Marine Organic Geochemistry

Analytical Methods – II.

Mass Spectrometry

What is Mass Spectrometry?
• The separation of matter according to atomic and molecular mass.
• Used in analysis of organic compounds of molecular mass up to 200,000 Daltons.
• Most versatile, sensitive and widely used analytical method available today.

Principle:
• Mass spectrometers use the difference in the mass-to-charge ratio (m/e or m/z) of ionized atoms or molecules to separate them from each other.
• MS is useful for the quantification of atoms or molecules, and also for determining chemical, structural and isotopic information about molecules.
• Molecules have distinct fragmentation patterns that provide chemical information (structural elucidation).
Basic Components of a Mass Spectrometer

General operation:
1. Create gas-phase ions
2. Separate the ions in space or time based on their mass-charge ratio.
3. Measure the quantity of ions of each mass/charge ratio.

Since MS systems create and manipulate gas-phase ions, they operate under high vacuum.

Magnetic-sector, quadrupole and time-of-flight mass analyzers also require extraction and acceleration ion optics to transfer ions from the source region to the mass analyzer.

Ionization Methods

- **Electron Impact (EI) ionization**
  - An EI source uses an electron beam, usually generated from a tungsten filament, to ionize gas-phase atoms or molecules.
  - An electron from the beam knocks an electron off the analyte to create ions.

- *EI is the most common ionization method for routine GC/MS analysis*
- **EI is a relatively harsh ionization technique and can lead to extensive fragmentation of the molecule (good and bad).**

- Typical ionization conditions 35-70 electron volts (eV)
- 12-20 eV = "low eV" (less fragmentation).
Ionization Methods

Chemical Ionization (CI)

- CI uses a reagent ion to react with the analyte molecules to form ions by either proton or hydride transfer:
  - \( \text{MH} + \text{C}_2\text{H}_5^+ \rightarrow \text{MH}_2^+ + \text{C}_2\text{H}_4 \)
  - \( \text{MH} + \text{C}_2\text{H}_5^+ \rightarrow \text{M}^+ + \text{C}_2\text{H}_6 \)
- The reagent ions are produced by introducing a large excess of methane or another gas (e.g. ammonia) relative to the analyte into an EI source. Electron collisions produce \( \text{CH}_4^+ \) and \( \text{CH}_3^+ \) which react further with methane to form \( \text{C}_2\text{H}_5^+ \).

CI is a softer ionization technique than EI.

The method is often used to derive complementary information to EI in GC/MS analysis.

Fast-atom Bombardment (FAB)

- In FAB a high-energy beam of neutral atoms, typically Xe or Ar, strikes a solid sample causing both desorption and ionization.
- The atomic beam is produced by accelerating ions from an ion source through a charge-exchange cell. The ions pick up an electron in collisions with neutral atoms to form a beam of high energy atoms.

FAB causes little fragmentation and usually gives a large peak corresponding to the molecular weight (molecular ion).
Atmospheric pressure ionization (API)

Atmospheric pressure ionisation (API)
- Used in conjunction with LC/MS techniques. Ions are formed at atmospheric pressure.
- Very soft ionization forming molecular ion and minimal fragmentation.
- There are two common types of atmospheric pressure ionisation: ESI and APCI.

Electrospray ionization (ESI)
- The ESI source consists of a very fine needle and a series of skimmers. A sample solution is sprayed into the source chamber to form droplets. The droplets carry charge when the exit the capillary and as the solvent vaporizes the droplets disappear leaving highly (multiply) charged analyte molecules.
- However, the sample must be soluble in low boiling solvents (acetonitrile, MeOH, CH₃Cl, water...) and stable at low concentrations, i.e. 10⁻² mol/l.

ESI characteristics
- Soft ionization method, provides molecular weight information.
- Suitable for analyzing large bio- or synthetic polymers that are difficult to vaporize or ionize, or have molecular weights that are beyond the mass range of the analyzer.
- Sensitivity depends strongly upon the analyte.
- Suitable for analyzing polar and even ionic compounds (e.g. metal complexes).
- Less fragmentation.
- Ideal for LC / MS coupling.

