Training on the Agilent Open Access GC/MS

Questions?

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Analytes suitable for GC/MS

- In general, GC/MS is used to analyze non-polar compounds soluble in non-polar solvents such as the following:
  - Hexane, Ethyl Acetate, Methanol, Acetonitrile, Acetone
  - High-boiling solvents should be used only with appropriate methods
  - **Pyridine and Chlorinated Solvents MUST be avoided.
- The compound(s) to be analyzed should be both volatile and chemically stable at the operation temperature of the injector (280° Celsius)
  - Thermally labile compounds may be pyrolyzed at the max temperature of injector and/or column (320° Celsius)
- Water solutions, and organic solutions at pH lower than 2 and greater than 8 are NOT allowed for GC/MS analysis, as they may damage the column

Sample Preparation

- Concentration of the analyte: 1 ng/uL
- The solvent used MUST completely dissolve your compound – no cloudy solutions or precipitate!
- Use 2 mL autosampler vials: Agilent PN 5182-0553
  - Recommended minimum sample volume is 500 uL
- For limited volume samples, use 300 uL inserts: MicroSolv PN 9502S-02CP
  - Minimum sample volume using inserts is 30 uL

Instrument Prep Checklist

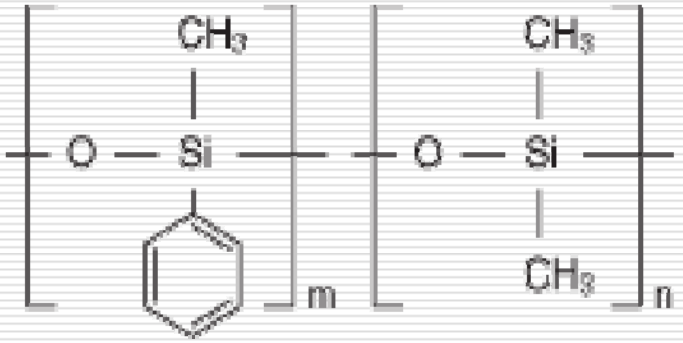
- Make sure the solvent vials have enough solvent as indicated by the blue line on the vial.
- Solvent A: turret position 11
  Acetonitrile, b.p. 82° C
- Solvent B: turret position 8
  Ethyl Acetate, b.p. 77° C
- Waste: positions W, A5
  vials present and empty

Solvents are stored under the fume hood; glass pipets are attached to the bottles

GC-MS Column

- All methods use a capillary column with 3 uL of stationary phase HP-5, (5%-phenyl)-methylpolysiloxane which is equivalent to USP Phase G27
- Column dimensions: length 30 m, ID 250 um, film thickness 25 um
- More application information is available online at

http://www.chem.agilent.com/en-US/Products/consumables/columns/gcandgc-ms/jwhp-5/pages/gp42676.aspx



Methods

Open Access GC/MS

GC/MS Methods

- General survey method
  - **SUMS_Standard** (1 ng/uL) (methanol, hexane)
- Focus on compounds by boiling point
  - **SUMS_HighBP** (heptane, iso-octane, toluene)
  - **SUMS_MedBP** (ethyl acetate, acetonitrile, acetone)
  - **SUMS_LowBP** (methanol, pentane, hexane)
- Higher sensitivity methods – same temperature program as Standard method
  - **SUMS_StdMedSens** (0.5 ng/uL)
  - **SUMS_StdHighSens** (0.1 ng/uL)

• ***Method_Name*** *(recommended solvents or concentrations)*
• *All methods inject 1 uL of sample*

Standard

Methods

- SUMS_Standard — Split ratio 1:100
  SUMS_StdMedSens — Split ratio 1:50
  SUMS_StdHighSens — Split ratio 1:25

- Oven Temperature
  Start: 35° C, hold for 3.75 min
  Ramp: 20° C/min from 35° C to 320° C
  End: 320° C, hold for 7 min

- Injector Temperature 280° C

High Boiling Point Methods

- SUMS_HighBP Split ratio 1:100

- Oven Temperature

Start:	75° C, hold for 2 min
1st Ramp:	40° C/min from 75° C to 120° C
Isotherm:	120° C, hold for 5 min
2nd Ramp:	20° C/min from 120° C to 320° C
End:	320° C, hold for 7 min

- Injector Temperature 280° C

Medium Boiling Point Methods

- SUMS_MedBP Split ratio 1:100

- Oven Temperature

Start:	50° C, hold for 2.00 min
1st Ramp:	30° C/min from 75° C to 100° C
Isotherm:	100° C, hold for 4 min
2nd Ramp:	20° C/min from 100 C to 225 C
Isotherm:	225° C, hold for 1.7 min
3rd Ramp:	40° C/min from 225° C to 320° C
End:	320° C, hold for 7 min

- Injector Temperature 230° C

Low Boiling Point Methods

- SUMS_LowBP Split ratio 1:100

- Oven Temperature

Start:	35° C, hold for 5 min
1st Ramp:	20° C/min from 35° C to 180° C
Isotherm:	180° C, hold for 2.25 min
2nd Ramp:	40° C/min from 180° C to 320° C
End:	320° C, hold for 7 min

