Rapid Drug Analysis and Research (RaDAR) Standard Operating Procedures: *Sample Extraction and Analysis*

Version 4
Date: 09/09/2025

Disclaimer: *Certain commercial products are identified in order to adequately specify the procedure; this does not imply endorsement or recommendation by NIST, nor does it imply that such products are necessarily the best available for the purpose.*

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1. Purpose

The purpose of this document is to provide procedures for sample preparation and analysis of Rapid Drug Analysis and Research (RaDAR) samples using the Bruker DART-SVP and JEOL JMS T100-LC-4G mass spectrometry (DART-MS) system.

2. Sample Collection

2.1 Samples are collected by trained collaborators using either a meta-aramid wipe, cotton swab, test strip, or vial. Syringe plungers may also be submitted.

2.2 Collaborators send wipes, swabs, test strips, vials, and/or syringe plungers in individual barcoded coin envelopes. The coin envelopes are mailed using either USPS, FedEx, or UPS.

2.3 Paraphernalia (excluding plungers) is not analyzed. If paraphernalia is received, immediately bag, and dispose of in accordance with waste disposal procedures. If broken glass/paraphernalia is received, place in a rigid plastic container and dispose of as fentanyl waste. If sampling material is received with visible blood, immediately place the sample and envelope in a red biohazard bag and dispose of it as biohazard waste.

3. Safety Notes

3.1 For complete safety procedures, refer to associated Hazard Reviews.

3.2 Sample preparation must be done in a hood.

3.3 Proper PPE must be worn at all times and includes:

3.3.1 Nitrile gloves.

3.3.2 KN95 or better mask.

3.3.3 Eye wear with side shields.

3.3.4 A fume extractor must be used during DART-MS analysis of samples.

4. Sample Intake

4.1. Open the *RaDAR Results* file.

4.2. Under the **Results** tab enter the following information:

4.2.1. **ID** – Select the next available cell then scan the barcode on the submitted coin envelope using the barcode scanner. After entering the barcode ID's, several other fields will autofill from the **MailedEnvelopes** tab, these include:

4.2.1.1. **Wipe_Swab** – Sampling material substrate (*e.g.*, wipe, swab, vial, test strip).

4.2.1.2. **Agency** – Submitting agency.

4.2.1.3. **Site** – Specific submitting site from the agency.

4.2.1.4. If any of the information is incorrect, update it in the **MailedEnvelopes** tab. This will automatically update it in the **Results** tab.

4.2.2. **Site_ID** – Site provided identifying number. Some sites will add additional numbers for their own purpose on the coin envelope, record that here. Leave blank if not applicable.

4.2.3. **Item** – Item sampled. Located on the submitted coin envelope. Match provided information to best fit from dropdown. If item sampled is not provided, then select "Unknown". If item is included but not listed in the dropdown, select "Other" and include the information in the **Notes** column. If the substrate is a test strip, select "Test Strip Solution" and if it is a vial, select "Powder". If specific information is provided (*e.g.*, color or details), include that information in the **Notes** column.

4.2.4. **Collection_Date** – Date sample was collected. Located on the submitted coin envelope. If no date is present, leave blank.

4.2.5. **Run_Date** – Date qualitative DART-MS analysis was completed. If a DART-MS analysis is not completed, leave blank.

4.2.6. **Notes** – Any additional information including but not limited to additional paraphernalia information (*e.g.*, color of paraphernalia), specific site information, and any other information that does not fall within the previous columns.

4.2.7. **Position –** This number corresponds to the position in the vial storage box. This number increases by 1 for each sample moving down the sheet.

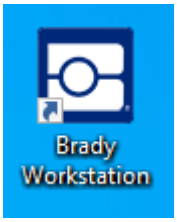
5. Vial Printing

5.1. Individual vials are made for each sample (expect samples received in quant vials) and are labelled with a barcode sticker corresponding to the ID.

5.2. Using the *Vials* Excel file, update the file by copying the new barcode numbers from the *RaDAR Results* Excel into column A ("Barcode") and the corresponding vial storage position in column B ("Position"), starting with row 2. Leave the text "Barcode" in cell A1 and "Position" in Cell B1. Save the file.

