4, 785.4, 786.4, 787.4, 788.4, 789.4, 790.4, 791.4, 792.4, 793.4, 794.4, 795.4, 796.4, 797.4, 798.4, 799.4, 800.4, 801.4, 802.4, 803.4, 804.4, 805.4, 806.4, 807.4, 808.4, 809.4, 810.4, 811.4, 812.4, 813.4, 814.4, 815.4, 816.4, 817.4, 818.4, 819.4, 820.4, 821.4, 822.4, 823.4, 824.4, 825.4, 826.4, 827.4, 828.4, 829.4, 830.4, 831.4, 832.4, 833.4, 834.4, 835.4, 836.4, 837.4, 838.4, 839.4, 840.4, 841.4, 842.4, 843.4, 844.4, 845.4, 846.4, 847.4, 848.4, 849.4, 850.4, 851.4, 852.4, 853.4, 854.4, 855.4, 856.4, 857.4, 858.4, 859.4, 860.4, 861.4, 862.4, 863.4, 864.4, 865.4, 866.4, 867.4, 868.4, 869.4, 870.4, 871.4, 872.4, 873.4, 874.4, 875.4, 876.4, 877.4, 878.4, 879.4, 880.4, 881.4, 882.4, 883.4, 884.4, 885.4, 886.4, 887.4, 888.4, 889.4, 890.4, 891.4, 892.4, 893.4, 894.4, 895.4, 896.4, 897.4, 898.4, 899.4, 900.4, 901.4, 902.4, 903.4, 904.4, 905.4, 906.4, 907.4, 908.4, 909.4, 910.4, 911.4, 912.4, 913.4, 914.4, 915.4, 916.4, 917.4, 918.4, 919.4, 920.4, 921.4, 922.4, 923.4, 924.4, 925.4, 926.4, 927.4, 928.4, 929.4, 930.4, 931.4, 932.4, 933.4, 934.4, 935.4, 936.4, 937.4, 938.4, 939.4, 940.4, 941.4, 942.4, 943.4, 944.4, 945.4, 946.4, 947.4, 948.4, 949.4, 950.4, 951.4, 952.4, 953.4, 954.4, 955.4, 956.4, 957.4, 958.4, 959.4, 960.4, 961.4, 962.4, 963.4, 964.4, 965.4, 966.4, 967.4, 968.4, 969.4, 970.4, 971.4, 972.4, 973.4, 974.4, 975.4, 976.4, 977.4, 978.4, 979.4, 980.4, 981.4, 982.4, 983.4, 984.4, 985.4, 986.4, 987.4, 988.4, 989.4, 990.4, 991.4, 992.4, 993.4, 994.4, 995.4, 996.4, 997.4, 998.4, 999.4, 1000.4. The inset spectrum (top) shows the molecular ion peak (M+) at m/z 1866.68812, with other peaks at 1867.69142, 1868.69862, 1869.70238, 1870.70568, 1871.71344, 1872.71461, 1873.70948, and 1874.74100. The chemical structure of compound 10 is shown on the right, featuring a phthalocyanine core with a central metal atom (M) coordinated by four nitrogen atoms. The structure is substituted with a long alkyne chain and a phthalocyanine derivative.



Laser Desorption/Ionization - LDI

- energy of laser is directly absorbed by analyte
 - without matrix
- spectra without matrix ions
- instrumentation is same as for MALDI
- for small molecules only
- better reproducibility than MALDI
- ! harder ionization than at MALDI – fragments in the spectra
- ! sensitivity depends on analyte

Thank you for your attention