- Injector Temperature 180° C

Data Acquisition

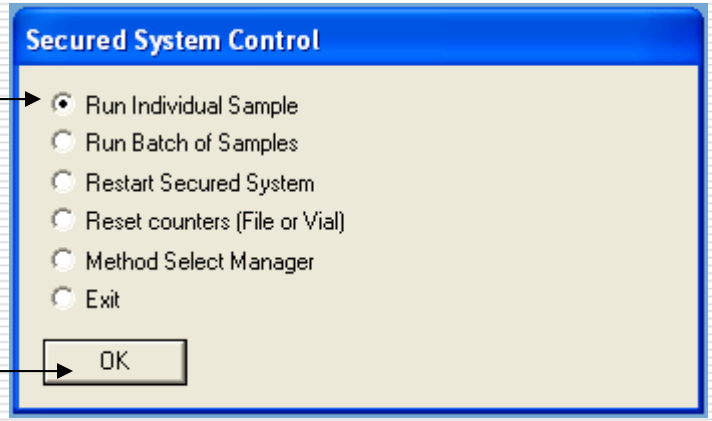
Queuing an Individual Sample: Start with “Queue Mode Enabled”

Click the “Queue Mode Enabled” button at the bottom of the screen

The “Secured System Control” Window will appear

Choose
1) Run Individual Sample

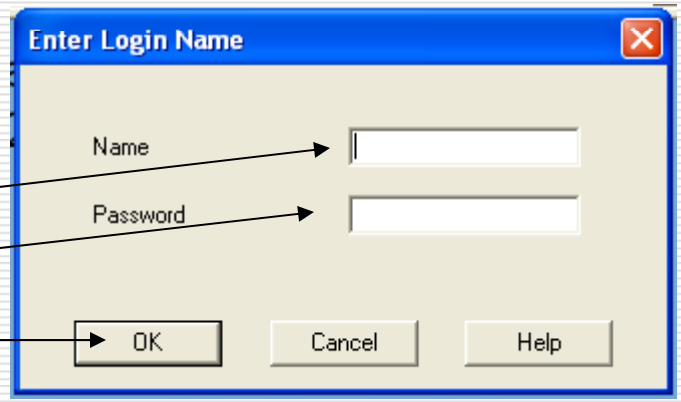
2) Click “OK”



Individual Sample:
Log in, choose a “method”

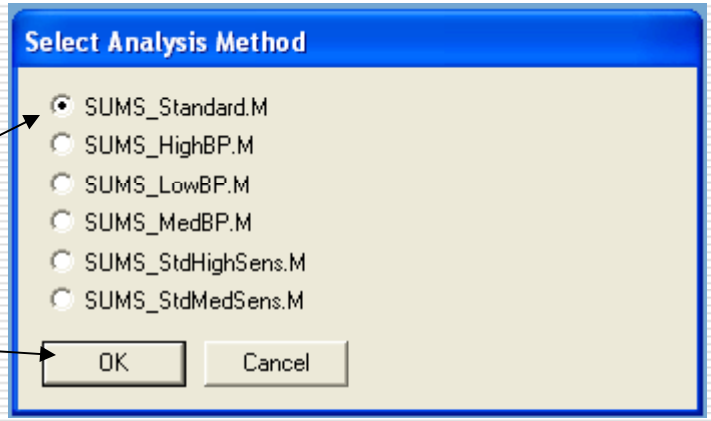
The “Enter Login Name” Window will appear

1) Enter your “e-mail” ID
2) Enter your “Password”
3) Click “OK”



The “Select Analysis Method” Window will appear

1) Choose one method
2) Click “OK”



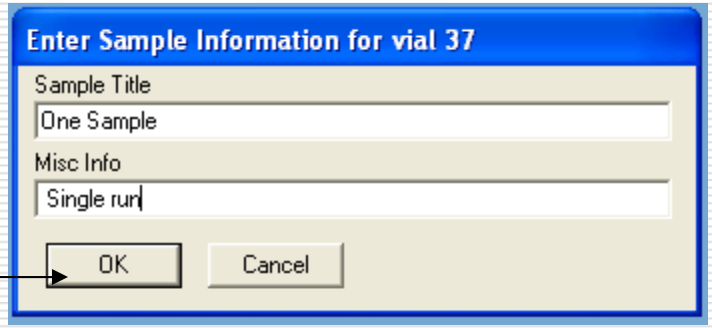
Individual Sample:
Submit the Sample

The “MDS ChemStation ”
Window will appear

Display the Vial position
Place your vial in the position #
Do not overwrite the position
1) Click “OK”

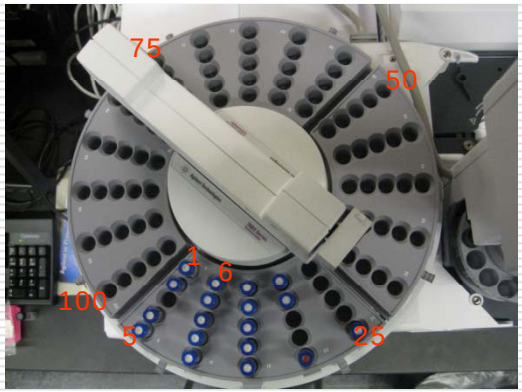
The “Sample Information”
Window will appear

1) Enter your “text” Sample Title
2) Enter your “text” Information
3) Click “OK”