5.3. Open the Brady Workstation program located on the desktop.

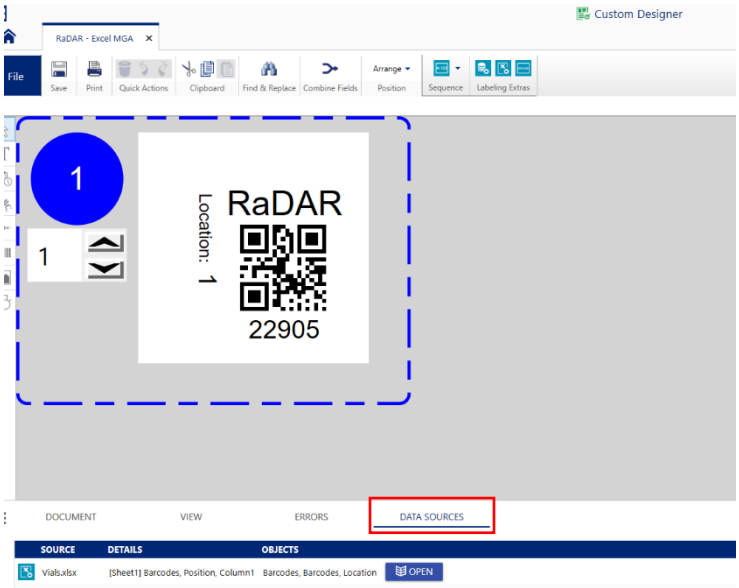
Brady Workstation Icon



5.4. Open your existing RaDAR print file.

5.5. The label editing screen will open with a premade label template.

Label editing screen with premade label template



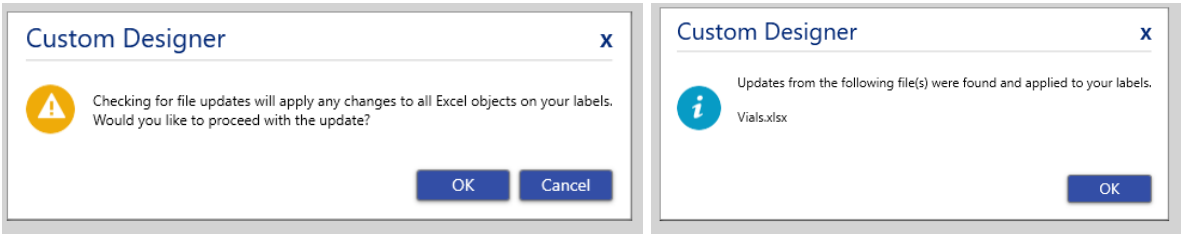
5.6. Select *Data Source* tab (red box). Make sure that the source is set to *Vials.xlsx*. Select *OPEN*.

5.6.1. If the source is not set to *Vials.xlsx,* within the *Labeling Extras* at the top select *Excel Import* (middle icon). Then select *Select Excel File* and choose the *Vial.xlsx* file from the desktop folder.

5.7. Next select *Check for updates* in the bottom left corner.

5.8. A window will open named *Custom Designer* select “OK”. Another window will open, select “OK”.

Custom Designer windows for data source update



5.9. Check that the numbers on the labels now show the barcode numbers needed to be printed along with the corresponding vial storage position number.

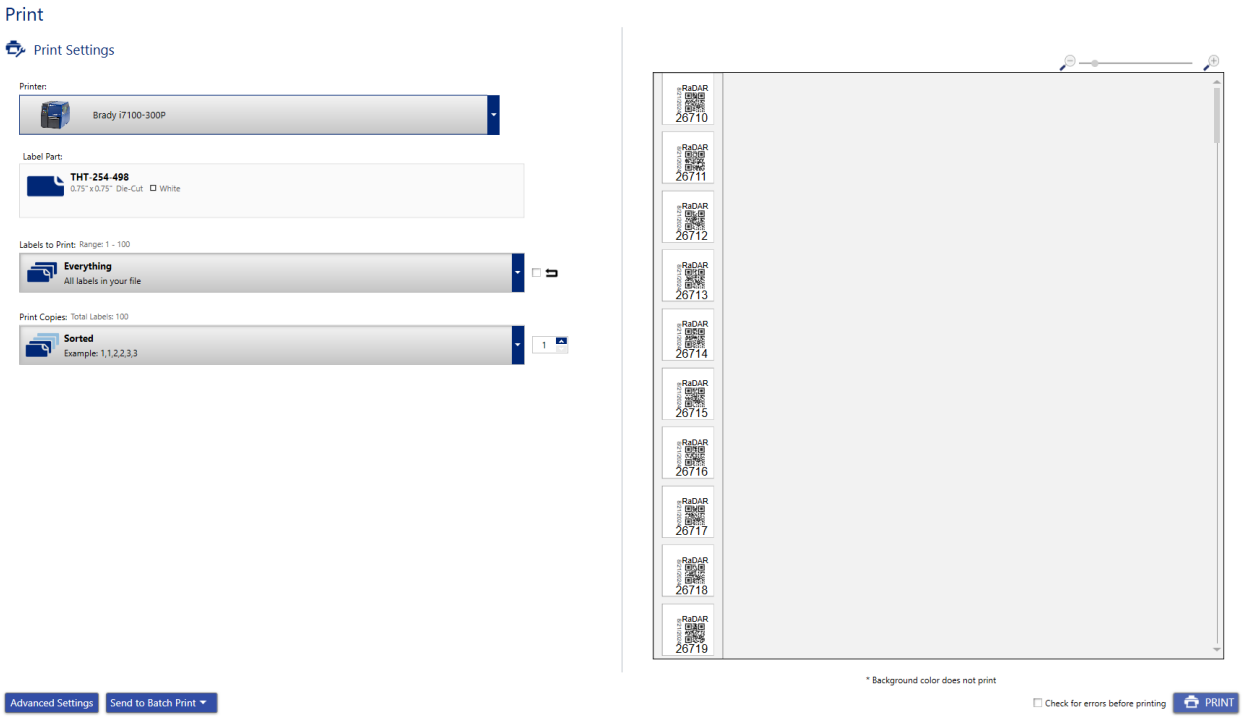
5.10. Once all the information on the label is correct, select *Print* at the top of the screen. A new window will appear.

5.10.1. Make sure the correct options are selected.

5.10.1.1. Printer: Brady i7100-300P

5.10.1.2. Label part: THT-254-498 (0.75” x 0.75” Die-Cut White)

Print window



5.10.1.3. Labels to Print: Everything

5.10.1.4. Print Copies: Sorted

5.11. Press *Print* in bottom right corner.

5.12. The screen on the printer will illuminate with the prompt “Printing 1 of 1”.

5.13. Place a 2 mL amber glass vial in the Brady printer with the opening of the vial to the right.

5.14. Press the yellow button (with the picture of a finger). Label will be printed onto the vial then the vial will be dropped into the tray below. Remove the vial and place in vial tray.

