MANUAL FOR ESI-TOFMS

This guideline is intended for experienced user who works together with the operator of the instrument. In order to perform the analysis correctly, the instrument has to be calibrated prior to the work. User can verify this in the log sheet and talk to the operator.

Records

- Each user needs to record his/her name and general condition in the log sheet
- More detailed information about samples and their origin should be recorded in the "Walk-In user log book".
- Before the TOF use, users must prepare a sheet with structures of chemicals, their molecular formulas (or monomeric unit) and exact masses (monoisotopic masses). This sheet must be left for the record in the lab in the binder for Walk-in users.
- Each user is also expected to prepare a PowerPoint document that includes the resulting spectra and the sample information (file name, conditions such as solvent, electrolyte (ionization agent) and voltages, and ions used for compound identification) for the record of MS lab and leave it in the Walk-in binder and send via E-mail to Dr. Kubatova and the TOF operator. The users will not be allowed to use the instrument next time until this information is provided.

Sample Preparation

- The suitable solvents for the electrospray ionization (ESI) are 50% methanol (MeOH) or acetonitrile (ACN) in water. Although it is possible to work with tetrahydrofuran (THF), this solvent will suppress ionization. In addition, THF analysis MUST be performed with stainless steel connections. THF cannot be used with APCI ionization source.
- The electrolyte (ionization agent) enhances ionization. In order to achieve efficient evaporation, volatile electrolytes such as formic and acetic acid or ammonium salts can be used at concentration up to **10 mM (typically 2.5 mM).** Some application show higher concentrations of acids up to 160 mM of acetic acid. More common is use of 0.1 % e.g., 16 mM acetic acid. If stronger basis or acids are needed ammonium hydroxide or trifluoroacetic acid (TFA) can be used. However, user should minimize use of TFA as it leads to suppressions of samples even for consecutive analysis. Sodium iodide (NaI) is known to be widely used for the ionization of polymers. Its frequent use may lead to the undesired system contamination.
- The sample preparation should be started with *stocks in ACN or MeOH (THF may be used for polymers)* if sufficient amount of material is available. These solutions must be diluted in the MS lab as suggested in table in aqueous solvent (MeOH/H_2O or ACN/H_2O) and the electrolyte. The stocks are prepared so dilutions in various solvents with different electrolytes can be tested.

Sample preparation

	Small Molecules		
	Analyte	Electrolyte	Solvent
Initial concentration	100 ppm	100 mM	
Volume to take	10 µL	25 µL	965 µL
Final concentration (in 1mL solution)	1 ppm	2.5 mM	

Polymers		
Analyte	Electrolyte	Solvent
10,000 ppm	100 mM	
10 µL	25 µL	965 µL
100 ppm	2.5 mM	

Blank preparation

	Sample	Ionization agent	Solvent
Initial concentration	—	100 mM	
Volume to take	—	25 µL	975 µL
Final concentration (in 1mL solution)	—	2.5 mM	

Working with MassHunter – main operation features

The Mass Hunter has four major panes:

- **Instrument status**
  Showing which module is on. The (MS, pumps) modules may be turned on individually by right click
- **Real time analysis window**
  Showing with chromatogram and mass spectrum
- **Instrument operation tab**
  Allowing to setup voltage and other operation features
- **Worklist**
  Used for LC analysis sequence

Operation tabs

The walking users will typically work in direct infusion mode, the instrument is operated by two major tabs "Sample" and "MS TOF" located on the third panel from top in the software.

Tab "MS TOF"

Selecting of MS TOF gives larger menu with further options
On the left side of menu

- Check the type ion source (ESI vs. APCI)
- Check the ion polarity (positive or negative mode)

On the right side in menu "Data"

- Set time to 0.5 min
- Data storage of mass spectrum in profile
- The mass range is preset range, you can if needed expand the mass range up to the up to 3,200 m/z. For polymers i.e. higher mass range you need to contact the operator to recalibrate instrument up to 9980 m/z.