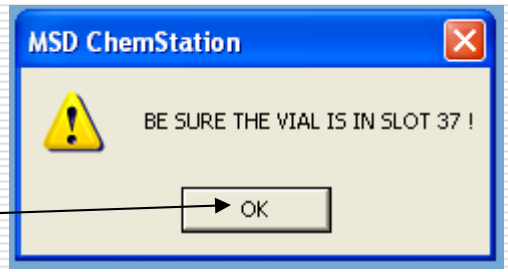


Individual Sample:
Submit the Sample

The “MSD ChemStation ” window will remind you of the vial position:

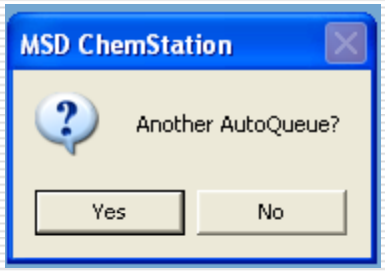


Your Vial should be in position #
Be sure your vial is in position #



1) Click “OK”

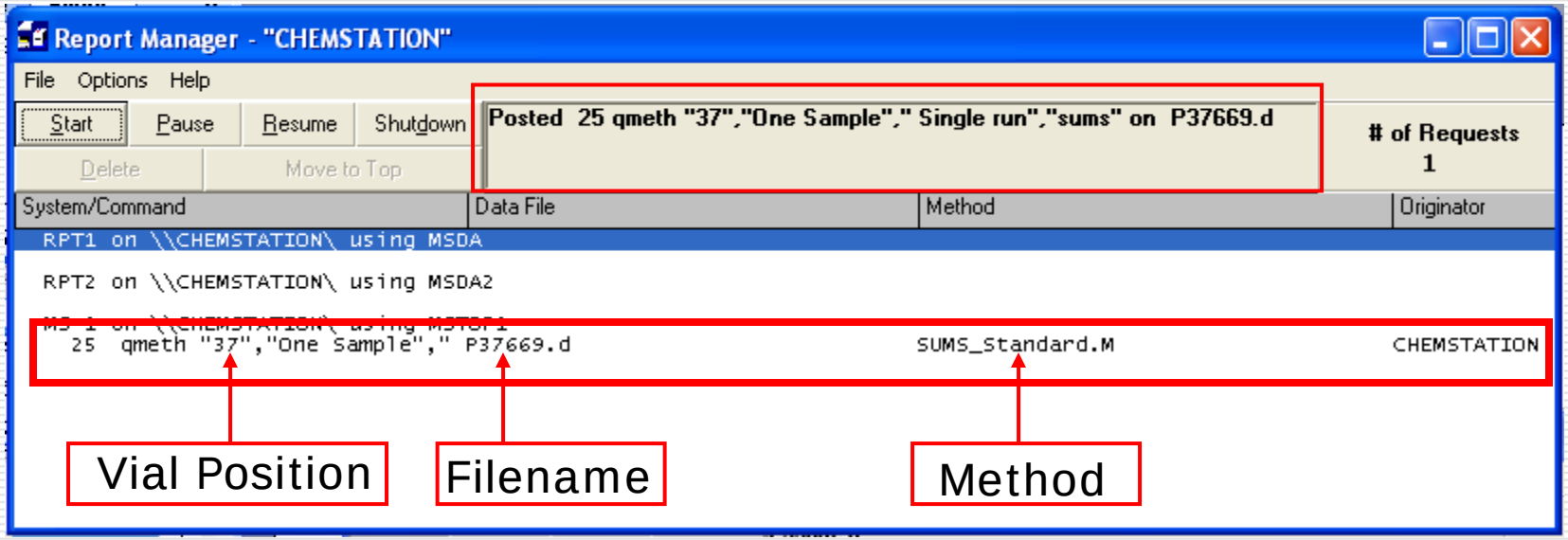
If you need to submit another sample:
Click “Yes”, re-start from slide #13
(no login needed); otherwise Click “No”



Individual Sample:
Verify Sample Submission

After you submit the sample request and your sample vial has been placed in the assigned autosampler position:

- Click on “Report Manager” and verify that your sample posted



- Important! Write the Filename in your lab notebook

Individual Sample:
Important reminders

- Never close windows on the instrument control computer! Only minimize them
  - Closing/exiting Chemstation windows will stall the queue and aggravate your colleagues

- All queued analyses are posted in the “Report Manager
  - Never remove a vial in a position which is still listed in the “Report Manager”
  - If a vial position is not listed in the “Report Manager”, a vial present in that position may be replaced with your sample vial

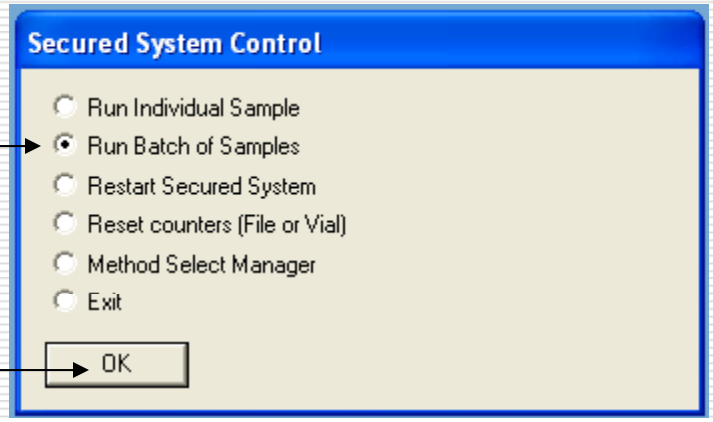
Queuing a Batch of Samples: Start with “Queue Mode Enabled”

