

Advion CMS User Guide

Columbia University
Department of Chemistry

I. Introduction	1	V. Running ASAP Samples	5
II. Getting Started (Mass Express)	2	VI. Instrument Standby & Log Off	6
III. Running FIA Samples	3	VII. Troubleshooting	6
IV. Running TLC/MS Samples	4	VIII. TLC Plate Ordering Guide	7

I. Introduction

Welcome to the Advion Compact Mass Spectrometer (CMS) in the Shared Materials Characterization Lab in 544 Havemeyer. In order to obtain swipe access to 544 Havemeyer and to get an account on the instrument, you must be trained by a super-user and pass a test given by the mass spec facility manager, Brandon Fowler. If you would like to receive training on the instrument, sign up on the sheet outside 544 Havemeyer. Speak to your lab's super-user (see below) or Brandon Fowler (brandon.fowler@columbia.edu) if you encounter any problems with the instrument that are not addressed in these instructions.

Lab	Super-User	Lab	Super-User
Campos Lab	Sam Sanders	Nuckolls Lab	Madhav Neupane
Ju Lab	Jianyi Ren	Owen Lab	Iva Rreza
Lambert Lab	Barry Cohen	Sames Lab	Tom Fiala
Leighton Lab	Isaac Hughes	Stockwell Lab	Pieter Bos
Min Lab	Chen Zeng		

What is the Advion CMS? This multi-functional, compact mass spectrometer is a single quadrupole mass spectrometer (10–2000 Da) equipped with a variety of methods for analyzing a wide range of sample types. The instrument includes two interchangeable sources: atmospheric pressure chemical ionization (APCI), typically used for less polar compounds in less polar organic solvents, and electrospray ionization (ESI), typically used for more polar compounds in polar/protic solvents. The APCI source includes an atmospheric solids analysis probe (ASAP); this allows users to swab solid or liquid samples with a glass capillary, which is then inserted into the APCI source where the sample is vaporized by a stream of hot nitrogen gas. Both the APCI and ESI sources can receive samples for flow injection analysis (FIA) and thin-layer chromatography/mass spectrometry (TLC/MS). FIA allows for sample solutions to be loaded into an injection loop before opening the loop to a flow of solvent headed for the mass spectrometer. TLC/MS allows users to analyze TLC spots on a developed TLC plate. In this method, an elution head lowers itself onto the spot of interest, presses firmly into the plate to form a seal, then flows solvent over the spot to extract the sample and sends this solution to the mass spectrometer.

What is the Advion CMS instrument good for? Chemists will find the Advion CMS instrument useful in all aspects of preparative chemistry. Crude reaction mixtures or extractions can be analyzed using FIA. The TLC/MS function allows for simple reaction monitoring using any type of stationary phase (silica, alumina, C₁₈-reversed phase, cellulose, etc.) with or without a fluorescent indicator. Column chromatography fractions can be identified quickly using either FIA or the ASAP. Insoluble solids, precipitates, oils, films, and surfaces can be swabbed and analyzed using the ASAP. The mass spectra produced on this instrument are considered low-resolution (within 0.5–1.0 Da) and are good for qualitative analysis of a sample; whereas, high-resolution data are required in most publications for proof of molecular formula. If you would like to submit a sample for accurate mass/high-resolution mass spec (HRMS) analysis, please follow the instructions on the Chemistry Department's Research Facilities website: <http://chem.columbia.edu/resources/facilities>.

II. Getting Started (Mass Express)

In most cases, the APCI/ASAP source will work for your sample. If you require ESI, ask for help from your lab's super-user or speak with Brandon Fowler to receive special instructions for switching sources. Always check that the correct source is installed before logging into the computer. If you use the ESI source, you **MUST** switch back to the APCI/ASAP source when you are finished.

When you log into your account, Mass Express will open automatically to the Manual Run page. In the top right corner, click the green I/O icon to switch the instrument into "Operate." This will begin warming up the instrument and start the flow of nitrogen gas. Navigate to the Ion Source page by clicking on the tab on the left. Load the ion source settings for the desired ionization(s) by clicking on the folder in the top left and choosing a ".ion" file (for example, "APCI_POSITIVE_DEFAULT_Ion_Source.ion"). You can load one positive and one negative ion source file at a time. For ASAP samples, use APCI ion source files. These settings adjust the temperatures and voltages in the source to fit your needs.

