

Work Guideline for TOF

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

Records

1. Each user needs to record his/her name and general condition in the log sheet
2. More detailed information about samples and their origin should be recorded in the "Walk-In user log book".
3. 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, ionization agent and voltages, and ions used for compound identification) for the record of MS lab and leave it in the binder.

Sample preparations

- The suitable solvents for the electrospray ionization (ESI) are 50% methanol (MeOH) or acetonitrile (ACN). Although it is possible to work with tetrahydrofuran (THF), this solvent will suppress ionization. In addition, THF analysis needs to be performed with stainless steel connections.
- The ionization agent enhances ionization. In order to achieve efficient evaporation, volatile salt such as formic and acetic acid or ammonium salts can be used at concentration up to **10 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.
- The sample preparation should be started with *100 ppm stocks in ACN or MeOH* if sufficient amount of material is available. These solution must be diluted in the MS lab to 1 ppm in 50/50 aqueous solvent (MeOH/H₂O or ACN/H₂O) and ionization agent. The stocks are prepared so dilutions in various solvents with different ionization agents can be tested.

Table 1. Sample preparation

	<i>Sample</i>	<i>Ionization agent</i>	<i>solvent</i>
<i>Initial concentration</i>	100 ppm	0.1 M	
<i>Volume to take</i>	10 μ L	100 μ L	890 μ L
<i>Final concentration (in 1mL solution)</i>	1 ppm	10 mM	

Work in direct infusion mode

- 1) Software Agilent Mass Hunter Workstation is open
- 2) The system must be calibrated (check with operator)
- 3) Check vacuum (top left panel) and compare to previous values (recorded in log sheet). If you find discrepancies inform the operator.
- 4) Open Analyst and create working directory:
Tools / Create project and name it in the following format: *YY-MO-DD-IN* where IN stands for the initials of the user (check with older directories).
- 5) Go to Software Agilent Mass Hunter Workstation:
File / Open method / D:/PE Sciex Data/Projects/calibration Methods/ and in “Name” window select *Infusion_POS.m* or *Infusion_NEG.m*.
- 6) Check that TOF is on (green), if not contact the operator.

In 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

- 1) Check the ion source (ESI)
- 2) Check the ion polarity (positive or negative mode)

In the tab “Data”

- 1) Set time to 0.5 min
- 2) Data storage of mass spectrum in profile
- 3) Keep mass range in preset range, for other ranges you need to contact the operator to recalibrate instrument (your compound molecular mass should be in the operated range).

In the tab “Acquisition”

- 4) Set gas temperature to 300–350 °C, drying gas to 12 L/min, nebulizer gas to 25 psi.
- 5) Capillary energy is the ionization energy, and the typical starting point is 4000 V, with possible operating range in between 2000 and 6000 V. However, voltages > 5500 V should be limited to prevent arching.
- 6) Fragmentor is a voltage for collision induced ionization (CID) and can be set between 150-250 V.

Capillary and Fragmentor are parameters which should be optimized for each compound.

Tab “Sample”

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

- 1) Name your file, use coding which you use for your own lab documentation (01_comp_01.wiff).
- 2) In Sample Name, fill in further specifications such as solvents, ionization agents, voltages (compound_MeOH/H2O_ac.acid_150/4000V)
- 3) In “*Run*” tab, select Manual start
- 4) Hit Apply

Measurements

- 1) First measure blanks (solvent and solvent + ionization agent).
- 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). One mass spectrum can be used in full range and the other can be zoomed to see your ions.
- 3) On the syringe pump set syringe volume and flow rate to 5 μ l/min (0.3 mL/h).
- 4) Set the project name, file name and sample name (see above).
- 5) When the signal is stable, acquire data by hitting “START” (RED VIAL) on the very top of the Workstation window.
- 6) To measure your samples, follow the procedure for the blank (see above): set syringe volume and flow rate, project, file and sample names.
- 7) 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.exe* to calculate the exact m/z values.
- 8) Maximize the adduct ions of interest by varying capillary and fragmentor voltages.
- 9) Acquire data, when you see you compounds at max abundance and the signal is stable.
- 10) 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 you samples.
- 11) If data are good, proceed to the next sample, if lower accuracy > 15 ppm is achieved, perform the analysis with the reference or ask operator to recalibrate.
- 12) 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.