Polynuclear Aromatic Hydrocarbons (PAHs)

PAHs are a class of organic compounds produced by incomplete combustion or high-pressure processes. Polycyclic aromatic hydrocarbons (PAHs) are hydrocarbons consisting of three or more fused benzene rings. PAHs form when complex organic substances are exposed to high temperatures or pressures. Hundreds of such compounds exist and they are ubiquitous in the environment. At room temperature, PAHs are solids with low volatility. They are soluble in many organic solvents and are relatively insoluble in water. The more common PAHs include benzo(a)pyrene (B[a]P) and benzo(e)pyrene. Benzo(a)pyrene is the most carcinogenic PAH studied. Certain B[a]P metabolites are believed to interact with DNA, causing malignancies and heritable genetic damage.


PAH Metabolism

Incomplete combustion produces complex mixtures of PAHs. When someone is exposed to PAHs, they can collect in fatty tissues & organs. Many kinds of metabolites are formed as the body tries to eliminate them.
Ionization is KEY to MS

- The kind and quality of information provided by an MS measurement depends critically on the type of ionization method used. You will learn about 4 of the many available methods.
- Hard Ionization
  - Electron impact usually produces many ion fragments
- Soft Ionization
  - Chemical ionization uses gas to ionize with less fragmentation
  - MALDI volatilizes and ionizes large molecules with minimal fragmentation
  - Electrospray ionization aerosolizes and multiply ionizes large molecules with little fragmentation

Ionization determines ion fragmentation

1. Depending on the energy imparted to an ion by the ionization process and its molecular structure, an ion may dissociate into smaller ions and neutral fragments.
2. The abundance and mass to charge ratio of the fragment ions provide a “fingerprint” of the parent compound.


http://www.specmetcrime.com/New%20metabolite%20of%20BaP.htm
Electron Impact Ionization/
Chemical Ionization

for volatile molecules -

- Electrons produced by hot filament (W, Rh), accelerated to high NRG by anode
- Gas phase analyte molecules ionized by e⁻ impact
  \[ M + e^- \rightarrow M^+ + 2e \]
- Small potential on focusing plate propels beam of ionized analyte to mass analyzer
- Ions in excited states, relax by fragmentation producing complex spectra; can have low M⁺
- Chemical ionization uses similar source but mixes reagent gas (e.g. CH₄, NH₃) with gaseous sample (10⁻³-10⁻⁴ excess) so that ionized reagent ionizes analyte:
  \[ \text{CH}_4^+ + M \rightarrow MH^+ + \text{CH}_4, \text{etc.} \]


Section 20B-1-2

MALDI

for large molecules

- solid A is mixed w/ solid UV absorber (mix sol’ns & evaporate)
- solid mixture introduced into ionization chamber on a sample probe
- high NRG UV hv (λ=266nm) absorbed by the matrix (absorber)
- matrix molecules vaporized by heat from VR, etc*
- A molecules vaporized “sympathetically” as ‘solvent’ molecules vaporized
- UV hv ionize vaporized A

matrices - dihydroxybenzoic acid
  nicotinic acid
cinnamic acid

http://www.chm.bris.ac.uk/ms/theory/maldi-ionisation.html

Section 20B-4
Steps of MS/MS Analysis of PAH in Urine

1. **Method Selection** - MS/MS measurements can be carried out for many PAHs and their metabolites in aqueous samples with appropriate sample preparation.

2. **Sampling** - PAHs and their metabolites typically are not highly soluble in water and are analyzed by extracting them into more suitable solvents often by capturing them onto solid phase resins or chromatographic materials (modified silica) first.

