Analyzing Semi-Volatiles by GC-MS

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Overview

- GC-MS Instrumentation Description
- GC-MS Method Sections In Detail
- Sequence Programming
- Data Analysis
- Tips and Tricks

Quick Description

- Agilent 7683B autosampler and tray
  - Can handle up to 100 samples at a time
  - 0.2-10 uL injection volumes
  - 2 different wash solvents can be used
- Agilent 6890n Gas Chromatograph
  - He carrier gas
  - Split/splitless injector
    - 500:1 split ratio or higher
  - -60 ℃ to +450 ℃ temperatures possible @ 20 ℃/min
    - Liquid CO_2 cryo option available
- Agilent 5973 inert mass selective detector
  - EI source (default) or CI source available
  - 10-800 m/z range
  - Positive and negative ions (CI mode) can be analyzed
- All instrument functions controlled by MSD Chemstation 1701D.02 software

GC-MS Availability

- The undergraduate labs paid half the cost for the new instrument
- Thus, A315 and A314 lab sections have absolute priority for instrument time
  - A315 uses GC-MS 2 or 3 afternoons throughout the fall semester
    - Their runs last from 2:00 pm – 10:00 pm
  - A314 uses GC-MS for 2 weeks in spring semester
- Check the sign on the GC-MS for their schedule

Sample Guidelines

- Sample needs to be volatile or semi-volatile
  - All analytes MUST pass through a GC column
  - Good rule of thumb, under 350 Da
  - Salts will most likely not work
  - Extremely volatile samples (e.g. CFCs) work great
- Sample should be 1-20 mg/L
  - More dilute sample can use splitless injection
  - If possible, use a volatile solvent
    - Ether, chloroform, methylene chloride, hexane, ethanol, or something more volatile
  - Non-volatile solvents (e.g. DMF) can be used, but are not preferred
- If possible, remove non-volatile components
  - Non-volatiles foul up inlet liner and first few cm of GC column

GC-MS Overview

- GC-MS is really two experiments in one
- Gas chromatography separates compounds by hydrophobicity/volatility
- Mass spectrometry separates compounds by mass/charge
- One can utilize the advantages of both techniques in a single analysis
- However one must make sure sample is compatible with both GC and EI-MS

Gas Chromatography

- Volatile molecules equilibrate between stationary phase and carrier gas
  - $M_{ads} \leftrightarrow M_{vap}$
  - Carrier gas: He
  - Default column: 30 m long x 0.25 mm i.d. DB-5MS
    - 95% dimethlysiloxane, 5% phenylsiloxane, 0.25 µm dF
- Affinity for stationary phase favors adsorption, slowing transit through column
- Temperature increase favors volatilization, accelerating transit through column
  - Oven temperature is the major experimental variable
  - Stationary phase chemistry is another variable
- Retention time is the measured quantity

Other Column Chemistries

- The MSF owns a handful of columns other than the rather non-polar DB-5
  - DB-1 (100% dimethylsiloxane, very non-polar)
  - DB-VRX (special volatiles column)
  - CarbonPLOT (carbon stationary phase for permanent gases)
  - DB-17ms (50% phenylsiloxane, 50% dimethylsiloxane, medium polarity)
  - DB-WAX (ethylene glycol, polar phase)
- Column changeover requires ~2 hr
  - Let MSF staff know 1 day in advance
  - Users may provide their own columns as long as they are stable enough for GC-MS

EI-QMF Mass Spectrometry

- As molecules elute from column, they pass into an electron ionization source
- 70 eV electrons ionize molecules ($M^{+\bullet}$)
  - EI often results in extensive fragmentation
- Ion and fragment m/z ratios analyzed by quadrupole mass filter
  - Only centroid or "stick" data are recorded
    - Height of centroid peak corresponds to area of raw data peak
- Full scan takes ~0.3 seconds
  - Default range: 40-500 m/z
  - Scanning wide m/z range can hurt sensitivity
    - (0.22% duty cycle for 40-500 m/z example)
  - SIM mode can increase sensitivity 10-200 fold

Policies In Brief

- All samples should be run as a sequence
  - Users can add more samples during a run
- All methods and data must initially be stored in the "Default" directory
  - MSD ChemStation only allows one directory for methods and data to be specified during a sequence
- Samples are discarded each morning unless we are told in advance

