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Flir Systems AI-MS 1.2 Training Manual for Grant-Related Methodologies

As prepared for

Grant No. NIJ 2011-DN-BX-K552

PI: Dr. Christopher C. Mulligan (Illinois State University)

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CHAPTER 1 – GENERAL PROCEDURES

SECTION 1.1 – SOLVENT PREPARATION

1.1.1: Positive-Ion Mode Calibration Mixture

1. 75% methanol
2. 25% water
3. 0.1% formic acid
4. 600 ppb dimethyl methylphosphonate (DMMP) (125.03675 m/z)
5. 400 ppb triethyl phosphate (TEP) (183.07862 m/z)
6. 400 ppb tributyl phosphate (TBP) (267.17251 m/z)
7. 1200 ppb tritolyl phosphate (TTP) (369. 12556 m/z)
8. 2000 ppb tris(2-ethylhexyl) phosphate (TEHP) (435.36030 m/z)

The list above comprises the components of the positive-ion mode calibration mixture used during the five-step tuning procedure. Typically, only Compounds 4 through 6 are seen in the mass spectrum. However, the higher mass compounds may also be seen in some instances. Compounds 4 through 6 must be present for a successful calibration.

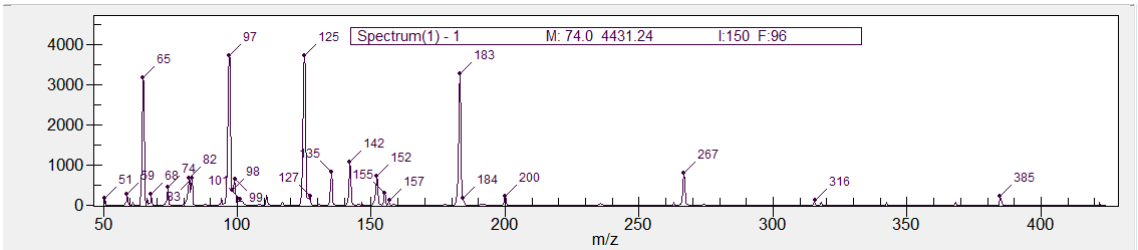

Figure 1.1 – Mass spectrum of positive-ion mode calibration mixture

1.1.2: Positive-Ion Mode (Standard) Spray Solvent

1. 50% methanol
2. 50% water
3. 0.1% formic acid

The typical spray solvent utilized for most positive-ion mode studies is 1:1 MeOH:H_2O with 0.1% formic acid. However, depending on the desired acidity and ionization efficiency of the target analyte(s), the mixture can easily be altered.

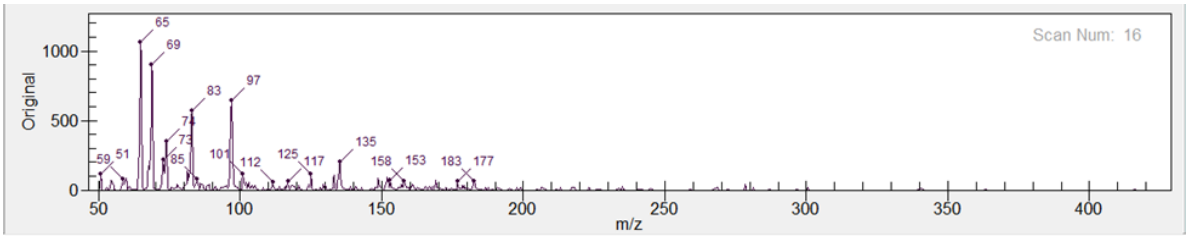

Figure 1.2 - Mass spectrum of positive-ion mode spray solvent

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1.1.3: Negative-Ion Mode Spray Solvent

1. 50% methanol
2. 50% water

A mixture of 1:1 MeOH:H_2O is typically used for negative-ion mode studies. This mixture can be altered in order to increase ionization efficiency, including an increase in basicity.

SECTION 1.2 – FILLING HAMILTON SYRINGE

All syringes are labeled for a particular analysis (DESI spray solvent (Red), ESI analyte (Green), and calibration syringe (colorless)). Each syringe can either be filled from a solvent bottle or by attaching a needle to the end of the syringe.

1.2.1: Solvent Bottle

1. Wash out the syringe with clean methanol multiple times.
2. Ensure that the syringe is empty and the plunger is completely down.
3. Attach the syringe to the solvent bottle using the syringe fitting (Luer Lock) located on the top of the bottle cap. Turn the valve so it is parallel with the syringe to allow for solvent to escape from the bottle (Figure 1.3).

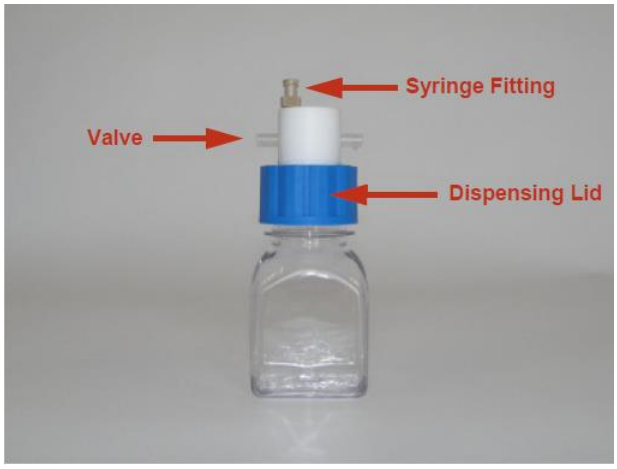

Figure 1.3 – Solvent bottle

4. Turn the bottle upside down and pull the syringe plunger out (solvent should fill the syringe) (Figure 1.4).

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Figure 1.4 – Pulling solvent into syringe

5. Detach the bottle from the syringe.
6. Tap the syringe with a finger to move the resulting air bubble to the entry point of the syringe.
7. Expel the air bubble by depressing the plunger.
8. Attach the syringe to the solvent transfer line and place it in the Harvard pump (Figure 1.5).

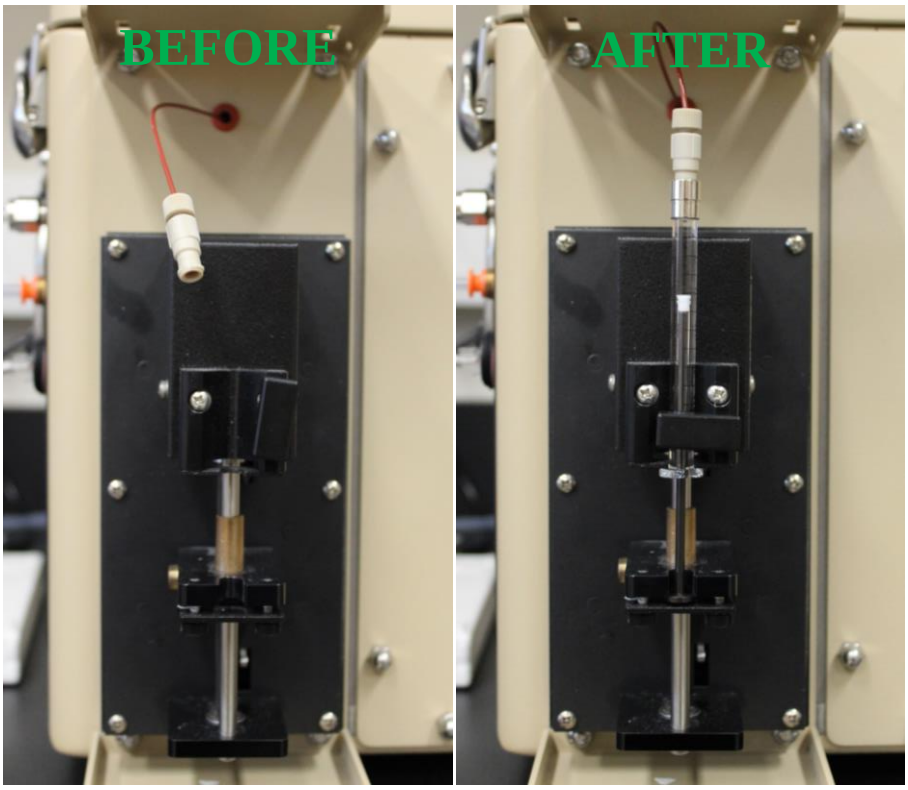

Figure 1.5 – Syringe attachment to solvent transfer line

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1.2.2: Needle-Based Syringe

1. Wash out the syringe with methanol multiple times.
2. Ensure that the syringe is empty and the plunger is completely down.
3. Attach a needle to the end of the syringe.
4. Place the needle in the desired solution and pull the plunger out to collect solution.
5. Detach the needle from the syringe.
6. Tap the syringe with a finger to move the resulting air bubble to the entry point of the syringe.
7. Expel the air bubble by depressing the plunger.
8. Remove the needle.
9. Attach the syringe to the solvent transfer line and place it in the Harvard pump.

SECTION 1.3 – POWERING-UP THE AI-MS

Power-Up Procedure

The following list should be tentatively followed when powering-up the AI-MS.

1. Make sure the electrical cord on the left side of the AI-MS is connected to an appropriate AC electrical outlet. Flip the electrical switch on the left side of the AI-MS to the "On" position. The green light on the front panel confirms that the AI-MS has power. Wait 10 minutes to allow the instrument to warm-up (Figure 1.6).

Figure 1.6 – AI-MS power switch and green light power indicator

2. Connect the appropriate laptop computer to the AI-MS using an Ethernet cable.
3. On the computer desktop, open the "Griffin System Software" (GSS) icon.
4. Connect the instrument by pressing the "Connect" icon in the upper-left corner of the start page screen. The system is successfully connected when the current status is labeled as "ready" and all subsystems are operational (Figure 1.7).

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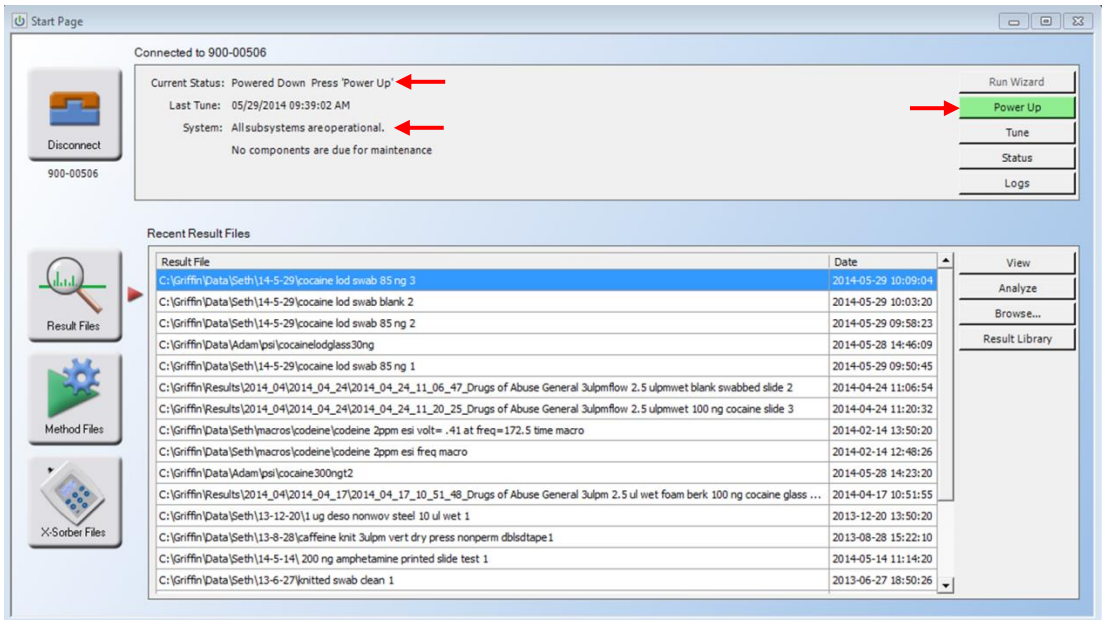

Figure 1.7 – AI-MS power-up/status window

5. To power-up the AI-MS 1.2, click the "Power Up" icon to bring up the auto power control window (Figure 1.7). This process will take approximately 5 minutes to complete as the system vacuum needs to reach the desired pressure of ~1.0 x 10^{-5} Torr (viewable in the bottom right corner of the screen).
6. The AI-MS has been successfully powered-up when the auto power control progress bar reaches 100% (Figure 1.8).
7. Close out of the window once the progress bar has reached 100%.

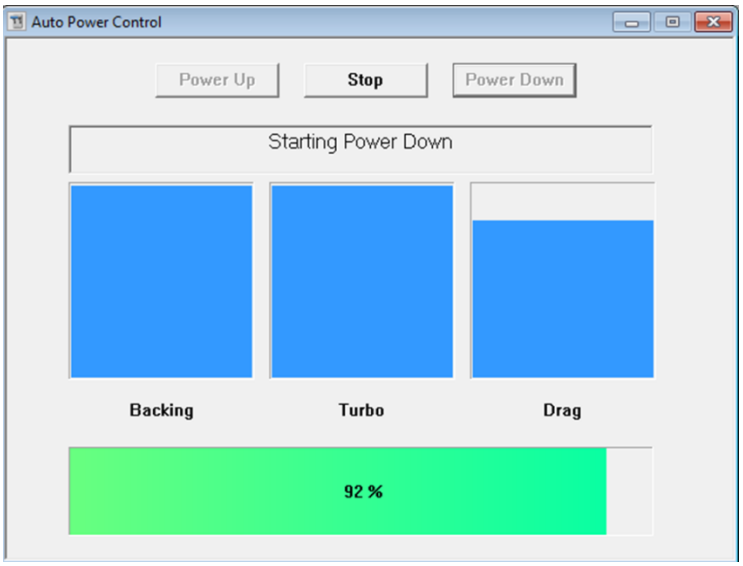

Figure 1.8 – Auto Power Control status window

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SECTION 1.4 – SWITCHING THE POLARITY OF THE AI-MS

Changing Ionization Polarity

1. If the instrument is powered-up, the polarity can be changed. To change the polarity, select "Status" under the "Instrument" drop-down menu at the top of the screen (Figure 1.9).

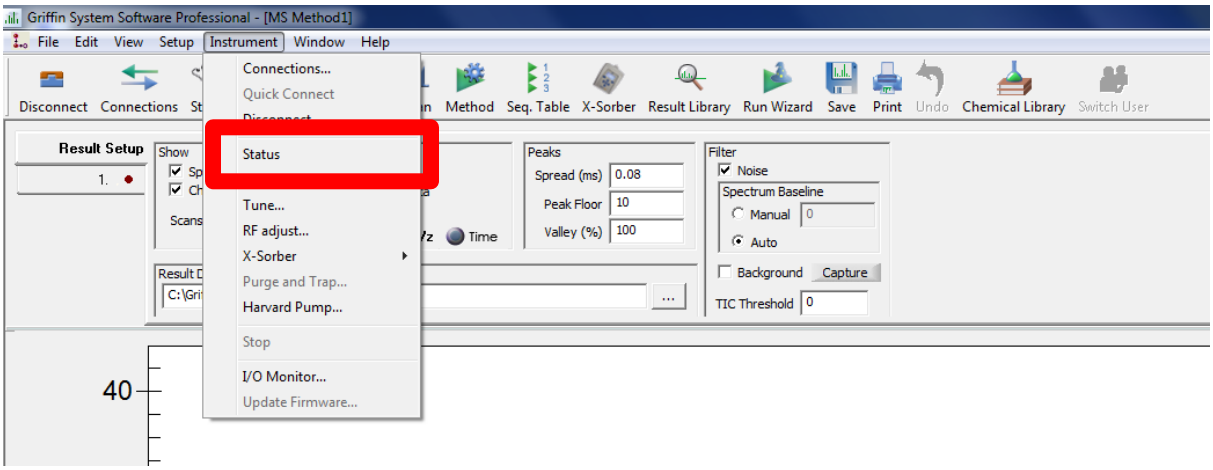

Figure 1.9 – Accessing instrument status

The instrument status box will appear (Figure 1.10). Under the "MS" tab, there will be 4 toggle buttons at the top right corner. One will read "Positive" (or "Negative" depending on the most current instrumental settings). This is the current ionization polarity of the instrument. The current ionization polarity can also be determined by looking at the instrument status bar at the bottom of the main program window.

2. Click the toggle button that reads "Positive." The button should change and now read "Negative." (Figure 1.10). Confirm the ionization polarity has changed by referencing the instrument status bar at the bottom of the main program window. To return to positive mode, simply repeat the procedure above.

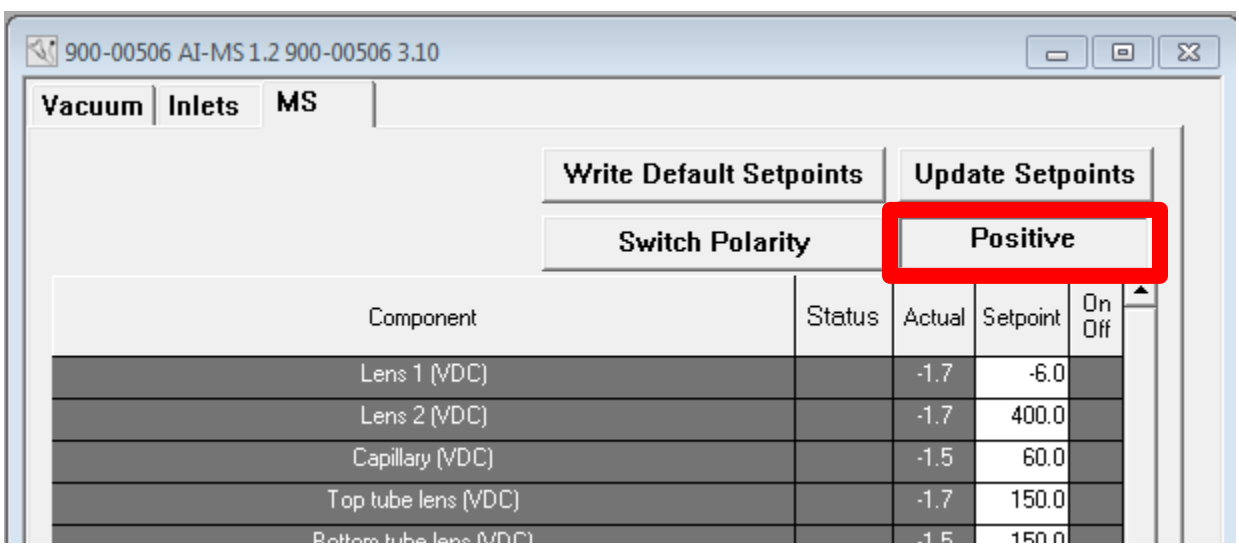

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Figure 1.10 – Polarity switching interface

Note: The I/O settings the instrument pulls from the currently loaded Configuration file are those settings stored in the "Standby" column.

