Operation Procedure for Sciex 4500/5500

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General Policy

1. Please email dma6@uthsc.edu before you start using the LC/MS the first time. You must be trained in order to use the LC/MS. No one is allowed to use other people's account unless authorized by SIF staff.
2. You may book time using Faces Scheduling website.
3. If you find any problem with instrument, **DO NOT CONTINUE** and please inform SIF staff asap.

I Operation Using Autosampler with Established Method

I-a Representative Biological Sample Preparation Procedure (reference only)

1. Place 50 µl of plasma or serum or other type of biological matrix in a plastic vial (0.5 mL).
2. Add 150 µl of cold (refrigerated) organic (ACN or MeOH) and vortex for 10 seconds.
3. Ice-cool the sample for 30 mins.
4. Centrifuge the sample to pellet out the precipitated proteins.
5. Take 150 µl of the supernatant and transfer to a HPLC vial or insert.
6. 10 µl on 4500 or 1 µL on 5500 is suggested inject volume to start with for LC/MS/MS analysis.

I-b LC-MS Preparation

1. When preparing mobile phase solution, degassing ahead of time is highly recommended.
2. Use a HPLC column in good working condition.
3. Make sure there are sufficient mobile phase solution and wash solution
4. If wash solution is low, please inform SIF staff

I-c Logging on the Workstation and Startup in Configure Mode

1. Enter your username and you password.
2. Click on "**Analyst**" icon on the desktop to start the program.
3. Open or create a user's Project folder by clicking on **Tools→Project→Create Project**
4. Activate the instrument. **Double click** the **Hardware Configuration** and **activate** the **LC-MS** profile. After a green check appears for selected profile, **Close** the window.

I-d Acquire Mode Operation

1. Enter Acquire mode and open Queue Manager window by clicking '**View Queue**' icon
2. Click '**Ready**' icon to make the LC-MS hardware ready
3. Click '**Equilibrate**' icon and choose the LC-MS method to start the LC flow.
4. Place samples in the autosample, using 1mL vial, 1.5mL vial, or 96-well plate.

5. Build Acquisition Batch using the Batch Editor window
    a. Under Sample tab, choose LC-MS method from Method Editor
    b. Click **Add Set** and then **Add Samples**. Build and save the Batch.
    c. Under Submit tab, highlight the samples that need to be run, and click **Submit**
6. Check the **Queue Manager** window and click '**Start Sample**' icon to start the LC-MS run
7. When finished, stop the Queue, and **Standby** the instrument.
8. Data can be processed under **Explore** mode or using **MultiQuant** software

II Manual Compound Optimization Using Syringe Pump Infusion (Method Development).

II-a Sample Requirements

1. For compound profiling, prepare your sample solution at or less than 1µg/ml in methanol or acetonitrile. (***NOT 1 mg/ml, WHICH IS TOO MUCH AND WILL CLOG THE ION PATH***).
2. Sample purity plays an important role in speed and accuracy of analysis as well as method ruggedness. Endogenous matrix component such as proteins, lipids, salts, and extracellular materials should be removed via precipitation, extraction, filtration, centrifugation, or any methods possible.
3. Compounds to be avoid
    ◊ Salts can interfere with ionization and can cluster to complicate spectrum (but also aid in identification).
    ◊ Strong based or quaternary amines can interfere with positive mode analytes, e.g. Triethylamine (TEA).
    ◊ Acids-sulfuric/sulfonic acids and TFA interfere in negative mode experiments
    ◊ Phosphate buffer and non-volatile ion pairing agents (e.g. SDS) can cause severe suppression and complex spectra.
    ◊ Dimerization ($[2M+H]^+$) can occur at high concentration, leading to non-linearity during quantitation.
    ◊ Dimer signal at m/z=(MW*2)+1 can cause non-linearity at high concentrations.

II-b Logging on the Workstation and Startup

1. Enter your username and you password.
2. Click on "**Analyst**" icon on the desktop to start the program.
3. Create a user's Project folder by clicking on **Tools→Project→Create Project** (enter a project name).
4. Activate the instrument. **Double click** the **Hardware Configuration** and **activate** the **Mass Spec Only** profile. After a green check appears for selected profile, **Close** the window.

II-c Introducing Your Sample (direct infusion)

1. Fill the 1.0mL Hamilton syringe with methanol to clean infusion tubing.

2. Fill the syringe with your sample (1ug/mL or lower); place the syringe on the built-in syringe pump; turn on the syringe pump from Tuning mode window or press the button next to the syringe pump. The default syringe flow rate is **7 or 10 µl/min**. **Start the Syringe pump**.