5.15. Repeat placing vials in printer and printing labels until all vials are prepared.

Printer instructions showing printer display and how to place vials into printer

6. General Sample Extraction Protocols

6.1 Samples are prepared in the hood.

6.1.1 The hood should be wiped down with methanol and a Kimwipe (located in the hood) before starting sample preparation and after preparation is completed.

6.1.2 Set up an unlabeled zip-lock waste bag in a beaker inside the hood.

6.2 Scissors and tweezers are needed to prepare the samples. Clean scissors and tweezers with methanol before and after each sample by applying methanol to a fresh Kimwipe and wiping all surfaces.

6.3 Caffeine-d_3 in methanol (10 mg/L) is used as the internal standard/extraction solvent.

6.3.1 If all solvent bottles have been consumed, additional solvent should be prepared using the following procedure.

6.3.1.1 Calibrate the balance following the protocols in the ***Balance Calibration*** document.

6.3.1.2 Place a clean weigh boat on the balance and allow it to settle to zero.

6.3.1.3 Using a spatula cleaned with a Kimwipe and methanol, place 10.0 mg of caffeine-d_3 into the weigh boat and document the exact mass weighed.

6.3.1.4 Rinse a clean, dry 1 L volumetric flask with HPLC grade methanol. Then add about 400 mL of HPLC grade methanol to the flask.

6.3.1.5 With a glass Pasteur pipette and bulb, gently add HPLC grade methanol to the weigh boat to dissolve the caffeine-d_3. Transfer the solution to the 1 L volumetric flask and repeat this step until all caffeine-d_3 has been dissolved and transferred to the volumetric flask.

6.3.1.6 Once all caffeine-d_3 has been transferred, fill the volumetric flask to the 1 L meniscus line with HPLC grade methanol, cap the flask and invert approximately 20 to 30 times to mix thoroughly.

6.3.1.7 Once solvent is thoroughly mixed, transfer to four clean 250 mL bottles and cap them. Ensure the bottles contain proper chemical labels and store them in the refrigerator (at or below 4 °C).

6.4 Substrates or samples that should **NOT** be analyzed.

6.4.1 Paraphernalia/ pills/ powders/ etc.

6.4.1.1 Immediately bag and dispose. If broken glass/paraphernalia is received, place in a rigid plastic container and dispose of as fentanyl waste.

6.4.1.2 Note in the *RaDAR Results* Excel sheet under **Compound_1** column use the entry “Sample Not Tested”. In **Notes** column, give explanation that paraphernalia was received.

6.4.2 Substrates (swabs/ syringe plungers) with visible dried blood contamination

6.4.2.1 Immediately return substrate to envelope, place envelope in a small red biohazard plastic bag and dispose of in the biohazardous waste bin.

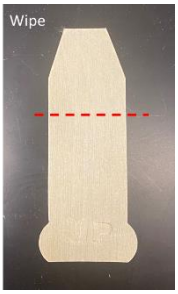
6.4.2.2 Note in the *RaDAR Results* Excel sheet under **Compound_1** column use the entry “Sample Not Tested”. In **Notes** column, give biohazard explanation.

7. Wipe Extraction

7.1 Add 1 mL caffeine-d_3 in methanol to the barcoded 2 mL amber glass vial.

7.2 Trim the wipe, with clean scissors, as shown in the photo on right. Using clean tweezers, place the tapered portion of the wipe into the corresponding vial. Place the unsampled portion of the wipe in the zip-lock waste bag located in the hood and clean the scissors with methanol and a Kimwipe.

Where to trim wipe for optimal recovery of residue



7.3 Cap the vial and vortex for 10 s.

7.4 After vortexing the sample, remove the wipe from the vial using clean tweezers to prevent reabsorption. Dispose of the substrate in the zip-lock waste bag located in the hood. Wipe the tweezers clean with methanol and a Kimwipe in between the removal of each substrate from the vial.

7.5 After the sample has been prepared, store the coin envelopes in an envelope storage box.

8. Cotton Swab Extraction

8.1. Add 1 mL caffeine-d_3 in methanol to the barcoded 2 mL amber glass vial.

8.1 If a wooden-shaft cotton swab is received, place the swab into the corresponding vial and trim the swab shaft to a length where the vial can be capped (see photo). Place the remaining wood piece in the zip-lock waste bag.

Where to trim swab for optimal recovery of residue

Note: If a plastic-shaft swab is received, extract the sample the same way but make note that a plastic swab was received in the *RaDAR Results* Excel sheet under the **Notes** column.

8.2 Cap the vial and vortex for 10 s.

8.3 After vortexing the sample, remove the swab from the vial using clean tweezers to prevent reabsorption. Dispose of the substrate in the zip-lock waste bag. Wipe the tweezers clean with methanol and a Kimwipe in between the removal of each substrate from the vial.

8.4 After the sample has been prepared, store the coin envelopes in a storage box.

Note: If both a wipe and swab are received, first, prepare, extract, and analyze the swab. If after analysis by DART-MS, no compounds are detected using the DIT, prepare and extract the wipe in the sample solution and reanalyze by DART-MS. For this additional DART-MS run, include a sequential letter directly after the barcode number in the filename (*e.g.*, sample 0327 would be rerun as 0327a_30 and 0327a_60).