On the right side in menu "Acquisition"

- Set gas temperature to 250–350 °C (depending on the expected polymer stability), drying gas to 12 L/min and 4 L/min for small and large molecules, respectively
- For nebulizer gas pressure the typical starting point is 15 psi, with possible operating range in between 5 and 40 psi – ***needs to be optimized*** (when optimizing nebulized pressure, keep capillary and fragmentor energies at 3500 and 150 V, respectively)
- Capillary energy is the ionization energy (potential), and the typical starting point is 3500 V, with possible operating range in between 2000 and 5500 V in the positive mode. Higher voltages should be limited to prevent arching – ***needs to be optimized***
- Fragmentor is a voltage for focusing and CID and can be set between 110–250 V – ***needs to be optimized***

Capillary and Fragmentor are parameters which should be optimized for each compound, for polymers and larger molecules also nebulizer needs to be evaluated.

Tab "Sample"

In *Datafile* window, set project name to one you have created.

- Name your file, use coding, which you use for your own lab documentation, however number all your analysis runs in sequence of performance (01_comp_01_solvent_electrolyte_capillary-fragmentor voltages.wiff), this is critical if later troubleshooting is needed.
- In Sample Name, fill in further specifications such as solvents, electrolytes (ionization agents), voltages (compound_MeOH/H2O_ac.acid_150/4000V).
- In *"Run"* tab, select Manual start.

Data Acquisition

Initial setup for work in a direct infusion mode

Direct infusion involves analysis by direct injection

1) Software Agilent MassHunter Workstation is open.
2) The system is **calibrated** (check with the operator).
3) **Check and record vacuum** (top left panel) and compare to the previous values in the log sheets. If you find discrepancies inform the operator. The high vacuum should be ~4 x 10^{-7} Torr.
4) Open Analyst and create working directory: Go to Tools/Create project and name it in the following format: **YY-MMDD-IN** where IN stands for the initials of the user.
5) Go to Software Agilent Mass Hunter Workstation, load method C:/PE Sciex Data/Projects/calibration Methods/ and in "Name" window select Infusion_POS.m or Infusion_NEG.m. **Save the method in your project method directory before any further work.**
6) Check that TOF is ON (green), if not contact the operator.
7) **Record required information** (initial and throughout the work) in the log sheet and walking user notebook (as specified in record section).

Blank Analysis

1) First measure all blanks (solvent and solvent + electrolyte) at 15 psi, 3500 V/150 V) with drying gas set to 4 L/min and nebulizer gas pressure 15 psi at 300 °C, in both positive and negative mode.
2) Before running sample check MS spectra to make sure there is not cross contamination of ion of interest, look at the abundance of ions present. The mass spectra in second panel should be both changed to profile (right click/TOF spectrum/profile. In MassHunter one mass spectrum pane can be used in a full range and the other can be zoomed to see targeted ions.
3) On the syringe pump set syringe volume and flow rate to 5 µl/min (0.3 mL/h), the syringe pump is typically preset for a 1-mL syringe (*additional setting must be changed for work with different volume syringes*).
4) Set the project name, file name and sample name in the sample tab (see above).
5) When the TIC signal is stable, acquire data by hitting "START" (RED VIAL) on the very top of the Workstation window.

Sample Analysis

To measure your samples, follow the steps in procedure for the blank: set syringe volume and flow rate, project, file and sample names.

FOR SMALL MOLECULES <1000 *m/z*

1) The typical sample should be prepared at concentration of 1 ppm; 2.5mM electrolyte.
2) The initial TOF conditions are described under "operation tabs" in the previous section, the good starting point is drying gas at 12 L/min, nebulizer 15 psi, capillary voltage of 3500 V and fragments of 150 V.
3) View the spectra of samples, use profile to find your analytes. The common adduct ions to look for are $[M+H]^+$ (M+1) and $[M+Na]^+$ (M+23). Use *mass calculator.xls* to calculate the exact *m/z* values.
4) Maximize the response of adduct ions of interest by varying capillary and fragmentor voltages and record the response values in the walking user logbook along with the tested conditions.
5) Acquire data, when satisfactory mass spectra (upon testing positive/negative mode, capillary and fragmentor voltages) are obtained in the MassHunter.
6) In the top window hit "START" (RED VIAL) to start data acquisition.
7) After acquisition is completed, open the data file in Analyst (refer to the work guideline for Analyst software) and verify whether you obtained high mass accuracy <10 ppm error for your samples.
8) If data are good, proceed to the next sample, if higher mass accuracy error > 15 ppm is obtained, consult with the operator to recalibrate.

