

Work Guideline for TOF

This guideline is indented for 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

Each user needs to record his/her name and general condition in the log sheet and more detailed information about samples and their origin in the “Walk-In user log book”. Each user is also expected to print the resulting spectra with appropriate information 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. 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 will be diluted in the MS lab to 1 ppm in 50% aqueous solution and ionization agent. The stocks are prepared so dilutions in various solvents can be tested.

Work in direct infusion mode

Direct infusion involves analysis by direct injection

- 1) Software Agilent Mass Hunter Workstation is open
- 2) The system is calibrated (check with operator)
- 3) Check vacuum (top left panel) and compare to previous values. If you find discrepancies inform the operator.
- 4) Open Analyst and create working directory: Go to 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, load 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.

MS TOF

Selecting of MS TOF gives larger menu with further options

On the left side option whether you operate in positive or negative mode is available

On the right side in menu “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.

On the right side in menu “Acquisition”

- 4) Set gas temp to 300–350 °C, drying gas to 12 L/min, nebulizer gas to 25 psi.
- 5) ESI capillary is ionization energy, the typical starting point is 4000 V, and the operating range is between (2000–6000). However, higher voltages > 5500 V should be limited to prevent arcing.
- 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.

First measure your sample without reference.

- 7) 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.
- 8) On the syringe pump set flow rate to 5 µl/min (0.3mL/h).
- 9) View the spectra of samples, use profile to find your analytes.
- 10) Acquire data, when you see you compounds, this is performed in Sample menu.

Sample

- 1) In Datafile window, set project name to one you have created.
- 2) Name your file, use coding which you use for your own lab documentation.
- 3) In Sample Name, fill in further specifications such as solvents, ionization agents, etc.
- 4) In Run window, select “Manual start”
- 5) In the top window hit “START” (RED VIAL)
- 6) After acquisition is completed, Open the data file in Analyst and verify whether you obtained high mass accuracy <10 ppm error for you samples (use the Analyst guideline).
- 7) 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.
- 8) 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 observed the reference masses as well as your analyte of interest acquire data under a new file name.