9. Syringe Plunger Extraction

9.1 Add 1 mL of caffeine-d_3 in methanol to a barcoded 2 mL amber glass vial.

9.2 Swirl the rubber end of the plunger in the solvent for 5 s and remove.

9.3 Place the plunger in the zip-lock waste bag located in the hood.

9.4 After the sample has been prepared, store the coin envelopes in a storage box.

Note: If a plunger is received along with a wipe or swab, swirl the plunger in the solvent first then extract the wipe or swab in the same vial.

10. Used Test Strip Extraction

10.1. Add 0.5 mL of caffeine-d_3 in methanol to a barcoded 2 mL amber glass vial.

***Note*:** This step is different from the other substrates.

10.2. Place the test strip in the corresponding vial with the white absorbent side down and trim the test strip (with clean scissors) to a length where the vial can be capped. Place the remaining unused portion of the test strip in the zip-lock waste bag.

10.3. Cap the vial and vortex for 10 s.

10.4. After vortexing the sample, remove the test strip from the vial using clean tweezers to prevent reabsorption. Dispose of the substrate in the zip-lock waste bag located in the hood. Wipe the tweezers clean with methanol and a Kimwipe in between the removal of each substrate from the vial.

Where to trim test strip for optimal recovery of residue

10.5. After the sample has been prepared, store the coin envelopes in a storage box.

11. Quant Vial Preparation

11.1 Pre-weighed vials are provided to sites containing 0.5 mL of caffeine-d_3 in acetonitrile. Vials are prepared by sites by adding a 1 to 2 microscoops of powder material to the vial and submitting it for quantitative testing.

11.2 These samples are ready for DART-MS analysis upon receiving, no sample preparation is needed.

11.3 Following analysis by DART-MS, remove and dispose of the caps and place the vials in a hood for evaporation.

11.4 Proceed to *RaDAR Quant SOP* for full quant protocol.

11.5 After the sample has been prepared, store the envelopes in a storage box in.

12. Waste Disposal

12.1 Mailing envelopes.

12.1.1 Mailing envelopes can be disposed directly in a trash bin.

12.2 Sample envelopes (barcoded coin envelopes).

12.2.1 Discard of envelopes from two months and prior by placing them in a zip-lock bag and discard as hazardous waste.

Note: Envelopes are kept for two months in case a discrepancy is found.

12.3 Substrates.

12.3.1 Ensure that all used substrates collected after sample extraction were disposed of in the zip-lock waste bag within the labeled fentanyl waste beaker.

12.3.2 Dispose of full unlabeled zip-lock bag as hazardous waste.

12.4 Vials (extracted, confirmatory, and quant).

12.4.1 All vials are to be stored according to the position identified in the *RaDAR Results* Excel position column which is listed on the vial.

12.4.2 Store the vial in the appropriate box with proper hazard documentation.

12.4.3 Once a box is filled, start a new box.

12.4.4 Vials within the previous six months must be kept for additional sample testing or confirmatory testing.

12.4.5 Vials older than six months must be disposed of as hazardous waste.

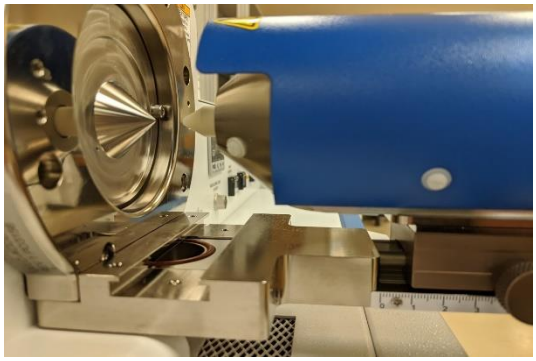
12.4.6 Quant vials are stored along with the extraction samples in their respective vial positions. Use a sharpie to write the position on the vial before storing but after quant analysis.

13. Setting up and Turning on the DART Source

13.1 Open the helium and nitrogen gas cylinders connected to the corresponding instrument. Ensure that there is sufficient gas (500 psi on right hand dial) remaining in the tank and that the pressures (left dial) are set to between 75 psi and 80 psi for helium and 95 psi to 100 psi for nitrogen.

13.2 The DART source is set up by ensuring it is securely screwed into the baseplate, and the front of DART baseplate is at the 1.0 cm to 1.5 cm mark on baseplate ruler, as shown below. Remove the cap covering the mass spectrometer inlet.

Image of DART-MS configuration. Note how the front of the baseplate is aligned with the 1.0- 1.5 cm mark on the rail.



13.3 Ensure that the DART controller box (blue box) is turned on and that the DART power cable (black cable) and the DART gas supply lines are appropriately connected.

13.3.1 Open Google Chrome to access the DART control panel.

13.3.2 If the DART control screen does not automatically appear, type in the following IP address: 192.168.10.111 (this is the IP address for the server within the DART box).

13.4 The main screen on the DART control panel has three operating modes:

13.4.1 **Off:** Both the heater and the high voltage are turned off. This is how the source should be left when the operator is finished running samples or plans to leave the instrument for more than one hour.

13.4.2 **Standby:** The DART gas temperature is maintained at the selected temperature and the system is automatically switched to nitrogen. Under this setting, the high voltage is not on. This setting should be used when warming up the instrument or between runs.