If you are using FIA or TLC/MS modes, check that the desolvation nitrogen flow is set to 3 bar, check that the tubing from the injection port is connected to the inlet on top of the source, and turn on the solvent pump by pressing the Play/Pause button so that a green light appears next to "RUN" (*the instrument MUST be in "Operate" before starting the flow of solvent*). If you are using ASAP, check that the *black* plastic fitting is plugging the inlet on the source, check that the solvent pump is off, and turn the desolvation nitrogen flow to 0.

In Mass Express, navigate to the Manual Run page by clicking the tab on the left. Set the Ion Source Settings that you will use for your run. Since you already loaded the settings earlier, you can set these to "Use Current Positive/Negative Settings." If you are running both positive and negative, set Ion Source Settings 1 to positive and Ion Source Settings 2 to negative. Under the Ion Source Settings, load the Tune Settings for each ion source you are using — use the drop-down menu to choose the settings that (1) match your source, (2) match the polarity (+/-) of the corresponding ion source, and (3) have the latest date (for example, November 19, 2015 = 20151119). To run both polarities, you must also check the box for "Use two ion sources" further down the page under Acquisition Method. If you are running only one polarity, set Ion Source Settings 1 to this polarity, and leave the latter box unchecked. Also under Acquisition Method, set your mass range; the minimum (start) is $m/z = 10$ and the maximum (end) is $m/z = 2000$. Change the scan time to 500 ms.

Start your acquisition by clicking the blue Play button at the top of the screen. You will be prompted to specify a user (once you enter a user, it will be available from a drop-down menu — this will also be the name of your data folder), a sample group (for example, the date), and a sample name. The instrument will then start collecting data to establish a baseline before you load your sample. You may load multiple samples during this acquisition; keep in mind that all of the samples run during the same acquisition will be under the same sample name. Check out the sections on FIA (pg. 3), TLC/MS (pg. 4), or ASAP (pg. 5) to see the ways you samples can be loaded into the mass spectrometer. Only one method of loading a sample can be used for any one acquisition.

Key Points

1. Check for or install the correct source and connect/disconnect any tubing as needed
2. Log in and turn the instrument to "Operate"
3. Load the default ion source settings (positive, negative, or both)
4. Check desolvation nitrogen flow (0 bar for ASAP, 3 bar for FIA or TLC/MS)
5. Check the source inlet (plug for ASAP, tubing for FIA or TLC/MS)
6. Turn on solvent flow at the pump (FIA or TLC/MS)
7. Specify your run settings (1 or 2 ion sources, mass range, scan time) in the Manual Run page
8. Click Play to name your sample and start your acquisition, then load your sample (see below)

III. Running Flow Injection Analysis (FIA) Samples

Resting state of injection port and injection syringe. The injection port should be cleaned of any prior sample by the last user and switched to the “Load” position. The syringe should be cleaned of any prior sample by the last user, left empty between the computer and the mass spectrometer, and should NEVER be left in the injection port (not even for a few seconds).

Sample preparation. Sample solutions for FIA should be **no more than 0.1 mg/mL** in any volatile solvent (avoid high-boiling solvents such as DMF, DMSO, etc.). If your material doesn’t dissolve in acetonitrile, please also bring an extra 5–10 mL of neat solvent for washing the injection loop (see below). Higher quality solvents such as HPLC grade or LC/MS grade will provide better results. The majority of molecules can be analyzed using the APCI source, which also works better with less polar solvents (such as methylene chloride, hexanes, ethyl acetate, toluene, acetonitrile, ethers, etc.). Very polar molecules in polar solvents (such as water, alcohols, acetonitrile, etc.) may require the ESI source.

From Section II, the tubing that connects the injection port to the source is in place, you have solvent flowing at 0.2 mL/min (pressure 500–750 psi), the desolvation nitrogen is set to 3, and you have started your acquisition. The injection loop for FIA only holds 5 μL , but plan on injecting 10–20 μL /injection. To load your sample, switch the injection port to the “Load” position (if not already). Using **ONLY the 50- or 100- μL glass syringe at the instrument**, pull up 20–100 μL of neat solvent and inject it into the waste container to rinse out the syringe. Then pull up 10–20 μL of your sample solution, wipe the needle with a KimWipe (to avoid contamination of the injection port), gently insert the needle into the injection port (all the way until they syringe pushes against the port), empty the contents of the syringe into the injection loop, switch the port position from “Load” to “Inject” (turn knob clockwise), remove the needle from the injection port, and check the computer to see the spectra being collected.

