Running Samples

Michael H. Hiatt
U.S. Environmental Protection Agency
National Exposure Research Laboratory
Environmental Sciences Division
P.O. Box 93478, Las Vegas, Nevada 89193-3478

Topics for Running Samples

- Preparing standards and samples for distillation
- Setting up the Sequence for batch analyses
- Setting up a calibration curve
- Reviewing the vacuum distillation log files
- Decontamination procedures

Preparing standards and samples for distillation

- The following slides are meant as a guide.
- Standard solutions and sources may vary at user's discretion (more sensitive systems may use less concentrated materials or spike smaller aliquots into samples)
- Concentrations of individual compounds contained in stock solutions may be varied to meet user needs

Preparing Surrogates

- Spiking solution is prepared from a stock surrogate mix (Supelco Custom Mix #20323485 or equivalent**)**
- Add 10uL of the stock surrogate mix is added to 990 uL methanol in an 1.4 mL septum vial
- The resultant solution is 50 to 2500 ngs/uL, pyridine-d5 being the more concentrated
- **note: the relative concentrations of the components may be made different to accommodate different system sensitivities**
- Store solutions in volatile organics standard refrigerator

Preparing Standards

- Spiking solution is prepared from a stock “75 compound mix” (Supelco Custom Mix # 20323943 or equivalent), Nitrogen VOC Mix (Supelco Custom Mix # 20323943 or equivalent), and the volatile organics “gases” mix
- 50uL of the stock “75 compound mix”, 50uL of the stock “Nitrogen VOC Mix ”, and 25uL of the gases mix are added to 875 uL methanol in an 1.4 mL septum vial
- The resultant solution ranges from 50 to 1000 ngs/uL **note: the relative concentrations of the components may be made different to accommodate different sensitivities**
- Store solutions in volatile organics standard refrigerator

Preparing Sample Vessels

- Samples are put into 100 mL round bottom pyrex vessels that have a size 15 o-ring connector
- Vessels are attached to apparatus with a Viton o-ring, and o-ring clamp
- Glassware for method 8261 analyses should be cleaned in accordance with laboratories Volatile Organic Analyses procedures, including methanol rinse and oven drying
- Viton o-rings should be rinsed with methanol, then soaked in a methanol bath overnight, and finally dried in the glassware oven

Introducing Sample Aliquots, Spiking, and

- Tare sample vessel and add sample (normally 5 mL water or 5 g soil)
- Record sample size (method 8261 performs quantitation based upon actual sample size)
- Remove any material in o-ring groove
- Spike sample with surrogate mixture (5 uL of surrogate solution). Spike can be introduced directly into sample or at boundary between glass vessel and sample

Sample Vessel

Attaching Sample Vessel

- Inspect o-ring and o-ring connectors and remove any matter that could break seal
- Put clean o-ring in sample vessel connector grove and clamp sample vessel to autosampler port.
- Tighten clamp so that vessel is firmly in place. Sample vessel can still be turned while attached
- The vacuum distillation log (later topic) can be inspected after a distillation to identify an improper seal

Attached Sample Vessel

Setting up the GC/MS

- Set up the GC/MS method for acquisition of the vacuum distillate as described in the following slide
- Be sure the GC/MS is in the slave mode as the vacuum distiller will send the start signal when the GC/MS is ready
- If multiple runs are being distilled in batch mode the GC/MS acquisitions must be in batch mode
- After chromatography conditions are optimized vacuum distiller and GC/MS cycle times (time between samples) should be close
- **Note: if vacuum distillation cycle time is too short the number of flushing cycles can be increased**