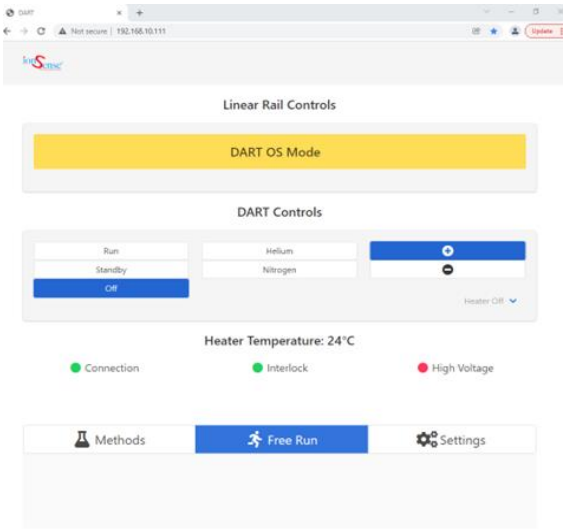
Note: There will be no signal when using this setting because the voltages inside the DART source are off.

13.4.3 **Run:** The DART gas temperature and high voltages are on. The source will switch to helium under this setting. This setting should be used for collection of data. Due to the high consumption of helium in this setting, use ***Standby*** when not collecting data.

13.5 Select ***Standby*** and set the heater temperature to 400 °C.

13.6 Ensure the positive polarity is chosen on the DART control panel.

DART Software Interface



13.7 Ensure the snorkel is placed over the sampling region and turned on to prevent unintended inhalation of samples.

Snorkel Placement

14. Setting up and Turning on the Mass Spectrometer

14.1 Open the flight tube isolation valve (silver valve in the center of the top of the MS) by pushing down on the valve, turning it counterclockwise, lifting it up and turning it clockwise to lock it in the open position.

Note: When there is no signal in the system, it is most commonly because the isolation valve was not opened or did not stay open.

14.2 If not already opened, open the msAxel@LP program.

msAxel Icon

14.3 Make sure that the correct project is open. The project information is located in the top left corner. The project name should reflect the current year and month (*e.g.,* 2025-01).

14.3.1 If the project needs to be changed, select File > Open Project. Select the appropriate file.

14.3.2 If a new project is needed (e.g., at the start of a new month), select File > New Project. Change the project name to reflect the current month and ensure that the

msAxelData folder is selected. Check *Copy Project* and select the previous month as the template. Do not select the *Acquisition Data* box.

14.4 Load method for sample analysis by selecting ***Load Method*** then selecting ***2024_RaDAR_30***.

14.5 On the second dropdown on the drop banner, which reads “Evacuation Ready”, select ***Operate*** (This will turn on the high voltages within the mass spectrometer).

14.6 Allow the system to reach temperature and stabilize for at least 10 min prior to collecting data, including tuning and QC check.

15. Daily Mass Spectrometer Calibration & QC Check

15.1 The mass spectrometer must be calibrated and QC checked daily, prior to analyzing samples.

15.2 Refer to ***AccuTOF Calibration and QC*** document for instructions on how to tune and QC check the instrument.

15.3 If the calibration and QC checks have been completed for the day, go directly to setting up your run.

16. Setting Up a Run

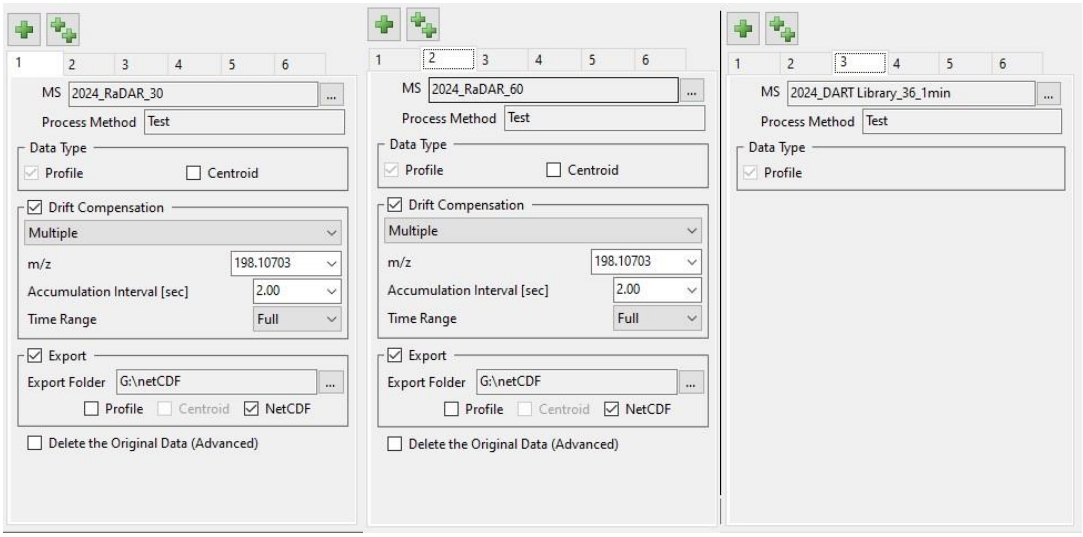
16.1 To setup a data collection sequence, click the ***Monitor/Sequence*** button in msAxel.

16.2 Ensure the following method is set in the method editor box (on the right of the screen).

16.2.1 ***2024_RaDAR_30*** should be Method 1, ***2024_RaDAR_60*** should be Method 2, and ***2024_DART_Library_36_1min*** should be Method 3. To change the methods, select the three dots next to the method name then select the appropriate method.

16.2.1.1 The two RaDAR methods should have Drift Compensation and Export checked. The drop down within Drift Compensation should be selected to

Method editor within msAxel



Multiple, the *m/z* set to 198.10703, the Accumulation Interval to 2 s and the Time Range to Full. The Export Folder should be set to the desired location.

