Analyzing Small Molecules by EI and GC-MS

July 2014
Samples Appropriate for GC-MS

- Volatile and semi-volatile organic compounds
  - Rule of thumb, <250 u will likely work by GC-MS
  - Highly polar or ionic compounds may not be sufficiently volatile for GC-MS
  - PNP ligand @ 429 u has been analyzed by GC-MS

- Mixtures of semi-volatiles
  - Crude reaction mixtures
  - Stable isotope labeled samples

- Solvent should be in volatile matrix
  - Please remove TBAF, NaCl, urea, or other involatiles if at all possible
Gas Chromatography Basics

- Vapor-phase molecules separate by their interactions with the inner wall of the column and the He stream.

- Oven temperature is raised to weaken interaction with stationary phase.

- Temperature profile can be altered to improve resolution of similar compounds or reduce analysis time.
Basic GC Diagram

A Sample → Gasflow (He) → Detector

High affinity to stationary phase
Low affinity to stationary phase

B

Image source: http://www.cubic.uni-koeln.de/research/horstmann/gas_chromatography2.jpg
Agilent 6890/5973 GC-MS
GC-MS Injection

• A 100-position autosampler feeds one of two split/splitless injectors

• Normal methods use “split mode” for concentrated samples
  – Usually aim for <50 ng on column
  – 1 uL injection, 50:1 split is default on most methods
    • 1 mg/mL solution gives 20 ng on column at 50:1

• Splitless mode is available for dilute samples

• Injector kept very hot (280 °C) to ensure instant vaporization of sample
Split Injection Mode Diagram

**Split Ratio**

Total Flow 104.25 ml/min
Septum Purge 3 ml/min

Split Vent 100 ml/min

Column Flow 1.25 ml/min

**Split Ratio Calculation**

\[
\text{Split Vent Flow} = \frac{100}{1.25} = 80 \text{ to } 1
\]

Image source: http://www.sge.com/uploads/yB/sx/yBsxaFxQS4C3FWzlvBJ6Uw/split_injection_2.gif
Default 6890 Settings

• Default column on front injector is an Rxi-5Sil MS
  – 95% dimethylsiloxane, 5% phenylsiloxane
    • Very non-polar stationary phase from Restek
  – 0.25 mm i.d., 30 m long, 0.25 μm film thickness

• Temperatures can range from -40-350 °C
  – <30° is available with liquid CO₂ tank
  – Up to 20 °C per minute ramp possible

• Default gradients
  – A “slow” gradient from 40-300 °C over 30 minutes
  – A “fast” gradient from 70-280 °C in 18 minutes
Other Column Chemistries

• There are entire catalogs full of GC columns with different chemistries
  – Any user-supplied column MUST be compatible with GC-MS (bonded phase, no particles, limited bleed, etc)
• MSF has the following columns available
  – DB-1 (100% dimethylsiloxane)
  – DB-17 (50% phenyl, 50% dimethyl siloxane)
  – DB-VRX (special dimethylsiloxane for volatile compounds)
  – DB-WAX (polyethylene glycol)
• Column change takes about 4 hours
  – Schedule this DAYS in advance with Dr. Karty
Detection of Compounds

• As compound come off the end of the column, they need to be detected in some way

• A 5973 inert mass spectrometer is coupled to the outlet of the 6890 GC
  – Provides both retention time AND electron ionization mass spectrometric information
Electron Ionization (EI)

• Gas phase molecules are irradiated by beam of electrons

• Interaction between molecule and beam results in electron ejection
  – $M + e^- \rightarrow M^{+\cdot} + 2e^-$

• EI is a very energetic process
  – Molecules often fragment right after ionization
Figure 6.5: Fragmentation of t-butyl ethyl ether.

Figure from *Mass Spectrometry Principles and Applications*  
E. De Hoffmann, J. Charette, V. Strooband, eds., ©1996
EI Advantages

• Simplest source design of all
  • EI mass spectrometers even go to other planets!

• Robust ionization mechanism
  – Even noble gases are ionized by EI

• Fragmentation patterns can be used to identify molecules
  – NIST ’11 library has over 240,000 spectra
  – Structures of novel compounds can be deduced
EI Disadvantages

• Fragmentation makes intact molecular ion difficult to observe

• Samples must be in the gas phase

• Databases are very limited
  – NIST’11 only has 213,000 unique compounds

• Interpreting EI spectra is an art
  – Suggest reading “Interpreting Mass Spectra” by McLafferty and Turecek as a reference
Oleic acid heated to 150°C

Retention Time (min)

0 4 8 12 16 20 24

pentane
DCM
hexane
pentanal
heptane
butanal
hexanal
octane
2-pentylfuran
3-methyl-2-heptanone
octanal
2-octenal
heptanal
2-nonenal
2-decenal
2,4-decedenedial
2-undecenal
Oleic acid heated to 150°C
Scan 5576 (17.680 min): BRON-OLEIC3.D

Mass Spectrum from 17.68 minutes
Scan 5576 (17.680 min): BRON-OLEIC3.D

Mass Spectrum from 17.68 minutes

#19126: Nonanal

Nonanal Library Mass Spectrum
A Word About Quantification

• GC-MS is a quantitative technique
• Use only 1 m/z when quantifying a compound
• Intensity is proportional to concentration
  – $I \propto [X]$
  – $\alpha$ is unique to each compound
  – The more two compounds differ chemically, the more careful one must be when comparing their intensities
• Ideally a calibration curve is constructed using multiple solutions of pure analyte at varying concentrations
Let MSF Staff Know

• If peaks look broad or non-Gaussian
  – Implies time to change liner or septum
• If the same unexplainable mass is seen in multiple injections
  – 207, 281, 355, 429 are septum bleed
  – 185 is tributylamine (from TBA-BF$_4$ or TBAF)
• Any time an error shows up on the computer
Training Times

• Angie and I usually train groups of 2 or 3
• Training takes about 1 hour
• Read through online training manual prior to your session to speed the process
• Email jkarty@indiana.edu or asorg@indiana.edu to make an appointment