GC-MS Method Sections

- Injector (sets volume to be injected)
- Inlet (sets split conditions and inlet temp)
- Column (sets flow rate and column type)
- Oven (contains temperature program)
- Aux (sets MS transfer line temperature)
- Sim/Scan (controls mass spectrometer)
- Other sections are not routinely used
- Configure buttons are only for MSF staff

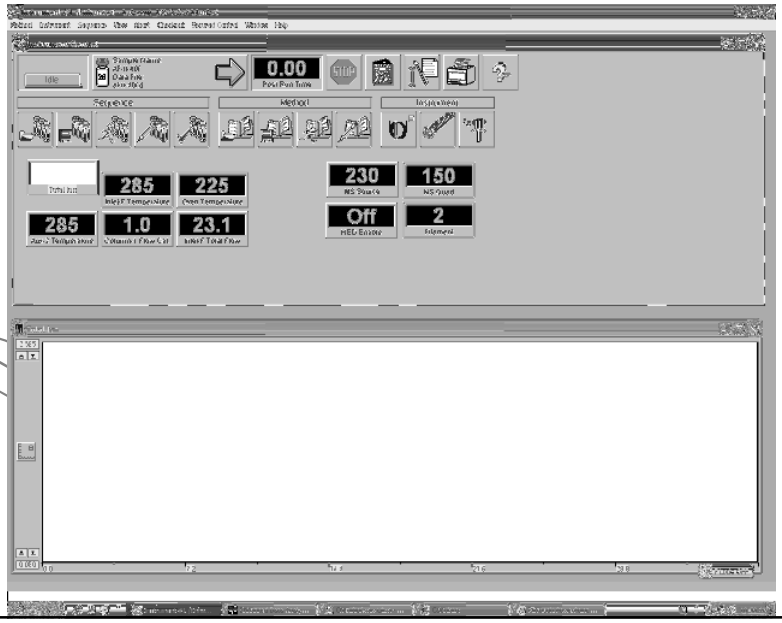
A Few Concepts to Remember

- Inlet temps above 285 ℃ accelerate septum degradation
- Solvent delay protects the source from high heat capacity solvent peak
  - Relatively large amounts of solvent are introduced during injection
    - CH_2Cl_2 is 15.6 M with itself; ether is 9.6 M
    - C_p and thermal conductivity of solvent vapors differ significantly from He
  - Rapid cooling of EI filament by solvent vapor rapidly degrades it
  - Large amount of solvent ions foul interior of the MS
- Initial temp should be at least 10 ℃ above bp of solvent, if possible

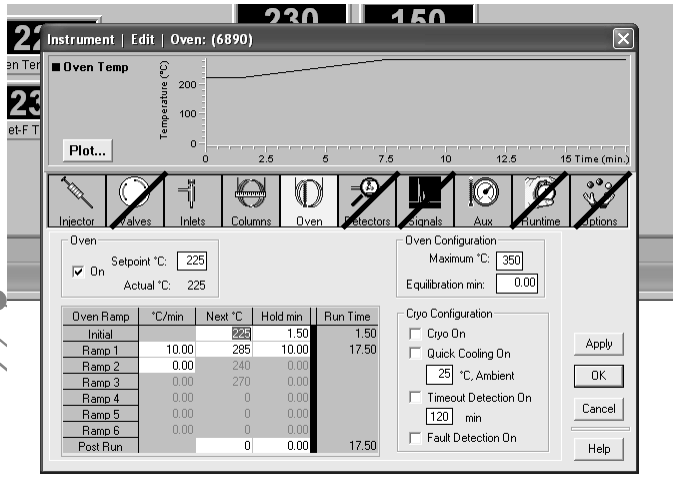
Instrument Control Window

- This window controls the GC-MS
- Sequences are controlled from sample tube icons
- Method is edited from the “Oven” icon
- Mass spectrometer is controlled from quadrupole icon
- A few instrument parameters are monitored in real-time

Instrument Control Screenshot



GC Method Control Screenshot



Injector Tab

- Injection volume is quantized
- Washes involve filling the syringe and dispensing it to waste
  - 2 wash solvents available (A and B)
  - One need not use both solvents
  - 4 washes are usually adequate
  - Be sure to use pre and post-injection washes
- Pumps flush the syringe with the sample
  - Again, 4 pumps are usually adequate