SECTION 1.5 – POSITIVE-ION MODE CALIBRATION OF THE AI-MS

Positive-Mode Calibration Protocol

The positive-ion five-step calibration should be carried out daily before any analysis has taken place and after the AI-MS has been powered-up. If the AI-MS is in negative mode, the polarity must be switched back to positive mode to accommodate the calibration solution. The desired Harvard pump flow rates should be 30 µL for purging and 10 µL for calibration using ESI, as set in the Configurator.

1. If not already open, open the "Griffin System Software" (GSS) icon and connect the AI-MS.
2. Click the "Method" icon and select "New" from the "MS Only" options.
3. The window that opens should be similar to the one below (Figure 1.11).

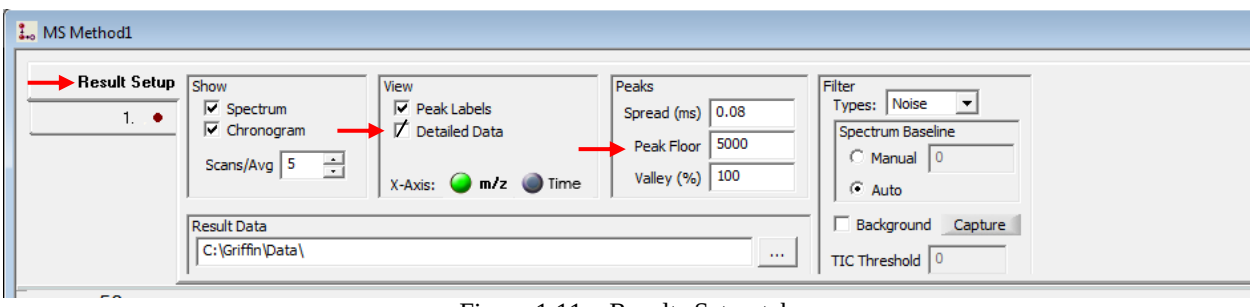

Figure 1.11 – Results Setup tab

4. Under the "Results Setup" tab, change the peak floor to **10** and make sure that detailed data is **check marked** (Figure 1.11).

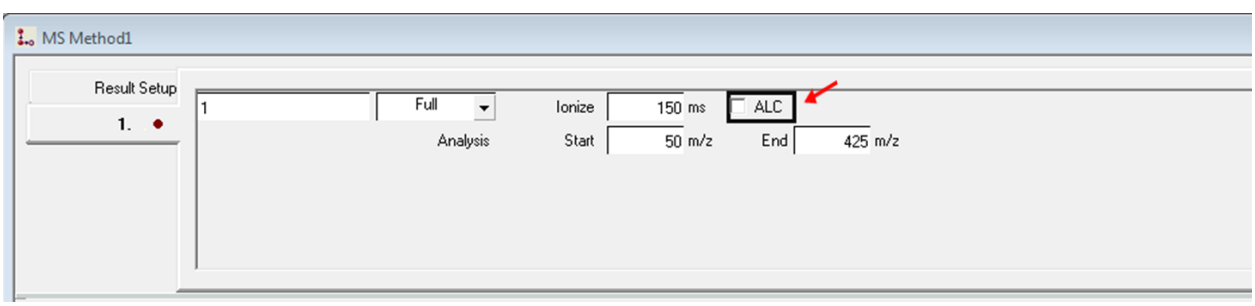

Figure 1.12 – Enabling automatic level control

5. Under the tab with the red dot, make sure that automatic level control (ALC) is not checked. Save these new configurations to the AI-MS by clicking the "Downloads Scan Configuration to Instrument" (blue arrow) icon in the right corner of the screen.

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6. Next, fill the calibration Hamilton syringe with ~300 µL of the positive-mode calibration mixture and attach to the solvent transfer line and syringe pump (see Section 1.2).
7. Before the five-step tune can be initiated, the solvent transfer lines must be purged. To purge the lines, make sure the Nitrogen gas is on and set to 100 PSI. Click the "Run" button (green arrow).

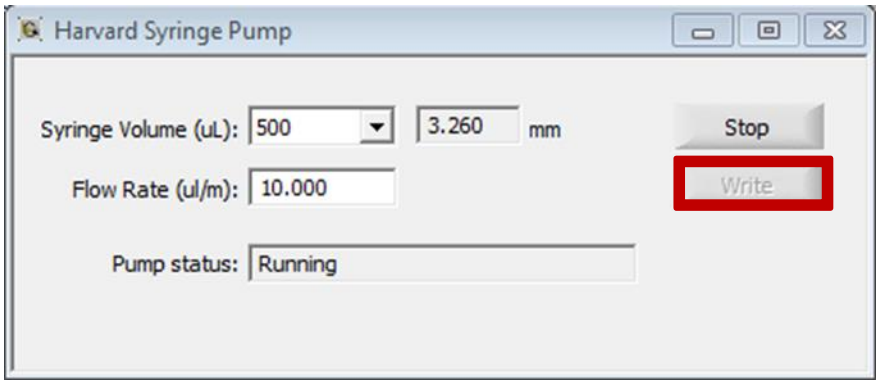

Figure 1.13 – Harvard Syringe Pump window

8. The AI-MS software will operate the syringe pump at 30 µL/min for a short period of time in order to purge the solvent transfer lines. However, there are instances where the syringe pump will need to be operated at this flow rate for a longer time period to successfully purge the lines of air. In this event, click the "Instrument" tab and select "Harvard Pump" from the drop-down menu. A syringe pump window will open in which the flow rate of the pump can be changed back to 30µL/min. If a new flow rate is inputted, **the "Write" button must be pressed to communicate the new flow rate to the instrument** (Figure 1.13).
9. Once the transfer lines have been successfully purged, change the flow rate to 10 µL/min. The mass spectrum generated should be similar to the positive-ion calibration spectrum seen in Figure 1.1.
10. After the flow rate and spectrum have stabilized, stop the current analysis by clicking the "Stop" button.
11. To perform the five-step tune, click the "Tune" icon in the top portion of the software followed by depressing the "Run" icon (green arrow).
12. The tune will then start by performing the tasks in the list below.

    1. **RF Tune** – equilibrated the RF electronics system
    2. **Low-Mass Tune** – optimizes for low mass ion intensity
    3. **MB Gain Tune** – optimizes ion signal/detection system
    4. **Quant Tune** – checks for reproducibility of ion signal
    5. **MS/MS Tune** – verifies that MS/MS operation is acceptable

13. In order for the tune to completely pass, all five steps must be passed in succession. If one step fails, the entire tuning process must be restarted. If one or more steps fail, restart the tuning process, Steps 7-12. In the case that failed steps are still present, refer to the CH.5 for troubleshooting procedures and Appendix A for corresponding flow charts.

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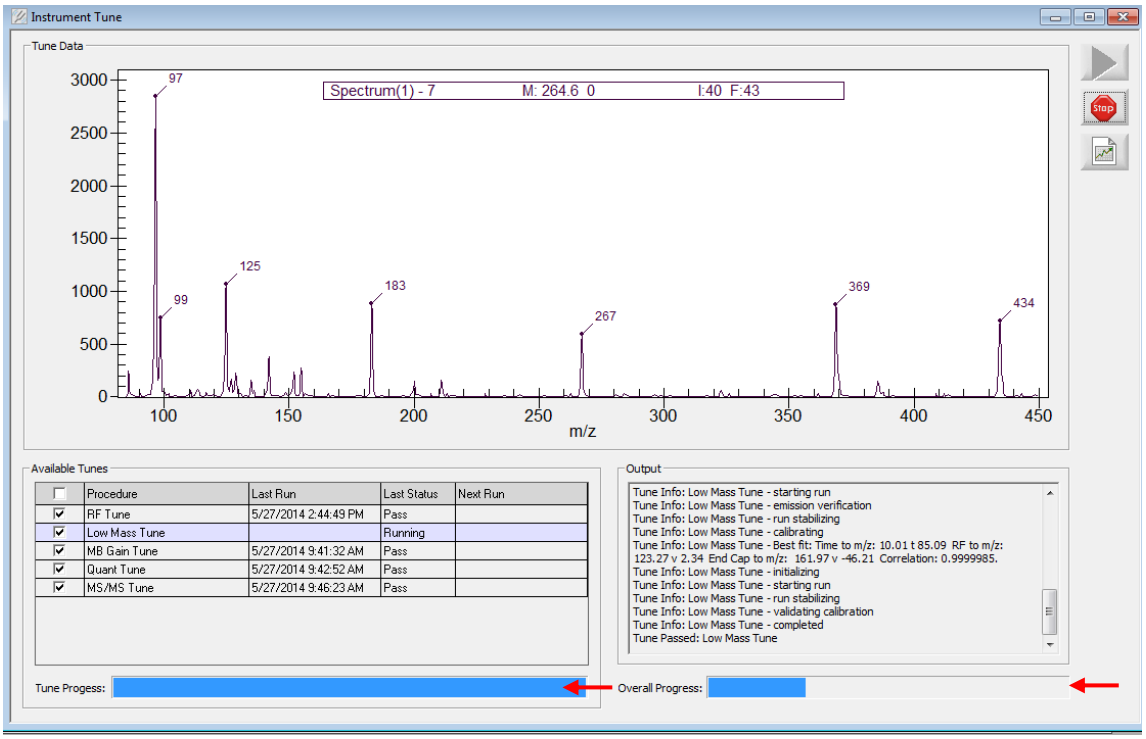

Figure 1.14 – Tune progress window

14. The five-step tune is complete once the tune status and overall progress bars have reached 100% (Figure 1.14).
15. Once complete, close out of the "Instrument Tune" window.

SECTION 1.6 – NEGATIVE-MODE CALIBRATION OF THE AI-MS

Negative-Ion Mode Calibration Protocol

Before analysis in negative-mode can be carried out on the AI-MS, the positive-ion mode calibration protocol (Section 1.4) must be carried out, followed by changing the instrument polarity to negative-ion mode, see Section 1.3 for polarity switching. A specific negative-ion mode calibration does not need to occur.

SECTION 1.7 – POWERING-DOWN THE AI-MS

Power-Down Procedure

The following list should be tentatively followed when powering-down the AI-MS.

1. On the computer desktop, open the "Griffin System Software" (GSS) icon.
2. Connect the instrument by pressing the "Connect" icon in the upper-left corner of the start page screen. The system is successfully connected when the current status is labeled as ready and all subsystems are operational (Figure 1.15).

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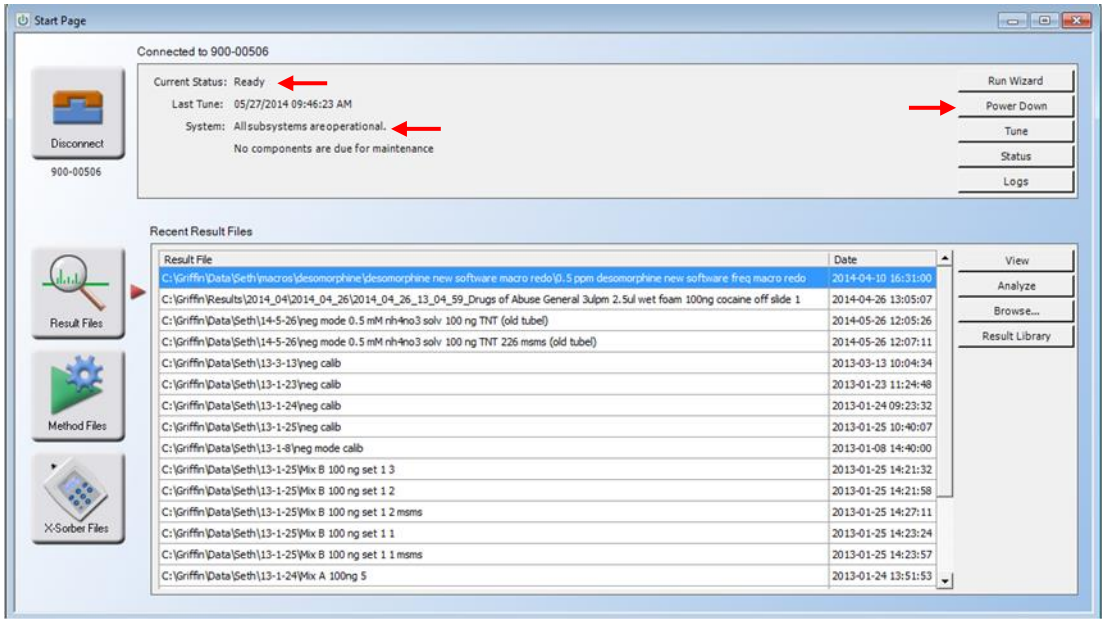

Figure 1.15 – AI-MS power-down/status window

3. Make sure there is no Nitrogen flow to the ionization source.
4. To power-down the AI-MS, click the "Power Down" icon in the top-right corner of the screen. The user will then be prompted to press the "Power Down" button again in a separate window.
5. The AI-MS has been successfully powered-down when the auto power control progress bar reaches 0%. Wait 5 minutes before proceeding to Step 6.
6. Disconnect from the instrument, flip the electrical toggle switch on the left side of the AI-MS to the "Off" position, and disconnect the power cord from the AC outlet (Figure 1.6). The AI-MS is now completely powered-down.

SECTION 1.8 – CHANGING THE ON-BOARD HELIUM TANK

Helium Changing Protocol

The AI-MS uses an on-board Helium supply for use in ion trapping and collision-induced dissociation in MS/MS analyses by means of a cartridge-based tank. The pressure regulator for this tank should be set to 30 PSI for all analyses. The tank is ready to be replaced when the regulator reads 0 PSI (Figure 1.16). Follow the procedure below to replace the tank.

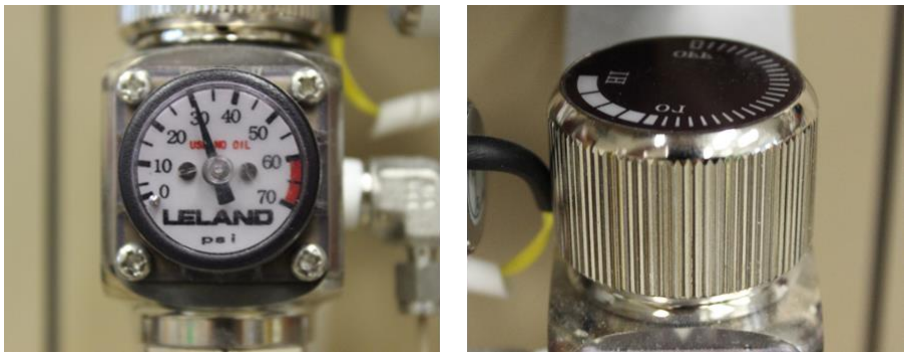

Figure 1.16 – Helium supply regulator

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1. Make sure the regulator gauge is set to 0 PSI, turned all the way to the left.
2. Unscrew the Helium cartridge from the receiver.
3. Screw new cartridge into place and adjust regulator to 30 PSI.

SECTION 1.9 – CLEANING THE AI-MS

1.9.1: Cleaning Solvent Syringes

All syringes used for solvent delivery can be cleaned/rinsed with clean methanol or another appropriate solvent. Multiple rinses are recommended to prevent carryover from previous analyses.

1.9.2: Changing the Inlet Capillary

If unwanted MS signal is witnessed due to contamination, the inlet capillary should be replaced with a clean capillary. The inlet capillary of the AI-MS can easily be changed without powering-down the instrument. In order to change the inlet capillary, the protocol below should be followed.

1. Make sure the instrument is not performing an analysis and the nitrogen carrier gas is off.
2. Unclasp the clip holding the capillary in place relative to the source (Figure 1.17).
3. Press in the circular release button on the left-side of the source and move the ESI/DESI source to the "up" position (Figure 1.17).
4. Use a ½ inch wrench to loosen the nut that secures the capillary in place until the inlet capillary can easily move.

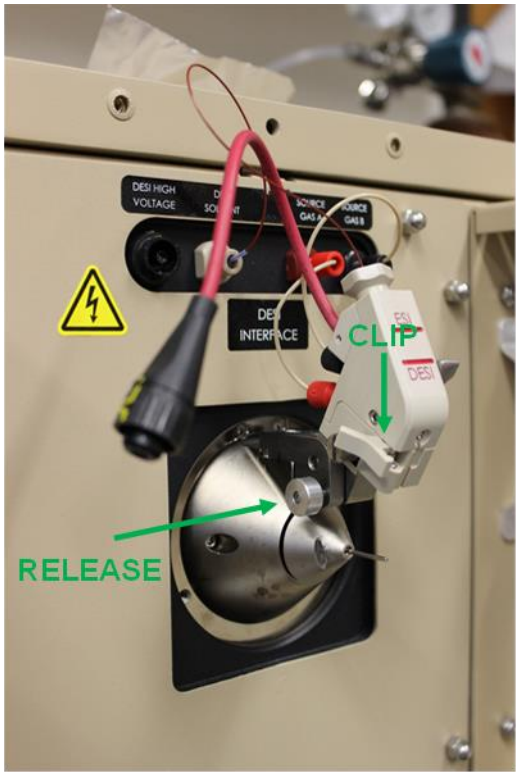

Figure 1.17 – "Up" position of ESI/DESI source

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Figure 1.18 – Changing the inlet capillary

5. Pull out the old capillary and immediately replace it with a new/clean capillary (Figure 1.18).
6. Use the ½ inch wrench to snugly tighten the nut that secures the capillary in place. Be careful not to overtighten during this step, as it could affect the vacuum seal.