Method of choice for proteins, oligonucleotides and metal complexes

Atmospheric pressure ionization (API)

Atmospheric pressure chemical ionisation (APCI)
- APCI is a relative of ESI. The ion source is similar to the ESI ion source. In addition to the electrohydrodynamic spraying process, a plasma is created by a corona-discharge needle at the end of the metal capillary. In this plasma proton transfer reactions and to a small amount fragmentation can occur. Depending on the solvents, only quasi molecular ions like [M+H]+ and M+ (in the case of aromatics), and/or fragments can be produced. Multiply charged molecules [M+nH]+, as in ESI, are not observed.
- APCI is suitable for the analysis of organic compounds with medium - high polarity.
### Ionization Methods

**Field Ionization (FI) and Field Desorption (FD)**
- Molecules can lose an electron when placed in a very high electric field.
- High fields can be created in an ion source by applying a high voltage between a cathode and an anode - called a “field emitter”. A field emitter consists of a wire covered with microscopic carbon dendrites, which greatly amplify the effective field.

*FI causes little fragmentation. Used extensively in characterization of humic and fulvic acids (soil science).*

**Laser Ionization (LIMS)**
- A laser pulse ablates the material from the surface of the sample, and creates a microplasma that ionizes some of the sample constituents.
- Laser can be tuned to selectively ionize certain molecular species.
- The laser pulse accomplishes both vaporization and ionization of the sample.

**Matrix-assisted laser desorption ionization (MALDI)**
- Macromolecules are dispersed in a solid matrix such as nicotinic acid or glycerol.
- A UV laser pulse ablates the matrix which carries some of the large molecules into the gas phase in an ionized form.

*MALDI is a LIMS method for vaporizing and ionizing large biological molecules (e.g., proteins, DNA fragments). See MALDI-TOF-MS*

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**Ionization Methods**

**Resonance Ionization (RIMS)**
- One or more laser beams are tuned in resonance to transitions of a gas phase atom or molecule to promote it above its ionization potential and create an ion.
- Solid samples must first be vaporized by heating, sputtering or laser ablation.

**Secondary Ionization (SIMS)**
- A primary ion beam - such as $^3$He$, ^16$O$, or $^{40}$Ar$^+$ - is accelerated and focused onto the surface of a sample and sputters material into the gas phase. Approximately 1% of the sputtered material comes off as ions, which can then be analyzed by the MS.

*SIMS has the advantage that material can be continually sputtered from a surface to determine analyte concentrations as a function of distance (spatial mapping and depth profiling).*

**Thermal Ionization (TIMS)**
- A sample is deposited on a metal ribbon, such as Pt or Re, and an electric current heats the metal to a high temperature.
- The ribbon is often coated with graphite to provide a reducing effect.

*TIMS is used for elemental or refractory materials.*
Mass Analyzers

- Magnetic-Sector MS
  - The ion optics in the ion-source chamber extract and accelerate ions to a kinetic energy (K.E.) given by:
    \[ \text{K.E.} = 0.5 \, m v^2 = eV \]
  - where:
    - \( m \) = mass of the ion
    - \( v \) = velocity of the ion
    - \( e \) = the charge
    - \( V \) = applied voltage of the ion optics.
  - The ion enters the flight tube between the poles of a magnet and are deflected by the magnetic field, \( H \). Only ions of m/e ratio that have equal centrifugal and centripetal forces pass through the flight tube:
    \[ m v^2 / r = Hev; \text{ centrifugal} = \text{ centripetal forces} \]
  - Where:
    - \( r \) = radius of curvature of the ion path:
      \[ r = m v / e H \]
  - Thus:
    \[ m/e = H^2 r^2 / 2V \]
  - This equation shows that m/e (mass-to-charge, also expressed as m/z) of the ions that reach the detector can be varied by:
    - Changing \( H \) (magnetic field) "magnet scan"
    - Changing \( V \) (accelerating voltage) "voltage scan".

Instrumentation:

- Single focus analyzers: A circular beam path of 180, 90 or 60 degrees can be used. The various forces influencing the particle separate ions with different m/e ratios.
- Double focussing analyzers: An electrostatic field is added to separate particles with different kinetic energies.

- Magnetic sector MS provides nominal to high mass resolution.
- Most common mass analyzer for determination of isotope ratios.
Mass Analyzers

**Quadrupole MS**
- A quadrupole mass filter consists of four parallel metal rods.
- Two opposite rods have an applied potential of $(U+V\cos(wt))$, and the other two rods have a potential of $-(U+V\cos(wt))$ where:
  - $U$ is a dc voltage
  - $V\cos(wt)$ is an ac voltage.
- The applied voltage affects the trajectory of ions travelling down the flight path centered between the four rods. For given ac and dc voltages only ions of certain m/e ratio pass through the quadrupole filter, others are thrown out.
- A mass spectrum is obtained by monitoring the ions passing through the quadrupole filter as voltages on the rods are varied.