16.3 Click green plus button above the ***Method Editor*** to add rows to the ***Sample List***.

16.3.1 The single green plus adds one line at a time and the double green plus button allows you to add multiple lines at one time.

16.3.2 Add the number of rows (twice the number of samples) needed.

16.4 Create your sequence in the ***Sample List***.

16.4.1 Samples are run in duplicate, one time using MethodSet 1 (***2024_RaDAR_30***) and one time using MethodSet 2 (***2024_RaDAR_60***).

16.4.1.1 Label the **Acquisition Data Folder** as the date using a YYYY-MM-DD format (*e.g.*, 2025-08-12). All datafiles collected on the same day can be placed into the same folder.

16.4.2 Under ***Acquisition Data*** scan the barcode on each vial twice.

16.4.2.1 Select the cell to be filled.

16.4.2.2 Scan the barcode located on the vial and add "_30" to the first run.

16.4.2.3 The selected cell will automatically move down to the next cell.

16.4.2.4 Rescan the vial to add a second run and add "_60" to the second run.

16.4.2.5 Repeat the above steps until all the samples have been scanned.

16.4.2.6 If it is an additional analysis of a previously analyzed sample, label with a letter after the barcode number (e.g., a rerun of sample 0001 would be labeled 0001a_30 and 0001a_60).

16.4.3 Set the ***Method*** to MethodSet 1 for all samples with "_30" and MethodSet2 for all samples with "_60".

An example of a typical sequence table.

Acquisition Data Folder	Acquisition Data	Method
2025-08-12	47567_30	MethodSet 1
2025-08-12	47567_60	MethodSet 2
2025-08-12	47568_30	MethodSet 1
2025-08-12	47568_60	MethodSet 2
2025-08-12	47569_30	MethodSet 1
2025-08-12	47569_60	MethodSet 2
2025-08-12	47571_30	MethodSet 1
2025-08-12	47571_60	MethodSet 2
2025-08-12	47573_30	MethodSet 1
2025-08-12	47573_60	MethodSet 2
2025-08-12	47574_30	MethodSet 1
2025-08-12	47574_60	MethodSet 2

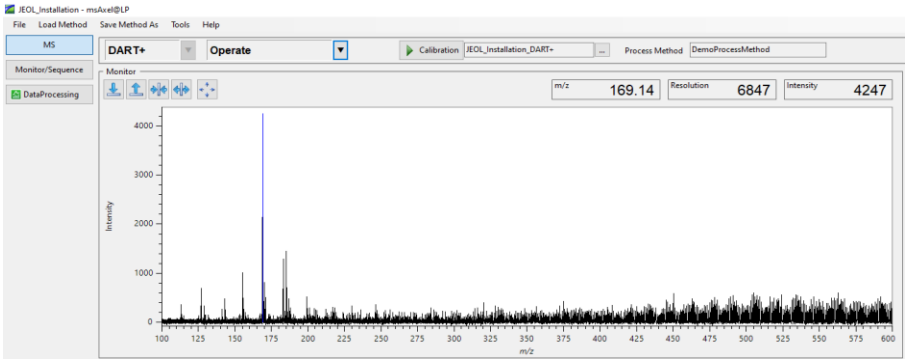
17. Initial Operation

17.1 Once the sequence is created, click ***MS*** in msAxel to go back the main screen.

17.2 Open the DART control panel (Google Chrome) and select ***Run***.

17.2.1 At this point a background mass spectrum should be visible in the spectrum monitor of the msAxel software under the Monitor screen. If nothing is showing, check that the flight tube isolation value is open and that the cap is removed from the inlet.

An example of a typical background mass spectrum that should be observed when entering Run mode.



17.3 Use the heated gas stream of the DART source to clean the necessary number of glass microcapillary rods by leaving them in the gas stream for approximately 4 s. Alternatively, they may be baked in an oven over 250 ºC overnight. Note: The rods have trace amounts of plasticizer from the plastic container in which they are stored. A peak at nominal *m/z* 371 will be present on uncleaned rods.

18. Completing a Run

18.1 Select ***Monitor/Sequence*** in msAxel to go back to the ***Sample List***.

18.2 When ready to begin collecting data, select the green ***Start*** button.

18.2.1 Make sure that the instrument is in ***Operate*** mode and that the DART gas source is set to ***Run*** with helium running.

18.3 A window will pop up to prompt data collection. When ready, select ***Go***.

18.4 In each run collect three replicate spectra for the sample. The rod should be dipped into the sample between each replicate. Dispose of this rod after collecting all replicates.

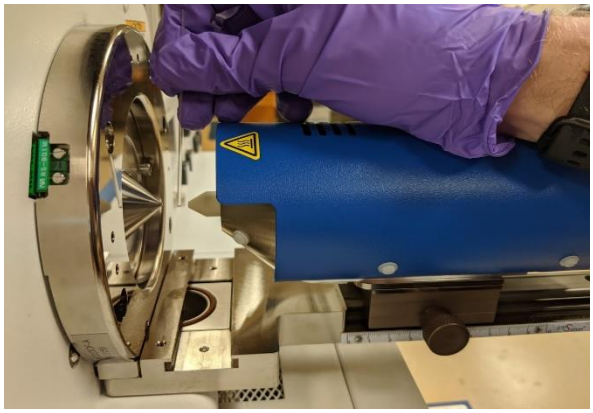
18.4.1 The same rod can be used for the 30 V and 60 V runs.

18.5 The run will automatically end after run time and a new ***Start Run*** window will pop up for the next sample in the sample list.

