GC-MS ChemStation Reference Guide

Last Updated July 2014
Overview

- GC-MS Instrumentation Description
- Sequence Programming
- Data Analysis
- Tips and Tricks
  - Method programming reference is for your enrichment
Policies In Brief

• Follow all signs/announcements
  – No matter how they inconvenience you

• ALL SAMPLES MUST BE LOGGED INTO THE ONLINE BILLING SYSTEM (on msf.chem.indiana.edu website)

• All samples should be run as a sequence
  – If you need manual injection, consult with MSF staff

• All methods and data must initially be stored in the “1” directory
  – Data is eventually archived in C:/msdchem/1/year/month

• Samples are regularly discarded unless we are told to save them in advance
  – Send email with sample location and filename to asorg@indiana.edu and jkarty@indiana.edu
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 to 1:1 split ratio or splitless injection
  – -50 °C to +450 °C temperatures, up to 20 °C/min ramp rate
    • Liquid CO₂ cryo option available
    • Make sure the column can handle the temperatures you specify

• Agilent 5973 inert mass selective detector
  – EI source (default) or CI source available
  – 10-800 m/z range
  – Positive (EI or CI) and negative ions (CI mode only) can be analyzed

• All instrument functions controlled by MSD Chemstation 1701E.02.02 software
Sample Guidelines

• Sample needs to be volatile or semi-volatile
  – All analytes MUST pass through a GC column
  – Good rule of thumb, under 300 Da
  – Extremely volatile samples (e.g. CFCs) need special methods or sub-ambient temperatures

• Sample should be 1-20 mg/L
  – More dilute samples 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 inlet liner and first few cm of column
  – Remove tetrabutyl ammonium salts, triphenylphosphine conjugates as these foul the inlet as well
Improving GC Peakshape

- Amines, alcohols, and acids have polar groups that can lead to poor GC peak shapes
- Conversion to ether, amide, ester derivatives improves volatility and reduces polar interactions
  - Suggest using straight BSTFA or BSTFA+TMCS (99:1)
    - Add reagent mix right to sample film
    - MSF has BSTFA+TMCS for purchase, buying one 0.1 mL kit counts as running one additional sample
    - 1 kit can derivatize 1 sample
  - Methylchloroformate in 0.1 M NaOH also works
Instrument Control Screenshot
Instrument Control Buttons

- Load
- Save
- Run
- Edit
- Validate

Sequence Icons

Method Icons
Programming a Sequence

• One of the more frustrating aspects of MSD ChemStation in an open access lab
• MSD 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
General Sequence Instructions

• If no sequence running,
  – Load a method from the Methods directory
  – Load sequence “0_blank.s” from Sequence directory

• All analyses are type “sample”

• Vial numbers must be 1-100

• Data file names must follow Windows 7 rules
  – Do not add .D (that gets added later by ChemStation)
  – Email/date-based names like jkarty_10jul14_01 work well
  – Email/notebook names like jkarty-2-117-a work well too
  – Total filename cannot exceed 38 characters
  – No “special” characters (@ * % ^ ! . , / ~ \ ”)
  – Be especially careful to avoid . (like jkarty_2.5) the .
    really confuses Chemstation

• **DO NOT CHANGE DATA or METHOD DIRECTORIES!!!!**
Sequence Instructions II

• Select a method from the list as follows
  – Right click on the cell in the “Method/Keyword” column
  – Select “Browse Methods” to pull up a list of available methods
  – Try the 18 minute method first, be nice to your colleagues
  – Use method labeled “FRONT” as these use the default Rxi-5Sil MS column. The Back injector is for special projects

• Sample name is a free text box for your use (special characters can go in it)

• Put your sample’s database number into the comment cell (new requirement 7/8/14)

• Each month, old data get moved to a directory named for the month and year they were recorded (e.g. July 2012)
  – C:/MSDChem/1/Year/Month
## Sample Log Table