Setting Up the GC/MS

- GC/MS conditions will vary and the following are guidance for settings, injector and column variations can cause differences
- MS: full scan mode,37 to 270 amu scans/sec
- Column: flow rate 1.5 ml/min, velocity 33 cm/sec, pressure 29.0 psi, mode constant flow
- Temperature Program: 6 min at -25 °C; 50 °C/min ramp to 40 °C, 5 °C/min ramp to 120 °C, 22 °C/min ramp to 220 °C, and isothermal at 220 °C for 7.15 min, resulting in a total run time of 35 min
- Injector: Split/Splitless type injector, mode is splitless. Split at 5:1 ratio at constant flow, temp 220 °C, pressure 29.0 psi, split flow 7.5 ml/min, total flow 11.5 ml/min, gas saver off

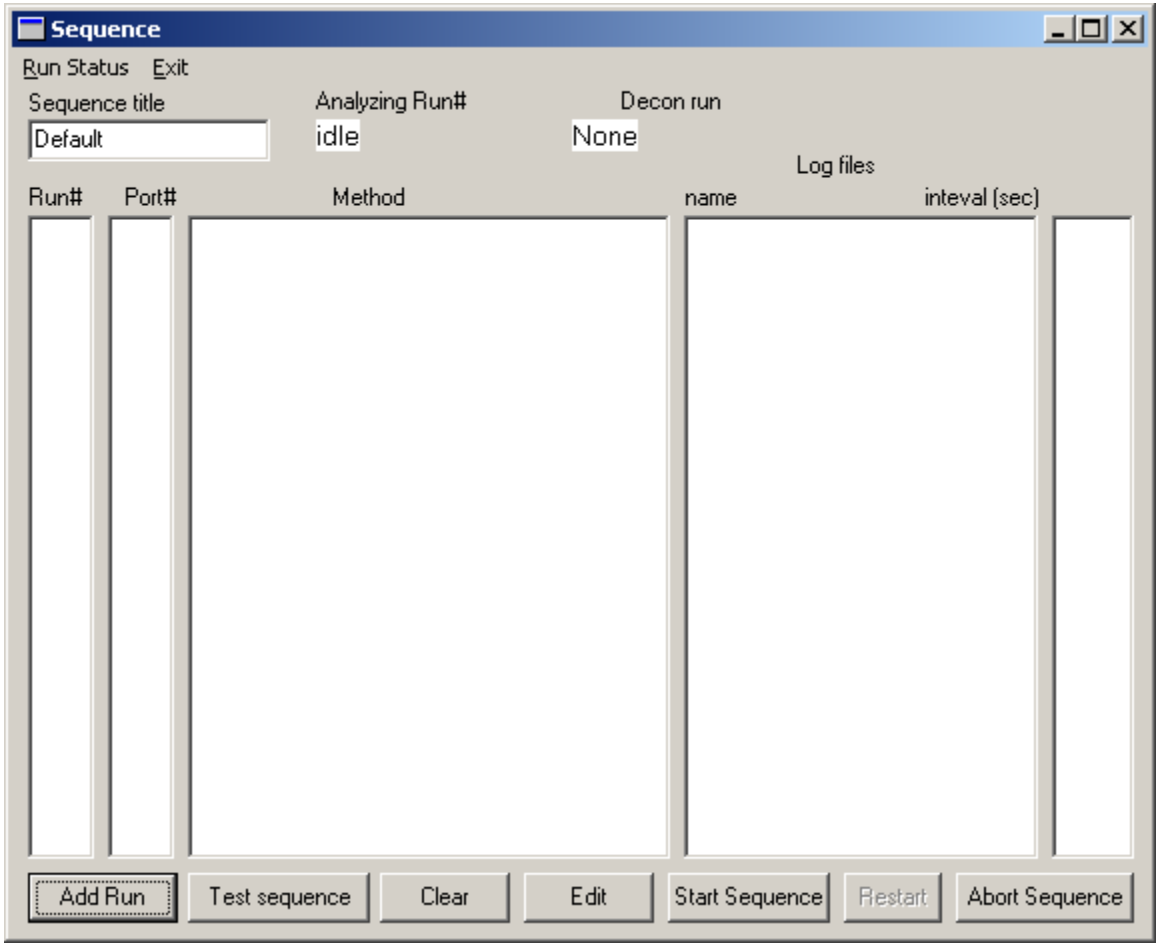
Batch Distillations

- Multiple vacuum distillations can be performed with the sequence of ports and the distillations methods differing (very handy for tuning system)
- Be sure the number of distillations match the number of GC/MS acquisitions being set up
- Verify helium, nitrogen (flush gas), and liquid nitrogen (LN2) are ample. Separate 160 liter dewars of LN2 for GC/MS and vacuum distillers should last 2-3 days of use
- Vacuum distiller has a time-out function to turn-off LN2 should GC not come to “ready”. The waiting time is accessed Main->Config->Set Time Limits