Analysis with Reference

In MSTOF window keep parameters the same, but in "Tune" check REF A and in Reference masses check "Enable ref mass correction" and "Use bottle A". Click "Select masses" select ESI POS ref (or neg) and see in spectra if you can find masses of 118 and 922 (for positive mode). Note those have to be of sufficient abundance (500) and only small error of 50 ppm. If those are not correct MS cannot use those ions for correction, you can modify required parameters in the reference mass window. If you can observe the reference masses as well as your analyte of interest, acquire data under a new file name.

FOR POLYMERS

3) The typical polymer sample should be prepared in concentration 10-100 ppm with 2.5mM electrolyte.
4) The initial TOF conditions are described under "operation tabs" in the previous section, the good starting point is drying gas at 4 L/min, nebulizer 15 psi, capillary voltage of 3500 V and fragments of 150 V.
5) View the spectra of samples, use profile mass spectrum to find ions, which belong to the polymer pattern, which typically resembles Gaussian distribution.
    a) Look at the spectrum and make sure you see polymer distribution
        - If the distribution is observed go to step (b)
        - If you do not observe the polymer or if TIC is not consistent:

        Make sure the sample is well nebulized: drying gas may be increased to 7 L/min, nebulizer may be increased to 25 psi, T may be lowered to 250 °C.
    b) Find a repeating unit

    In MS Excel open mass calculator and calculate the estimated mass of repeating unit based on number of C, H, O and see if you can find the repeat in the mass spectrum
    c) Try to determine the charge from isotopic pattern (refer to the question in your take-home-exam) by scaling the mass spectrum to a specific cluster. Repeat for several clusters within different repeating until chains.

6) Maximize the response of main ion in one of the clusters in 75% polymer Gaussian distribution toward a higher m/z end (use left MS window). Keep in the right MS window full spectrum to assure that the polymer distribution is not affected
    a) Nebulizing conditions nebulized (5-40 psig), when you change settings make sure the actual pressure is stabilized. While optimizing make sure you see polymer distribution besides the main ion. (keep capillary and fragmentor potentials at 3500 and 150 V)
    b) Capillary voltage 2500–5500 V in positive mode
    c) Fragmentor voltage 100–250 V
7) Acquire data, when the highest abundance (area) for a selected ion is achieved without losing polymer distribution and the TIC signal is stable (upon optimizing nebulizer, capillary and fragmentor voltages) are obtained in the MassHunter.

Data Conversion

After all the samples are analyzed, the user should convert ("translate") the data.
For work with MassHunter the data have to be converted by WIFF Translator located on the desktop.

- In the right pane create folder in which the new files will be stored (should in the same project but new folder "Data-MH."
- Uncheck "convert profile data to peak format" Make sure to select/enable profile spectra in main window of WIFF
- In the left pane right click on the directory with data to be converted (data folder) and select translate.

Upload/Download data

Connect computer to the internet
WinSCP File Transfer client
Host name: 134.129.184.35
Port: 22
MS user name: akgroup
The password will be provided by the instructor
The left screen MS data projects are in a local directory "PE Sciex Data/Projects" (find the project (MH) and copy to right screen under root folder directory/TOF MS, ensure you have select "profile"
Disconnect the computer from the internet

For data processing use "Sharing Data and working with MassHunter" instructions.
Each user is also expected to prepare a PowerPoint document that includes the resulting spectra and the sample information (file name, conditions such as solvent, electrolyte (ionization agent) and voltages, and ions used for compound identification) for the record of MS lab and leave it in the Walk-in binder and send via E-mail to Dr. Kubatova and the TOF operator. The users will not be allowed to use the instrument next time until this information is provided.