Quadrupole MS provides nominal mass resolution.
Most common mass analyzer for routine GC/MS applications (“Bench-top” GC/MS).

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**Ion-Trap MS**
- The ion trap MS uses three electrodes to trap ions in a small volume. The mass analyzer consists of a large ring electrode separating two hemispherical electrodes.
- A mass spectrum is obtained by changing the electrode voltages to eject the ions from the trap.

The advantages of Ion Trap MS include compact size, the ability to trap and accumulate ions to increase signal-to-noise, and the ability to perform MS-MS, or MS$^n$ experiments.
- Common benchtop MS for GC or LC.
- Ion Trap MS provides nominal mass resolution.
Mass Analyzers

Fourier-Transform Ion Cyclotron Resonance MS (FT-ICR)

- FT-ICR MS takes advantage of ion cyclotron resonance to select and detect ions.
- Ions are trapped within a cubic cell under the influence of small trapping potentials and a constant magnetic field. The frequency of the signal measured at the receiver plate is proportional to ion mass.

FT-ICR MS provides extremely high-resolution (accurate) mass measurement, even for very large molecules.

The more powerful the magnet, the greater the resolution

Currently the most powerful mass analyzer available.

Time-of-Flight (TOF) MS

- A TOF MS system uses the differences in transit time through a drift region to separate ions of different masses.
- It operates in pulsed mode so ions must be produced or extracted in pulses.
- An electric field accelerates all ions into a field-free drift region with a kinetic energy of $qV$, where $q$ is the ion charge and $V$ is the applied voltage.
- Since the ion kinetic energy $= 0.5mv^2$, lighter (smaller) ions have a higher velocity than heavier ions, and reach the detector at the end of the drift region sooner.

The advantages of TOF-MS are the ability to measure very large masses, and fast MS acquisition rate.

TOF-MS provides nominal to medium resolution.
Ion Detectors

- Channeltron
- Daly detector
- Electron multiplier tube (EMT)
- Faraday cup (used in isotope ratio mass MS)
- Microchannel plate (used in TOF-MS)

Interpretation of Mass Spectra

Important Features of Mass Spectra

Molecular Ion ($M^+$)
- Intensity will depend on stability of molecular structure and ease of fragmentation

Base Peak ($B^+$)
- May be molecular ion or favored fragment ion, depending on structure

Fragment Ions
- May be formed by cleavage, loss of neutral fragments or by structural rearrangement
- May be many or few

The mass spectrum (EI) of acetone, $CH_3COCH_3$, contains many fragment ions as well as the molecular ion at m/z 58
Interpretation of Mass Spectra

Major Influences on Mass Spectral Fragmentations of Organic Compounds

- 1. Ring Structures
- 2. Branching points
- 3. Double bonds
- 4. Aromaticity
- 5. Stereochemistry
- 6. Functionality

Mass spectrum of 4α-methyl-24-ethyl-5α(H)-cholestan

Relative intensity

m/z

Interpretation of Mass Spectra

Ephedrine (mw 165)

El (70 eV)

M⁺ (absent)

Cl (CH₃)
Interpretation of mass spectra

Mass Resolution

- R = resolution required to baseline separate a pair of ions having the same nominal mass:
  \[ R = \frac{M}{\Delta m} \]
- Where:
  - M = nominal mass of ions to be separated
  - \( \Delta m \) = difference in mass
- e.g. CO⁺ (27.995) and N₂⁺ (28.006), nominal mass = 28
  \( \Delta m = 0.011 \), R = \(~ 2500\)

<table>
<thead>
<tr>
<th>Element</th>
<th>Symbol</th>
<th>Nominal Mass</th>
<th>Precise Mass</th>
<th>Abundance</th>
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<tr>
<td>Phosphorus</td>
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<td>31</td>
<td>30.9738</td>
<td>monoisotopic</td>
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</tbody>
</table>

Mass Defect: the difference between the nominal and exact mass. The mass defect can assume both positive and negative values.
Interpretation of mass spectra

Highest base-line resolved mass for selected doublets at a resolution of 1 part in 25000