Setting up Vacuum Distillation Batches

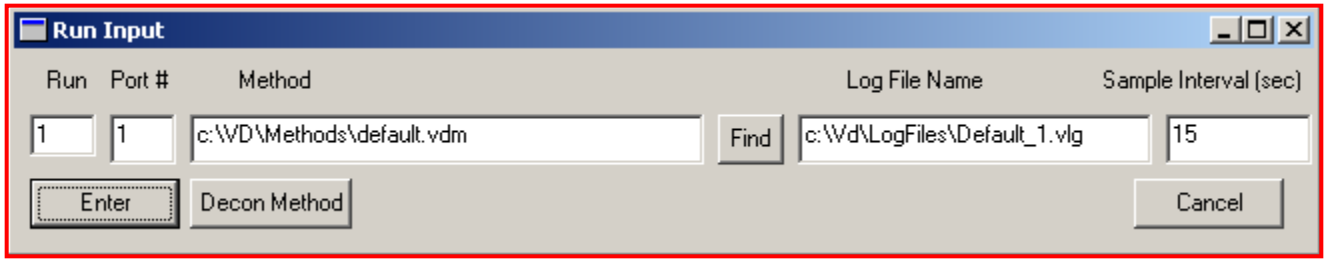
- Vacuum distillation batches are created in the Sequence window Main->Run->Set up Sequence.
- Note the sequence title (next slide) entry box at the top left. There should be a unique name entered as all log files will use that name (adding run #)
- For each run entered in the sequence, sample port, vacuum distillation method, and time interval for log file entries can be varied.