Inlets Tab

- Only use front inlet and He gas
- Split mode is good for most samples
- Splitless can be used for dilute or vapor samples
  - Purge flow to split vent should be 30 mL/min @ 1.00 min
- Try to keep heater to <285 ℃
- Gas saver should be set to 15 mL/min 2 minutes after injection
- All 4 boxes should be checked on this page
- Pulsed split and pulsed splitless modes are not normally used

Columns Tab

- Make sure installed column in method matches column indicated on sign
- Always specify:
  - Column: 1
  - Inlet: Front
  - Detector: MSD
  - Outlest PSI: Vacuum
- Flow rate should be ~1 mL/min
  - Pressure and velocity are calculated
- Ramping of flow rate is not commonly used

Oven Tab

- Setpoint is the temperature at start of run
- ℃/min is the temperature ramp rate
- Next is the temperature to be attained at end of ramp step
  - Up to 6 ramp steps can be specified
- Hold time denotes time spent at “Next” temp prior to proceeding to next ramp step
- All the boxes on right should be unchecked
- Users must NOT change oven configuration

Aux Tab

- Specifies temperature of transfer line between GC oven and EI source
- Usually set to same temperature as injector port
- Temperature can be ramped if your analyte is particularly labile

GC Method Hints

- Try to keep runs under 60 minutes
- Try to include 5-10 minutes at the end of a run @ 250-300 ℃ to clean the column
- Initial temps below 40 ℃ can take a long time to reach
  - Try to have initial temperature as high as possible for fast run times
- Inject solvent vapor when making a new method to figure solvent delay
- If two peaks won't separate, try lowering the temperature ramp
  - Isothermal steps are possible

GC Tabs Not Used

- The tabs for the following GC method parameters are not used
  - Valves
  - Detectors
  - Signals
  - Runtime
  - Options
- These control hardware we do not own

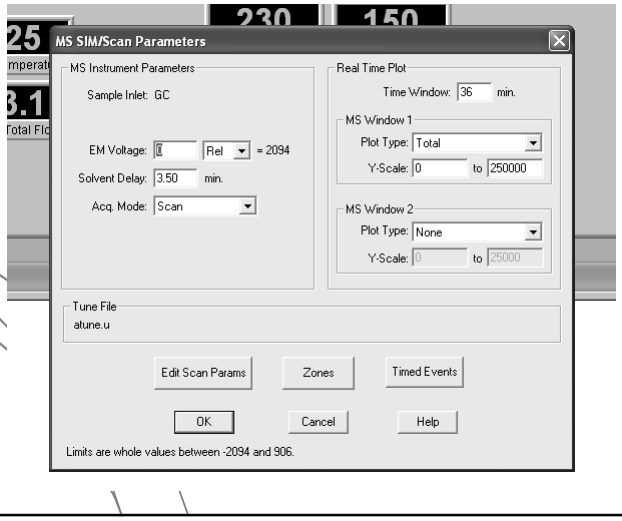
Mass Spectrometry Method

- Mode (Scan or SIM)
- Solvent Delay
  - Ensures MS is off when solvent arrives
  - For high BP solvents (e.g. DMF) or volatile analytes, event table can be used to turn MS off during a run
- EM voltage is always set to 0 (uses value in the tune file)
- Masses monitored
  - A full range in scan mode (e.g. 40-500)
  - A series of discrete masses in SIM mode
  - Multiple time segments in which different masses are monitored at different times can be employed
- Real-time window setup
  - Specify time and vertical scale for real-time MS monitor

Mass Spectrometry Modes

- Scan mode
  - Monitors all m/z's in a given range by scanning the quadrupole
  - Must be used if database searching is needed
  - Suffers from low duty cycle
- SIM mode
  - Specify an m/z and a length of time to monitor it
  - Up to 60 m/z's can be monitored in a group
  - Each m/z can be monitored for up to 100 msec at a time
- For either mode, try to have scan rate be greater than 3/sec
  - Ensures narrow GC peaks are adequately sampled