<table>
<thead>
<tr>
<th>Doublet</th>
<th>$\Delta$ Mass</th>
<th>Highest Resolved Mass (25000 x $\Delta$Mass)</th>
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</thead>
<tbody>
<tr>
<td>C - $\text{H}_2$</td>
<td>0.0939</td>
<td>2347</td>
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<tr>
<td>$\text{C}_2\text{H}_4 - ^{12}\text{S}$</td>
<td>0.0905</td>
<td>2263</td>
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<tr>
<td>$\text{CH}_4 - \text{O}$</td>
<td>0.0364</td>
<td>910</td>
</tr>
<tr>
<td>$^{32}\text{S} - \text{O}_2$</td>
<td>0.0277</td>
<td>692</td>
</tr>
<tr>
<td>$^{13}\text{CH} - \text{N}$</td>
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<tr>
<td>$\text{C}_3 - ^{32}\text{SH}_4$</td>
<td>0.0034</td>
<td>85</td>
</tr>
</tbody>
</table>

Interpretation of mass spectra

High resolution mass spectrum (FT-ICR-MS) of carbon monoxide and nitrogen
Gas Chromatography-Mass Spectrometry (GC/MS) and Liquid Chromatography-Mass Spectrometry (LC/MS)

Objective:
• Identification and Quantification of components in complex mixtures.
• GC/LC: Separates components of complex mixture according to molecular size, shape, polarity.
• MS: Permits recognition of individual components as they sequentially elute from GC.

Approach

Compound Identification
• Mass Spectra
• Mass Chromatography

Compound Quantification
• Total (Reconstructed) Ion Current (TIC/RIC)
• Mass Chromatography

• MS scans across a given mass range (e.g. 50 - 500 amu) at a set rate (e.g. 1 scan/sec).
• Spectra are collected ("acquired") for each scan over a time (usually corresponding to the length of the GC run).

Mass Chromatography/Mass Fragmentography

• Use: single ion monitoring
• multiple ion monitoring

• Can select ions characteristic of:
• compound type
• carbon number
• stereochemistry

[Graph of Mass Spectra over Retention Time]
Mass Chromatography/Mass Fragmentography

**Example 1**
- Mass Spectra are collected for unrelated compounds A, B and C separated from a mixture by GC
- Mass x, y and z are found to be uniquely characteristic for compounds A, B and C respectively.
- Can perform mass chromatography using diagnostic ions

**(Example continues with diagrams showing mass spectra and TIC plots for compounds A, B, and C)**

**Example 2.**
- For related compounds A and A' can select a common ion to study their distributions in complex mixtures
- This is a very good method for recognition, characterization and “fingerprinting” of homologous series.

**(Example continues with diagram showing TIC plots for compounds A and A’)**

*Example: n-alkanes (m/z 85)*
Quantitative mass spectral analysis of the dimethyl derivative of 5-flourouracil using isotopically labeled standard

Advantages
Use of standards with similar retention times and ionization efficiencies to analytes.
(= more accurate quantification).

Mass Chromatography/Mass Fragmentography

- m/z 85 (alkanes)
- m/z 257 (diasterenes)
- m/z 355 (C27 sterenes)
- m/z 191 (hopanes)
- m/z 219 (C32 hopanes)
In MS/MS, the first mass analyzer selects one m/z value for fragmentation; the second mass analyzer produces the mass spectrum of the fragments.

In ion trap and FT-ICR systems, this process can be repeated multiple times (MS^n).

This approach is very popular for sequencing of amino acids within peptides for protein characterization.
MALDI-TOF/MS of high mass organic compounds

Mass Spectrometry of high mass organic compounds

MALDI-TOF/MS

Laser ablation of C_{70} soot

C_{60}^{+} C_{70}^{+} C_{116}^{+}

M/Z

600 800 1000 1200 1400 1600

Mass Spectrometry of high mass organic compounds

MALDI-TOF/MS of bovine serum albumin (protein)

Monomer

Doubly charged

Dimer

Trimer

m/z

24000 100000 200000

33,220 66,430 132,860 199,200

(M)^[+] (M)^[2+] (2M)^[+] (3M)^[+]

DNA ABUNDANCE

1 2 3 4
Mass Spectrometry of high mass organic compounds

ESI/MS of bovine carbonic anhydrase (enzyme protein, mw 28,000 Da, 12.5 pmol)

Electrospray ESI/MS of large biomolecules results in an array of multicharge ions with moderate m/z values.