1.9.3: Cleaning the ESI/DESI Interface and Changing Capillary O-Ring Seal

Once a month, the ESI/DESI interface should be cleaned and the capillary O-ring that maintains/seals the vacuum should be changed. The ESI/DESI interface may also need to be cleaned if unwanted MS signal or contamination is present. The protocol below should be followed with the AI-MS completely powered down.

1. Make sure the AI-MS is completely powered down by following the procedure in Section 1.7.
2. Un-plug the DESI high voltage cable from the instrument interface and un-clasp the clip that secures the ESI/DESI source to the inlet capillary (located on lower-left portion of ESI/DESI source) (Figure 1.17).
3. Push in the release button (located on the upper-left side of the ESI/DESI source) and gently raise the source up to the highest position, as depicted above (Figure 1.17).
4. Un-tighten the capillary nut that secures the inlet capillary with a ½ inch wrench and completely remove the nut as well as the inlet capillary (Figure 1.18).
5. Make sure that all DESI voltage cables, solvent transfer lines, and gas delivery lines are disconnected from the front of the instrument.
6. Using a 7/64 in. Allen wrench, completely unscrew the bolts located on the left and right side of the interface and carefully remove the entire ESI/DESI source assembly (Figure 1.19).

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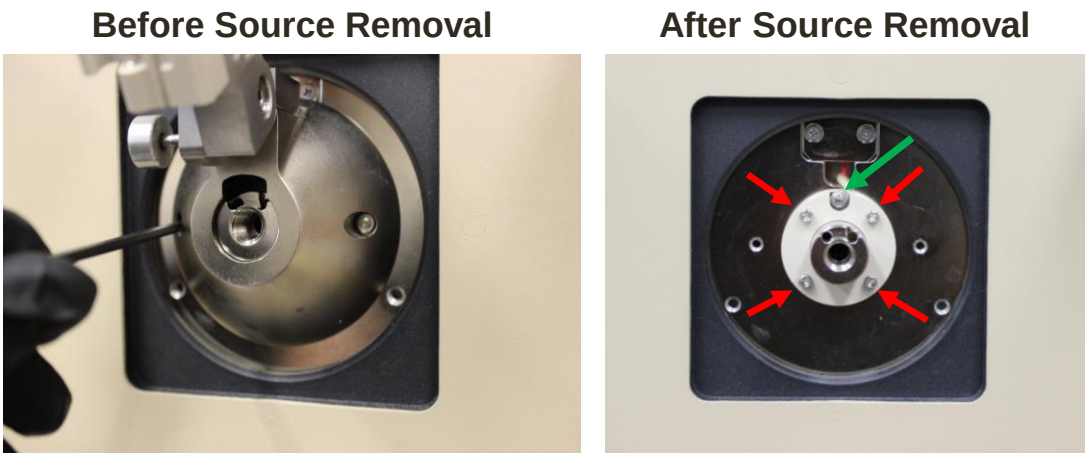

Figure 1.19 – Source Removal

7. After the ESI/DESI source has been removed, use a 3/32 in. Allen wrench to remove the bolt that secures the small red voltage wire, as depicted above by the green arrow (Figure 1.19).
8. Use the same wrench to remove the bolts that are arranged around the inlet of the AI-MS, as depicted above by the red arrows (Figure 1.19).
9. Once the four screws are removed, remove the inlet, (Figure 1.19 and 1.20).

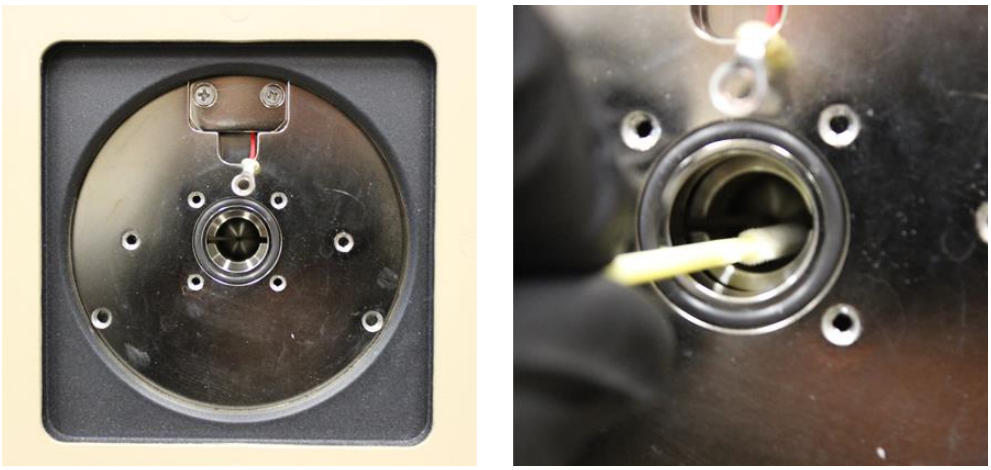

Figure 1.20 – Removal of capillary housing and skimmer cleaning

10. Use methanol and a swab to gently clean the inside of the AI-MS, focusing on the tube lens (U-shaped pieces) and the skimmer (cone-shaped piece). Extra care should be taken to not contact the orifice entrance of the skimmer with the swab as this may cause damage to the skimmer and leave material that would impact ion transfer (Figure 1.20).
11. Now, use a new swab to clean the removed ESI/DESI source with methanol while avoiding contact with the fragile spray emitter tip (Figure 1.21). The ESI/DESI source may also be cleaned with methanol while still attached to the instrument if a "Quick Cleaning" is desired. In this case, the instrument would not need to be powered down, as

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the ESI/DESI source would still be attached and the AI-MS system would still be under vacuum.

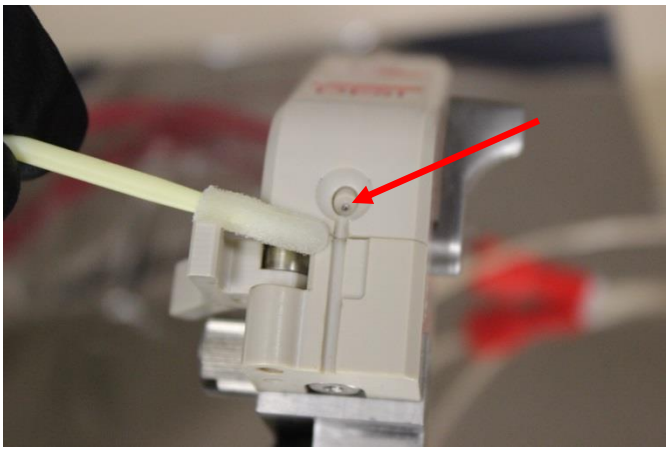

Figure 1.21 – Cleaning of the ESI/DESI Source, emitter tip (RED)

12. After the skimmer, tube lens and ESI/DESI source have been cleaned, secure the inlet that was removed in "Step 9" with the four bolts that were previously removed. These bolts should be gently tightened in diagonal order starting with bolt #1 (Figure 1.22).

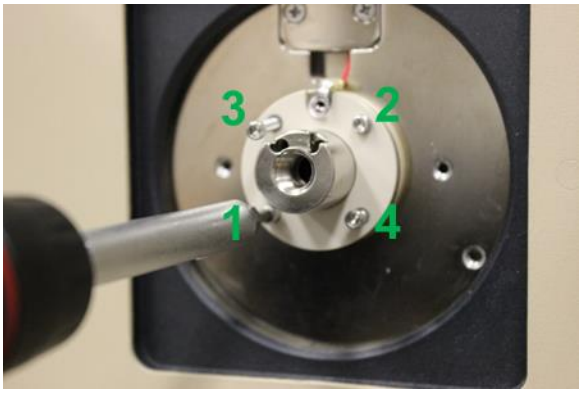

Figure 1.22 – Attaching the inlet capillary housing

13. Gently tighten the bolt that holds the red voltage cable (Figure 1.23).
14. Use the inlet capillary that was previously removed in Step 4 to remove the old black o-ring, as depicted below. After removal, use a 7/64 in. Allen wrench to secure the ESI/DESI source back to the AI-MS (Figure 1.23).

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Removal of old O-ring **Insertion/reseating of new O-ring**

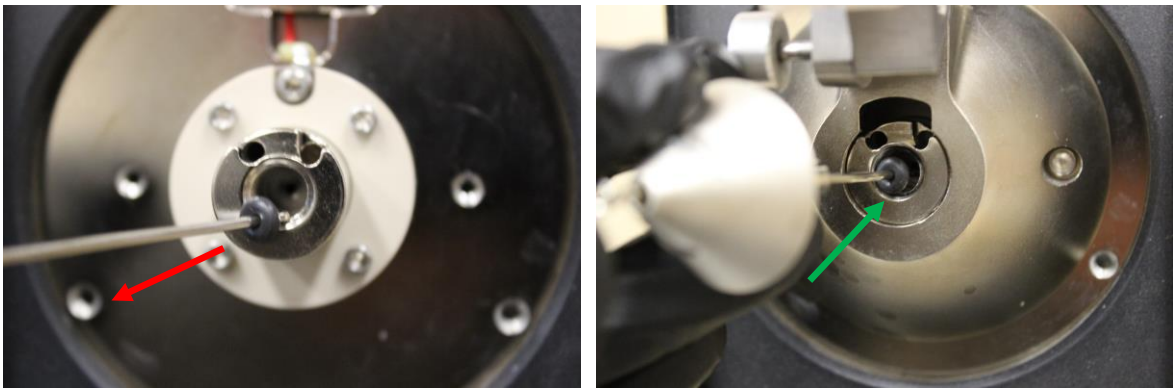

Figure 1.23 – Removal and replacement of inlet capillary O-ring

15. Use a clean capillary, inserted into the capillary nut, to place a new o-ring into the inlet, as shown above (Figure 1.23). Tighten the nut with a ½ inch crescent wrench until it is snug, but not excessively tight.
16. Make sure that the solvent transfer line (Figure 1.24), DESI voltage cables, and gas delivery lines are connected to the front of the instrument (Figure 1.25) and move the ESI/DESI source to the analysis position as seen in Figure 1.25
17. The AI-MS is now ready to be powered-up according to the protocol in Section 1.3.

Figure 1.24 – Reattaching solvent transfer line

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Figure 1.25 – ESI/DESI source and lines in working position

1.9.4: Changing the ESI/DESI Spray Head

The ESI/DESI spray head may need to be switched out for cleaning or carryover purposes, as stated in the flowcharts provided in Appendix A. To change the ESI/DESI spray head, the procedure below should be followed.

1. Disconnect the solvent transfer line from the front of the instrument by un-screwing the fitting represented in Figure 1.26.
2. **CAUTION!** The sprayer should be turned in a clockwise direction. Turning the sprayer counter-clockwise can cause the front and back PEEK polymer sections to separate from the center metal piece. To prevent this from happening **only turn the sprayer clockwise**. Remove the sprayer by pulling straight out. If the sprayer does not pull out easily, turn it clockwise while gently pulling (Figure 1.26).

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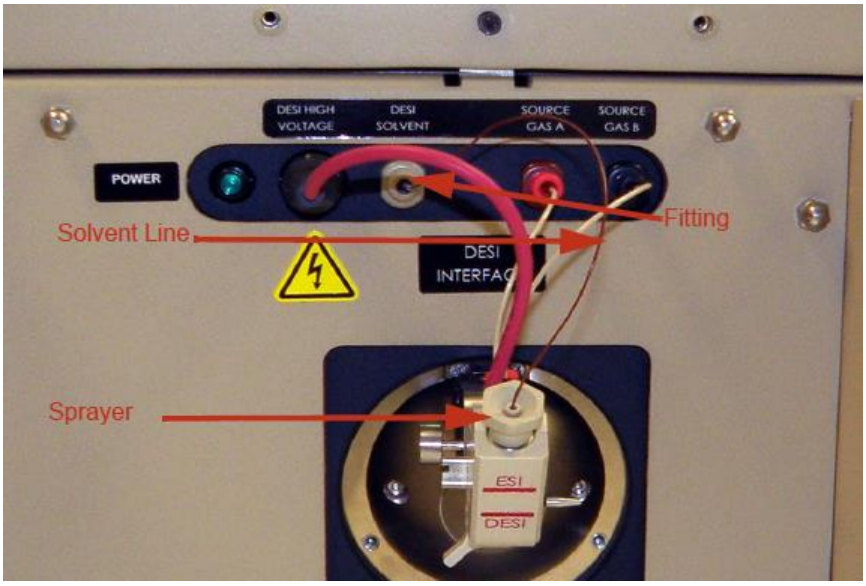

Figure 1.26 – Removing the solvent line

3. Remove the protective cap from the new spray head before inserting into the ESI/DESI source opening (Figure 1.27). Lightly press the new spray head into the ESI/DESI source body. If the new sprayer does not seat correctly, turn it clockwise while pressing (Figure 1.28).

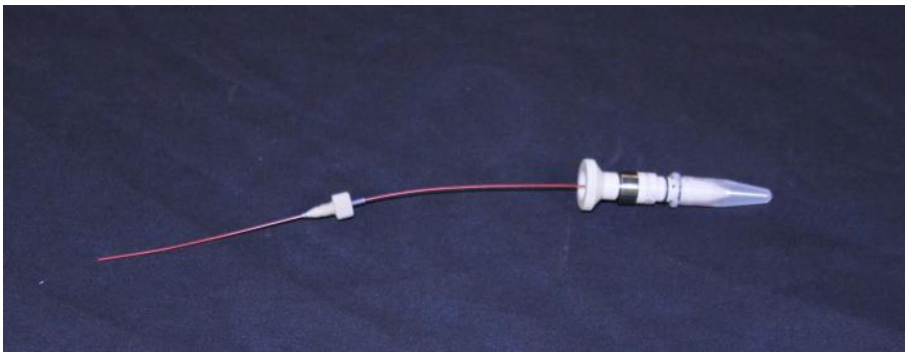

Figure 1.27 – ESI/DESI spray head

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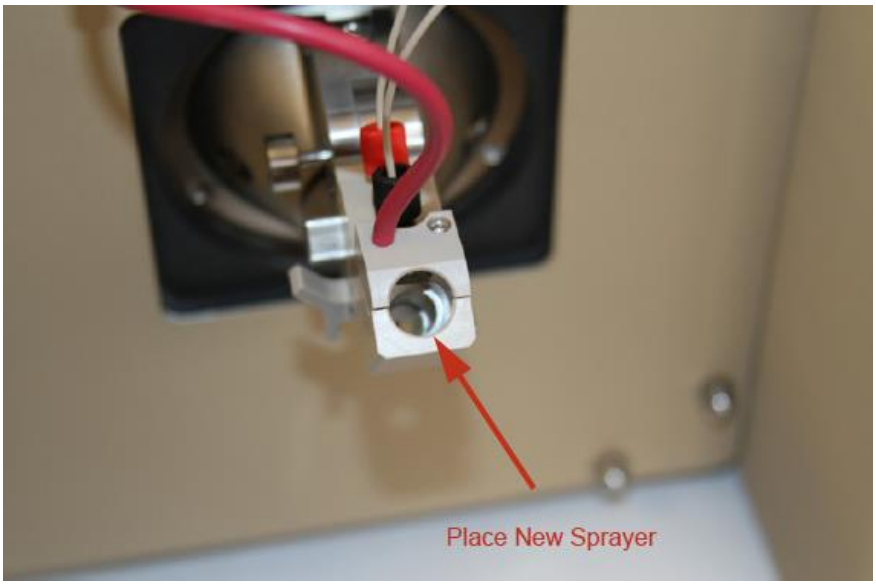

Figure 1.28 – ESI/DESI source body

4. Replace the solvent transfer line. When placing the solvent transfer line, ensure that an excess line of approximately ¼" extends beyond the fitting. This excess will ensure that the line reaches all the way into the instrument to prevent leakage. The user should grasp both the fitting and the red line to ensure the line does not pull away during tightening (Figure 1.24).

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CH. 2 – OPERATION OF IONIZATION SOURCES

SECTION 2.1 – ELECTROSPRAY IONIZATION (ESI)

2.1.1 Electrospray Ionization Protocol

ESI is primarily used for the analysis of liquid analyte(s) or solutions. The following protocol should be followed when performing ESI on the AI-MS in either positive or negative-ion mode.

1. Fill the specified "ESI-only" 500 µL Hamilton syringe with at least 300 µL of the analyte(s) of interest. A screw-on needle can be easily attached to the end of the syringe to aide in the filling process.
2. After filling, make sure no air bubbles are present inside the syringe, as this will cause the electrospray to become unstable. Air bubbles can be expelled by tapping the syringe with a finger and pressing the plunger in to remove the trapped air, see Section 1.2.

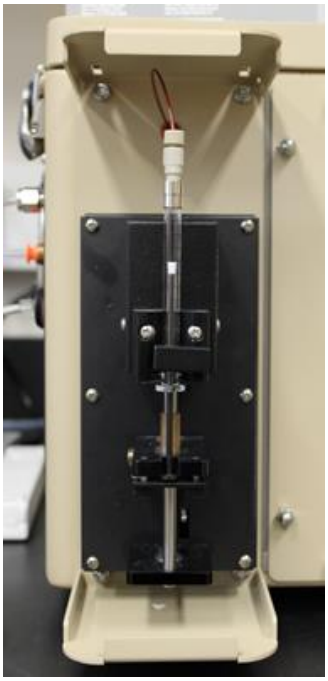

Figure 2.1 – Harvard Pump

3. Remove the needle, if used, and screw the syringe into the solvent transfer system and place it into the Harvard pump (Figure 2.1).
4. Make sure the lever on the side of the ESI/DESI source is in the ESI position (Figure 2.2).

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Figure 2.2 – ESI/DESI source in the ESI position (lever up)

5. Confirm that the solvent transfer lines have been purged, resulting in an uninterrupted and stable MS spectrum of the analyte(s) of interest. Refer to Section 1.5 for the purging process.
6. Follow Level 1 or Level 2 operating procedures, as discussed in Chapters 3 or 4.