MS Method Screenshot



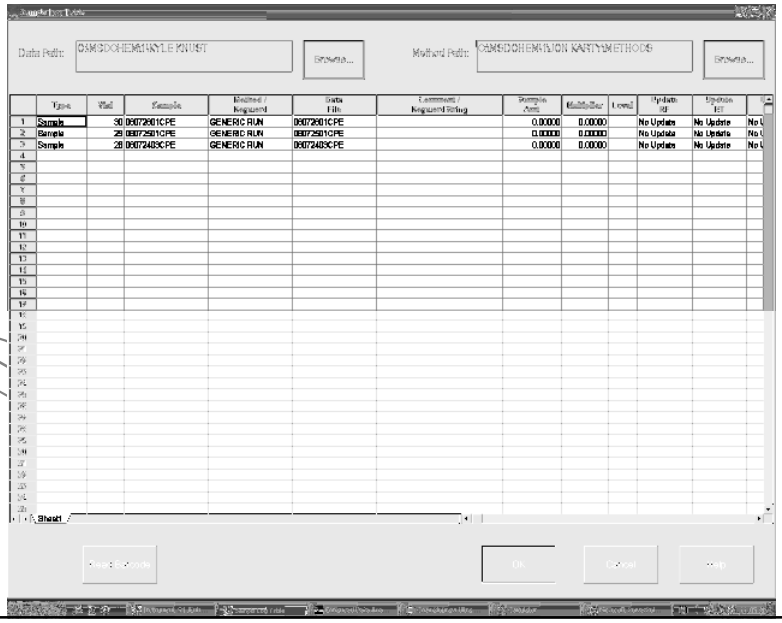
Programming a Sequence

- One of the more frustrating aspects of MSD ChemStation in an Open Access lab
- ChemStation allows 1 directory each for methods and data storage in a sequence
- Thus, all methods and data are initially stored in the Default/Methods and Default/Data directories
- Data and methods can be copied to user-specific directories after analysis
- Cut and paste don't work reliably in the sequence editor

General Sequence Instructions

- If no sequence running, load a method from the Default/Methods directory
- If no sequence currently running, load sequence “blank.s” from Default/Sequence directory
- All analyses are type “sample”
- Vial numbers must be 1-100
- Data file names must follow WinXP rules
  - Do not add .D (that gets added later by ChemStation)
  - Suggest using email/date-based names like jkarty_jul312008_001
- Select a method from the list obtained by double clicking in the method cell
- Sample name and comment can be left blank
- All fields to the right of comment are only for automated quantitation experiments

Sequence Screenshot



If a Sequence is Already Underway

- Click the “Edit” button in the box along the bottom of the Instrument Control Window
- Enter new samples to the sequence then click OK
- New samples are added the queue
- Remember, only 1 directory can be specified methods and/or data storage

Sequence Helpers

- “Fill and Increment”
  - Useful for file names and vial numbers
  - Will increment numbers in successive rows
    - vial numbers 1, 2, 3, 4
    - File names e.g. jak_001, jak_002, jak_003
  - Do not use in “method” column
  - Cannot skip a number
- “Fill and No Increment”
  - Useful for method names and sample types
  - Merely copies top entry into all successive cells

Finalizing a Sequence

- Click the OK icon when finished
- Click the Save Sequence icon
  - Give it a file name based on the date
    - Jul312008_a
  - DO NOT OVERWRITE BLANK.S
- Click the Validate Sequence icon
  - This is one last chance to make sure you entered everything correctly
  - Be sure “Run entire method” is checked
- If something is wrong, click Edit Sequence icon and fix it, the resave and validate