Rapid mass spectral acquisition via GC/TOF-MS

Acquisition rates up to 500 spectra per second.
Fig. 2. ESI-FT-ICR positive ion mass spectrum of the humic acid extract of a degraded wood sample from Mt. Rainier, WA, acquired on a 7 T FT-ICR MS. The sample was prepared in 25:75 water/methanol at a concentration of 1.25 mg ml$^{-1}$. The spectrum represents the average of 18,000 scans. The inset is an expansion of the region around 321 m/z where the mass resolving power was approximately 80,000.
Fig. 2. Comparison of expanded spectra of Mt. Rainier hemic acid. The ESI QqTOF spectrum (bottom) is an expansion of the 771–774 m/z range from Fig. 1. The ESI FT-ICR spectrum (middle) was acquired on a V. I. FT-ICR MS and the mass resolving power in this region was approximately 60,000. The top two mass spectra are further expansions of the FT ICR spectrum. The resolution of peaks in these regions was sufficient to allow the assignment of unique molecular formulas derived from proposed structures (values and errors in Table 1).

Fig. 4. ESI FT-ICR positive ion mass spectrum of dissolved organic matter (DOM) from Suwannee River. The spectrum was acquired in the same manner as that in Fig. 2.
High performance liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry (HPLC/APCI-MS) of intact tetraether lipids

Hopmans et al. 2000

High performance liquid chromatography/electrospray ionization-mass spectrometry (HPLC/ESI-MS) of intact polar lipids

Sturt et al. 2004
Isotope Ratio Mass Spectrometry

Principle
- Isotope ratios can be precisely measured using a sector mass spectrometer (Faraday cup detectors).
- The MS precisely measures the ratio of currents from ion beams corresponding to different isotopes (e.g., for $^{13}$C/$^{12}$C, measure $^{13}$CO$_2^+$ (m/e = 45) and $^{12}$CO$_2^+$ (m/e = 44))
- Ratio is compared to a standard reference gas.

Conventional Method
- Introduction of gases via dual viscous inlet.

Continuous-flow mass spectrometry
- Elemental analyzer – irMS (EA-irMS).
- Isotope ratio monitoring-Gas Chromatography-Mass Spectrometry (irm-GC-MS, GC-irMS).

<table>
<thead>
<tr>
<th>Element</th>
<th>Isotope</th>
<th>Measured as</th>
<th>Reference Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>$^{13}$C/$^{12}$C</td>
<td>CO$_2$</td>
<td>PDB</td>
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<tr>
<td>Nitrogen</td>
<td>$^{15}$N/$^{14}$N</td>
<td>N$_2$</td>
<td>Atm. N$_2$</td>
</tr>
<tr>
<td>Oxygen</td>
<td>$^{18}$O/$^{16}$O</td>
<td>CO$_2$</td>
<td>SMOW</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>D/H</td>
<td>H$_2$</td>
<td>SMOW</td>
</tr>
<tr>
<td>Sulfur</td>
<td>$^{34}$S/$^{32}$S</td>
<td>SO$_2$</td>
<td>CDT</td>
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</tbody>
</table>
Isotope ratio Mass Spectrometry

HIGH-PRECISION CONTINUOUS-FLOW ISOTOPE RATIO MS

Magnetic Sector

Universal Faraday Cups

Hydrogen Detection

CO₂, N₂, SO₂ Detection

Ion Source

FIGURE 1. Diagram of a generic isotope ratio mass spectrometer, not including inlets. A tight electron impact ion source efficiently generates positive ions that are mass-analyzed by a single magnetic sector. Multiple Faraday cups permit simultaneous and continuous monitoring of the analyte major isotope masses in a split flight tube for hydrogen or for other gases. Universal collectors with a central narrow cup and two outer wider cups are used for capturing higher mass gases such as CO₂ and N₂.

GC-irMS or irmGC/MS

Gas Chromatograph

Combustion Furnace

Water Separator

Open Split

Isotope-ratio-monitoring-Mass-Spectrometer

Computer
GC-irMS or irmGC/MS

m/z (45/44)/100

m/z 44

CO2 standard pulses
internal standards of known isotopic composition

Fig. 1. Partial m/z 44 and 45/44 chromatograms from irmGC-MS of phenolic standards. Composed names are as listed in Table 1. In addition, 2-chloroethylation, 3-chloroaniline, and 3-chloroethylbenzene were used as internal standards.
Accelerator Mass Spectrometry

- Direct measurement of the proportion of $^{14}$C atoms (relative to $^{13}$C or $^{12}$C) by accelerator mass spectrometry (AMS)
- Measurements are typically made on graphite (sometimes CO$_2$).
- Graphite formed by combustion of sample to CO$_2$ and reduction to graphite.
- Cs sputter source (SIMS) generates C$^-$ ions ($^{14}$N does not make negative ions)
- Accelerator removes isobaric interferences (e.g. hydrides such as $^{13}$CH$^-$) by electron stripping.
- Sample size requirements: now as low as 25 $\mu$g C and measurement times as short as 20 min.