Sequence Window Display



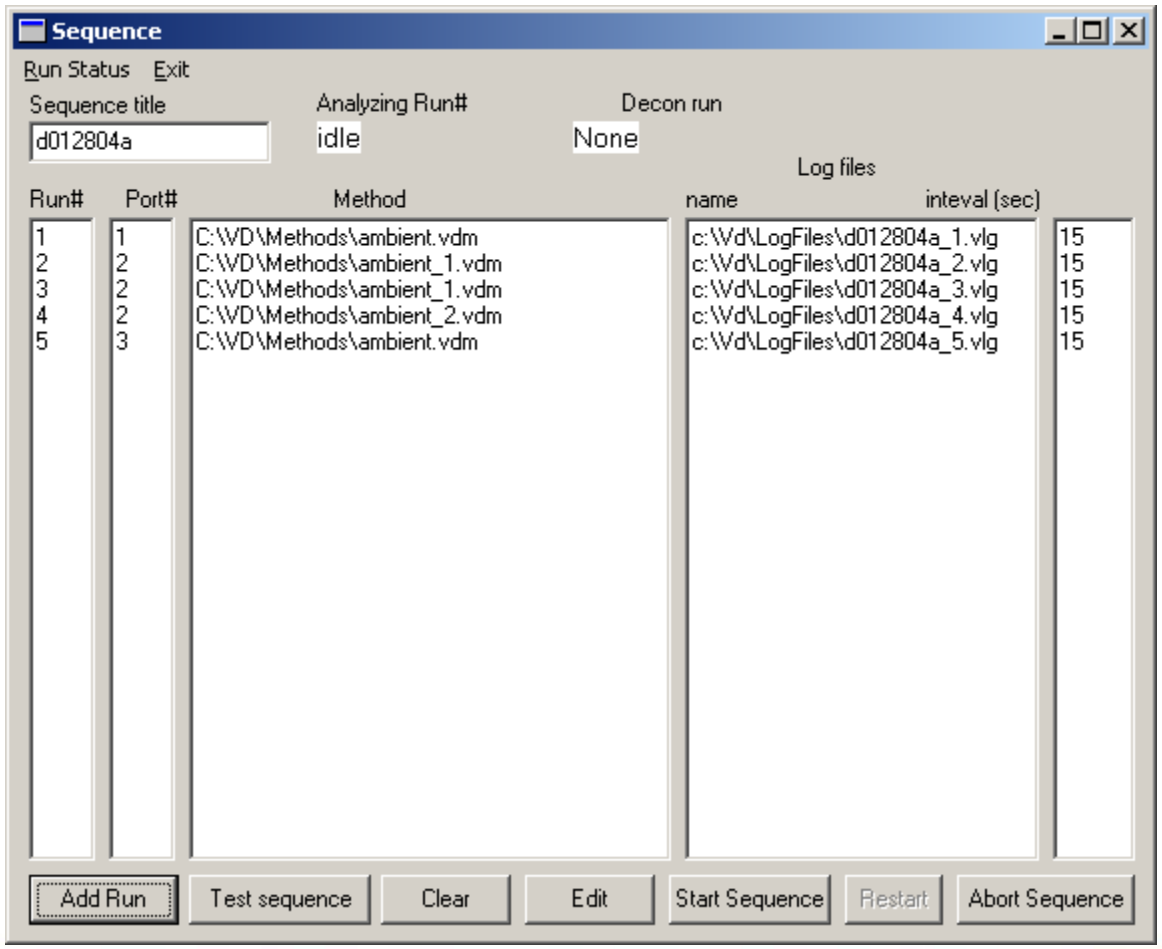
Adding Runs to the Batch

- The batch is built by adding runs (press Add Run button). The following is displayed



- Enter the port number of the first sample in the port entry box. For following run entries ports will be incremented upward but can be overwritten with another port (or the same port rerun).
- Find the vacuum distillation method to be used (press “Find” and navigate to desired method). Unless manually changed all following method will be the same
- If a logfile sampling interval different from 15 s is desired it can be changed by entering the appropriate amount in seconds
- After samples for distillations are complete the Sequence window will look like the following slide