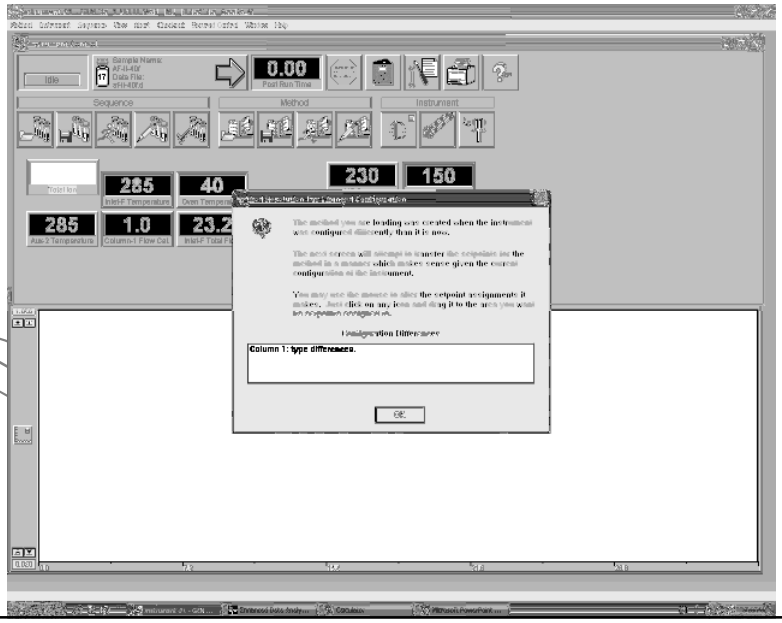
Running a Sequence

- Make sure autosampler wash vials A and B are filled with appropriate solvents
  - 4 mL vials for washing
  - Some have been labeled with solvent names
  - Fill the up to the neck
  - Wash A goes in “A11”, Wash B goes in “B8” on turret
- Make sure waste vials are in place
- When everything is OK, click Run Sequence icon
  - Be sure “Run Entire Method” is checked
  - Check “Overwrite existing files” at your own risk
  - However, if a file already exists, sequence will stop

Common Pop-up Messages

- Method Resolution for Changed Configuration
  - Usually column or oven max temperature
  - Review the method in the window that pops up
  - Make sure column in the method is the right chemistry and matches column installed on system
- Override solvent delay
  - Unless you injected solvent vapor to find the solvent delay, **ALWAYS ANSWER NO**
- Filename already exists
  - Change filename in sequence, rerun the row in question
  - Avoid this by using Validate Sequence and CAREFULLY checking the report

Method Resolution Screen



Override Solvent Delay Screen

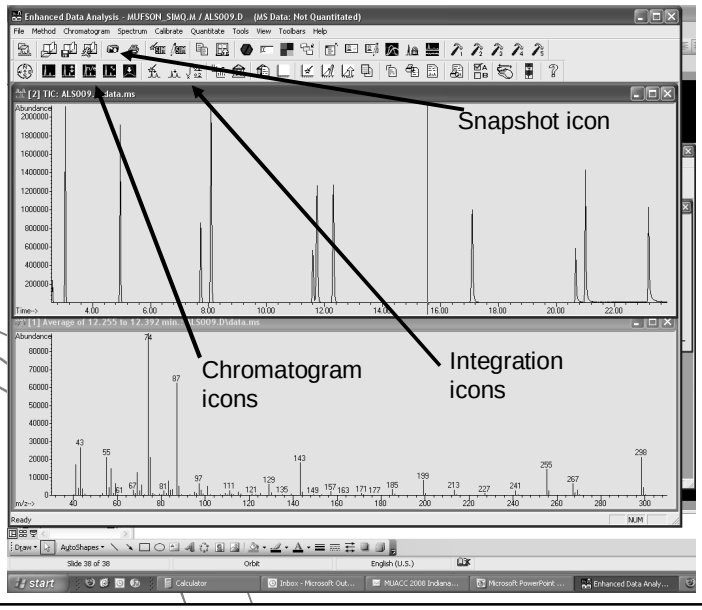
Analyzing Data

- Once recorded, data are processed in the Data Analysis
- The basics of using Data Analysis are covered in the document "How to access GC-MS data from outside the MSF and ChemStation Tutorial" on the MSF website
- NIST 02 library can be used for database comparisons of EI data
- Snapshot icon can be used to look at data currently being recorded

Data Analysis Icons

- The chromatogram icon at far left redraws the TIC
- The m/e icon allows one to create up to six extracted ion chromatograms
  - These plot intensity of a particular ion throughout the run
- Integration icons are used to compute peak areas
- Output can be printed either to paper (MSF2420) or pdf (deskpdf)
  - Printer must be specified as Windows default printer PRIOR to submitting the job

Data Analysis Screenshot



Walk-Up Training

- The GC-MS is so easy to use, training will be done as you bring up samples
- Make sure Angie or Jon is around to conduct the training
- Labmates ARE NOT authorized to train each other