Once you see your sample has come through, you can repeat the above steps to analyze more samples. Once you’ve finished with all of your samples, **YOU MUST CLEAN THE SYRINGE AND INJECTION PORT**. To do this, switch the port back to “Load,” and rinse the syringe out 3 \times into a waste container with an appropriate wash solvent (if your sample dissolves in MeCN, there is a wash vial of MeCN provided; if your sample does not dissolve in MeCN, please bring 5–10 mL of a solvent that will dissolve your sample to use as your wash solvent). Rinse the injection loop with wash solvent by injecting >50 μL (for extra cleaning, you may use the 2.5-mL glass syringe at the instrument) into the injection loop *while the port is in the “Load” position*. Switch the port to “Inject,” remove the needle, and check the spectra that are collected to make sure your sample is no longer coming out. If you still see your peaks, repeat the syringe and injection loop rinses and check the spectra again. Repeat until no more of your sample shows up after rinsing. Once your sample has been completely washed away, switch the port back to the “Load” position and leave it this way for the next user. Stop the acquisition by clicking the blue Stop button in Mass Express.

Key Points

1. Pre-prepare a sample solution with a concentration of 0.1 mg/mL, set the port position to “Load”
2. Rinse the syringe with the acetonitrile provided (or a cleaning solvent you bring)
3. Pull up 10–20 μL of the sample solution, wipe the needle with a KimWipe
4. Insert the needle into the injection port, empty the syringe into the injection loop
5. Switch the port position to “Inject,” remove the needle from the port
6. After collecting your data, rinse the needle 3 \times with the appropriate wash solvent
7. Switch the port back to “Load” and wash sample loop with >50 μL of wash solvent
8. Switch the port to “Inject” to check that all sample has been rinsed out, if not, repeat #7
9. Switch the port position back to “Load” for the next user and stop your acquisition

IV. Running TLC/MS Samples

Resting state of the Plate Express. When not in use, the Plate Express module should be left (1) with a “dummy” TLC plate directly underneath the elution head and (2) with the clear, plastic cover slightly open so that the LEDs are purple/pink indicating the Plate Express is not ready for acquisition. These requirements are to protect the elution head from coming down onto the metal surface without a TLC plate present.

Sample preparation. Your sample should be spotted on a TLC plate at an approximate concentration of 1 mg/mL. TLC plates MUST be aluminum or plastic-backed, and the thickness of the silica/alumina/etc MUST be 200–250 μm to avoid leaking. **Glass-backed plates are FORBIDDEN** because they can shatter under the pressure of the elution head. All TLC plate chemistries (silica, alumina, reversed phase, cellulose, etc.) are compatible. See page 7 for ordering information for aluminum or plastic-backed silica, alumina, and C₁₈-reversed phase TLC plates (200–250 μm thickness) from various vendors. If your spots are UV-active, find the spots under a UV-lamp and mark any spots of interest with a pencil before bringing the plate to the instrument. TLC plates must not be stained because (1) stains react with your compound (it won't be your compound of interest any more!); (2) the stain itself will dominate the mass spectrum; and (3) most stains are very harsh and destructive to the instrument. If your spots require staining for visualization, you can run two TLC plates simultaneously — one for staining, and one for mass spec analysis. Speak with a super-user if you have any questions or require any help preparing your TLC/MS sample.

From Section II, the tubing that connects the injection port to the source is in place, you have solvent flowing at 0.2 mL/min (pressure 500–750 psi), the desolvation nitrogen is set to 3, and you have started your acquisition. If the Plate Express overhead and laser lights are not on, close the plastic cover and turn them on in the “Control and Status” page in Mass Express. Open the clear, plastic cover on the Plate Express, move the “dummy” TLC plate aside, and align the spot of interest on your TLC plate with the laser light. Gently close the cover — the LEDs above the cover will blink blue, then become solid green. To initiate the elution head (the part that probes the TLC spot), either (1) press the blue button connected to the plate express or (2) click the Plate Express icon in Mass Express. The elution head will lower down to the TLC plate, press firmly down to form a seal, then flow solvent over the TLC spot for 1 minute to elute the sample and send it to the mass spectrometer. During this time, the LEDs above the cover will blink blue. After 1 minute, the elution head will clean itself with a burst of nitrogen and the LEDs will change back to green indicating the Plate Express is ready to run another sample. At this time, you can move your TLC plate to the next spot of interest and initiate the elution head again (you can run multiple spots during the same acquisition, just be sure to keep track of the order in which the spots were run). Once you've run all spots of interest, stop the acquisition by clicking the blue Stop button in Mass Express. Return the Plate Express to its resting state (explained above) by placing the “dummy” plate directly under the elution head and leaving the cover slightly ajar.