SECTION 2.2 – DESORPTION ELECTROSPRAY IONIZATION (DESI)

DESI can be used for the analysis of dried liquids or solids from an array of substrates. Liquid samples can be spotted onto printed Teflon slides or swabbed from a surface using the swabbing protocol below. Solid samples can be dissolved and spotted onto printed Teflon slides using µL aliquots or swabbed using the same procedure utilized for swabbing liquids from surfaces. The following protocol should be followed when performing DESI on the AI-MS in either positive or negative-ion mode.

2.2.1: Printed Teflon Protocol

1. If the sample of interest is in the solid state, a variable amount of the solid can be dissolved in methanol. If the sample of interest is already in the liquid state, proceed to Step 2.
2. Once the sample is in the liquid state, an Eppendorf pipette can be used to spot, at most, 3 µL of the liquid sample onto a well of a printed Teflon glass slide. If more sample deposition is desired, the liquid sample can be spotted multiple times with drying between independent spotting events. In either event, the spot must be dry before DESI analysis.
3. Similar to the protocol used for ESI (Section 2.1), the specified DESI spray solvent syringe must be filled with the appropriate spray solvent (positive-ion or negative-ion)
4. Make sure the lever on the side of the ESI/DESI source is in the DESI position.
5. Before introducing the sample, confirm that the solvent transfer lines have been purged resulting in an uninterrupted and stable MS spectrum of the spray solvent of interest (See Section 1.1).

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6. You are now ready to perform DESI analysis.
7. Follow Level 1 or Level 2 software operation procedures, as discussed in Chapters 3 or 4.

Figure 2.3 – Introduction of Printed Teflon Slide to DESI Source

8. Introduce the printed Teflon slide to the DESI source (Figure 2.3). Special care should be taken to not contact the spray emitter tip. The slide should not be moved around vigorously when in contact with the MS inlet, as glass shards may be pulled into the capillary by the vacuum system. If all spectral signal is lost, this event likely occurred and the capillary should be changed/cleaned (Section 1.8).
9. If all of the analyte has been removed by spray solvent (loss of analyte signal), a new spot on the printed Teflon slide can be analyzed.
10. It is possible that contamination of the inlet capillary or DESI source may occur if analytes with high concentrations or in bulk solid form are analyzed. In the case that analyte signal is seen in the mass spectrum upon introducing a blank slide (no analyte added) between sample analyses, the inlet capillary should be changed according to the procedure explained in Section 1.9.2.
11. The DESI source should also be cleaned with a methanol swab similar to the procedure in Section 1.9.3, see Step 11 – "Quick Cleaning" option. The DESI source would remain attached and the instrument would not be powered down in this case.
12. If analyte signal is still witnessed upon introducing a blank slide after changing the inlet capillary and cleaning the DESI source, the ESI/DESI interface should be cleaned according the procedure in Section 1.9.3. The entire AI-MS system would be powered down in this case.

2.2.2: Swabbing Protocol

When sampling a surface of interest with a swab, the following procedure should be followed using a polyurethane foam swab.

1. Using the visible seam on the swab as a divider between two faces of the swab, apply 2.5 µL of methanol to each face of the polyurethane swab
2. Swab the surface of interest horizontally with a zig-zag motion using one face of the swab
3. Next swab the surface of interest in a vertical zig-zag motion using the opposite face of the swab.

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2.2.2.1: Swab Introduction

When analyzing a swab with DESI, the positioning of the swab in relation to the sprayer and inlet capillary is crucial in regards to obtaining the optimal signal. To assist with positioning of swabs, a swab guide can be attached to the ESI/DESI source.

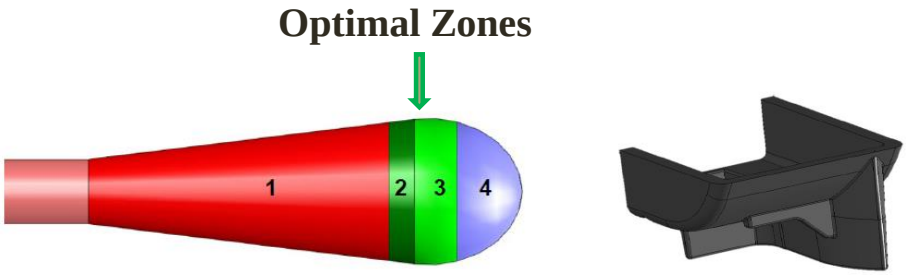

Figure 2.4 – Optimal swabbing zones and black swab guide

Use of a swab guide allows for the analysis of zones 2 and 3. Zones 1 and 4 are not readily accessible when using a swab guide. The swab guide is a black, molded plastic piece which attaches to the bottom of the ESI/DESI source (Figure 2.4). The protocol depicted below should be followed when analyzing Q-Tip-Style swabs with DESI.

1. Confirm that the ESI/DESI source is in the DESI position (lever down). The swab guide cannot be successfully attached to the ESI/DESI source in the ESI position (lever up). (Figure 2.5)

Figure 2.5 – Swab guide installation with source in DESI position (lever down)

2. The swab guide can now be installed by centering it under the DESI source, with the curved portion of the guide facing out or towards the user. Slide the swab guide onto the bottom of the DESI source until it is firmly in position (Figure 2.6).

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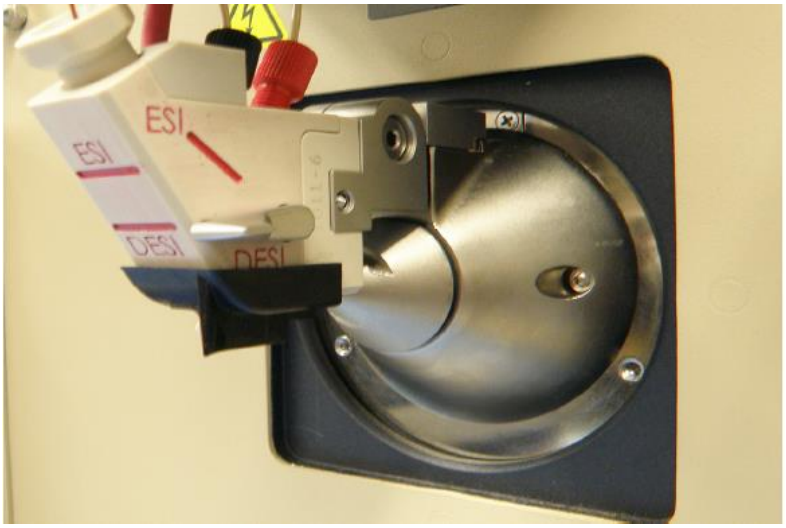

Figure 2.6 – Successful installation of swab guide

3. Once the swab guide is installed, check the placement of the guide to insure the correct installation. The swab guide should be flush against the bottom of the DESI source.
4. The source is now ready for swab presentation. The swab guide allows the user to present the swab from a side orientation (Figure 2.7).

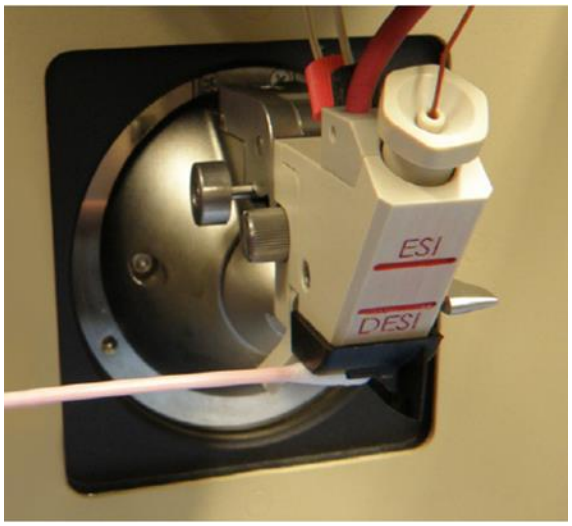

Figure 2.7 – Analysis from side of swab

5. In order to present a sample from the side, position the swab so it is making contact with the side stop portion of the swab guide and placed in the alignment groove (Figure 2.8).

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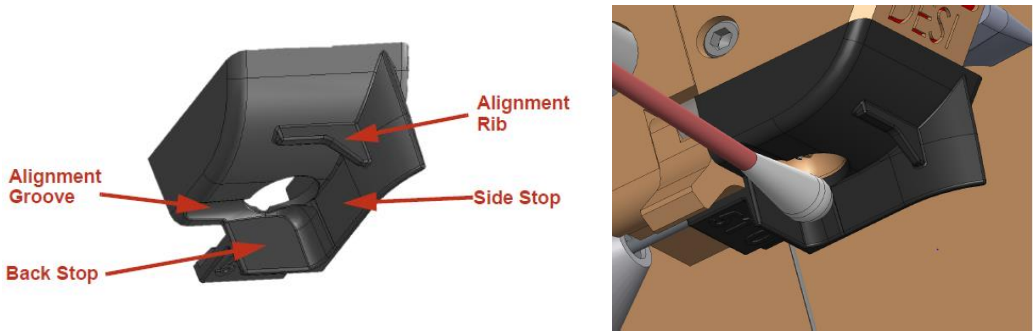

Figure 2.8 – Side presentation of swab

6. Care should be taken to keep the swab parallel with the bottom of the source. The swab should not be introduced at an angle that allows it to contact the sprayer tip. Doing so will block the MS inlet capillary and potentially damage the emitter tip.
7. If all of the analyte has been removed by spray solvent (loss of analyte signal), a new swab can be analyzed after analysis of a blank swab (no analyte present).
8. It is possible that contamination of the inlet capillary or DESI source maybe occur if analytes with high concentrations or in bulk solid form are analyzed. In the case that analyte signal is seen in the mass spectrum upon introducing a blank swab (no analyte added) between sample analyses, the previously utilized swab guide should be exchanged for a clean guide and the inlet capillary should be changed according to the procedure explained in Section 1.9.2.
9. The DESI source should also be cleaned with a methanol swab similar to the procedure in Section 1.9.3, see Step 11 – "Quick Cleaning" option. The DESI source would remain attached and the instrument would not be powered down in this case.
10. If analyte signal is still witnessed upon introducing a blank swab after changing the inlet capillary, changing the swab guide, and cleaning the DESI source, the ESI/DESI interface should be cleaned according the procedure in Section 1.9.3. The entire AI-MS system would be powered down in this case.

SECTION 2.3 – PAPER SPRAY IONIZATION (PSI)

PSI can be used for the analysis of dried liquids or solids from an array of surfaces. Liquid samples can be directly spotted onto the paper substrate or swabbed from a surface using the paper substrate as a swab. Solid samples can be dissolved in methanol and spotted onto the paper substrate or swabbed using the same procedure utilized for swabbing liquids from surfaces. The following protocol should be followed when performing PSI on the AI-MS in either positive or negative-ion mode. Note: no solvent should be present in the solvent transfer lines. An empty Hamilton syringe can be pulled out 500 µL and placed in the Harvard pump to allow only air to flow through the transfer lines.

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2.3.1: Cutting the Paper Substrate

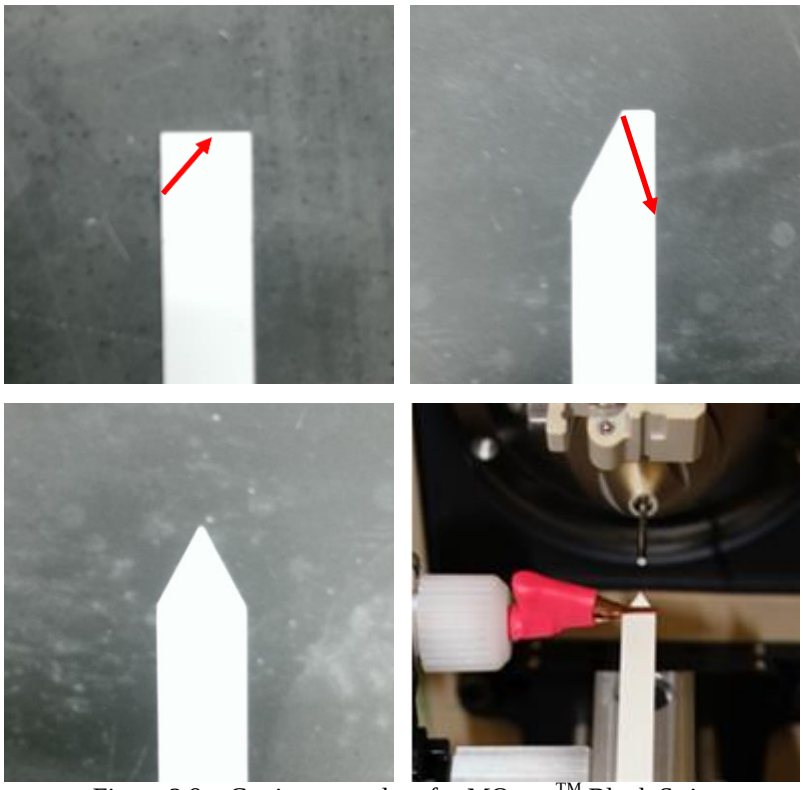

Figure 2.9 – Cutting procedure for MQuant™ Blank Strip

When cutting an MQuant™ Blank Strip for PSI, make two scissor cuts as represented by the red arrows in Figure 2.9. After making the two cuts, a variable amount of exposed plastic backing should remain for stabilization of the paper, as seen in Figure 2.9 and 2.10

2.3.2: Direct Spotting Method

1. Cut an MQuant™ Blank Strip into a triangular shape.
2. If the sample of interest is in the solid state, a variable amount of the solid can be dissolved in methanol. If the sample of interest is already in the liquid state, proceed to Step 3.

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Figure 2.10 – Addition of liquid via Eppendorf pipette

3. Use an Eppendorf pipette to apply a desired aliquot of liquid analyte (less than 3 µL) to the center of the paper substrate (Figure 2.10) and allow the liquid analyte to dry.

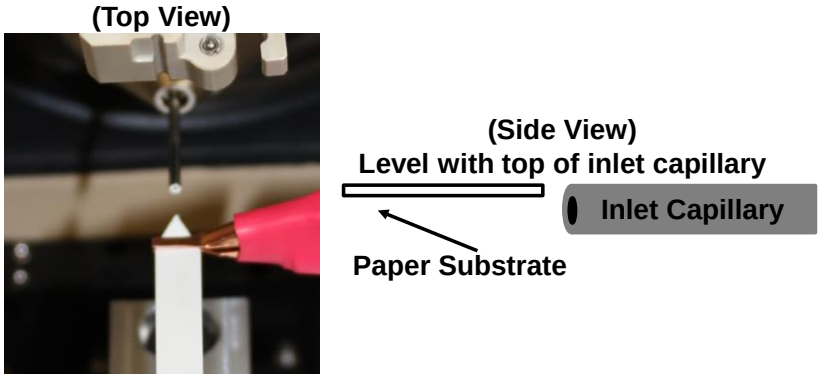

Figure 2.11 – Correct positioning of the paper substrate

4. Attach the paper substrate to the high voltage clip (Figure 2.9). Confirm that the positioning of the paper in regards to the capillary is optimized (slightly above capillary and ~2 mm away from the entrance of the capillary) (Figure 2.11).
5. After confirming the position of the paper, use an Eppendorf pipette to spot, 1.7 µL of spray solvent to one of the bottom corners of the paper substrate. Special care should be taken to not contact the paper to the inlet capillary, as this will result in an electrical discharge and could cause damage to the instrument. Correct positioning will result in a mass spectrum of the typical spray solvent peaks resulting from either a positive-ion or negative-ion spray solvent mixture (Figure 1.2) along with the analyte of interest at high intensity depending on the amount spotted on the paper substrate.
6. An additional aliquot (1.7 µL) of spray solvent can be added if desired analyte intensity is not witnessed in the mass spectrum.
7. In the case that all spectral signal is lost, the paper substrate likely ran out of analyte(s) or spray solvent. In this case, the procedure should be repeated with a new paper substrate.

2.3.3: Surface Swabbing Method

1. Cut an MQuant™ Blank Strip into a triangular shape, as discussed in Section 2.3.1.
2. Saturate the paper portion of the strip with 2 µL of methanol to assist in efficient analyte transfer to the swab.

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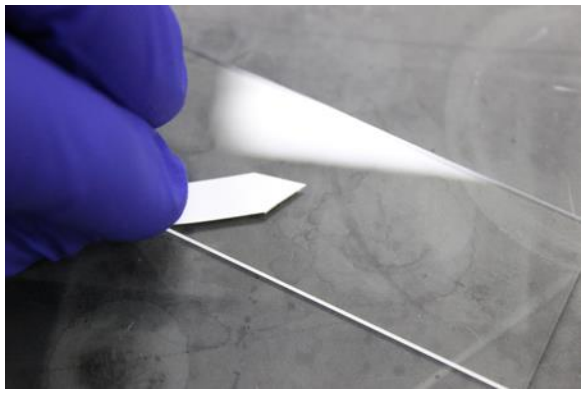

Figure 2.12 – Swabbing of glass slide

3. Proceed to swab the surface of interest with the paper portion of the strip in a circular motion (Figure 2.12). Allow the swab to dry, and attach the swab to the voltage source (Figure 2.9).
4. Before applying spray solvent to the paper substrate, confirm that the positioning of the paper in regards to the capillary is optimized (slightly above capillary and ~2 mm away from the entrance of the capillary) (Figure 2.11)
5. After confirming the position of the paper, use an Eppendorf pipette to apply 1.7 µL of spray solvent to one of the bottom corners of the paper substrate. The entire substrate should be wetted with spray solvent after this addition (Figure 2.10).
6. An additional aliquot (1.7 µL) of spray solvent can be added if desired analyte intensity is not witnessed in the mass spectrum.
7. If all spectral signal is lost, the paper substrate likely ran out of analyte(s) or spray solvent. In this case, the swabbing procedure should be repeated with a new paper substrate.

SECTION 2.4 – ATMOSPHERIC PRESSURE CHEMICAL IONIZATION (APCI)

APCI allows for the sampling of a variety of analyte(s) present in a gaseous state (e.g. accelerants or compounds related to clandestine methods. The APCI source utilized with the AI-MS allows direct sampling of gaseous headspace via a sampling line or direct vapor injection (Figure 2.14). The following procedure should be followed when using APCI.