3. **Sample Preparation** - 10 mL of urine were acidified and buffered with 20 mL of 1 M HCl/0.1 M NaOAc/HOAc buffer (pH 5). After spiking with 20 μL of glucuronidase/aryl sulfatase, the mixture was incubated overnight at 37°C. The hydrolyzed sample was loaded onto a solid phase extraction (SPE) cartridge, which was preconditioned with 5 mL of methanol, then 10 mL distilled water. The SPE cartridge was washed with 3 mL distilled water, then 3 mL of 50% MeOH/HOH and the analytes were eluted with 10 mL of MeOH. The eluent was concentrated by rotary evaporation of the methanol extracts to 0.5 mL at 60°C, and frozen (-10°C) in darkness until analysis.

4. **Measurement** - MS/MS used to analyze complex mixtures
   Ten μL of the extracts were injected into the LC system.

5. **Processing** - Under the right conditions, ion current depends linearly on concentration.

6. **Interpretation & Report**

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**Molecular MS vs Molecular Abs**

- The mass spectrometer measures ions sorted by mass/charge (m/z)
- The spectrophotometer measures photons sorted by wavelength (λ)
Tandem Mass Spectrometer

Q-TOF MS/MS (Quadrupole/Time-of-flight Tandem Mass Spec)

Tandem mass spectrometers (MS/MS or MS^n) have 2 or more mass analyzers. MS^n provide structural information about complex molecules monitoring ion chemistry of molecular ion and important fragments.

Many combinations of analyzers have been used, e.g., QQQ & Q-TOF

Inlet -
solution: syringe delivery

Ion source -
Electrospray Ionization (ESI)

Mass Analyzers -
Quadrupole mass filter
Collision Chamber (selected ions fragmented by Ar/He impact)

Time-of-flight

Transducer -
microchannel plate

Signal Processing -
1->V converter
ADC/CPU

Electrospray Ionization

for large molecules

- large potential difference between needle and cylindrical electrode
- buffer solution charged by electrode assembly
- charged solution aspirated (capillary) to form charged mist
- charged droplets exit capillary
- drying gas reduces drop diameter
- M^n desorb from drops to reduce electrostatic repulsion
- ion beam formed by sampling and skimmer cones
- spectra complicated by multiple charge species: M^+, M^2+, ..., M^{11+}

Hewlett Packard ESI Interface
**Time of Flight Mass Analyzer**

- ions accelerated into drift tube by grids poised at \( V > 0 \)
- All accelerated ions should have same KE
  \[
  E_{\text{kin}} = \frac{1}{2} m v^2 = Z e V_\text{grid} s
  \]
  \[
  \frac{v}{2} = 2 e V_\text{grid} \left( \frac{s}{2} \right)^2
  \]
- drift tube is field free, ion flight time to detector depends on \( m/Z \)
  \[
  v = D / t \_\text{flight}
  \]
- Small differences in KE reduce R at detector. Reflectron added to drift tube to reduce differences.

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**Microchannel Plate**

made from highly resistive material, such as a semiconductor or lead glass

many tiny tubes “microchannels” go through the plate from one face to the opposite. The microchannels are densely packed over the whole device, are typically ~10 micrometer in diameter and have ~15 micrometer spacing between them.

microchannels are parallel to one another and tilted at a small angle to the surface (~8° from normal). The angle insures that a particle that enters one of the channels will hit the channel wall.

since the multiplication takes place under the presence of a strong electric field (applied between metal plating on the surfaces), the impact of a charged particle (ion, e-) on a microchannel wall starts a cascade of electrons that travels through the channel and amplifies the original signal by several orders of magnitude depending on the electric field strength and the geometry of the micro-channel plate.

electrons exit the channels on the opposite side where they are detected often by a single metal anode measuring total current.

http://www.dmphotonics.com/MCP_MCPImageIntensifiers/microchannel_plates.htm
MS/MS Acquisition Modes

Product or daughter ion scanning:
- Ions transmitted by analyzer 1 ions are fragmented by bombardment with a gas in the collision cell. These fragment ions are separated by m/Z in analyzer 2. The series of spectra constitute a fingerprint pattern specific to the mixture/compound under investigation. Used in structural analysis and analysis of complex mixtures.