Key Points

1. Bring your aluminum/plastic-backed, unstained, pencil-marked TLC plate to the instrument for TLC/MS
2. Turn on the overhead and laser lights for the Plate Express (if not already on)
3. Move the “dummy” TLC plate aside and place your plate such that the spot of interest is under the laser
4. Close the clear, plastic cover on the Plate Express and wait for the LEDs to turn green
5. Press the blue button or click or the Plate Express icon in Mass Express to probe your TLC spot
6. Wait 1 minute while the elution head probes the TLC spot, then cleans itself (LEDs will blink blue)
7. Once the LEDs are green again, move the TLC plate to start any additional TLC spots as needed
8. Once you have run all TLC/MS samples, stop the acquisition
9. Return the Plate Express to its “resting state,” explained above

V. Running Atmospheric Solids Analysis Probe (ASAP) Samples

Resting state of the ASAP. When not in use, the probe should be pulled partially out from its fully inserted position (~1 inch or 2 cm), and the nut should be gently tightened to hold the probe in place.

Sample preparation. You may bring your sample as a solid, oil, solution (0.1–0.2 mg/mL in a volatile solvent), or solid matrix (for example, shavings from a prep-TLC plate). Also, ASAP may be used to analyze TLC spots that are not compatible with the elution solvent (95:5 acetonitrile:water with 0.1% formic acid) that is used with the Plate Express for TLC/MS. Glass capillaries can be cleaned as described below. For solids/oils/matrices, use the *closed* tip of the glass capillary to swab the cap or the rim of the sample vial — DO NOT insert the probe into the vial. Gently wipe the metal probe and glass capillary with a KimWipe to remove excess sample. If you can see the sample on the capillary, you've loaded too much — wipe gently with a KimWipe again until you cannot see the sample. For solutions, use a microsyringe to apply a 2–5 μL drop to the *closed* tip of the capillary and allow the solvent to evaporate (do not use higher boiling solvents for this as it will take a long time). Be sure to rinse the syringe when you are finished (see page 3).

From Section II, the opening on top of the APCI source has been plugged with the black plastic plug, the solvent pump is off, the desolvation nitrogen is set to 0, and you have started your acquisition. In ASAP mode, a stream of 350 °C nitrogen flows through the source, across the rounded tip of a glass capillary, and into the mass spectrometer. This nitrogen stream will clean the glass capillary of any trace volatiles within 30–60 seconds. To clean the *closed* tip of the capillary, simply loosen the nut that holds the probe in place and slide the probe all the way into the source (you do not need to re-tighten the nut until you are finished with your acquisition). After 60 seconds, slide the probe all the way out of the source, let it cool for ~10 seconds, and load your sample onto the *closed* tip of the capillary as described above. Wipe the probe and capillary with a KimWipe and gently slide it all the way back into the source. You will see a spike in the ion count and new peaks will appear in the mass spectrum. Leave the capillary in the source for 30–60 seconds to allow all of the sample to be vaporized from the capillary (the ion count will return to baseline and you will no longer see your peaks in the mass spectrum live feed).* You can run multiple ASAP samples within this same acquisition. When you finish running your samples, click the blue Stop button in Mass Express to stop the acquisition, pull the probe partially out of the source (~1 inch or 2 cm), and tighten the nut to keep the probe in place.

Key Points

1. Clean the capillary by inserting the probe into the source as far as possible for 30–60 seconds
2. Remove the probe, allow to cool, load the sample onto the capillary (above), and clean with KimWipe
3. Insert the probe into the source for 30–60 seconds or until the sample is not visible in the MS live feed*
4. Run additional ASAP samples as needed, none of your sample should be left on the capillary at the end
5. Stop the acquisition, pull the probe partially out of the source (~1 inch or 2 cm), and tighten the nut to hold it in place

* If the sample is overloaded or is not very volatile, it may take longer than 60 seconds for the ion count to return to baseline or for your new mass peaks to disappear from the mass spectrum live feed. If it is taking a long time for the signals to return to baseline/background, remove the probe from the source, let it cool for 10–20 seconds, then remove the capillary from the probe by partially unscrewing the black handle from the metal probe and pulling the capillary out (discard in glass waste). Replace the capillary with a new one (*closed* tip sticking out), tighten the capillary into the probe, insert the probe into the source, and wait for all signs of your sample to disappear from the ion count and mass spectrum live feed (this may take several minutes).