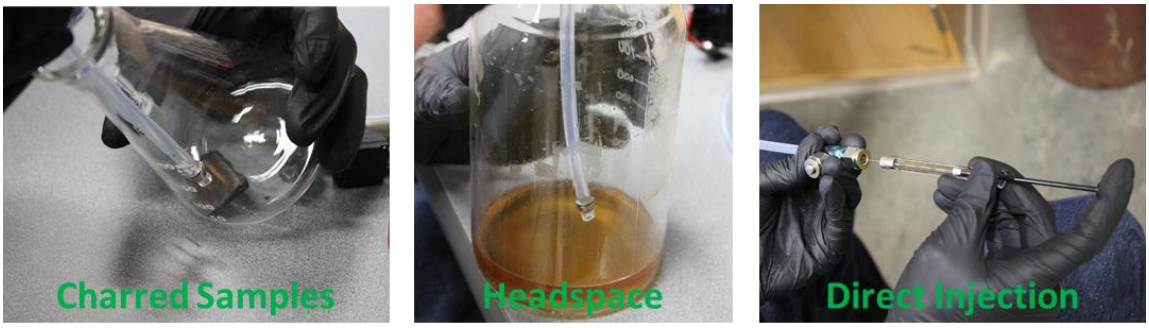

Figure 2.13 – Common ways of sampling using APCI

1. Ensure that the ESI/DESI source is in the “up” position. Confirm that the discharge needle is aligned correctly, ensuring a 3 mm gap is present between the end of the discharge needle and the inlet capillary (Figure 2.14).

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2. Attach the PFA exhaust line connecting the auxiliary pump to the APCI source (Figure 2.14 and 2.15).
3. Attach the PFA sampling line to the source inlet (Figure 2.14).
4. Adjust the ESI/DESI source to the up-position and slide the APCI source over the inlet capillary. Secure the APCI source using the mounting bracket.
5. Attach the high voltage cable to the source (Figure 2.14).

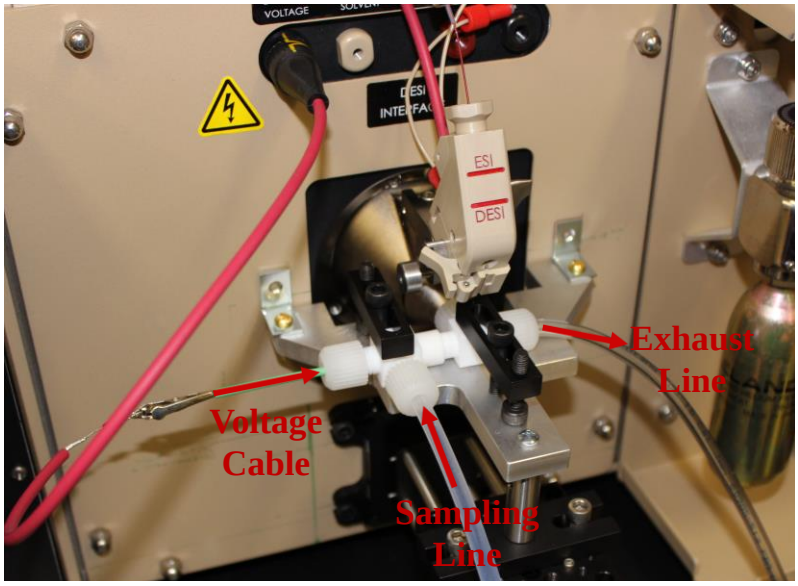

Figure 2.14 – APCI source setup

6. Connect the AC power adapter to the auxiliary pump and plug in to an outlet (Figure 2.15).

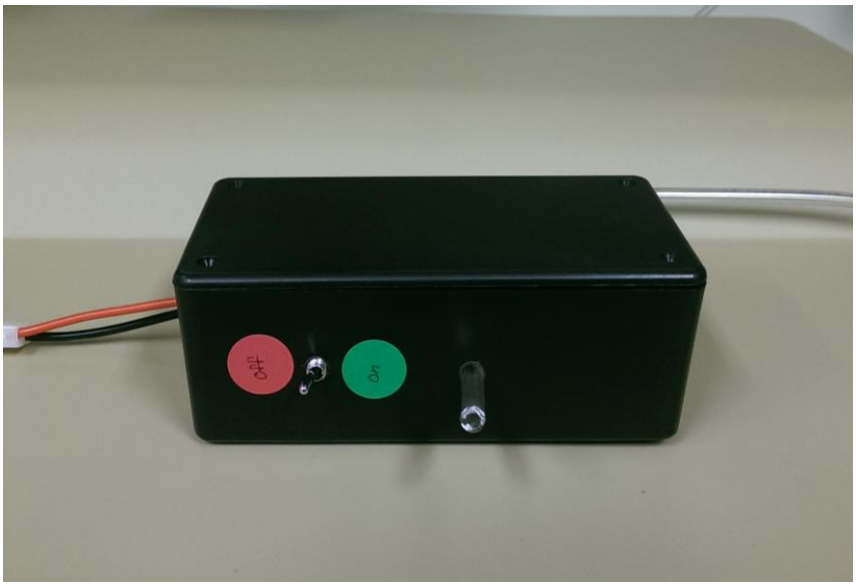

Figure 2.15 – AC power auxiliary pump

7. Turn on the auxiliary pump in order to perform analysis with the sampling line or by direct injection. This pump will pull vapor/gaseous sample of interest into the APCI

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source for direct analysis. Note that it will take a few seconds to a few minutes to fully evacuate the sample from the source prior to introduction of a new one.

8. Correct positioning will typically produce spectra featuring a low to moderate intensity peak at m/z 73 originating from a protonated water trimer (Figure 2.16). However, additional peaks may be present and intensities may vary greatly depending upon the local humidity and presence of any additional substances in the ambient air.

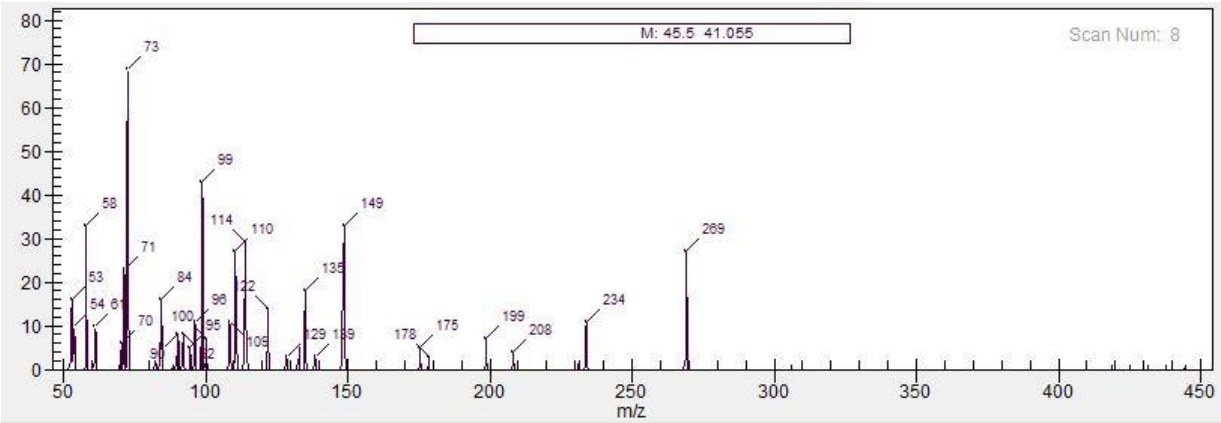

Figure 2.16 – Sample blank APCI mass spectrum

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CH. 3 – LEVEL 1 OPERATION

SECTION 3.1 – GENERAL INFORMATION

Level 1 operation allows the user to obtain sample data which is compared to a pre-installed library to give real-time chemical identification. The pre-installed library contains mass spectral information (MS and MS/MS modes) and algorithm information for many analytes of interest. Chapter 2 should be followed in addition to Chapter 3 when performing a Level 1 analysis. The cleaning and carryover flowchart (Figure A1) in Appendix A should also be followed to prevent analyte carryover.

3.1.1 Detection Tab

When a sample is introduced to the AI-MS while a method is running, the sample data being obtained is compared to the pre-installed library in order to find indicators of possible threats. Three color-coded indicators are present to assist the user by signaling either the absence, possible presence, or confirmed presence of a threat. The meanings of these color-coded indicators are further explained below.

Green Light

A green light indicates that the method is running and no threats have been detected. If the pre-determined method analysis time expires with no threats detected, the method will stop and remain green (Figure 3.1).

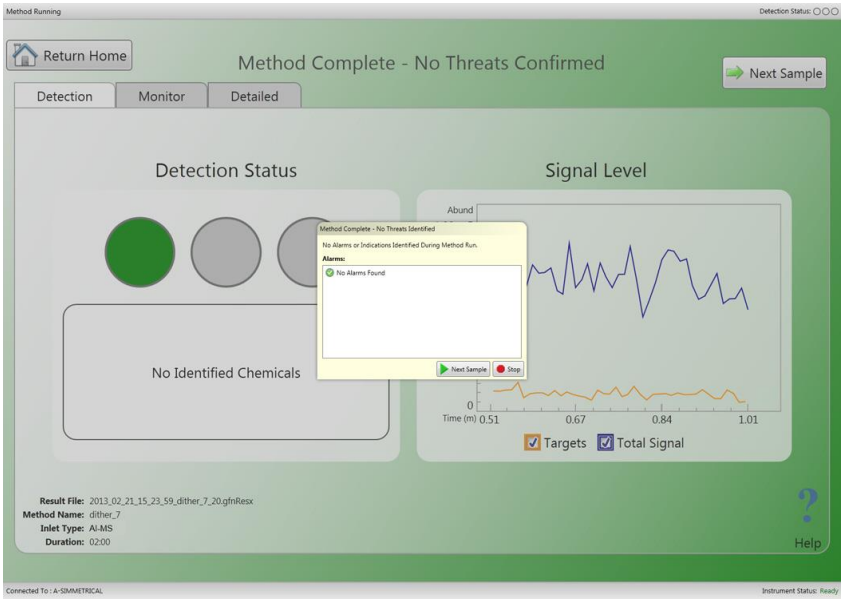

Figure 3.1 – No threats/method running

Yellow Light "Warning"

When a threat is found, molecular ion of library compound confirmed with MS mode, the software will indicate a "warning" with a yellow light (Figure 3.2). Consequently, the instrument will analyze the threat with MS/MS mode, while simultaneously analyzing for other potential threats.

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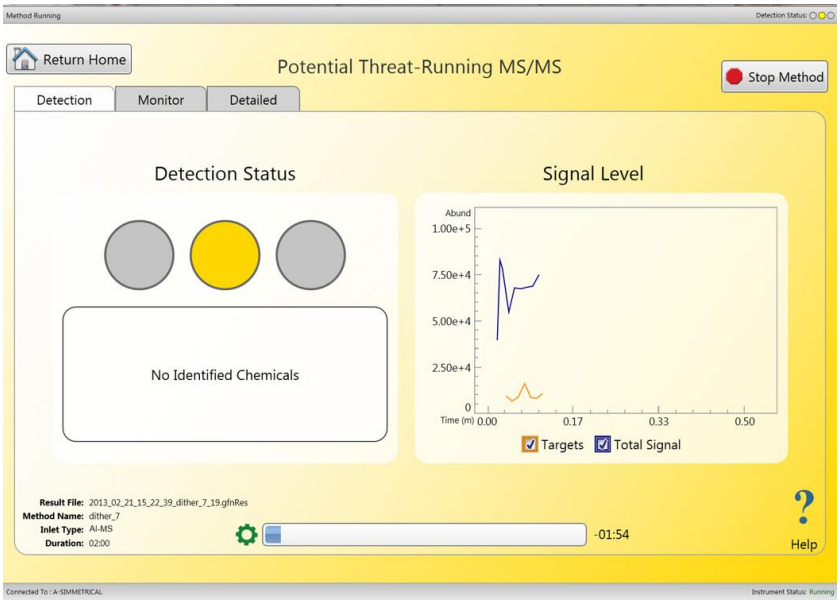

Figure 3.2 – "Warning" – Potential threat

<u>Red Light "Alarm"</u>

A red light "Alarm" will result if the MS/MS mode detects the presence of compound specific fragments for a particular threat that match information specified in the Level 1 method being carried out (Figure 3.3). The method will stop if the confirmed threat was assigned a high priority. A pop-up will then appear summarizing all alarms (Figure 3.4).

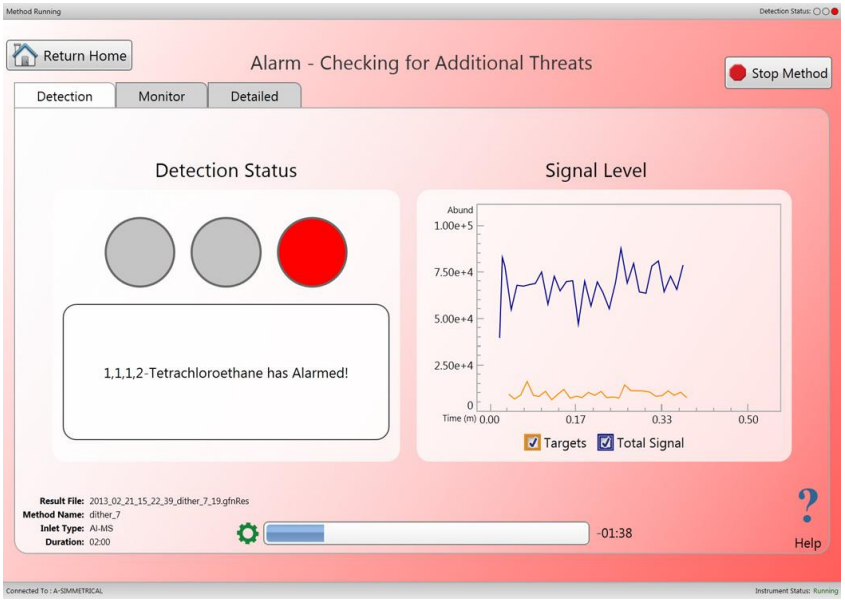

Figure 3.3 – "Alarm" – Confirmed threat

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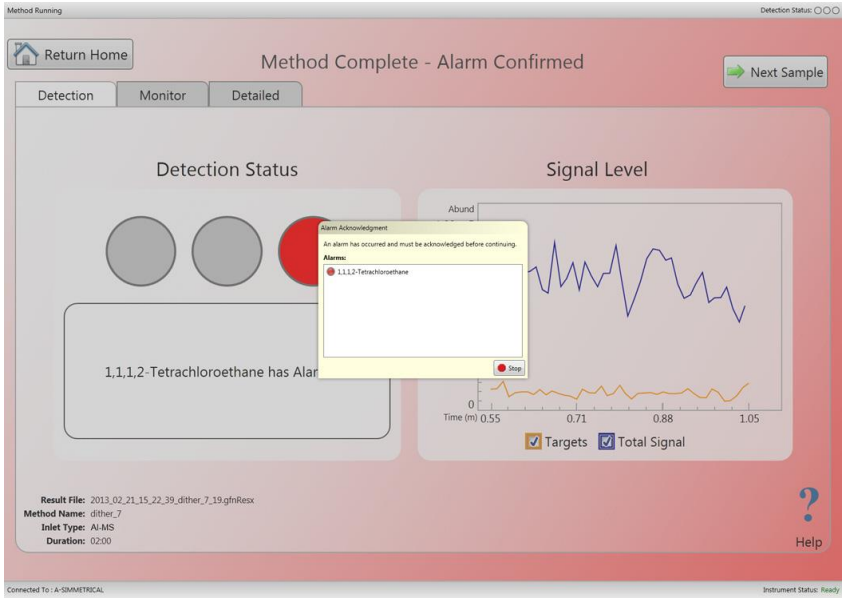

Figure 3.4 – Alarm Summary

In the case that the MS/MS scan does not confirm the presence of any threats before the method time stops, the method will remain yellow. If the method times out before the confirmation of any threats, a pop-up will open stating that no threats were confirmed.

3.1.2: Monitor and Detailed Tab

While performing a selected method, the user may view the alarm monitor (Figure 3.5) or alarm detailed (Figure 3.6) tabs.

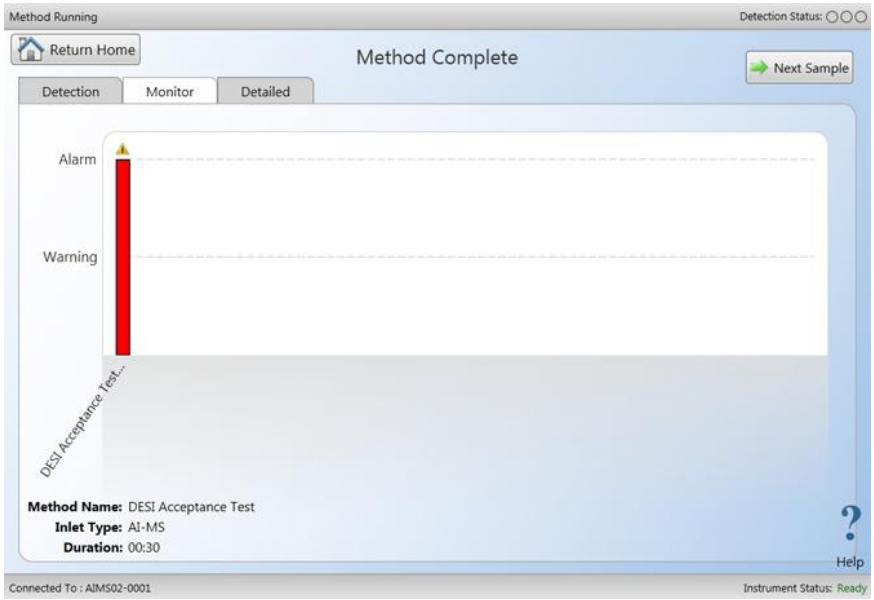

Figure 3.5 – "Monitor" tab

The "Monitor Tab" gives general information about the data being collected during the method of interest, while allowing the user to move on to the next sample or return to the home screen.

