Mass spectrometry

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Note on 2012.11.16 by James: On-site training is required for actually using the mass spec, but these protocols may provide a good overview of the software.

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Using the AB Sciex QTRAP (LC-MS-MS) for quantification of MEP pathway metabolites

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Materials

Put all of the following on cart and bring down to the MS facility:

- 1.5ml amber vials (Agilent)
- 400ul vial inserts (Agilent)
- Whatman syringeless filters
- Standards of MEP pathway metabolites. Standards are located in K9/K10/N11 boxes in the -80 fridge.
- Samples (i.e. leaf extract)
- HPLC buffer. At this time the buffer is 80:20 acetonitrile:50mM ammonium acetate.
- Pipette
- Pipette tips
- Waste box
- Eppendorf tubes
- Tube rack
- Lab book
- Gloves
- Key card
- Some paper towels
- A pair of tweezers

Wear gloves when handling samples and using equipments.

1. Extract plant samples with liquid nitrogen and 80:20 ACN-aqueous buffer. The ACN-aqueous buffer may simply be 80:20 ACN:H2O, or the mobile phase used on LCMS, usually 80:20 ACN:50mM ammonium acetate.
2. Change filter on LC.
    a) Take the filter column out
    b) Open up the column with a set of 7/16'' wrenches, take the filter out and examine under a dissecting scope.
    c) Place it in a beaker with methanol and sonicate for 10min. If the beaker is too small and there is a risk of it tipping over in the sonicator, place the small beaker in a larger one and fill the larger beaker with some water.
    OR
    a) Replace with a new filter.
3. While it is sonicating change the running buffer (mobile phase)
    a) Change to A to ammonium acetate and B to acetonitrile.
    b) Rinse the tube and filter with DI H2O when changing aqueous buffer, when changing to

acetonitrile rinse with acetonitrile
   c) If buffer levels are low make up new buffer
4. Open up valve to the drain (counterclockwise and parallel to the ground) on both pumps, then press "purge". Wait until the line is purged (~1 min) where it will automatically come to a stop. Close valve to the drain.
5. Place the filter back and tighten the screws with hand. Put the HILIC column in place.

6. Open the Analyst software on desktop on the computer
7. Go to Hardware Configuration → LCMS → Activate Profile
8. Go to Create Project to create project. → create subproject
9. Set up Methods. To load a method from a previous one: Go to the project used before, go to File → Open → Select Acquisition Methods → Make changes as appropriate → Save as → Close method.
10. Tune instrument at least once at the beginning of the day. Go to "Manual Tuning" → Open the previous method → Start tuning. Watch for leaks. If there is a leak around the column or the filter, unscrew the leaked part, clean the hand screws and put the screws back on.
    Watch the pressure. Make sure the pressure does not exceed the absolute maximum the column can handle, i.e. 2900psi or 20Mpa. Take action when it is over 2500 psi and keep rising. For the record take down the initial pressure (or the final pressure, when there is no gradient) in lab book so we know what the normal range of pressure is (abnormal pressure would indicate leaks (too low) or blocked column (too high)).
11. The instrument needs to be tuned or equilibrated immediately before sample injection since the instrument will shut down in ~ 5 minutes if not in use. Tune or equilibrate more times as needed before loading the sample onto the LC-MS.
12. Make sample list:
    a) Build Acquisition batch
    b) Add set
    c) Select method, add sample, enter # of new sample, check auto increment, uncheck sample # and set #.
    d) Change sample names
    e) Use plate 2
    f) Take out the plate from LC and put vials in place. Use a blank in between samples to flush out any residues (2 if needed) from the previous runs.
    g) Change vial #
13. Save as a template. Make up samples, load the vials on the plate and hit "submit". Use the filter vials if there are suspensions in the sample (e.g. typically in a reconstituted plant extract). The filter vials cannot hold more than 400uL so 390uL is typically used.
14. Go to Acquire → Start Sample
15. To look at data in real time go to Explore → Open data file. To look at data for each trace go to Explore → Extract ions → Open dialogue.

16. To minimize degradation of metabolites in buffer, it is best to make up the sample and load it immediately onto the rack before injection. An example procedure would look like this:
    a) Run standards
    b) As the standards are running make up the sample list. At 9 mins into the last standard sample, make up the plant extract sample (takes about 3mins) and load onto the plate, hit "submit". This way the machine would be running continuously and we do not need to equilibrate, thus achieving the best consistency. Shake the sample lightly ("agitate") to mix up the layers immediately before loading.
    c) Repeat for every sample, make up the sample immediately before loading and running on the LC-MS.

17. Save and analyze data. There is a built-in peak analysis module where area under curve can be quantified and plotted.

18. When the experiment is over take out the samples and dispose of them properly.
19. Go to Hardware Configuration → Deactivation.
20. Fill in the log book and the form for charging to the account. Log out on the computer.
21. Take out the HILIC column and put a union in place on the LC. Wash the column as the following:
    a) If the filter is in MeOH first rinse the filter and tube with water and place it in the dH2O bottle.
    b) Purge the pump
    c) Rinse the HILIC column with water at 0.1 ml/min for 15 mins.
    d) Replace buffer with 80:20 ACN:50mM AmAc and purge the pump.
    e) Equilibrate the HILIC column with 80:20 ACN:AmAc at 0.1 ml/min for 15mins.