18.5.1 Do not use a rod for more than one sample.

18.5.2 When analyzing, the rod should be positioned approximately **0.5 cm** from the DART source. A slight twirling motion while slowly dipping the rod through the gas stream for approximately 4 s will help in sample desorption.

Example of where to position the glass rod for sampling.



19. Putting the Instrument in Standby Mode

19.1 Once a sequence is completed, put the instrument into a standby state if it will be idle for more than 10 min.

19.2 In the DART interface software (Google Chrome), change the DART source state by selecting ***Standby*** or ***Off***.

19.2.1 Use ***Standby*** if additional data collection will take place in the next hour. Otherwise, turn the DART source to ***Off***.

19.2.2 If the DART source is turned off, a flow of nitrogen gas will continue to prevent overheating of the source until it cools below 200 °C. This flow will be automatically turned off once the source temperature drops below 200 °C and the blue highlighted box under the gas tab in the DART interface software turns grey.

Note: To prevent damage to the source, do not close the nitrogen gas cylinder prior to the DART source shutting off nitrogen.

19.3 If you are going to continue data collection within an hour, keep the mass spectrometer in ***Operate*** mode. If not, under the ***MS*** tab in msAxel, change the instrument status from ***Operate*** to ***Evacuation Ready***.

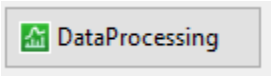
19.4 When done collecting data for the day, close the silver flight tube isolation valve on the top of the mass spectrometer.

19.5 When done collecting data for the day, close the helium and nitrogen tanks once the DART source has shut off the gas flow.

20. Data Processing – Manually Extracting Mass Spectra

20.1 To begin data processing click on the ***Data Processing*** tab on the left side of the msAxel program.

The Data Processing icon.



20.2 Once the Data Processing software is open, locate the desired datafile in the respective Data Acquisition Folder on the left side of the screen. Once located, right click on the 30 V file (e.g., 12345_30) and select ***Open TICC*** for the file. This will open the chronogram in the ***Chromatograms*** pane.

20.3 Operations in the ***Chromatograms*** and ***Spectra*** panes:

20.3.1 The pane with a blue box around it represents the active pane. A single left click will allow you to switch between panes.

20.3.2 Left click and drag or left click and drawing a box will allow you to zoom into the data.

20.3.2.1 A double left click will zoom out of the data.

20.3.3 In the ***Chromatograms*** pane, hold the **Control** button and then left click and drag over the peak(s) of interest to open the mass spectra. For samplings containing three peaks, all three peaks should be included to obtain the mass spectra of the samplings.

20.3.3.1 Holding the **Shift** button, left click and drag over an area of background directly before or after the peak(s) to perform background subtraction.

20.3.4 To delete a Chromatogram or Spectrum from view click on the desired pane and hit the **Delete** key. Alternatively, you can right click and select ***Close Chart*** or ***Close All Charts***.

20.4 Extract the mass spectrum of the sample by extracting all three replicate peaks at one time using the time of no sampling as the background subtraction (*i.e.*, press and hold **Ctrl** then highlight three peaks then press and hold **Shift** then highlight the area associated with no sampling).

20.5 The method used to collect the data allows for automatic drift compensation using caffeine-d_3 (*m/z* 198.10703). To check if the drift compensation was applied right click on the mass spectrum and select [Acquisition Data] *m/z* Calibration Viewer. Multiple should be selected under Drift Compensation with “0: 198.10703” selected.

20.6 Save the mass spectrum.

20.6.1 Save the file as a centroided file by selecting the spectrum and clicking ***File → Export → Plain Text (to Centroid)***.

20.6.2 Save the file in the appropriate folder (ensure folder naming is YYYY-MM-DD format).

20.6.3 Label the spectrum with the sample number followed by "_30" (*e.g.*, 12345_30).

20.6.3.1 If this is a sample rerun, also include the appropriate letter in the filename (*e.g.*, 12345a_30).

20.7 Right click on the 60 V data file (e.g., 12345_60) and select ***Open TICC*** for the file. Extract the mass spectrum of the sample by extracting all three replicate peaks at one time using the time of no sampling as the background subtraction (i.e., press and hold **Ctrl** then highlight three peaks then press and hold **Shift** then highlight the area associated with no sampling). This sample is also automatically drift compensated and can be checked in the same manner as above. Save the file in the same folder as above but with "_60" following the sample number (*e.g.*, 12345_60) also including additional information after the sample number but before the voltage information (*e.g.*, 12345a_60) if required.

20.8 Repeat this process for all samples in the sequence.

21. Data Processing – Searching Mass Spectra

21.1 To search the saved mass spectra, open the DART-MS Data Interpretation Tool (DIT) by opening ***DIT_v3-22*** and selecting ***run-DIT.bat***.

Note: Additional instructions and detailed overview of the DIT can be found in the DART-MS Data Interpretation Tool User Manual.