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"Warnings" and "Alarms" are indicated in this tab using a color-coded bar chart utilizing the same colors from the "Detection Tab," see Section 3.1.1.

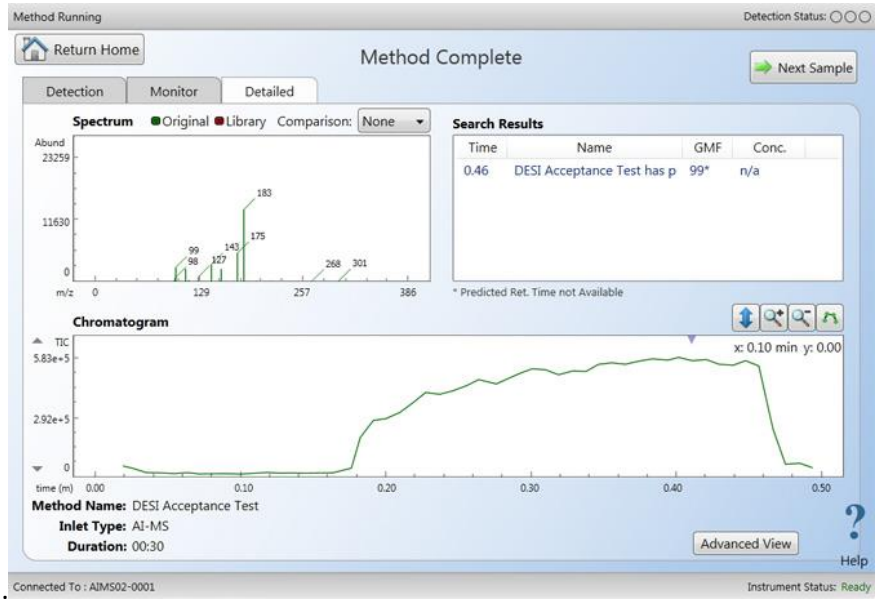

Figure 3.6 – "Detailed" tab

The "Detailed Tab" provides a more detailed picture of the alarm (MS and MS/MS scans) obtained in real time. This allows the user to view the spectrum, chramatogram, and search results. Users can also move onto the next sample or return to the home screen from this tab.

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SECTION 3.2 – EXPLANATION OF PRE-LOADED METHODS

Pre-developed methods have already been included with the software that scan for selected analytes. These analytes have been categorized into two main methods, a general purpose method primarily containing controlled substances and a general purpose method primarily contraining cutting agents common. Selected compounds are included in both methods.

3.2.1: Drugs of Abuse General Method

The Drugs of Abuse General method includes multiple common drugs of abuse, abused pharmaceuticals, and related compounds that may also be present such as synthetic precursors. Additionally, a small number of the most common cutting agents have been included to provide the ability to identify possible impurities that may be present in a sample.

Chemical	Parent Ion	Main Fragment Ions
Alprazolam	309	281, 274
Amphetamine	136	119, 91
Cathinone	150	132
Cocaine	304	182
Cocaethylene	318	196
Codeine	300	215, 243
Desomorphine	272	215, 197
Diazepam	285	222
Dextromethorphan	272	215, 147
Fentanyl	337	188
Heroin	370	268, 328
Hydrocodone	300	199
Hydromorphone	286	185
Ketamine	238	220, 207
LSD	324	281, 223
MDMA	194	163
MDPV	276	205
Mephedrone	178	160
Mescaline	212	195
Methadone	310	265
Methamphetamine	150	119, 91
Methylone	208	190, 160
Morphine	286	201
Oxycodone	316	298
PCP	244	159
Pentedrone	192	174
Phenylephrine	168	150
Phenylpropanolamine	152	134
Pseudoephedrine	166	148
Triazolam	343	308

Table 3.1 –Target analytes in “Drugs of Abuse General Method”

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3.2.2: White Powder and Cutting Agents Method

The White Powder and Cutting Agents method includes controlled substances commonly encountered as white powders and multiple pharmaceuticals frequently employed as cutting agents. The method is intended to identify chemicals potentially used to adulterate cocaine in unknown powder samples.

Chemical	Parent Ion	Main Fragment Ions
Benzocaine	166	138
Caffeine	195	138
Cocaine	304	182
Diphenhydramine	256	167
Hydroxyzine	375	201
Levamisole	205	178
Lidocaine	235	86
Methamphetamine	150	119, 91
Phenacetin	180	138
Phenylephrine	168	150
Pseudoephedrine	166	148

Table 3.1 –Target analytes in "White Powder and Cutting Agents Method"

SECTION 3.3 – CHANGING THE INSTRUMENT CONFIGURATION

Instrument Configuration Alteration Protocol

Before carrying out any Level 1 analysis, the instrument configuration (i.e. solvent flow rates, DESI (3 µL/min) and ESI (10 µL/min)) must be changed depending on the ionization source being utilized. To change the instrument configuration flow rate setting for a particular ionization source, follow the steps below.

1. Check that there are no connections to the instrument with other programs.
2. Open the Toolbox.EXE program on the desktop.
3. Connect to the instrument using the "Quick Connect" button located in the upper left portion of the screen. When connected, click the "Configurator" button to open the "Configuration" tab (Figure 3.7).

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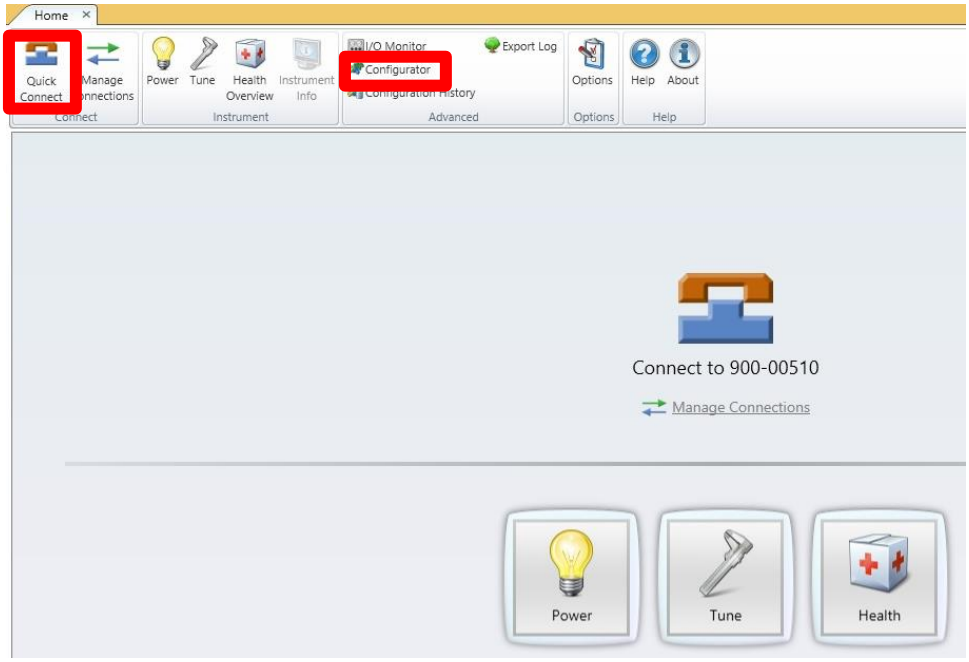

Figure 3.7 – Connection screen

4. To alter the flow rates for a particular ionization source, select "Harvard Pump" from the "Hardware Components" list on the left portion of the screen. After selecting the "Harvard Pump," a box displaying "Sample Flow Rate (µL/min)" will appear in the bottom portion of the screen (Figure 3.8).

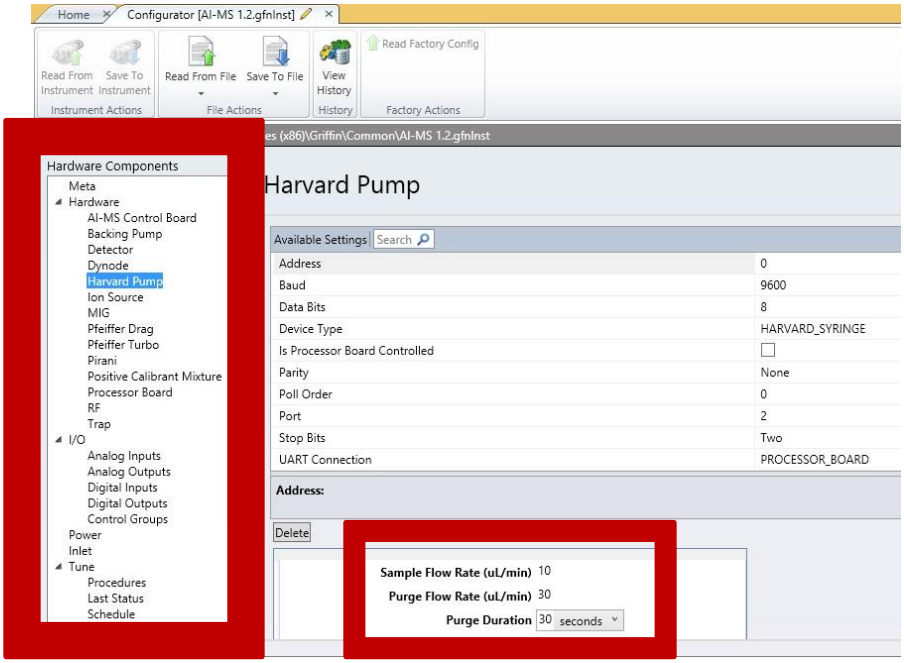

Figure 3.8 – Selecting "Harvard Pump" screen

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5. Click on the number next to "Sample Flow Rate" and change it to either 3 µL/min for DESI or 10 µL/min for ESI (Figure 3.9).

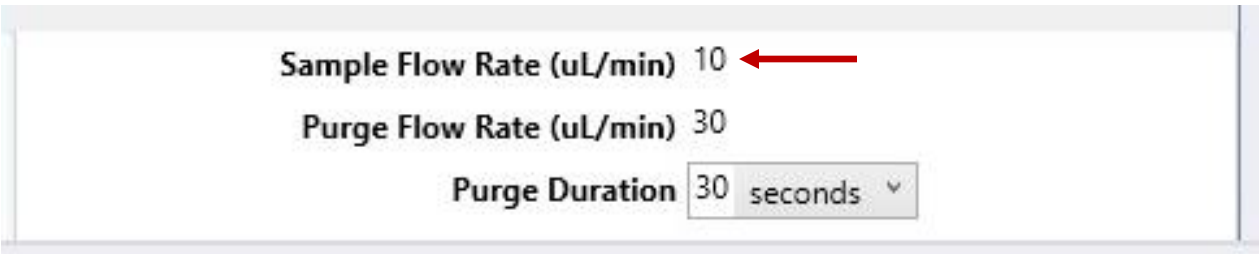

Figure 3.9 – Altering "Sample Flow Rate"

6. Once the appropriate flow rate has been inputted, press the "Save to Instrument" button. After saving to the instrument, click the "Home" tab to return to the software home screen and disconnect from the instrument (Figure 3.10)

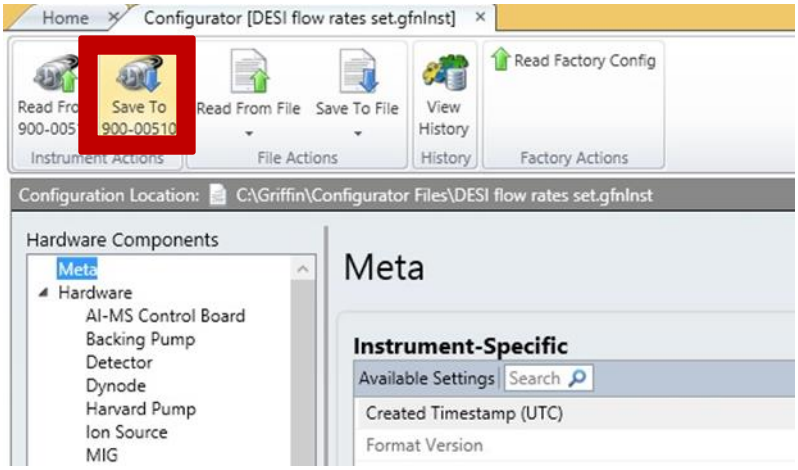

Figure 3.10 – "Save to Instrument" button

Caution: The configuration files contained in the instrument software are highly detrimental to the performance of the AI-MS. Further altering these files could cause serious issues in relation to AI-MS performance and thus data quality.

SECTION 3.4 – ANALYTE INTRODUCTION

Analyte Introduction Procedure

1. All ionization sources can be coupled with Level 1 operation. Before using one of the ionziaiton sources, the configuration settings for the specific ionization source must be saved to the instrument, see Section 3.3. The protocol used for operation of each ionization source should be followed when performing Level 1 analysis, as discussed in Chapter 2. Determine what ionization source will be utilized with Level 1. Follow the procedures detailed in Chapter 2.
2. After the ionization source has been set-up, open "GSS Level 1," connect the instrument, and select the "Methods" button (Figure 3.11).

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Figure 3.11 – GSS Level 1 – Methods icon

3. Choose the desired method from the list and select the "run selected method" button (Figure 3.12). Refer to Section 3.2 for pre-loaded method explanations.

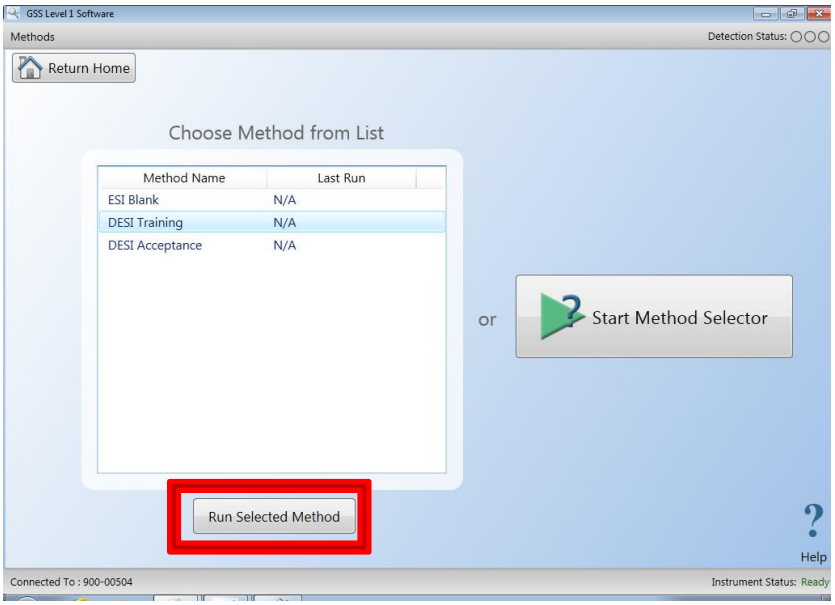

Figure 3.12 – Selecting a method

4. Before the method starts, it is optional to enter a sample name or add any comments pertaining to the sample. This information will be saved on the generated data files. To start the method, select the start method button. (Figure 3.13).

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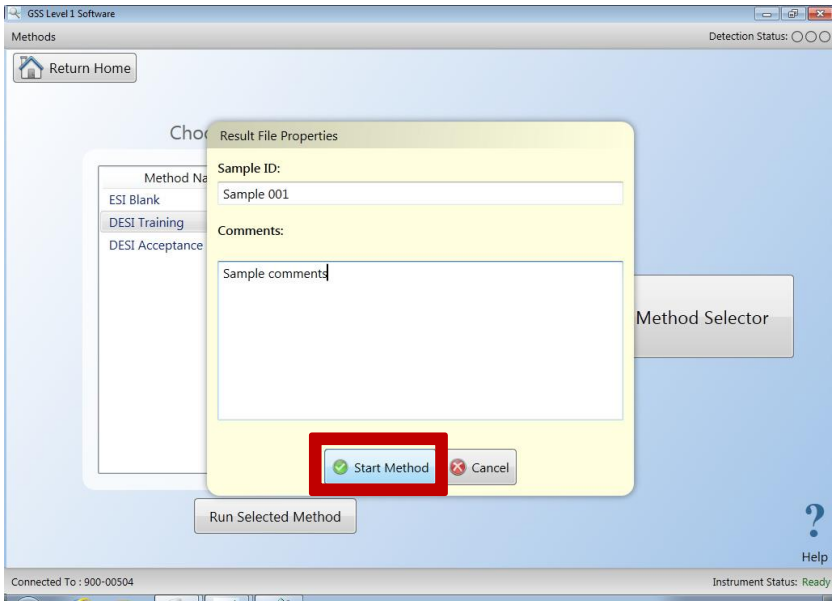

Figure 3.13 – "Result File Properties" window

5. When the method starts, a window with three tabs, consisting of "Detection," "Monitor," and "Detail" will open (Figure 3.1).
   Data will now start to be collected by the instrument. The specific protocol for the operation of a particular ionization source should be followed, as discussed in Chapter 2.

SECTION 3.5 – ACCESSING COLLECTED DATA

3.5.1 View Data Procedure

Collected spectral data can be accessed through the "View Data" button on the home screen (Figure 3.14). Data files can be sorted by date of creation, alarm status indicated by the color-coded indicators, as discussed in Section 3.1, or by a data file label consisting of the time the sample was analyzed, the method used to analyze the sample, and the sample name. Clicking a data file from the list then displays additional information, including any comments entered and a summary of any analytes that triggered or alarmed (Figure 3.15). The "Open Result File" button then opens the full method results containing the detection, monitor, and detailed views (Figure 3.16).