<table>
<thead>
<tr>
<th>Type</th>
<th>Vial</th>
<th>Sample</th>
<th>Method / Keyword</th>
<th>Data File</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>99 test-mix-070814</td>
<td>00_BACK_30MIN_40_300_SPLT</td>
<td>test-mix-070814-03</td>
<td></td>
</tr>
</tbody>
</table>
Sequence Cell Right Click Options

<table>
<thead>
<tr>
<th>Type</th>
<th>Vial</th>
<th>Sample</th>
<th>Method / Keyword</th>
<th>Data File</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>test-mix-070814</td>
<td>00_BACK_30MIN_300_SPI</td>
<td>test-mix-070814-03</td>
</tr>
</tbody>
</table>

- **Browse for Method**
- **Copy**
- **Cut**
- **Paste**
- **Insert Row**
- **Delete Rows**
- **Fill Column & increment**
- **Fill Column, NO increment**
- **Repeat Row & increment**
- **Repeat Row, NO increment**
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 during a “fill”

• “Fill and No Increment”
  – Useful for method names and sample types
  – Merely copies top entry into all successive cells

• Access both by right-clicking on a group of cells
  – Fill values based on entry in top-most selected cell
Finalizing a Sequence

• Click the OK icon when finished
• Click the Save Sequence icon
  – Give it a file name based on the date
    • Jul312013_a
  – 0_blank.S is read only
• Click the Validate Sequence icon (helpful for large sample sets)
  – 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
  – Fill the up to the neck (well above blue line)
  – Wash A goes in “A11”, Wash B goes in “B8” on turret
  – Make sure wash solvent matches solvent your sample is dissolved in

• Make sure waste vials (WA5, WA3, WB3, WB2) are in place

• When everything is OK, click Run Sequence icon (looks like 3 vials with a guy running)
  – Click “Full Method”
  – Check “Overwrite existing files” at your own risk
    – However, if a file already exists, sequence will stop
  – Click “Run Sequence” to start the instrument
Run Sequence Screen Shot
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 to the queue
• **THE SEQUENCE WILL NOT ADVANCE IF THE SAMPLE TABLE IS OPEN!!!!**
Sequence Running Screenshot
Common Pop-up Messages

• 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
Analyzing Data

• Once recorded, data are processed in the Data Analysis
• NIST 11 library can be used for database comparisons of EI data
• Snapshot icon can be used to look at data as it is being recorded
• Quantification can be done by integrating GC-MS data
Data Analysis Screenshot

Chromatogram

Mass Spectrum

Window Number
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 m/z throughout the run
• Integration icons are used to compute peak areas
• Output can be printed either to paper (Xerox Phaser 3250) or pdf (deskpdf)
  – Printer must be specified as Windows default printer PRIOR to submitting the job
  – Set default printer from the “Printers and Faxes” on the start bar
    – Right click on desired printer and select “Set as Default Printer”
• Hold mouse over any icon to learn its function
Data Analysis Icons Screenshot

- Take snapshot
- Adjust y-axis scaling
- Redraw TIC
- Create Extracted Ion Chromatograms
- Auto Integrate

Enhanced Data Analysis - DEFAULT.M / TEST-MIX-070814-03.D (MS Data: Not)

File Method Reprocessing Chromatogram Spectrum Calibrate Quantita
Navigating in Data Analysis

• Left mouse button is used to zoom in on data
  – Whatever is in the box fills the window after zooming
  – Double left click to zoom back out

• Right mouse button is used to extract mass spectra
  – I suggest extracting spectra from the inside of a peak to avoid background noise
  – Output is the average of the spectra selected
  – Double right click on chromatogram to extract a single spectrum
Data Zooming Example

Whatever is inside the zoom box will be what is shown after zooming in. Here, I selected the middle of 2 peaks, and those peaks are cutoff in the new view. Thus, be careful when zooming.
Auto Integration Example

Unless you are using your own method, answer “No: to the “Save Autointegration parameters to Method” question.
Extracting a Mass Spectrum

Spectrum is the average of all spectra in the region of retention time selected and this is shown in the title of the window.
Database Searching