Click at the bottom of the screen the “Queue Mode Enabled”

The “Secured System Control” Window will appear

Choose
1) Run Batch of Samples

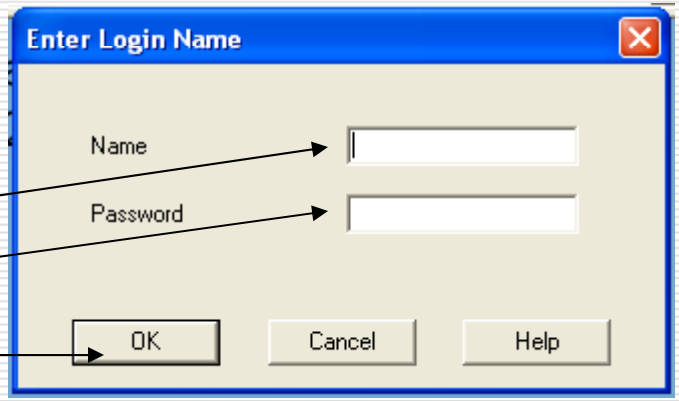
2) Click “OK”



Batch of Samples:
Choose the "method" only one method per batch

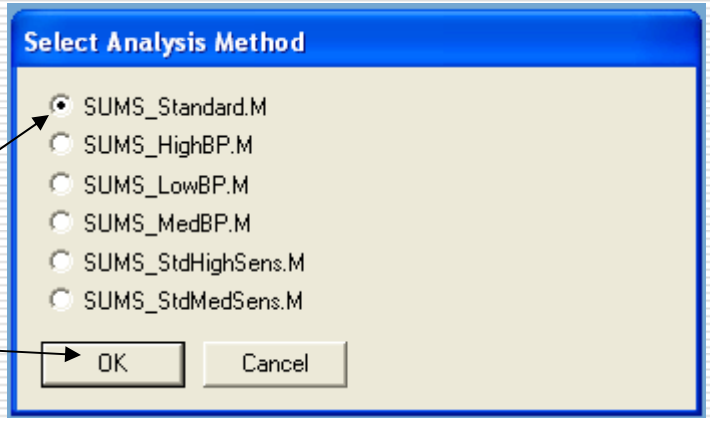
The "Enter Login Name" Window will appear

1) Enter your "e-mail" ID
2) Enter your "Password"
3) Click "OK"



The "Select Analysis Method" Window will appear

1) Choose only one method
2) Click "OK"



If you need to use other methods, repeat from slide #19 for each method (no re-login required)

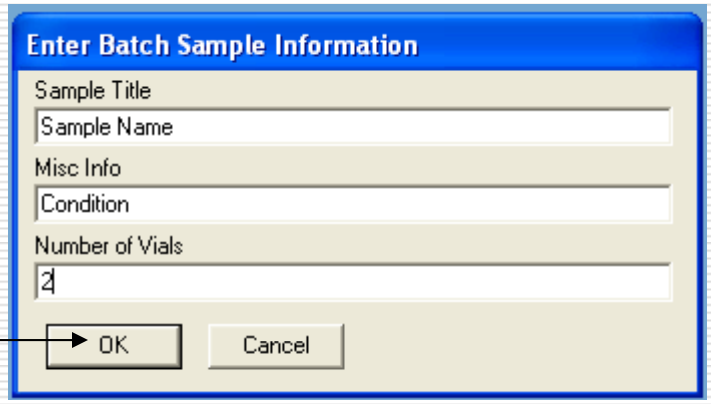
Batch of Samples:
Submit the Samples

The “MDS ChemStation” window will appear

Display the First Vial position
Place your vial in the position indicated
1) Click “OK”

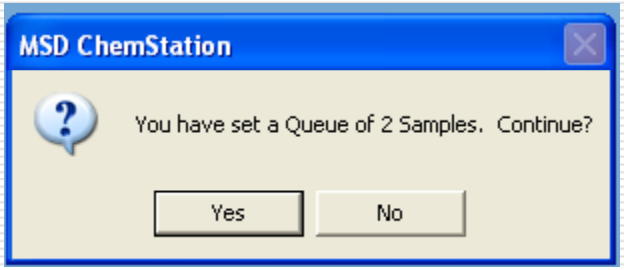
The “Batch Sample Information”
window will appear

1) Enter your “text” Sample Title
2) Enter your “text” Information
3) Enter the number of vials
4) Click “OK”



Batch of Samples:
Submit the Sample

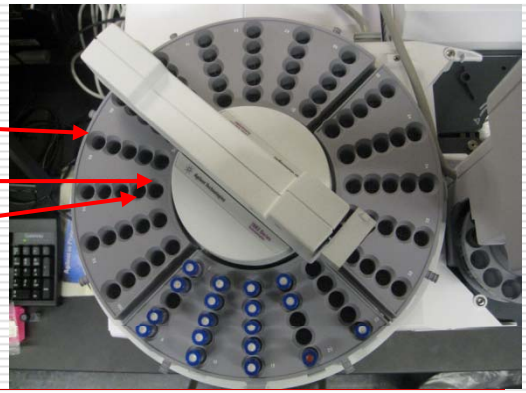
The “MDS ChemStation” window will reiterate the number of samples – click “Yes” or “No” as appropriate



Confirm the position of the first vial, – click“OK”