Batch Ready for Distillations



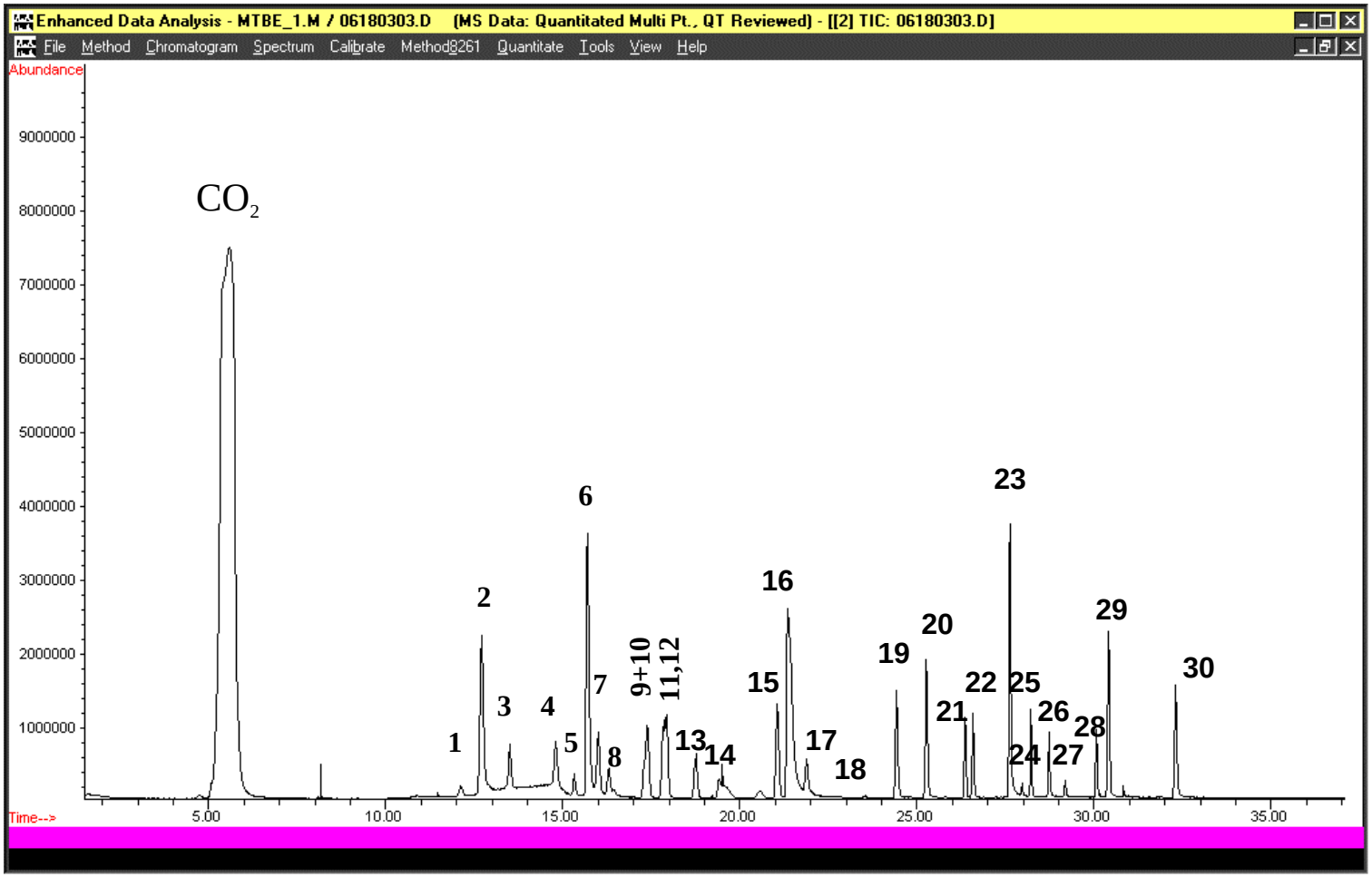
Starting the Batch

- Review the runs in the batch.
- Make sure gases are adequate and GC/MS system's batch mode is ready
- If everything is "go" correct press "Start Sequence"
- Note: Errors can be corrected before starting by pressing Edit button. You will then be prompted for which run# is to be edited and then can make changes

Identify Surrogates after the First Run!

- The analyte list for method 8261 covers a variety and large number of analytes
- Peak shapes can vary and relative retention time of polar compounds to non-polar compounds can vary by system
- Following slide shows surrogates in 5 mL water