• The MSF has a license for the NIST ‘11 library of over 240,000 EI mass spectra

• You can search your data against the library by double right clicking in the spectrum window
  – Be sure to have NIST’11 library selected
    • Under “Spectrum ➔ Select Library” menu
  – Dr. K often finds scores < 80 to be dubious
Select Library
NIST ‘11 Library Output

Your Data

Selected Library Spectrum
Library Matches on a Chromatogram
Library Labeled Peaks

Abundance

TIC: test-mix-070814-03.D.data.ms

Score

Top Match

2-Octanone: 91
Decane: 95
Octyl chloroformate: 91
Undecane: 95
Phenol, 2,6-dimethyl-: 97
Undecane, 2,5-dimethyl-: 95
Dodecane: 96
Tridecane: 97

Score

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2-Octanone: 91
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Integration and Quantitation

• Extract a single m/z using the m/e icon
• Autointegrate the resulting spectrum
  – Do NOT save integration parameters
• Click “Integration Results” on the Chromatogram menu to get the output
• Clicking Copy allows you to paste the integration data into Excel
• Data can be integrated manually as well
  – See Dr. Karty about this
Integration Results
Integration Output

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret Time</th>
<th>Type</th>
<th>Width</th>
<th>Area</th>
<th>Start Time</th>
<th>End Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.514</td>
<td>BB</td>
<td>0.021</td>
<td>49590249</td>
<td>6.476</td>
<td>6.573</td>
</tr>
<tr>
<td>2</td>
<td>6.675</td>
<td>BB</td>
<td>0.020</td>
<td>44620864</td>
<td>6.634</td>
<td>6.718</td>
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<tr>
<td>3</td>
<td>7.663</td>
<td>BB</td>
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<td>51716030</td>
<td>7.611</td>
<td>7.752</td>
</tr>
<tr>
<td>4</td>
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<tr>
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<td>7</td>
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<tr>
<td>8</td>
<td>10.583</td>
<td>BB</td>
<td>0.022</td>
<td>77753005</td>
<td>10.533</td>
<td>10.616</td>
</tr>
</tbody>
</table>
# Results Copied and Pasted in Excel

TIC: test-mix-070814-03.D\data.ms
test-mix-070814

<table>
<thead>
<tr>
<th>Peak #</th>
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<td>0.024</td>
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<td>10.616</td>
</tr>
</tbody>
</table>
Quantitation Guidelines

• Each molecule has its own ionization efficiency
  – Like molar absorption constant in UV-VIS
  – The more different two molecules are, the wider the variance

• Ideally, a 5 point calibration curve is made using the compound in question as the standard
  – Alternatively, a second compound can be added to the sample as an internal standard prior to analysis
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 trains you
  – Labmates ARE NOT authorized to train each other
• Walk-up GC-MS costs about $\frac{1}{2}$ what staff-run GC-MS rates are
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
Advanced Method Modification

This is for reference only, do not change methods without additional training by Jon Karty or Angie Hansen
A Few Concepts to Remember

• Temps above 285 °C accelerate septum and column degradation
  – Initial temp should be at least 10 °C above bp of solvent, if possible

• Solvent delay protects the source from high heat capacity solvent peak
  – Large amounts of solvent are introduced during injection
    • CH₂Cl₂ is 15.6 M and 1327 mg/mL with itself
    • Cₚ and thermal conductivity of solvent vapors differ significantly from He
  – Rapid cooling of EI filament by solvent vapor degrades it
  – Large amount of solvent ions foul interior of the MS
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
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
  - 3 or 4 washes are usually adequate
  - Be sure to use pre and post-injection washes
- Pumps flush the syringe with the sample
  - Again, 3 or 4 pumps are usually adequate
Inlets Tab

• Only use front inlet and He gas
• Split mode is good for most samples (50:1 ratio to start)
• 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 °C
• 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

• Always specify:
  – Column and inlet:
    • Column 1 uses front inlet, column 2 uses back inlet
  – Detector: MSD
  – Outlet PSI: Vacuum

• Flow rate should be 1-1.5 mL/min for 0.25 mm column
  – Make sure mode is “constant flow”
  – Make sure BOTH columns have at least 0.7 mL/min flowing through them

• Ramping of flow rate is not commonly used
Oven Temp

Plot...