21.2 Select the ***Search Tool*** tab from the top.

21.3 Select ***Interactive***.

21.4 Select the ***Settings*** tab.

21.4.1 Select ***Display only targets with potential matches***.

21.4.2 Set **Targeting Threshold (%)** to 1%.

21.4.3 Set ***m/z* Tolerance (± Da)** to 0.005.

21.5 Select ***Query Spectra*** tab.

21.5.1 Set **Reference Library**.

21.5.2 Upload 30 V spectrum of interest.

21.5.2.1 Select ***Browse*** for **Low (30 V) Spectrum**.

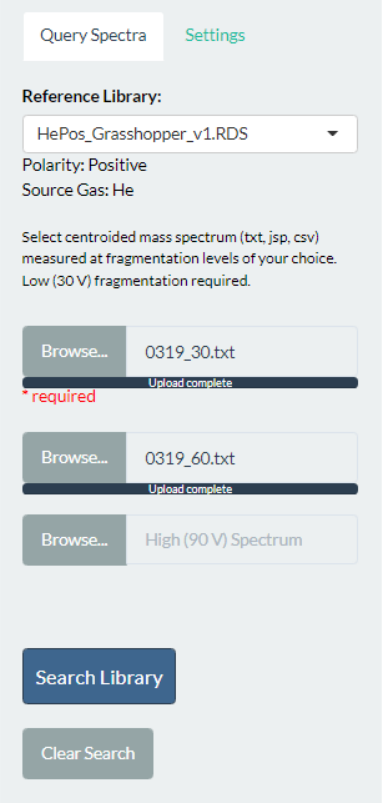
21.5.2.2 Select 30 V spectrum (*e.g.*, 0001_30) from the appropriate folder.

21.5.3 Upload 60 V spectrum of interest.

21.5.3.1 Select ***Browse*** for **Mid (60 V) Spectrum**.

21.5.3.2 Select 60 V spectrum (*e.g.*, 0001_60) from the appropriate folder.

Query Spectra tab within DIT



21.6 Search samples against the library by selecting ***Search Library***.

21.7 The results appear to the right.

21.7.1 Switch between viewing the 30 V and 60 V spectra by selecting the appropriate tab above the displayed spectrum.

21.7.2 The table on the right shows the *m/z* values detected with corresponding relative intensities and target assignments.

21.7.2.1 Targets are identified peaks above the targeting threshold %.

21.7.2.2 Targets are numbered based on decreasing relative intensity in the Low (30 V) spectrum.

21.7.3 Below the spectrum are the identified targets.

21.7.3.1 Only targets with potential matches are displayed.

21.7.3.2 To select different targets, click the appropriate tab.

21.7.3.3 Within each target tab the measured *m/z* of the peak and the relative intensity are listed.

21.7.3.4 The table contains the potential library match(es), the Δ*m/z* between the measured and theoretical values, the FPIE and RevMF spectral similarity scores, the isotope ratio difference, and the match type.

21.7.3.5 Possible match types include Protonated Molecule, Base Peak, +1 Isotope (PM), +1 Isotope (BP), and Major Fragment Ion.

22. Recording Results

22.1 Record the results in the *RaDAR Results* Excel file.

22.1.1 Under the **Results** tab enter the following information:

22.1.2 **Run_Date** – Date you completed the analysis.

22.1.3 **Compound_1** through **Compound_15** – Compounds identified by DART-MS analysis and DIT search.

22.1.3.1 Information to help identify commonly seen compounds can be found in the ***RaDAR Data Interpretation*** document. This document contains common *m/z* ratios, fragmentation patterns, general notes about the drug scoring, and helpful tips for proper identification using the DIT.

22.1.3.2 Some targets may be identifying the same compound (*e.g.*, base peak, major fragment, or isotope peak for one compound). For those cases, only record the compound once.

22.1.3.3 Only one compound should be entered in each cell.

22.1.3.4 Record results for targets over 3 % relative intensity in the **Compound** columns.

22.1.3.4.1 If a compound is detected between 1% and 3% add to the **Other Relevant Information** column with "BT:" (below threshold) before listing the compounds (e.g., BT: Cocaine; Xylazine).

22.1.3.4.2 Ensure that a ";" is used to separate all compounds listed as below threshold.

22.1.3.5 If no compounds are detected, then enter "No Compounds Detected" in the **Compound_1** column.

22.1.3.6 If the sample was not analyzed for any reason, enter "Sample Not Tested" in the **Compound_1** column and record why it was not tested in the **Notes** column.

22.1.4 **Other Relevant Information** – Only include compounds below threshold (BT) in this column.

22.1.5 **Confirmation Rule Outs** - If the compound requires confirmatory/additional testing, add the compound to this column with "*CP*:" (Confirmation Pending) (*e.g.*, CP: Cocaine). Highlight the cell **yellow**, highlight the **ID** cell in yellow, and add the information to the *Pending Confirmations* file in the RaDAR Teams General channel.

22.1.5.1 Once confirmatory testing is complete, change the highlight color to **purple** and change "*CP*:" to either "*CC*:" (Confirmation Complete) if the compound is confirmed or "*RO*:" (Ruled Out) if the compound is ruled out. If the

compound is confirmed, in addition to updating the **Confirmation Rule Outs** column add it to the appropriate Compound column and highlight purple. Highlight the **ID** column in purple as well.

22.2 Notify another RAD/RaDAR researcher to review the results. When results have been reviewed, enter an "*R*" in the **Reviewed** column.

22.3 For information on how to conduct confirmatory analysis, see the ***RaDAR Confirmatory Testing*** SOP.

23. Uploading Results

23.1 The results need to be uploaded to their respective submitting agencies.

23.2 Once the results are uploaded, change the "*R*" in the **Reviewed** column to an "*X*".

23.3 The following is the color key used for reporting results in Excel sheets of Google Drive sheets.

23.3.1 **Green**: All new samples are highlighted in green. If there are remaining green cells from previously uploaded data, use a different shade of green.

23.3.2 **Yellow**: Sample is undergoing confirmatory testing.

23.3.3 **Blue**: Confirmatory testing is complete, but result is not posted.

23.3.4 **Purple**: Confirmatory testing is complete, and result posted.