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Figure 3.14 – Home screen "View Data" button

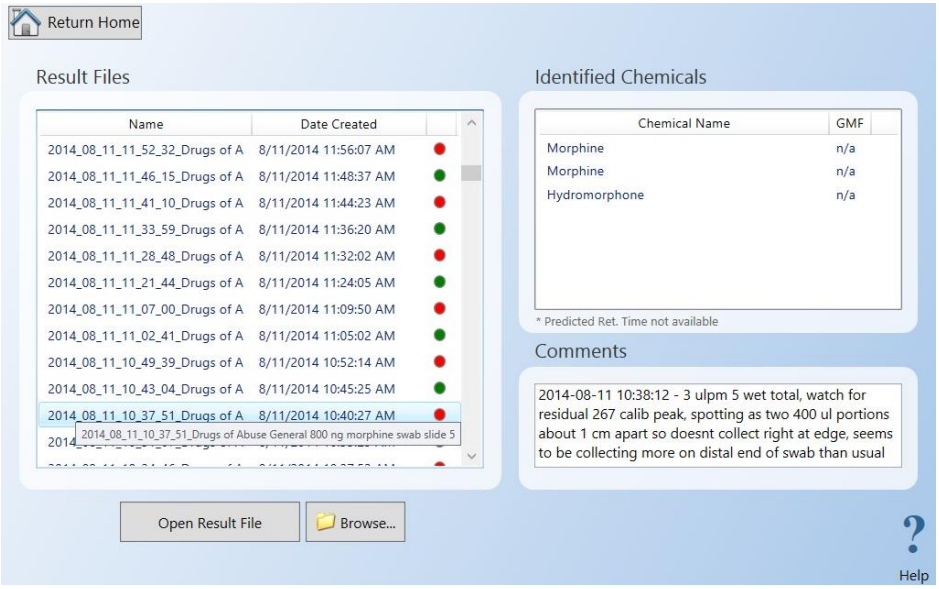

Figure 3.15 – "Data file List"

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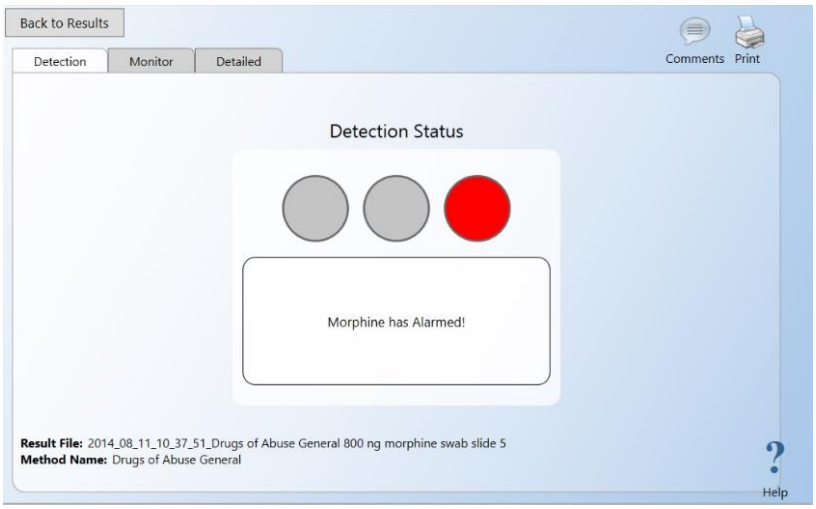

Figure 3.16 – "Detection" tab

When viewing results, the data file name and method name are displayed at the bottom of the screen. The "Back to Results" button returns to data file selection list, the "Comment" button displays any comments with the date they were recorded and allows the user to append the comment, and the "Print" button prints the screen.

Under the "Detailed" tab, the mass spectrum for a given scan is displayed in the top left of the screen and a summary of analyte warnings and alarms is displayed on the right with the time when each was triggered. The bottom half of the screen displays the chromatogram for the scan. Total ion intensity for the MS scan is displayed as a green line, while intensity for any triggered MS/MS scans are displayed in alternate colors (Figure 3.17).

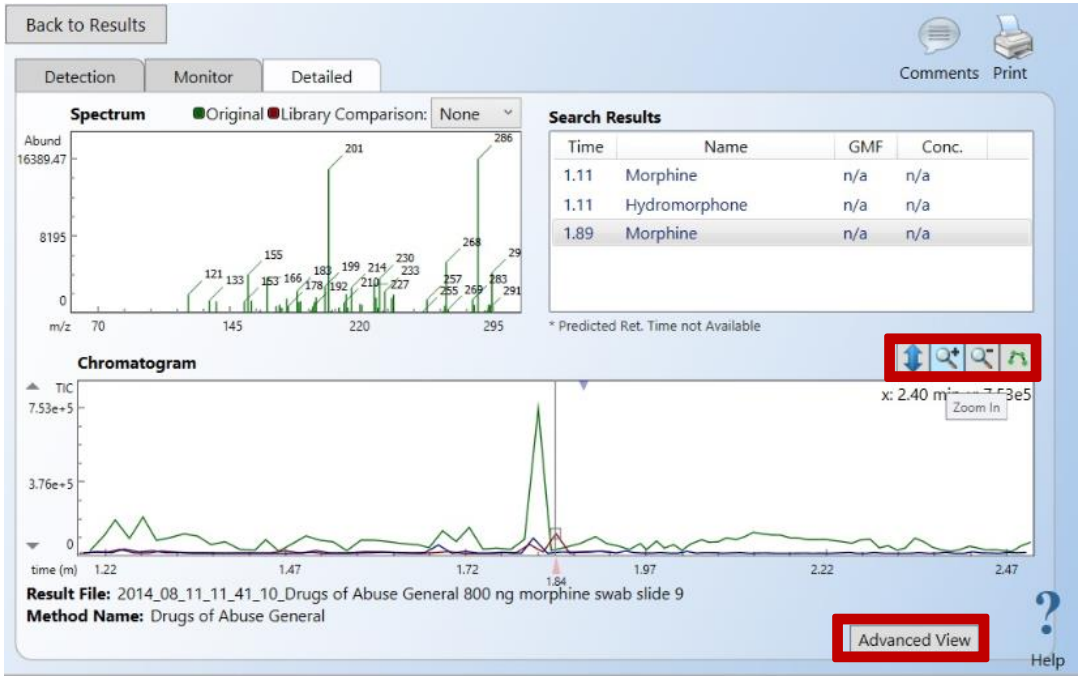

Figure 3.17 – "Detailed" tab

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Clicking on the chromatogram shows the mass spectrum obtained at a particular moment in time. The vertical line is movable and indicates the current region of the chromatogram selected and displayed in the mass spectrum portion of the screen. The arrow keys can then be used to navigate between different points in time with the left and right keys or between the MS scan and any triggered confirmatory MS/MS scans using the up and down keys.

The buttons located above the chromatogram allow the user to switch the larger display region from the chromatogram to the mass spectrum, zoom in or out on the chromatogram, and display points on the chromatogram trace corresponding to each scan. The "Advanced View" button sends the data file to GSS Level 2 for more analysis options such as averaging scans and creating chromatogram traces to display the signal intensity of additional peaks not recognized by the method itself. A full discussion of the Level 2 software is included in Chapter 4.

The "Monitor" tab displays which compounds in the specific method resulted in a warning (yellow) or an alarm (red) (Figure 3.18).

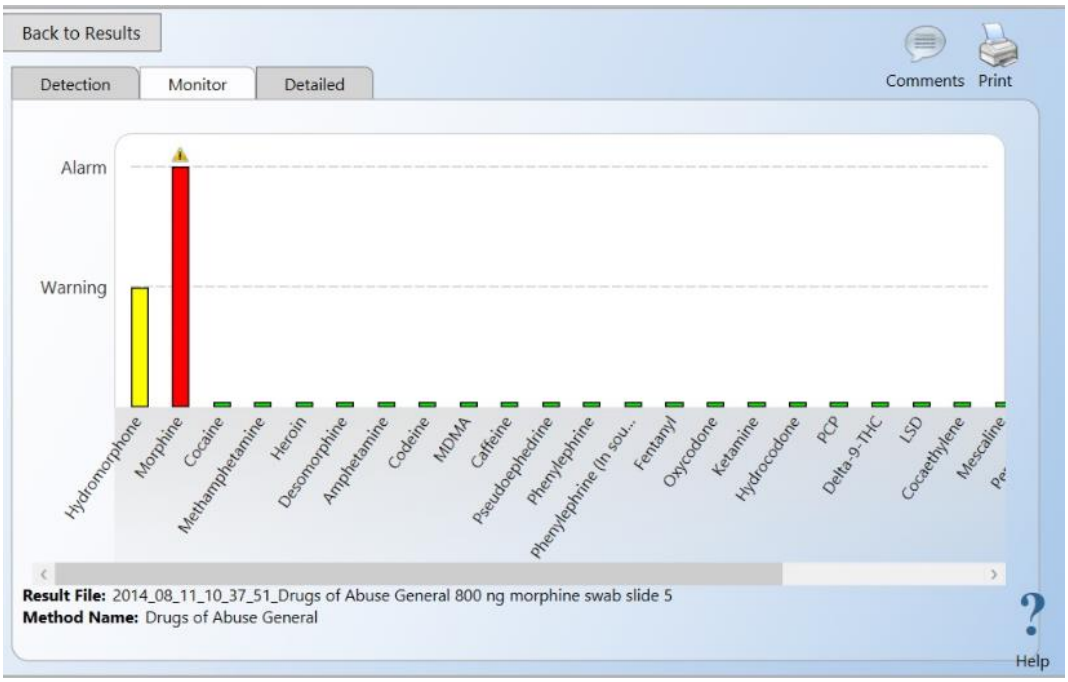

Figure 3.18 – "Monitor" tab

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CH. 4 – LEVEL 2 OPERATION

The AI-MS System also allows for the collection of data without coupling to the Level 1 or automated software. This is also how all of the pre-loaded methods were created for Level 1 analysis.

SECTION 4.1 - GENERAL DATA COLLECTION

4.1.1: MS Data Collection

1. Open Griffin System Software (*i.e* Level 2) (Figure 4.1).

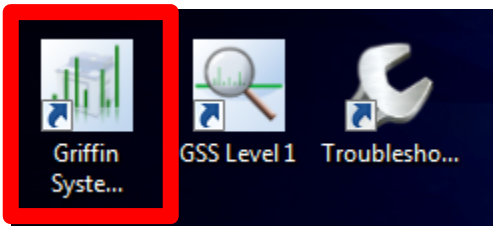

Figure 4.1 – Griffin System Software Icon

2. Click "Connect" on the top toolbar or the Start Page to establish the connection with the instrument (Figure 4.2). The Start Page can be closed at this point. However, recently saved data can be easily opened through the Start Page. How you open saved data files or open methods is of personal preference.

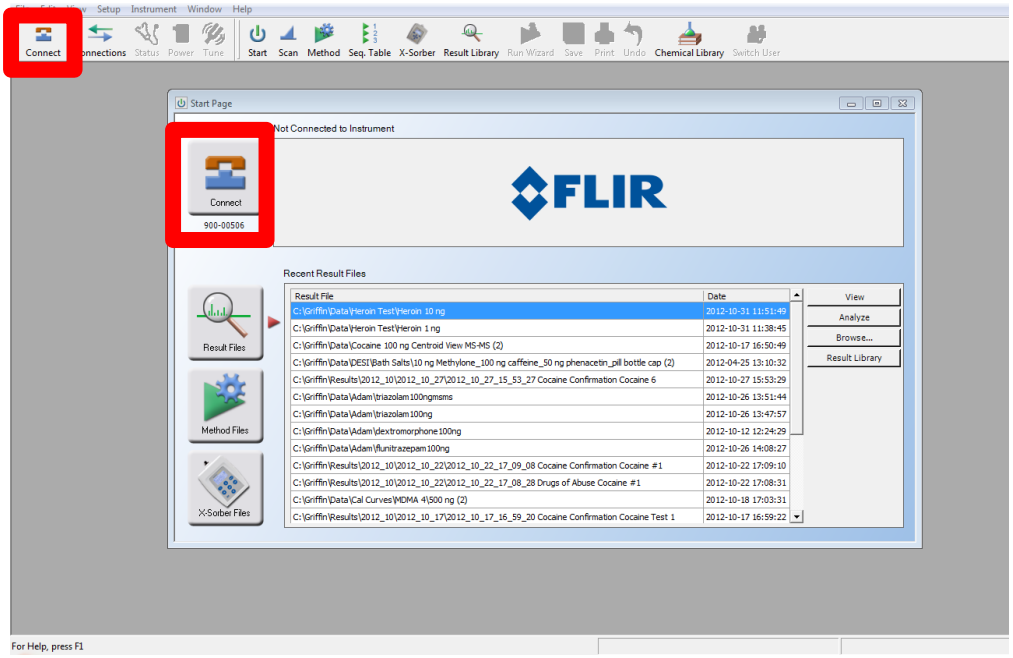

Figure 4.2 – Connecting the instrument

3. If a connection is successful, the "Connect" button will become a "Disconnect" button. The status bar at the bottom of the screen will also display the instrument serial number, instrument status, ionization mode (positive or negative), and ion trap vacuum pressure.

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When idle, the trap pressure should read <1.0e^{-5} Torr (Figure 4.3). This pressure may be slightly higher if the instrument has just been powered on. However, the idle pressure should be easily obtained within a few hours. If not, the system should be checked for leaks. When an analysis is being performed the trap pressure should be between 2.39e^{-4} and 2.49e^{-4} Torr.

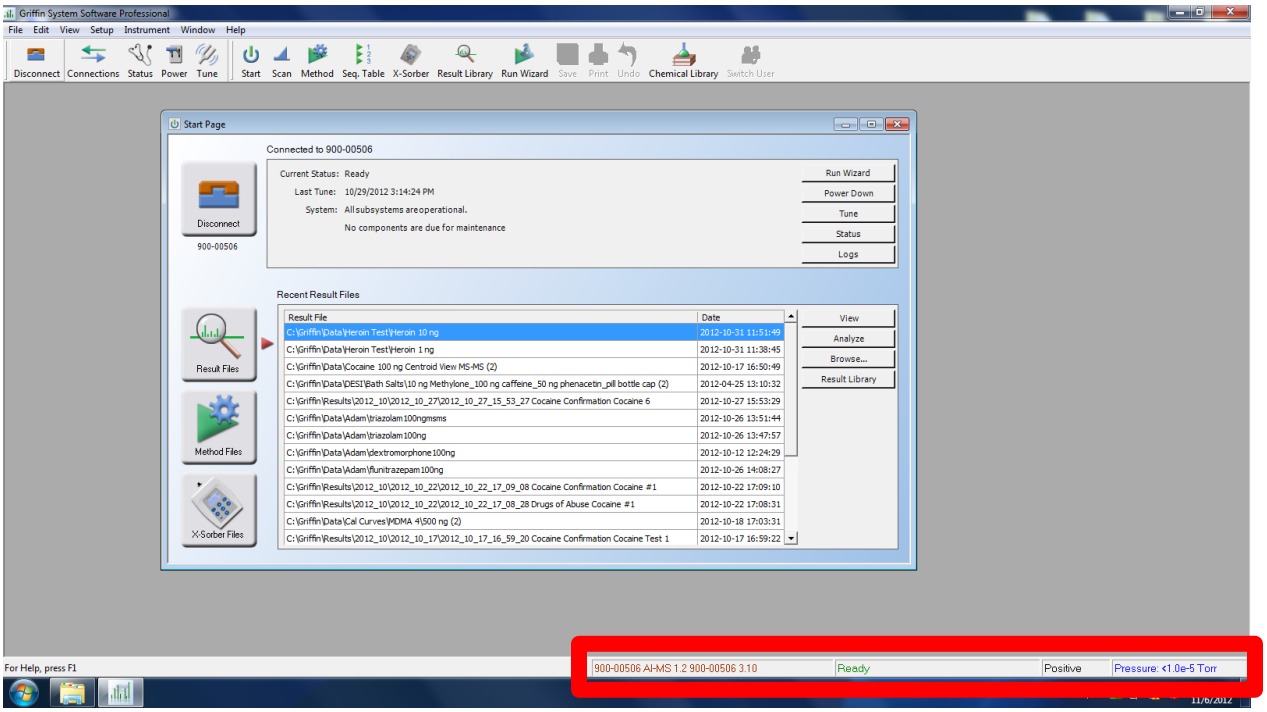

Figure 4.3 – Trap Pressure

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4. Open a new method by clicking the "Method" button on the top toolbar (Figure 4.4).

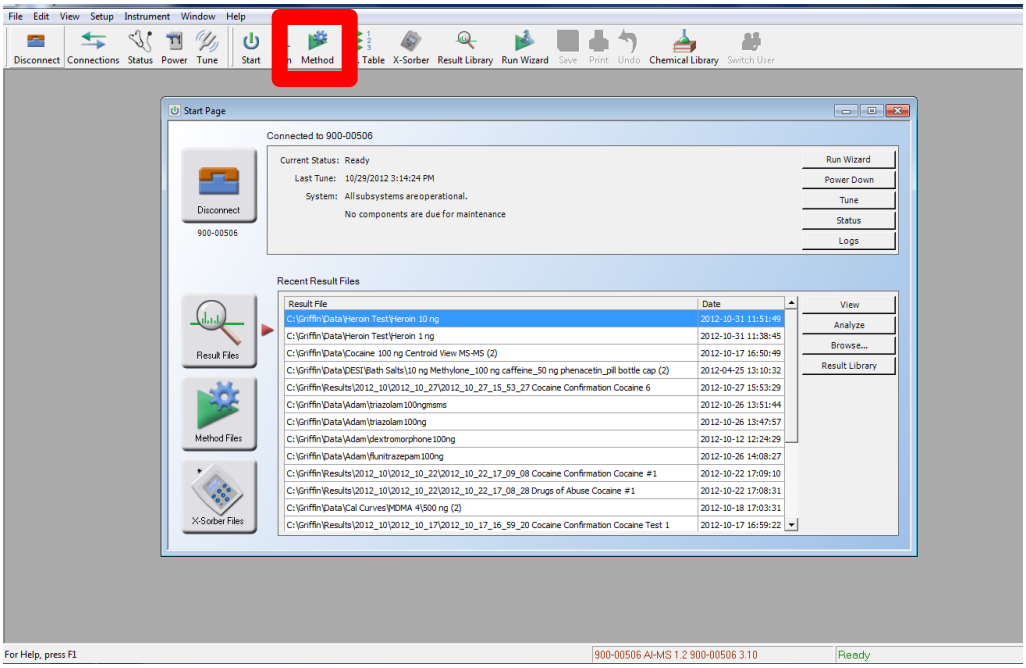

Figure 4.4 – Opening a method

5. Select "New" under the MS Only option (Figure 4.5).

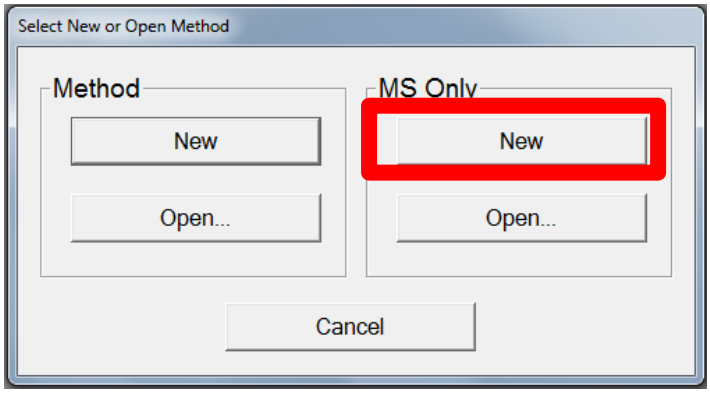

Figure 4.5 – Selecting an MS only method

6. The default method will load recommended starting conditions with the MS scan settings visible (Figure 4.6). The default mass ranges are 50 – 425 *m/z*. A default ionization time of 150 ms will also be set. Un-check the box for "ALC" (Automatic Level Control). "ALC" is also known as Automatic Gain Control (Figure 4.6).