Vial positions run radially from the interior to the perimeter of the vial tray

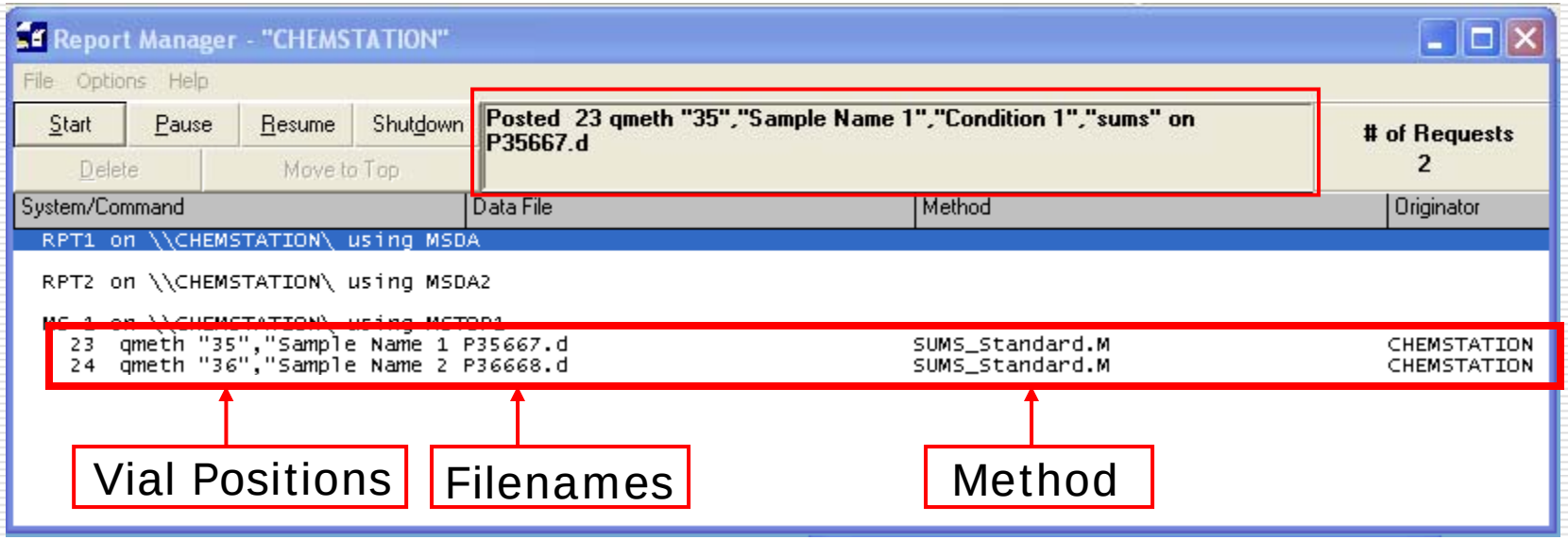
Example:
positions #, #+1, #+2



Batch of Samples:
Verify Sample Submission

After you submit the batch sample request and your sample vials have been placed in the assigned autosampler positions:

- Click on “Report Manager” and verify that your samples posted



- Important! Write the Filenames in your lab notebook

Batch of Samples:
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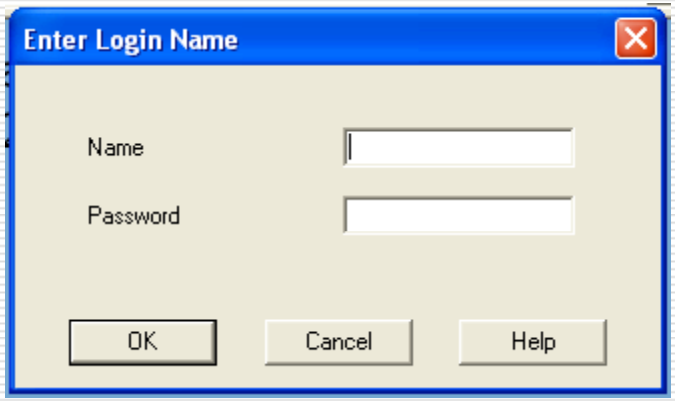
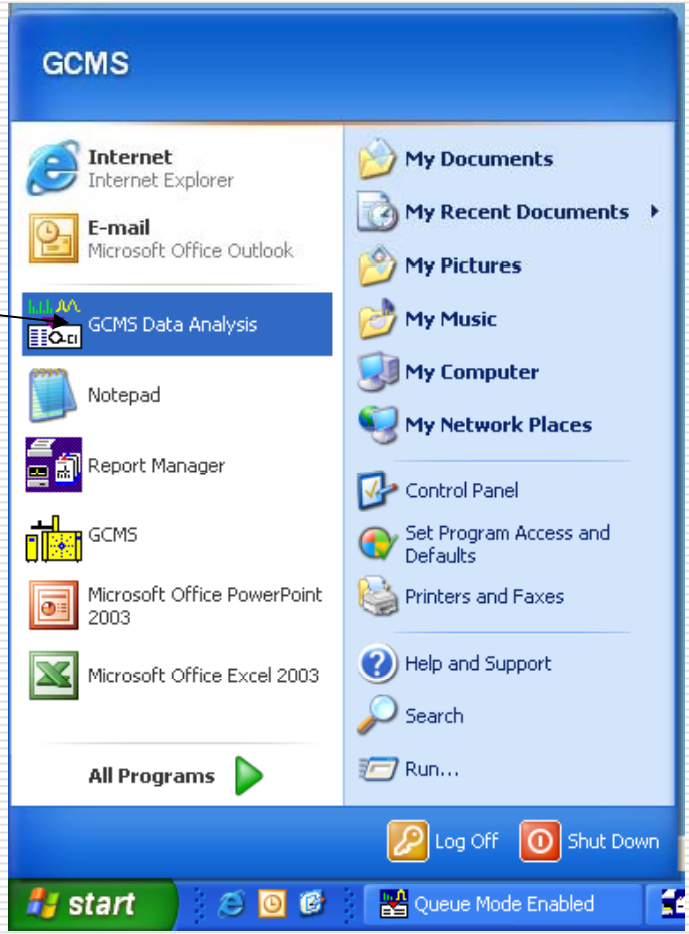
Data Analysis