VI. Instrument Standby & Log Off

After you have collected your data, stopped your acquisition, and processed your data, follow these steps *in this order*:

1. If the solvent pump is running, stop it by pressing the Play/Pause button on the pump. The green light next to “Run” will turn off, and the pressure read-out will gradually drop to zero.
2. In Mass Express, click on the red I/O icon in the top right corner to put the instrument in “Standby” — this will turn off any nitrogen flow and allow the instrument to cool down.
3. Close the Mass Express software by clicking the X in the top right corner of the window. You will be asked if you would like to exit the program — click “Yes.” You may be prompted to save your current method, tune settings, or ion sources settings. Always click “No” for these. If Data Express is also open, close this, too.
4. Log out of the computer by opening the Start menu in the lower left corner of the screen (Windows icon), then click “Log off” (DO NOT click “Lock” or “Switch User” as these will not log you out and you will continue to be charged for time).
5. If your sample required use of the ESI source, replace it with the APCI source for the next user.
6. Check that the injection port is set to the “Load” position.
7. Check that the “dummy” TLC plate is directly under the elution head and that the cover on the Plate Express is slightly ajar (the LEDs will be purple/pink).

VII. Troubleshooting (items to be added as needed)

1. *Solvent pump pressure.* If the solvent pump pressure is not reaching 500 psi after 1–2 mins of starting flow, check that the flow rate is set to 0.2 mL/min in the “Control and Status” page in Mass Express. If it is set to another value, change it to 0.2. If it is already set to 0.2 mL/min, there may be air in the line, and the line needs to be purged. With the pump running, loosen the black prime valve on the front of the pump by a couple of turns (the pressure will drop significantly). Use the plastic syringe in the pump tray to SLOWLY pull 5-10 mL of solvent out of the opening on the prime valve. Re-tighten the prime valve and check that the pressure gradually rises to 500–750 psi. Contact Brandon Fowler if this does not solve the issue.

VIII. TLC Plate Ordering Guide

The Plate Express is a powerful tool for synthetic chemists, and it is important to use the correct types of TLC plates to ensure consistent results, prevent leaks or shattered glass, and prevent unnecessary wear-and-tear on the elution head. Therefore, TLC plates **MUST** be aluminum or plastic-backed (**NO GLASS-BACKED TLC PLATES**) and have a silica/alumina/C₁₈/etc. matrix that is 200–250 µm thick (no more, no less). The TLC plates listed below match these criteria and include a fluorescent indicator for identifying the location of your TLC spots by UV-lamp. Prices are from November 2015.

Silica Gel 200-250 µm

- Aldrich:** 56524-25EA (20×20 cm aluminum sheets, 25 count, \$187)
99577-25EA (20×20 cm plastic sheets, 25 count, \$121)
70643-50EA (4×8 cm aluminum cards, 50 count, \$42)
- Fisher:** M1164840001 (20×20 cm aluminum sheets, 25 count, \$178)
M1055490001 (5×7.5 cm aluminum cards, 20 count, \$62)
05-719-864 (2.5×7.5 cm plastic strips, 200 count, \$333)
- VWR:** JT4449-2 (2.5×7.5 cm plastic strips, 200 count, \$167)
EM1.05735.0001 (20×20 cm plastic sheets, 25 count, \$122)

C₁₈ 200-250 µm (reversed phase)

- Aldrich:** none found
- Fisher:** M1055590001 (20×20 cm aluminum sheets, 20 count, \$406)
M1055600001 (5×7.5 cm aluminum cards, 20 count, \$88)
- VWR:** 1.05559.0001 (20×20 cm aluminum sheets, 20 count, \$478)
1.05560.0001(5×7.5 cm aluminum cards, 20 count, \$108)

Alumina 200-250 µm

- Aldrich:** 06408-25EA (20×20 cm aluminum sheets, 25 count, \$139)
89071-50EA (4×8 cm plastic cards, 50 count, \$43)
- Fisher:** NC0515121 (4×8 cm plastic cards, 50 count, \$61)
- VWR:** JT4467-2 (2.5×7.5 cm plastic strips, 200 count, \$166)
EM1.05551.0001 (20×20 cm aluminum sheets, 25 count, \$226)