Injector | Valves | Inlets | Columns | Oven | Detectors | Signals | Aux | Runtime | Options

Activated

- Column
  - Mode: Const Flow
  - Inlet: Front
  - Detector: MSD
  - Outlet psi: Vacuum

- He Flow
  - Setpoint: 1.3 ml/min
  - Actual: 1.3 ml/min
  - Pressure: 10.12 psi / 10.06 psi

Installed Column
- Inventory#: 1133739
- (not calibrated)
- Manufacturer's Specifications
  - Model No: Restek Rxi-5SilMS
  - 350°C Max
  - Capillary: 30.0 m x 250 μm x 0.25 μm nominal

Flow Table:

<table>
<thead>
<tr>
<th>Flow</th>
<th>ml/min²</th>
<th>ml/min</th>
<th>Hold min</th>
<th>Run Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td></td>
<td>1.3</td>
<td>1.3</td>
<td>30.00</td>
</tr>
<tr>
<td>Ramp 1</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Ramp 2</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Ramp 3</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Post Run</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>30.00</td>
</tr>
</tbody>
</table>

Notes:
- Change...
- Apply
- OK
- Cancel
- Help
Oven Tab

• Setpoint is the temperature at start of run
• °C/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
• See Dr. Karty about cryo cooling options
• 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
**Aux Channel**

- Thermal Aux #1
- Thermal Aux #2
- Pres Aux #3
- Pres Aux #4
- Pres Aux #5

**Heater**

- On
- Setpoint: 300 °C
- Actual: 300 °C

**Type**

- Valve Box
- MSD
- AED
- Nickel Catalyst
- Unknown

**Ramps**

<table>
<thead>
<tr>
<th>Ramp</th>
<th>°C/min</th>
<th>Next °C</th>
<th>Hold min</th>
<th>Run Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.00</td>
<td>300</td>
<td>0.00</td>
<td>30.00</td>
</tr>
<tr>
<td>Ramp 1</td>
<td>0.00</td>
<td>300</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Ramp 2</td>
<td>0.00</td>
<td>300</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Ramp 3</td>
<td>0.00</td>
<td>300</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
GC Method Hints

• Try to keep runs under 40 minutes
• Try to include 5-10 minutes at the end of a run @ 250-300 °C to clean the column
• Initial temps below 40 °C can take a long time to reach
  – Try to have initial temperature as high as possible for fast run times
  – If low temps are necessary, consider CO₂ cryo cooling
• Inject solvent vapor when making a new method to figure solvent delay
• If two peaks won’t separate, try lowering the temperature ramp rate
  – Isothermal steps/methods 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 gain is usually set to 1.00
• 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**
  – Improves sensitivity by up to 50-fold
  – 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
Setting the Mass Range

Low to High mass range must be in ascending order from 1.60 - 800.00.
Setting Threshold and Sampling

### Edit Scan Parameters

<table>
<thead>
<tr>
<th>Scanning Mass Range</th>
<th>Threshold and Sampling Rates</th>
<th>Plotting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Threshold (counts)</td>
<td>Sampling Rate (2^n)</td>
</tr>
<tr>
<td>Scan Group 1</td>
<td>500</td>
<td>2</td>
</tr>
<tr>
<td>Scan Group 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scan Group 3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Summary of Settings

<table>
<thead>
<tr>
<th>Group</th>
<th>Start Time</th>
<th>Low Mass</th>
<th>High Mass</th>
<th>Threshold</th>
<th>Samples</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.75</td>
<td>40.00</td>
<td>500.00</td>
<td>500</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Any mass abundances falling below this value will be ignored... valid values are 0 - 99999.