Enhanced Data Analysis

- Windows “start” menu
  (if data analysis module is not already open)
- Choose “GCMS Data Analysis”
- Enter “Name” and “Password”

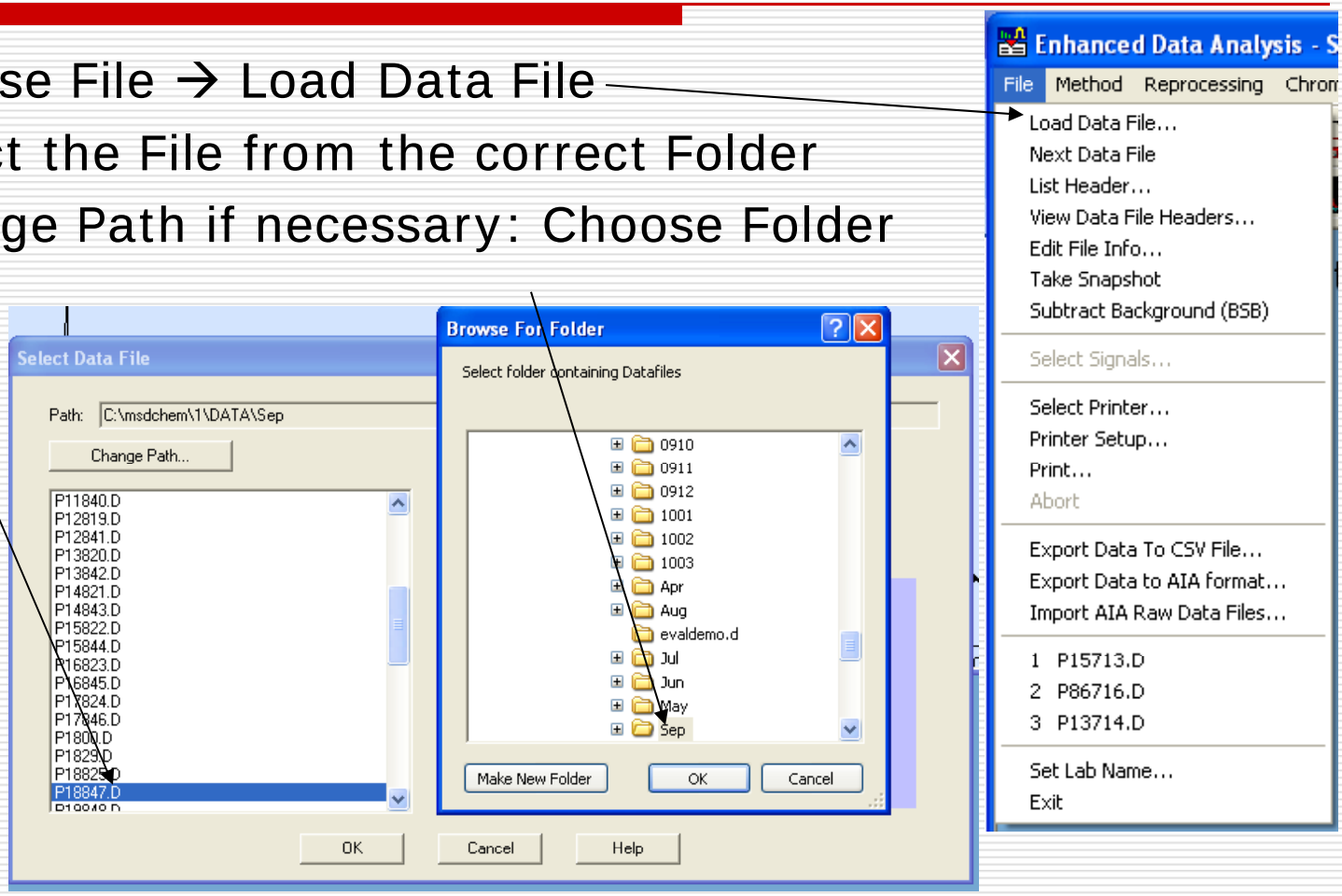




- Click “OK”

Open a File

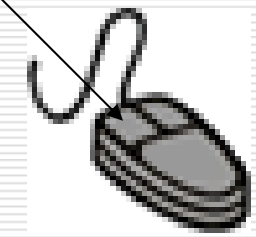
- Choose File → Load Data File
- Select the File from the correct Folder
  Change Path if necessary: Choose Folder "OK"
  and File "OK"

