

# IonSpec HiRes MADLI-FTMS

Updated May 28, 2008

## Cautions

1. Do not open the box (with key) that encloses the laser optics when the laser is firing.
2. Be careful when removing the probe. If you pull the probe out without closing the gate valve the instrument will vent to the atmosphere (VERY BAD!) If you accidentally pull the probe out (and you will hear the turbo pumps make noise) PUSH THE PROBE BACK IN – then find Beth.
3. If the instrument is pulled back from the magnet (for a bakeout), do not push it into the magnet unless you have been trained to do this (the cell filament may be on as part of the bakeout and you will destroy the filament if it passes into the magnet).

## Procedure for Running a MALDI Sample

1. Prepare sample (and calibration/blank if needed – see Calibration/Blanks below).
  - a. **Direct Laser Desorption** (no matrix; sample applied directly to the probe as a solid or solution) – sensitivity will not be as good, samples will be short-lived (only one or two shots before you need to move to a different spot on the sample).
    - i. To apply sample, either crush/smear a small amount of material onto the probe face (and brush/blow off excess) or deposit the sample on the probe surface in a solvent (be careful with organic solvents that wet the probe surface and spread onto other probe faces).
  - b. **MALDI Dried Droplet** (matrix and sample mixed and dried on probe) – good for peptides and water-soluble samples. Helps purify samples, if salts are present.

Note: Dihydroxybenzoic acid (DHB) is the matrix that has been used with most success for MALDI-FTMS. Other common MALDI matrices, like  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA) and sinapinic acid (SA) are too “hot” (produce too much fragmentation) on the FTMS. Also note that some sample preparation techniques that work well for MALDI-TOF are not always useful for MALDI-FTMS (because of the longer time frame for ion detection).

- i. Dissolve 40 mg of DHB in 125  $\mu$ L acetonitrile + 125  $\mu$ L water (use 0.1% TFA water for proteins). Make up fresh solutions after 2 days.
  - ii. Mix 0.5  $\mu$ L of matrix + 0.5  $\mu$ L of sample on probe. Try to keep spot size small and allow solvent to evaporate. Do not heat.
  - iii. In a small tube (better for a more homogeneous mixture) mix matrix and sample 1:1. Spot 0.5  $\mu$ L – 1  $\mu$ L on the probe face and allow solvent to evaporate.
- c. **MALDI Fast Evaporation** (thin layer of matrix applied first, sample applied on top) – good for samples soluble in organic solvents that do not dissolve the matrix ( $\text{CH}_2\text{Cl}_2$ , hexane, toluene – not acetone).

Note: See Note in Part B (MALDI Dried Droplet).

- i. Make a saturated solution of DHB in acetone (approximately 10-15 mg of DHB + 30  $\mu\text{L}$  acetone).
  - ii. If you want to promote cationization (formation of  $[\text{M}+\text{Na}]^+$ , for example) prepare a mixture of 10  $\mu\text{L}$  of the DHB solution + 2  $\mu\text{L}$  of 0.1 M NaAc (sodium acetate or other salt in MeOH – this solution is usually in the sample prep area – if you need to make it fresh, the NaAc/MeOH will need to be heated to dissolve).
  - iii. Place 0.5  $\mu\text{L}$  of matrix solution on the face. The goal is to produce a very thin homogeneous layer of tiny crystals. The sample, in an organic solvent **that does not dissolve the matrix**, is applied on top of the matrix. Don't apply too much sample (the spot should not look shiny, for example); multiple applications (for dilute samples) are OK.
2. Start computer.
3. Start programs (IonSpec99 and WinTV).
4. Turn on System Power.
5. Insert probe.
  - a. Insert to correct depth and tighten knurled nut.
  - b. Rough out inlet (open lower valve; pump to less than 10 mTorr; close valve).
  - c. Open gate valve fully (counter-clockwise about 15 turns – threaded rod protrudes slightly on top of the valve handle).
  - d. Insert probe – pressure on front digital gauge should fall to the low  $10^{-10}$  Torr range.
6. Rotate probe to desired position.
7. Note the focusing lens position (inside the laser box – 16 is a good starting point – adjust, if necessary using the forward/backward toggle by the computer monitor)
8. On the computer select a USER (keeps track of the instrument use) and the MALDI source.
9. Note: If you are the first user after the instrument is started you will need to do one dummy run before you can use the laser test mode to adjust the laser position.
10. Either go to the MALDI WIZARD icon and select an experiment, mass range, enter your target mass and polarity (positive or negative ions) or open an old file (experiment parameters are saved with all data files).
11. Click ACQUIRE to measure a spectrum and select  $n = 1$  (no average) – first may be dud if you haven't adjusted the laser position.
12. Use the MALDI WIZARD icon to change mass range, if desired.
13. Click on the TEST icon to slowly pulse the laser at low power. Adjust the laser position with the toggle controls left/right and up/down.
14. Select a laser power (100% = 100% power; 0% = 40% power) lower is generally better.
15. Save spectra in your folder. Include experimental details like the lens position (LP=16), the % power, the mass targeted (for example, LM=600 for low mass with 600 entered as the target mass).

