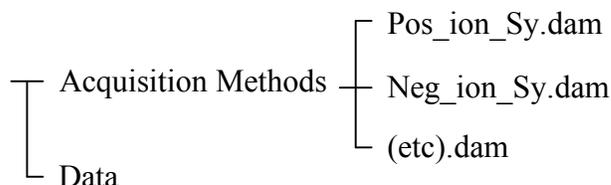


## Pulsar Instructions for Chem 5181 (ESI+, ESI-, MS/MS)

(Central Analytical Lab – Ekeley E266)

### **\*\*Your location on the computer\*\***

Data(E:)/PE Sciex Data/Projects/(Your Directory)/



### **1) Setting up the instrument**

**Important: Modify only parameters that are shown here!**

- Log on your account. (When you are done for the day, don't forget to log off)
- Click on "Analyst QS"
- Click on "**Hardware Configuration**" on the left pane
  - Uncheck Hardware Profiles other than "MS + Syringe2", by clicking on "Deactivate Profile"
  - Check "**MS + Syringe2**" by clicking on "**Activate Profile**"
  - Close
- Click on "**Manual Tuning**" to display the main panel
  - Make sure you see your directory (Chem5181\_2010) in the middle box on the second menu bar
- Open an instrument method (.dam)
  - File → Open → **Pos\_ion\_sy.dam** (for ESI+)
  - or
  - **Neg\_ion\_sy.dam** (for ESI-)
- "Do you want to save the current tuning acquisition method?" → **(always) No**  
**You NEVER save your method!**  
 (i.e., Don't change the original, optimized settings.)
- To change the polarity (ESI+ vs. ESI-) do NOT check the polarity button in the middle of CRT.  
**Always load** one of the methods above.

When you are switching to the other polarity, you will get a message prompting you to adjust parameters (specifically, the Offset Voltage)

→ Click on **OK**

→ Open the instrument door, and adjust the **OFFSET VOLTAGE** (red LED) on the power supply. *This is the only hardware setting that you need to change by hand.*

ESI+ → Use approx. **-10 V**

ESI- → Use approx. **-10 V**

h. Ion source settings (left pane: Source/Gas)

“**Ion Source Gas 1 (GS1)**” ≈ 8 (for **MeOH**)  
 ≈ 10 (for **ACN + trace H<sub>2</sub>O**)  
 ≥ 15 (for **water-rich samples**)

“**Ion Spray Voltage**” can go up to **+5200 V** (for ESI+)  
 can go down to **-4500 V** (for ESI-)

i. MS settings (right pane: MS – Advanced MS)

“**MS**” → “Scan Type” = **TOF MS**  
 → “TOF Masses (amu)” = Min (down to 50) - Max (up to 3,000)

“**Advanced MS**” → “Q1 Transmission Window” = “Suggest”  
 → “TOF Extraction Parameters” = “Suggest”  
 → Go back to the “MS” tab

## 2) Sample preparation

a. Put ~500 µL of solvent in an eppendorf tube  
 Solvent: MeOH (pure) or ACN (with 10-20 µL water added)

**IMPORTANT: Never use a solvent containing DMF or DMSO!** These compounds not only destroy your target signals but also cause persistent contamination to the ion source, requiring it be disassembled and cleaned.

b. *Completely* dissolve ~100 µg (~100 ppm) of neutral compound or ~10 µg (~10 ppm) of ionic compound

**NOTE: The smaller the amount of the sample, the better!**  
 (To *avoid* lost mass accuracy, peak saturation/splitting, cluster ion formation, instrument contamination, capillary tube clogging, etc.)

c. Wash the syringe (50 µL) 3-4 times with the same solvent

d. Wash the capillary line once or twice with the same solvent (each time using 50 µL)  
 Do this while the ESI voltage is ON (for “spray washing” the orifice)

e. Take 50 µL of sample solution, hook up the syringe, and start the measurement

f. Adjust the sample concentration s.t. the TIC (total ion current) reads 1 E04 – 2 E05

- g. Stop the measurement before the syringe drive hits the dead end stop  
(If it hits the stop, the computer aborts the program and you are in trouble. It is best to keep your eye and not let that happen.)
- h. When you are done with the sample, wash the syringe and the cap. line (c and d)

### **3) Running the instrument**

#### **a. Molecular Ion Measurement**

**“Start Syringe Pump”** → **“Acquire”**

You will be prompted to enter **“Sample Name”** and **“Data Filename”**  
(Only mouse-right-click works)

A **“Data Filename”** can store multiple data over successive runs

→ **“OK”** → Measurement starts automatically

→ Acquire stable signal → **“Stop”** (Acquisition) → **“Stop Syringe Pump”**

#### **b. MS/MS measurement**

**“MS”** → **“Scan Type”** = **Product Ion**

→ **“Product of”** = Your ion mass to be CID'd

→ **“TOF Masses (amu)”** = Min (down to 50) - Max

**“Advanced MS”** → **“Q1 Transmission Window”** = **“Suggest”**

→ **“TOF Extraction Parameters”** = **“Suggest”**

→ Go back to the **“MS”** tab

**“Compound”** (the second tab of the ion source pane)

→ **“Collision Gas (CAD)”** = 10

→ **“Collision Energy (CE)”** = 5 to 80 (ESI+) or -5 to -80 (ESI-), you improvise

**“Start Syringe Pump”** → **“Acquire”**

**Modify “Sample Name”** s.t. it indicates the mass of the CID'd ion while leaving **“Data Filename”** unchanged.

**When MS/MS is done, remember to reset the Collision Gas (CAD) to 3 (ESI+) or 4 (ESI-)**

#### 4) Data display and analysis

- a. Right-click on the TIC pane → “Open File” → Right-click on the MS pane (bottom) → “Delete ”
- b. Left click on the TIC pane and drag across to highlight a time region of interest  
→ Right click and “Show Spectrum”  
→ Left-click and drag across the mass axis to zoom in ←→ Double click on the axis to zoom out
- c. Go to File → Print “Pane”

#### 5) Making an electronic file

Right-click on the MS pane → “Save to Text File...”

#### 6) Exact Mass/Isotopic Distribution Calculator

- “Tool” (Menu Bar) → “Calculators” → “Isotope Distribution”
- Enter “Num of charges” → Enter “Formula”
  - “Calculate” → “Graph” tab → “Print”  
→ “Text” tab to read or print out exact masses