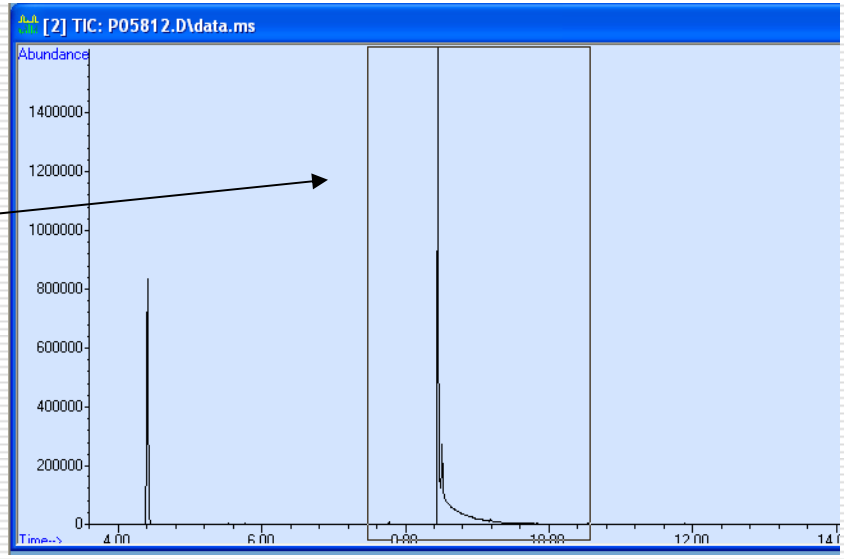


Total Ion Chromatogram

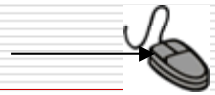
- When the file is opened in Enhanced Data Analysis, the Total Ion Chromatogram (TIC) is displayed
- To zoom in on a GC peak:

Click and drag the left mouse button to draw a box around the area of interest





- A double left click on the mouse will zoom back out to the original display



Viewing Mass Spectra

Every point on the chromatogram is associated with a mass spectrum

- To display a mass spectrum at a particular time on the TIC:
  - Position the cursor on the Chromatogram at the specific time
  - Double right click
  - The mass spectrum at that point in time will appear in a separate window

- To display an averaged MS across a chromatographic peak:
  - Right click and drag across the time range of interest

- You can toggle between windows just by clicking on them
- The active window will display a vivid blue bar at the top

Working with Mass Spectra

- To zoom in: left click and drag
- To zoom out: double left click

- The upper mass range limit on the mass spectrum is the highest mass detected during the scan
  - Thus, even though the mass spectrometer is set to scan to the upper limit (550 Da), the x-axis of the MS often does not display up to 550.

- To initiate a library search:
  - Double right click on the displayed mass spectrum
  - “Wiley275” should be selected as the library; this setting is in the “select library” option in the “Spectrum” menu

Integration

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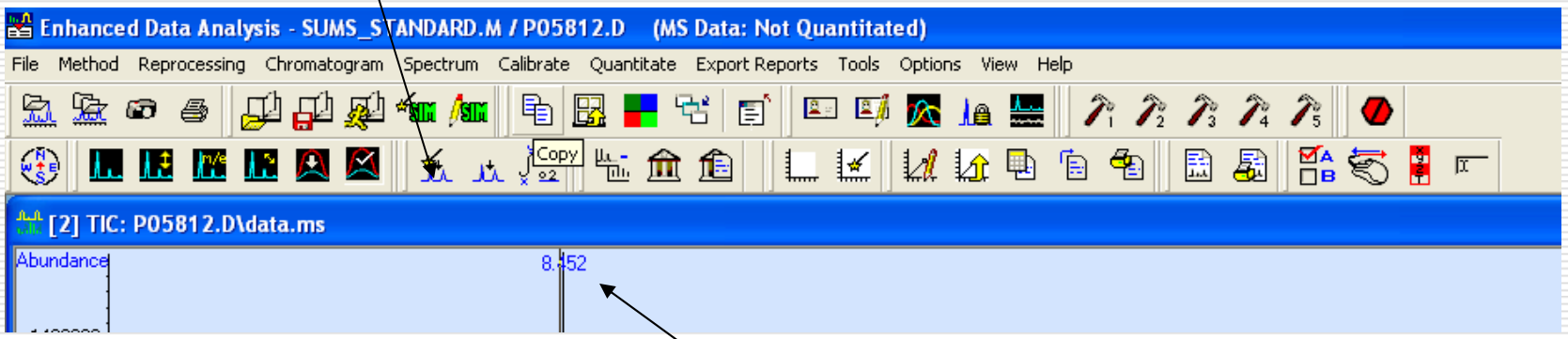
Integration of GC peaks

- Chromatogram integration calculates the area under the curve of the observed GC/MS peaks
- Areas for the same compound analyzed under the same experimental condition may be related to the concentration of compound in the sample.
- ****Appropriate controls, standards, and calibration curves should be used when quantitative measurements are desired.*
- Observed areas may be affected by sample matrix effects

Automatic Integration

- Click on the Auto Integration button



- In the Chromatogram window, the retention time of the integrated peaks will turn blue.
- A blue line across the base of each integrated peak indicates the baseline.