Chromatogram of Surrogates



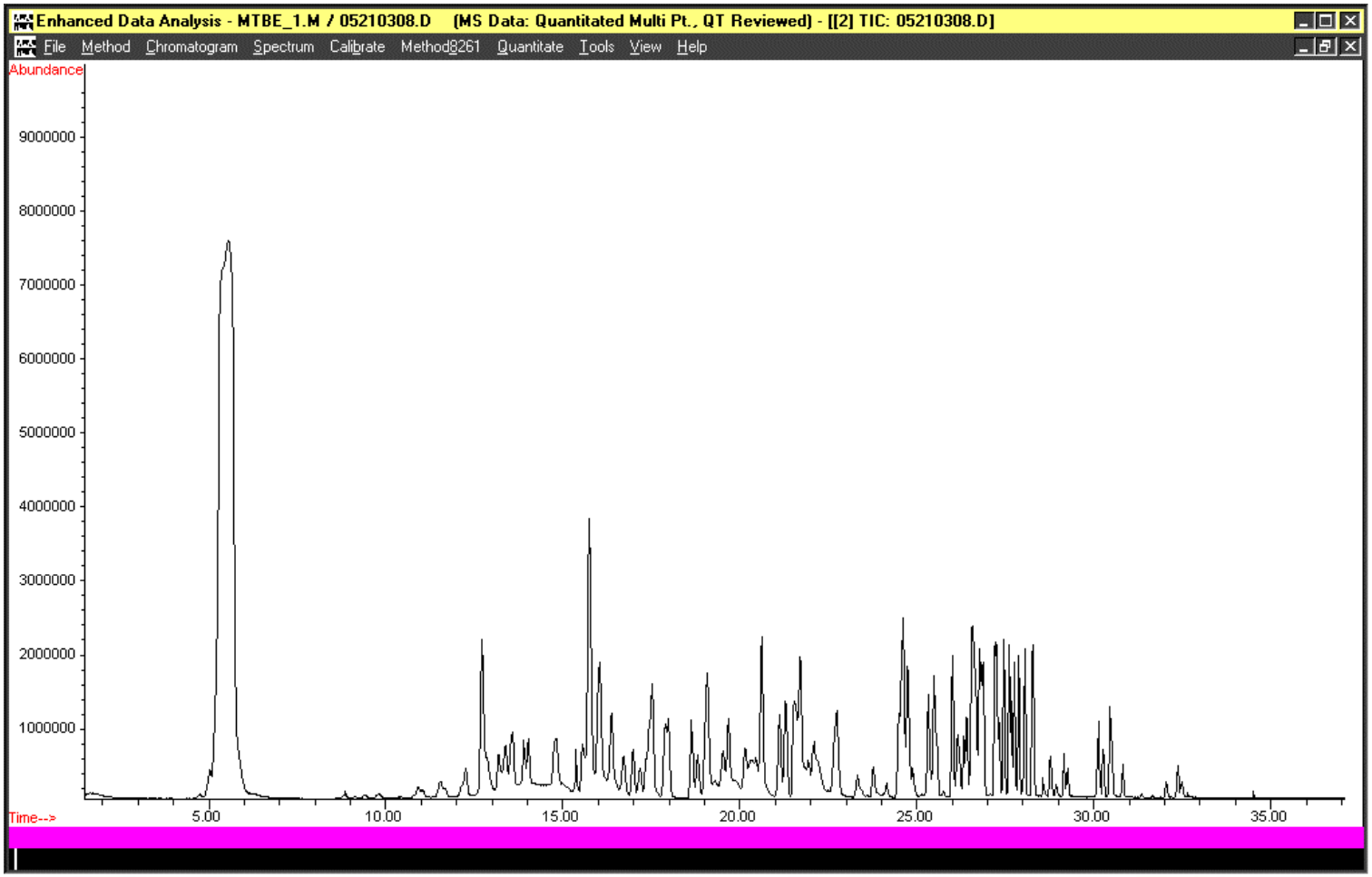
List of Surrogates

- 1. Diethyl ether-d10
- 2. Acetone-C13
- 3. Methylene chloride-d2
- 4. Hexafluorobenzene
- 5. Nitromethane-C13
- 6. Ethylacetate-C13
- 7. Pentafluorobenzene
- 8. Tetrahydrofuran-d8
- 9. 1,2-dichloroethane-d4
- 10. Benzene-d6
- 11. Fluorobenzene
- 12. 1,4-Difluorobenzene
- 13. 1,2-Dichloropropane-d6
- 14. 1,4-Dioxane-d8
- 15. Toluene-d8
- 16. Pyridine-d5
- 17. 1,1,2-Trichloroethane-d3
- 18. 1,2-Dibromoethane-d4
- 19. Chlorobenzene-d5
- 20. o-Xylene-d10
- 21. 4-Bromofluorobenzene
- 22. Bromobenzene-d5
- 23. Aniline-13C6 (optional)
- 24. Decafluorobiphenyl
- 25. 1,2-Dichlorobenzene-d4
- 26. Acetophenone-d5
- 27. Nitrobenzene-d5
- 28. 1,2,4-Trichlorobenzene-d3
- 29. Naphthalene-d8
- 30. 1-Methylenaphthalene-d10