Note:

1. My experience has been that even when leaf is directly extracted into 80:20 ACN:aqueous buffer the extract still often separates into two layers, a large top layer that is green and a much smaller lower layer that is colorless. We are still not sure why this happens but it could be that the natural [solutes] in the leaves are so high that it pushes acetonitrile out of aqueous solution.
2. At this point we have IDP, DMADP, DXP, MEP, CDP-ME and MEcDP standards in the lab.
3. The ideal internal standard to use is 13C-labelled compounds but they are either not-commercially-available or really expensive, but they may be artificially made or synthesized (e.g. 13C-DXP from 13C-pyruvate using DXS).
4. Chemicals with the same formula can be found using chemspider.com. (To identify isomers we can use GC)
5. If the sample is not clean a sample clean-up procedure can follow the extraction
    a) Extraction
    b) Adjust pH to ~3
    c) Run through an anion exchange column, or a TiO2 column or pipette tip, where phosphates will stick (TiO2 column/tip is how they do phospho-proteomics.)
    d) Elute
6. Extraction efficiency can be estimated by spiking the sample with DXP. Measure 3 samples on mass spec: (1) leaf extract; (2) leaf extract + standard DXP and (3) standard DXP at the same concentration.
7. To reserve a spot on the MS calendar, go to googleapps.msu.edu and use Google calendar.

Using the Waters Xevo G2-S QTof mass spectrometer

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1. The Xevo G2-S uses the MassLynx software which is also used on the Quattro Premier and MALDI.

2. Click on "MS Tune" to open the tune dialogue box. On the bottom right corner, click the "on" button (looks like a "1") to switch into operational mode.

3. Open the column manager and put in the column. While installing the column care should be taken that the elbow of the steel line is not bent too much. Use a guard column where necessary.
4. Switch the solvent bottle.
5. Open up the "MS Console" dialogue box on the left, click on "binary solvent manager", then "Control" menu > "Prime A/B solvents" to start purging. Set purging time (typically 2 min).
6. To check the pressure, go to "MS console". Typically a delta of < 10 psi over 1 minute means the pressure has stabilized.
7. To check the UPLC status, click on "Inlet Method" to open up the LC method dialogue box. This is where we build and load our LC method. Click the "Status" button to show current status. Click "Inlet button" to change gradient. "Autosampler" opens the autosampler dialogue, "Acquity CM" opens the colomn manager dialogue (e.g. change column temperature). The injection volume needs to be at least 10uL. Click on "LC" menu > "load method" to load a method. Once the column is loaded, click on "LC" menu > "run gradient" to start equilibrating. To equilibrate with only the initial mobile phase, simply click "LC" > "flow on".
8. To set up an MS method, click on "MS method". In the dialogue box that pops up ("MS method"), click on the "MS" button or "MS/MS" button depending on what we want to do. Turn on CE when constructing a MS/MS method. If lockspray is to be used, make sure scan region includes the molecular mass of Leu-enk (556).
9. To start a sample, go to main GUI which shows the sample list. Unlike the AB Sciex QTrap this is also where you make the sample batch list and run samples. Right click to "add" a sample, change file name, file text, MS file (MS method), Inlet file (LC method), bottle (sample position) and MS tune file (tune parameters, usually we'll leave as is) as needed.
10. Open up MS Console. Select + or – (type of ionization), sensitivity mode or resolution mode, MS or MSMS mode here. Turn on CE here if MS/MS mode is used. Turn on lockspray infusion. A 3uL / min infusion rate is a good starting point. (If lockspray fluid is low refill the syringe as needed.)
11. Place sample vial in the sample drawer.
12. On the main GUI, select the sample to run and click "Run" (looks like a play button), and then click OK.
13. To look at the data click on "spectrum" or "chromatogram" at the top of the GUI. To update data in real time click on the small clock button in the chromatogram or spectrum window.
14. When the experiment is over take out the samples and dispose of them properly.
15. When the run is over go to MS console and turn off CE, lockspray, then click on the "source standby" button to go into standby mode. Leave the LCMS and the software on at the computer.
16. Fill in the log book and the form for charging to the account.
17. Take out the column and put a union in place on the LC. Wash the column.

Note:

1. When changing the guard column take care not to lose the ferrule or the ring on the metal fitting, they are typically very small and easy to lose. If the column is short enough instead of replacing the metal union it is a better idea to place the guard column on the end of it.
2. The autosampler is typically set at 10C but can be changed if necessary.
3. The MS facility follows a certain way for organizing the file names in the batch list ("XT_today's date_serial #").
4. Sodium formate is used for calibration (by infusion) since the formate tend to "polymerize" and

form dimers and trimers and polymers and can be used to calibrate a very large range of m/z's.

5. Raffinol is used for calibrating ESI+ and reserpine is used for calibrating ESI–
6. What is referred at declustering potential on the Q-trap is referred to as cone voltage on Waters instruments. There is also a spray voltage (capillary potential) which is very high in the 2-3 kV range for driving the ions throughout the spray head hence the term electrospray ionization (the cone voltage is the voltage applied at the cone for *selecting* ions).
7. On Waters instruments both DP and CE are absolute numbers (therefore in negative ion mode it will also be a positive number).