- To generate a list of peak retention times and areas:
  - Select Integration Results from the Chromatogram menu
  - A Tabulated window will appear with Area of integrated peaks

Data Reporting

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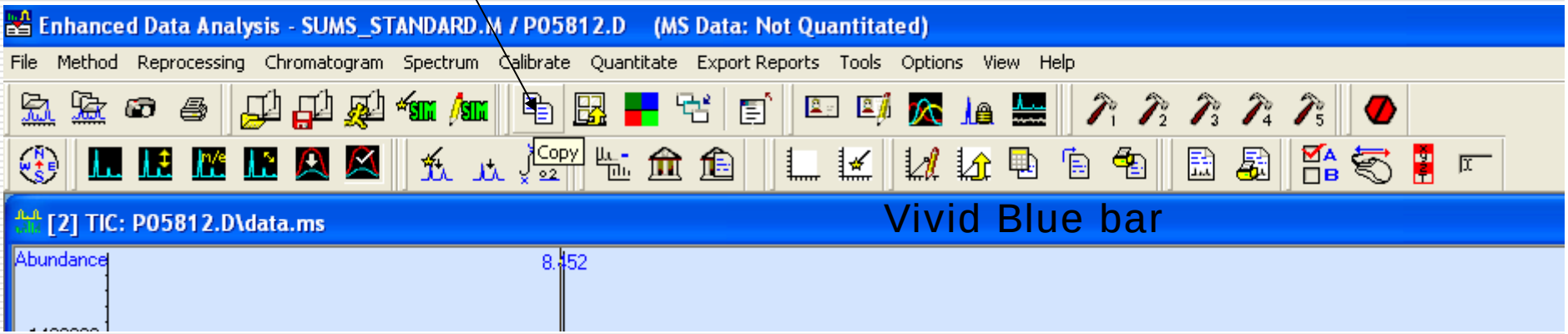
Datafile info

- Files are located in the folder C:\msdchem\1\DATA\"month" where "month" is the 3-letter month abbreviation.
  - Older files are in folders named "YYMM", e.g. 0912 for Dec. 2009
- Each GC-MS data filename begins with the letter "P"; the Windows extension is ".D"
  - The data "file" is actually a folder containing the files and results for each analysis.
- Users are responsible for backing up their own data. Data files are deleted periodically as needed to free up space on the hard drive.
- Data files and data report files can be easily transferred using a flash drive (USB2)

“Exporting” Data Views

- Select the window view that you would like to copy
- Click the copy button on the tool bar menu of the Enhanced Data Analysis



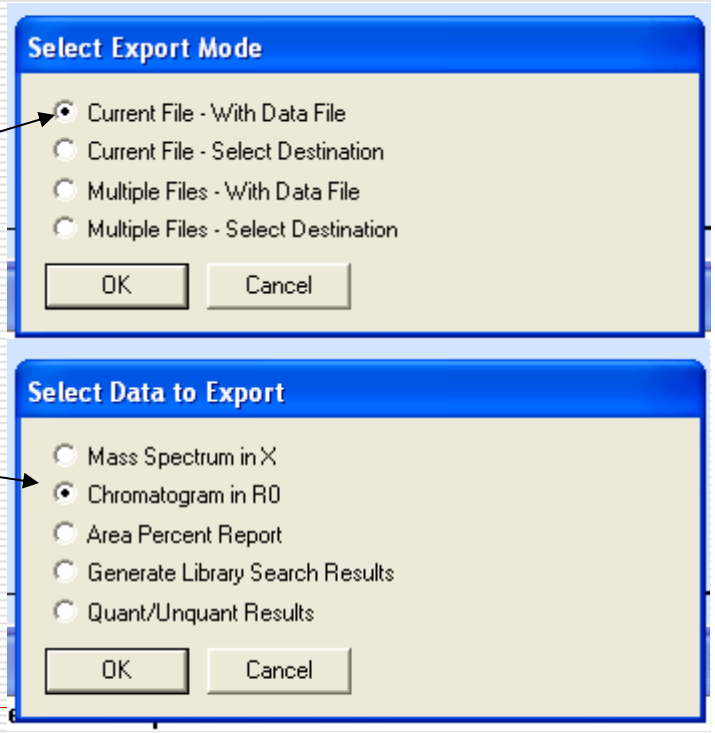
- Paste the clipboard on the report data file document of your choice, Office (Excel/PowerPoint/Word)

Printing Data Views

- Select “Print” from the file menu
- Either or both the TIC and MS windows may be printed.
- To generate .pdf files, select “Adobe PDF” as the printer.
- When printing hard copies, ensure that the blue ethernet cable is connected (the cables to connect are located to the right of the keyboard)
- It is good practice to generate electronic data reports, rather than printing hard copies which consume paper and are easily lost.

Exporting CSV Files

- CSV Files are comma separated value files that can be opened in Excel and saved as Excel files
- From the toolbar of the Enhanced Data Analysis window Choose: File → Export Data to CSV File
- On the Select Export Mode Window Select: Current File – With Data File
- Select the Data to export:
  Mass Spectrum
  TIC Chromatogram
  Area Percent Report (needs peak Integration)
- "OK" will generate the report data file in the datafile folder
  (see slide 32 for Windows file path)



If that doesn't work…

- Go ask Allis.
  - allis@stanford.edu
  - 650.723.0710

Copies of this training material may be downloaded from the SUMS website at

http://mass-spec.stanford.edu/Instruments-OpenAccess.html

Thanks to Maurizio Splendore for preparing version 1.0 of this guide, and Pavel Aronov for v 1.1.