Identify Analytes

- After identifying surrogate compounds perform an analysis of 5 mL water containing the surrogates and analytes (5 uL of the working solution)
- Perform the GC/MS analysis as described
- The following slide shows analytes + surrogates
- Be sure with identifications. The use of mixtures containing a subset of analytes may be necessary
- Hint: Look at homologous series and isomer groupings when identifying analytes the first time
- After all analytes and surrogates are identified and a GC/MS quantitation method is created a calibration curve should be developed

Chromatogram of All Surrogates and Analytes



Determine Method Calibration Range Needs

- Taking 5 uL of the analytes working standard yields 250 ng/5uL injection for most compounds
- If the calibration range is desired lower than the above amount, dilutions of the working standard are in order.
- Any dilutions of the working standard should yield between 5 uL and 1 uL injections
- Note: if the working solution is diluted remember to reflect these changes in the method 8261 quantitation library

Preparing a Calibration Sequence

- Without a specific calibration range specified it is recommended to start with a blank followed by standards of increasing concentration.
- It is recommended that 1:10 and 1:100 dilutions (aka 1:10 and 1:100) of the working analyte solution (aka 1:1)
- A sequence run of 12- 5 mL water samples spiked with surrogates + the amount of analytes shown below should be used to calibrate the first time to determine linear range as well as identify concentrations for a MDL study

- 1. no analytes
- 2. 1uL 1:100
- 3. 3uL 1:100
- 4. 5uL 1:100
- 5. 1uL 1:10
- 6. 3uL 1:10
- 7. 5uL 1:10
- 8. 1uL 1:1
- 9. 3uL 1:1
- 10. 5uL 1:1
- 11. no analytes
- 12. no analytes

Vacuum Distillation Log

- One attribute of the vacuum distillation procedure is there are log files generated that record all readings on user assigned intervals
- Log files are saved in a subfolder “logfiles” in the vacuum distillation folder created on installation
- Log files are ASCII (with .vlg extension) and can be read in “notepad”
- The log files are given a name combining the sequence name and the sample run number
- Use the log to identify problems

Log Information

- Log file records all pertinent information about a run, including
  - Port #
  - Distillation method used
  - Time
  - Temperatures of each zone
  - Vacuum pump pressure
  - Stage of the vacuum distillation
  - Errors that may have occurred
- Should a vacuum distillation yield poor results the log file may have the answer

Problems? Look at the Log

- If the final pressure reading during the “vacuum distilling” stage is typically 0.4 torr, then a reading of 2 torr or above is a strong indication there is a poor seal
- If recoveries of the higher boiling compounds has dropped, look for a temperature zone being too cold
- If during the “vacuum distilling” stage the pressure drops very quickly (accompanied by little weight loss in the sample and poor surrogate recoveries), the cryotrap is likely plugged. This occurs if there are excessive volatiles compounds (including CO2) that have been distilled (~.05g of material can plug the cryotrap). Also check for an incorrect method (too elevated of a condenser temperature during the distillation also would plug condenser)

Decontamination

- The vacuum distiller is very efficient in removing traces of analytes in the system between runs (the purge and flushing stages)
- Some analytes are resistant to removal and ~1 % carryover can still occur (aniline, pyridine and naphthalene). This can be a problem when going from high-level samples to low-level samples
- When extra steps are necessary to remove traces of contamination a “decontamination” method is used.
- The decontamination method is accessed by Main->Method->Decon Method
- The following slide shows the window

Decontamination Window

- Ports can be opened throughout the procedure
- Condenser temperature can be elevated
- The system can be pressurized (N2) an evacuated in up to 100 cycles
- A final evacuation can be held for an extended period
- After setting the parameters press buttons “Send” then “Run”
- After starting the procedure exit and monitor the system in “Run Status”