High Resolution Mass Spectroscopy Laboratory (YKL)

High Resolution Mass Spectroscopy Laboratory is housed at METU Central Laboratory. The laboratory's expertise and support are available to researchers throughout all universities, public and industrial sectors.

At this time, we have in operation Waters SYNAPT HDMS (High Definition Mass Spectrometry) Instrumentation that is hybrid quadrupole-time of flight, combined with ACQUITY UPLC, MALDI and nanoACQUITY UPLC Systems.

Software resources include instrument-specific package MassLynx; MetaboLynx, MarkerLynx and proteomics software. We routinely provide accurate mass analysis and currently developing protein and MALDI analysis.

Listed below are the types of analyses we routinely perform:

- **High Resolution ESI, MALDI:** High Resolution accurate mass measurement coupled with empirical formula or amino acid determination, molecular weight confirmation
- **LC-MS ESI:** Liquid Chromatographic (LC) separation combined with mass spectral analysis
- **LC-MS/MS:** LC separation coupled with the selected ion monitoring using tandem mass analyzers (MS/MS)
- **Intact Protein and Peptide Molecular Weight**: ESI or MALDI on Quadrupole-TOF spectrometers analysis

Figure 1 – HRMS system in Central Laboratory

Working Principle:

Mass spectrometry provides information about structure and exact molecular weight in Dalton. A mass spectrometer generates multiple ions from the sample under investigation, it then separates them according to their specific mass-to-charge ratio (m/z), and then records the relative abundance of each ion type. Generate ions by addition of a proton (hydrogen) to give $[M+H]^+$ by removal of a proton to give $[M-H]^-$. The first step in the mass spectrometric analysis of compounds is the production of gas phase ions of the compound, basically by electron ionization. This molecular ion undergoes fragmentation.

Each primary product ion derived from the molecular ion, in turn, undergoes fragmentation, and so on. The ions are separated in the mass spectrometer according to their mass-to-charge ratio, and are detected in proportion to their abundance. A mass spectrum of the molecule is thus produced. It displays the result in the form of a plot of ion abundance versus mass-to-charge ratio. In the spectrum of a pure compound, the molecular ion, if present, appears at the highest value of m/z (followed by ions containing heavier isotopes) and gives the molecular mass of the compound. The instrument consists of **ion source, analyzer and detector system.**

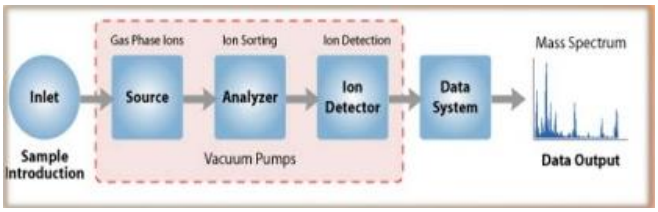

Figure 2 – Basic principles of a mass spectometer

A mass spectrometer works by using magnetic and electric fields to exert forces on charged particles (ions) in a vacuum. Therefore, a compound must be charged or ionized to be analyzed by a mass spectrometer. The choice of ionization method depends on the nature of the sample and the type of information required from the analysis.

Our instrumentation offers 2 types of ionization method:

Electrospray ionization (ESI): Transfer ions from the solution phase into the gas phase. Analyte is either pumped into the mass spectrometer via a syringe (infused) or is introduced after chromatographic separation (LC-MS).

Matrix-assisted laser desorption ionization (MALDI): It is initiated by mixing the sample solution with a large molar excess of the host matrix material and depositing the mixture on a specially designed MALDI sample target. After evaporation of the solvent, the sample-matrix crystals are irradiated with a laser beam of high irradiance power to simultaneously desorb and ionize the sample and matrix molecules into the gas phase. MALDI has become extremely popular as a method for the rapid determination of high-molecular-weight compounds (proteins and other molecules with masses in excess of 200 kDa).