Precursor or parent ion scanning:
- all sample ions transmitted by the analyzer 1, but only specific fragment ions, generated by bombardment with the gas in the collision cell are monitored by the analyzer 2. Used to monitor compounds that produce common fragment ions.

Constant neutral loss scanning:
- Analyzers 1 and 2 are simultaneously scanned with a fixed off-set in m/Z of transmitted ions. The off-set is equal to the mass of a neutral fragment, so analyzer 2 allows only monitors those ions from which the fragment has been lost. Used to monitor processes, e.g., CO₂ loss in carboxylic acid reactions.

Selected/multiple reaction monitoring:
- Analyzers 1 and 2 are set to transmit application specific ions and their fragments. Used to confirm the presence of a specific compound in a mixture e.g. drug testing of blood or urine samples; has excellent DL.

PAH Mixture Analysis by MS/MS

Tandem mass spectrometry (MS-MS) is used to produce structural information about compounds by fragmenting specific sample ions inside the mass spectrometer and identifying the resulting fragment ions. … Tandem mass spectrometry also enables specific compounds to be detected in complex mixtures [using] their specific and characteristic fragmentation patterns.

Given a complex mixture (soot, crude oil), MS/MS allows identification of the components by fragmenting selected ions (there are computerized lists of fragment masses):

http://www.astbury.leeds.ac.uk/facil/MStut/mstutorial.htm

Section 20C-5
**Molecular MS Quantitation**

In principle, MS signals are linear with analyte concentration –

\[ S_{MS} = k_c \cdot \text{analyte} + S_{MS}^{blk} \]

Moreover, molecular mass spectra at unit mass resolution have high ‘information content’ because MS peaks are narrow and well separated. Consequently, many mass spectra contain unique signals at specific m/Z for which calibration curves may be prepared.

The uncertainties associated with the small samples analyzed by MS are reduced by adding an internal standard to samples and calibration standards. For molecular analysis, isotopically labeled (²H, ¹³C or ¹⁵N enriched) analytes are often used as internal standards. Homologues of the analyte, e.g., pyrene for BaP, also are often useful internal standards.

Standard addition (‘spiking’) is widely used in MS measurements to avoid matrix affects.

Complex mixtures are typically quantitated by a combination of separation (chromatography or electrophoresis) and MS. Selected ion monitoring produces a chromatogram of species in the mixture that produce fragments that have a specific m/Z. Parent-ion MS/MS can also be used to separate and analyze the molecular ions of complex mixtures.

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**Mol MS Figures of Merit**

<table>
<thead>
<tr>
<th>Method</th>
<th>Accuracy</th>
<th>DL</th>
<th>High Mass</th>
<th>( R = m/Dm )</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI/EB</td>
<td>2-10%</td>
<td>1e-12g</td>
<td>500</td>
<td>~1e3</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>2-10%</td>
<td>1e-10g</td>
<td>1e5</td>
<td>~5e2</td>
</tr>
<tr>
<td>ESI-FTMS*</td>
<td>2-10%</td>
<td>1e-13g</td>
<td>1e4</td>
<td>&gt;1e6</td>
</tr>
</tbody>
</table>

* included for reference only
Molecular Mass Spectrometry: Analysis Examples

- Detect and identify the use of steroids in athletes
- Determine the composition of molecular species found in space
- Determine whether honey is adulterated with corn syrup
- Locate oil deposits by measuring petroleum precursors in rock
- Monitor fermentation processes for the biotechnology industry
- Detect mercury or dioxins in contaminated fish
- Determine gene damage from environmental causes
- Establish the elemental composition of semiconductor materials
- Determine how drugs are used by the body
- Perform forensic analyses such as quantitation of drugs of abuse
- Determine the age and origins of specimens in archaeology & art history

http://www.asms.org/whatisms/p1-p2.html

MS Exercise