### Problems? – Things to check

1. Can you see matrix peaks? Select Low mass range,  $m/z = 300$  from the WIZARD and look for matrix peaks (large  $m/z = 273$ , scale  $<100$  for DHB). If DHB shows up well, move around to find a “sweet spot” (spot that gives good analyte signals). If DHB doesn't show up check the next items.
2. Are you seeing much fluorescence from the matrix? If not check the items below – in addition, you may have a sample that is too concentrated (fast evaporation method – film that is too thick, for example).

3. Are you using enough power? Make sure you have tried 100% power (computer control of the laser power – icon on the right side of the menu). You may need to move the focusing lens back (toggle control at computer) to increase the irradiance (decrease the spot size).
4. Is the laser spot size large enough? Check the variables iris (in the box, where the laser light shines up to the first mirror). This is used to control the number of ions produced – open it more to create more ions.
5. Is the gas reservoir full? Check on the probe inlet side of the instrument. The pressure for inlet 1 should read 28-30 Torr.

### Calibration/Blanks

If accurate mass measurements are critical and your sample is largely uncharacterized you will need to prepare a sample for calibration. Currently, I have been using a sample of Ultramark 1621 for calibration purposes. A sample of Ultramark 1621 in  $\text{CHCl}_3$  should be in the sample preparation area. You can place 0.5  $\mu\text{L}$  – 1  $\mu\text{L}$  of this solution on a dried sample of DHB (either dried droplet or fast evaporation). Other compounds, (Substance P, for example) may be appropriate for other applications.

The best mass accuracy will be obtained when the calibration masses bracket the ions of interest and you make use of more than two calibration points. In addition, for external calibration, the total intensity of the measured signal (assessed by the scale and number of different types of ions) should match that of the calibration spectrum. Best calibrations are done internally (using ions that are in the same spectrum as the mass of interest).

1. Make sure you have ions covering a wide enough mass range. If you are calibrating with a peptide (like Substance P), you may need to play with the laser power (percentage and lens position) to induce fragmentation.
2. Try to get a spectrum with a scale of 200-500 (a crude measure of the number of ions in the cell). If the scale factor is too small (too many ions), decrease the size of the iris to limit the number of ions produced. When the scale is appropriate, go to Acquire from the pull down menu and pick 5 as the number of scans to average.
3. Calibrate the spectrum by going to the **Tools** menu and select **Calibrate**. If you are working with a previously used calibrant, select the **Delete All** button, followed by **Autocalibrate**.

### To Finish

1. Withdraw the probe – BE CAREFUL HERE – DON'T VENT THE INSTRUMENT!! Use the chain and bar to determine how far back to pull the probe and then fully close the gate valve (black/gold valve on top). Turn clockwise, DO NOT CRANK DOWN at the end.
2. Loosen the knurled nut (hold onto the probe) and withdraw the probe.
3. Close the programs and shut down the computer (unless someone else plans to use the instrument).
4. Turn off the instrument power.