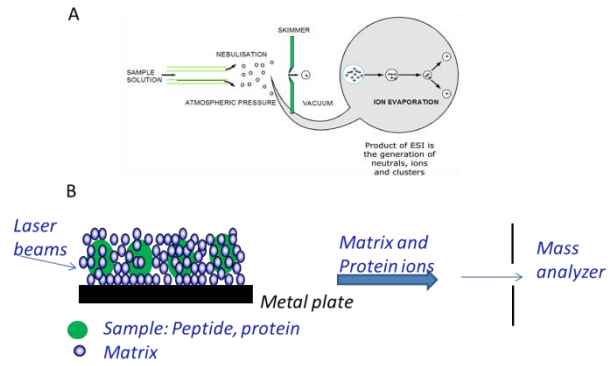

Figure 3 – A) ESI, B) MALDI theory

Detectors

***Quadrupole Theory*:** A quadrupole consists of four precisely matched parallel metal rods. The mass separation is accomplished by the stable vibratory motion of ions in a high-frequency oscillating electric field that is created by applying direct-current (dc) and radio-frequency (rf) potentials to these electrodes. Under a set of defined dc and rf potentials, ions of a specific m/q value pass through th geometry of quadrupole rods. A mass spectrum is obtained by changing both the DC and RF potentials while keeping their ratio constant.

Time of Flight Theory: TOF analyzer consists of a long field-free flight tube in which ions are separated on the basis of their velocity differences. The velocities, v, of ions are inverse function of the square root of their (m/q or m/z) values. Therefore, the lower m/q ions travel faster and reach the detector earlier than the higher m/q ions. The measured arrival times of all ions provides a time spectrum that is converted into a mass spectrum.

MS combined with chromotography

Liquid chromatography–mass spectrometry (LC-MS) combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry (MS). MS is combined with LC in order:

- To eliminate non-specific dedection limits,
- Better peak identification,
- To reduce the effects of ion suppression,
- Quantification

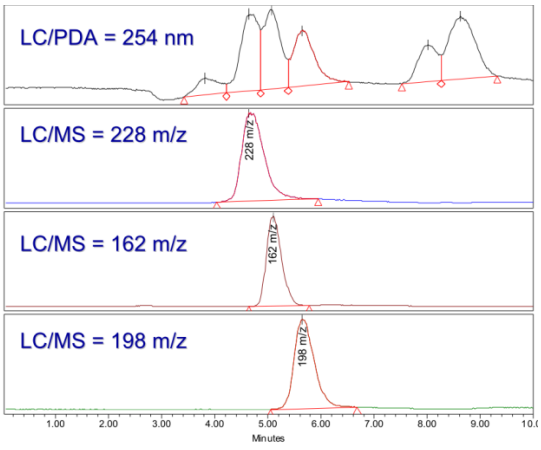

Figure 4 – LC combined with MS

Tandem Mass Spectrometry: Tandem mass spectrometry (also known as MS/MS or MS2) involves multiple steps of mass spectrometry selection, with some form of fragmentation occuring in between the stages. It is used for protein/peptide identification.

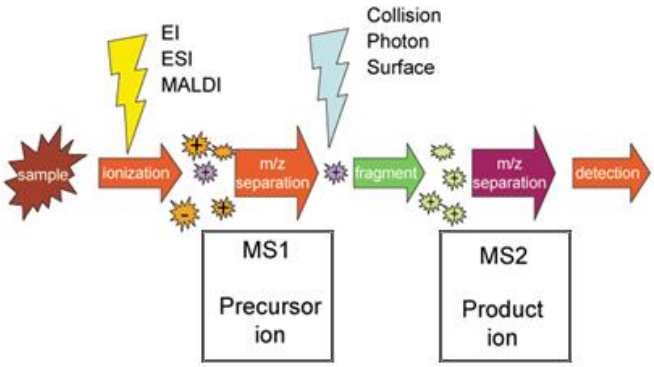

Figure 5 – Basic principle of tandem mass spectrometry

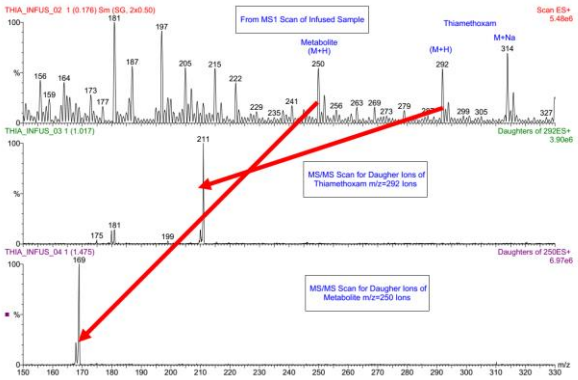

Figure 6 – Example for parent and daughter ion scan

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