II-d Run Q1 Scan

Q1 Scan is an MS full scan (start-stop) where the first quadrupole **Q1 acts as single MS analyzer** and the third quadrupole **Q3 transmits all ion toward the detector region**. Q1 scan is used primarily for identification of precursor/parent ion.

1. In the navigator bar, click on Tune. If open, close the Tune Method Editor Window.
2. Double click on **Manual Tuning** to re-open the Tune Method Editor Window. You should hear the gases turned on (depending on previous status, the gas maybe on already) as the instrument becomes active for use.
3. In **Scan Type**, select **Q1 Scan**
4. Enter a **Start (amu)** mass value, **Stop (amu)** value.
5. Select suitable **Polarity**. (Positive or Negative)
6. Set **Duration Time (min)** to 5.
7. In the **Source Gas** tab, set **NEB** to 8, **CUR** to 20.
8. Other parameters use default settings for now.
9. Click **Start** to monitor the MS spectra.
10. Click **Acquire** to store the MS spectra data in a file. You can open and check the data later.
11. Click **Stop** to finish before duration time is reached.

II-e Run Product Ion Scan

Product Ion Scan is an MS/MS scan where the first quadrupole **Q1 is fixed** and the third quadrupole **Q3 sweeps a range**. It is an experiment that will search for all of the products of a particular precursor/parent ion.

1. In the navigator bar, click on **Tune**. If open, close the **Tune Method Editor** Window
2. Double click on **Manual Tuning** to re-open the **Tune Method Editor** Window. You should hear the gases turned on (depending on previous status, the gas maybe on already) as the instrument becomes active for use.
3. In **Scan Type**, select **Product Ion Scan.**
4. In the Products of filed, enter one of the precursor ions observed in the Q1 scan above.
5. Enter a **Start (amu)** mass value of 40, **Stop (amu)** value of 10 mass units above the selected precursor/parent ion and **Time (sec)** of one scan cycle (such as:3).
6. Select suitable **Polarity**. (Positive or Negative)
7. Set **Duration Time (min)** to 5.
8. In the **Source Gas** tab, set **NEB** to 8, **CUR** to 20, set **CAD** gas to 4.
9. In the **Compound** tab, set the **DP** to 65, set **CE** as 10, use default for other settings now.
10. Click **Start** to monitor the MS/MS spectra.

11. After scaning has started, **Increase the CE 5v** at a time until 80v and observe how fragmentation patter shifts from high mass fragments to low mass fragments. The spectrum displayed will correlate to the CE entered. Choose a suitable CE for your compound. Take note of suitable product ions to be used for MRM transition.
12. Click **Acquire** to store the MS/MS spectra data in a file. You can open the file in the future.
13. Click **Stop** to finish before duration time is reached.

II-f Compound Optimization

MRM is an MS/MS technique where the first quadrupole **Q1 is fixed** and the third quadrupole **Q3 is also fixed**. MRM is used for quantitation. Analyst software provides Compound Optimization to optimize DP, CE, CXP parameters quickly for each MRM transition.

1. In the navigator bar, click on **Tune**. If open, close the **Tune Method Editor** Window
2. Double click on **Manual Tuning** to re-open the **Tune Method Editor** Window. You should hear the gases turned on (depending on previous status, the gas maybe on already) as the instrument becomes active for use.
3. Click Compound Optimization. Choose **Infusion** as Inlet; and **MS/MS Analysis;**
   MW Ion search window ± 0.5 Da; Resolution **Unit**;
   Product Ion choose **User Specified;** Resolution **Unit**
   Select Polarity: Positive or Negative suitable for the compound
   Input **Compound Name**, **Q1 Mass**, and **Q3 Mass**. Start automatic optimization
4. After it is done, it will indicate either optimization is successful or not. If successful, note the optimized DP, CE, CXP values and use the settings to create LC-MS method.
5. Stop the syringe pump.

II-g Explore MS Data

1. Select your project folder.
2. Click on Explore on the navigator bar. Open the data file to display your MS spectrum.

II-h Finishing Up

1. Stop the Queue. Put instrument at **Standby**
2. De-activate the instrument. **Double click** the **Hardware Configuration** and **de-activate** the **Mass Spec Only** profile. After a green check disappears for selected profile, Close the window.

Leaving the instrument

1. Exit the Analyst program.
2. Log out of the computer (**DO NOT shut down the computer**)
3. Clean up the instrument table, put the syringe in its box, and switch off the syringe pump.
4. **Please inform any problem to SIF staff**