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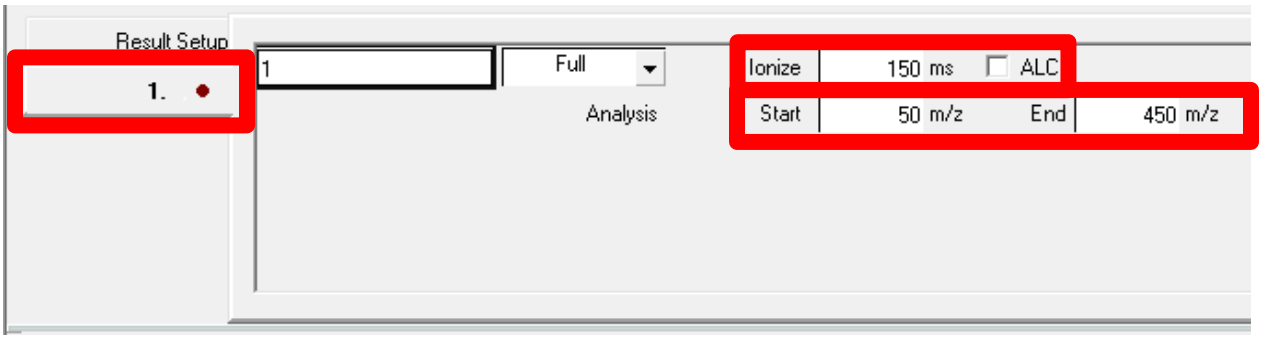

Figure 4.6 – MS scan settings

If any scan settings are to be changed, they must be written to the instrument after the change has been made. Do so by clicking the "Download Scan Configuration to the instrument" button (Figure 4.7).

Figure 4.7 – Downloading Scan Configuration

7. Click the "Result Setup" tab to change settings regarding how the data is collected and saved. All settings altered in this tab are immediate and do not need to be downloaded to the instrument (Figure 4.8).

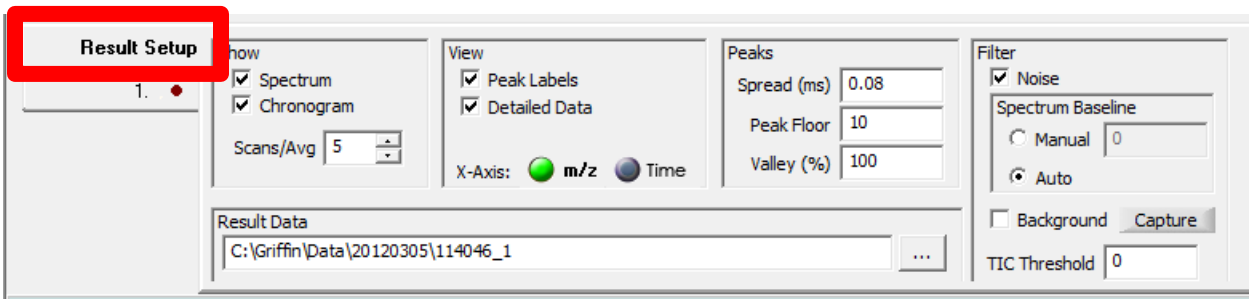

Figure 4.8 – Result setup screen

Default settings will produce decent-quality spectra. However, settings may be manipulated. Make sure both "Spectrum" and "Chronogram" are checked. This will make data interpretation much easier, as the MS spectra will be recorded for all time points.

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The "Scans/Avg" by default is set to 5. Lower than 3 Scans/Avg may result in lower quality spectra. No improvements are typically observed above 5 Scans/Avg; however, this may be chemical and/or matrix dependent.

Checking "Peak labels" will label each peak with the actual *m/z* value in the real-time view. If "Detailed Data" is checked, peak shapes will be observed. If unchecked, only peak centroids will be collected and observed (centroids convert the collected Gaussian peaks to a histogram centered at the maximum height of the curve).

"Peak Floor" will set the minimum abundance required to label a peak with its corresponding *m/z* value. Setting the Peak Floor to 10 usually allows analyte signals of low intensity to be observed while eliminating much of the background signal and random noise. The Peak Floor may be raised or lowered depending on personal preference or needs of a particular experiment.

The path under "Result Data" will be the physical path the data file is saved if the data is recorded for future interpretation. A path may be typed in directly or navigated by clicking the browse button to the right of the white box.

8. Before collecting (not recording/saving) data, ensure that the ionization source of interest is set-up correctly; see Chapter 2 for corresponding information.

9. To start collecting (not recording/saving) data, simply click the "Run" button (Figure 4.9). Spectral data should begin to appear in the spectrum window of the results. It should be noted that engaging the "Run" button enables all high voltage supplies and/or the on-board syringe pump. To save the data being collected, see Step 12.

Figure 4.9 – Run button

10. If the solvent syringe is being utilized (ESI/DESI), it may be necessary to purge the solvent transfer line to achieve a stable spray. To do this, click "Instrument" on the top menu bar of the screen and choose "Harvard Pump" (Figure 4.10).

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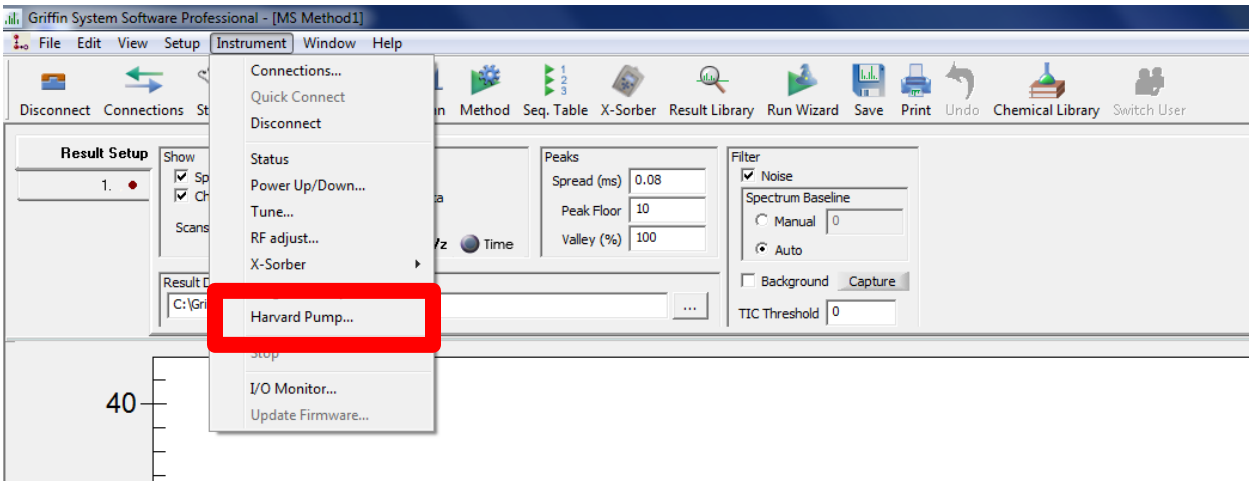

Figure 4.10 – Accessing the Harvard Pump

The Harvard Syringe Pump window should appear on the screen. The default syringe is 500 µL in syringe volume. Verify the syringe in the pump matches the setting in the software. To change the solvent flow, simply enter the desired flow in the Flow Rate window and click "Write." If "Write" is not clicked, no settings will be changed. When data collection is stopped and then started again, the solvent flow rate will revert back to whatever settings are stored in the Configuration File that is currently accessed by the instrument. To change the default settings of the pump, see Section 3.3 .

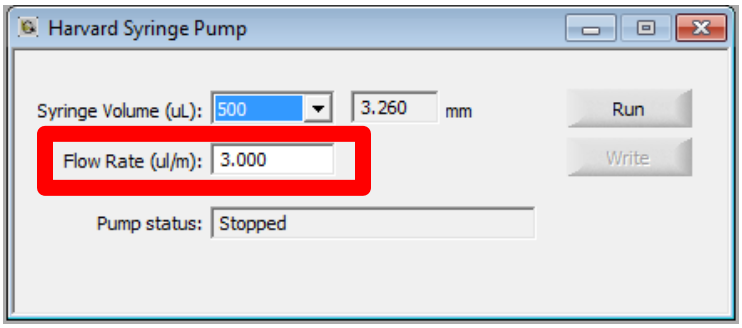

Figure 4.11 – Adjusting Harvard Pump flow rate

To purge the solvent transfer line, a flow rate of 30 µL/min is customarily set. When signal intensity of common background ions (and/or analyte ions if using ESI) rises sharply, return the flow rate to the desired flow rate typically 3 µL/min (DESI) or 10 µL/min (ESI) with a 500 µL syringe) (Figure 4.11).

The operator may notice that the signal intensities will rise sharply, but then all signal will appear to be lost. This is typical when the syringe is replaced and small air pockets enter the solvent transfer line. Repeat the purge process if this occurs (see Section 1.4).

With such high flow rates, solvent may begin to accumulate near the inlet capillary. A Kimwipe can be used to remove the excess solvent, being careful not to contact the ESI/DESI spray emitter or leave lint on the inlet capillary.

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11. At this point, analyte signals should be observed if performing an ESI experiment. If a DESI experiment is being performed, the surface may be introduced.

12. To record the data for later reference, simply click the "Record Data" button prior to introducing the sample.

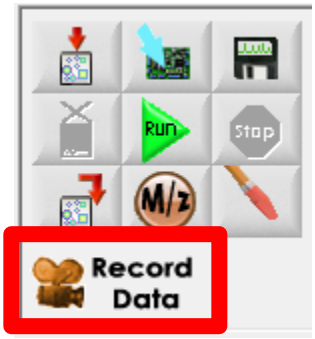

Figure 4.12 – Record data button

When you wish to stop recording, simply click "Record Data" again (this will be the same button that was clicked to begin recording).

4.1.2: Tandem MS (MS/MS) Data Collection

1. In order to perform MS/MS on the analyte of interest, select "MS/MS" from the drop down menu (previously labeled "Full" for MS anaylsis). Upon selecting MS/MS, a window of MS/MS parameters will be available. The values that typically remain unchanged are Ionize (150 ms), ALC (unchecked), and mass range (start 50 m/z and end 450 mz). Parameters that are altered consist of Mass (the m/z of the precursor or parent ion), Voltage (collision energy applied for dissociation/fragments), Frequency (accessed under the bottom "Adjust" menu), and Time (Figure 4.13). The values of these parameters are specific to the analyte of interest and result in idealized MS/MS signal. The values can be determined by entering the m/z of the precursor ion in for the "Mass" parameter and selecting "download scan configuration to instrument" resulting in software generated values for Voltage, Frequency, and Time. Another way of determining the values for these parameters is through semi-automated optimization of MS/MS method conditions, a process termed "macroing" or "compound-specific macroing." The compound-specific macro is detailed in Section 4.2.

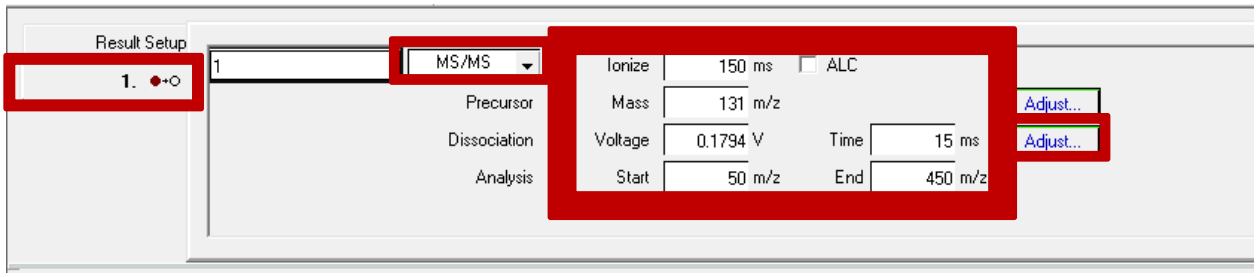

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Figure 4.13 – MS/MS scan settings

2. After entering specific values for the molecular ion and adjustable parameters (Frequency, Voltage, and Time), the values must be written to the instrument. This is done by pressing the "download scan configuration to instrument" button. MS/MS is now ready to be carried out.

3. The MS/MS scan may need to be turned on depending on whether the MS/MS settings were inputted while the full scan or MS scan was running. If the scan is stopped, press the green "Run" button to initiate the MS/MS scan. Data for MS/MS can be recorded in the same manner as described in Step 11 (see above).

SECTION 4.2 – SEMI-AUTOMATED OPTIMIZATION OF MS/MS METHOD CONDITIONS: "COMPOUND SPECIFIC MACRO"

Compound Specific Macro Procedure

1. To perform the compound specific macro, follow Steps 1-11, as necessary, to insure all settings are correct and a stable MS scan is present for the compound of interest. Change the scan from "Full" (MS scan) to "MS/MS," and enter the molecular ion seen previously in the MS scan for the "Mass" parameter, and click the "download scan configuration to instrument" button. If the scan is running, a stable MS/MS scan should be present displaying any compound specific MS/MS fragments.

2. While the scan is still running, press the "Scan" button and select "New File" from the resulting menu. Select "read scan configuration from the instrument" and press the "Save" button. In the save window, name the scan file with a descriptive name (scanfilecompoundnameconcentrationmacrovariable(frequency, voltage, time)) and select save. Stop the MS/MS scan in Level 2.

3. Close out of the scan file screen.Under "View" select "Macro" from the dropdown menu. A window will then open up displaying "Macro Detail" and "Result Setup" tabs. Under the "Macro Detail" tab, click the box next to "Scan Config". In the resulting window, load the previously saved scan file from Step 2, see above.

4. After loading the scan file, an individual macro parameter can be set up by accessing it from the "Variable" tab. In the "Variable" tab, "Amplitude" represents CID Voltage, "Duration" represents dissociation time, and "Frequency" is denoted as dissociation frequency. Under the "Output Register" the user can set specific starting/ending and increment values to be utilized for the variable of interest (Figure 4.14). Common ranges and increments for variables are listed below in Table 4.1.

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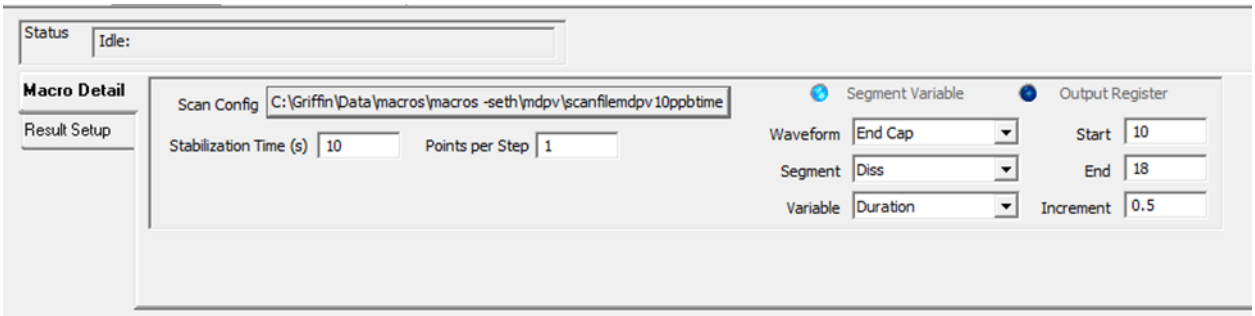

Figure 4.14 – "Macro Detail" tab

Table 4.1- Macro Value Ranges

Variable	Start	End	Increment
Frequency	167	180	0.5
Amplitude	(Instrument value - 0.05)	(Instrument value + 0.05)	0.005
Time	10	18	0.5

Note: "Instrument Value" is taken from Step 10

5. After entering values for a specific variable, click on the "Result Setup" tab. In this tab, name the data file under "Result Data". A unique name should be inputted to allow for the user to easily access that data after the macro is carried out and to prevent overwriting a preexisting data file.

6. Now that values and a file name have been inputted, a macro can be carried out for a certain variable using ESI, see Section 2.1 for the ESI protocol. It is recommended that the first variable be Frequency. Insure that the start, end, and increment values for the frequency variables match Table 4.1. Press the green "Run" arrow to start the macro.

7. After the Frequency macro has finished, the data can be accessed by following the file path specified in Step 5. Opening the data file displays the chromatogram and resulting mass spectra generated during each change of frequency settings. Under "RICs", enter the m/z of the highest intensity fragment from typical MS/MS spectra for this compound, and check the "Use Custom m/z Cal" box. The intensity of the fragment should now be able to be monitored over the entire chromatogram as specified by a differing color (Figure 4.15).

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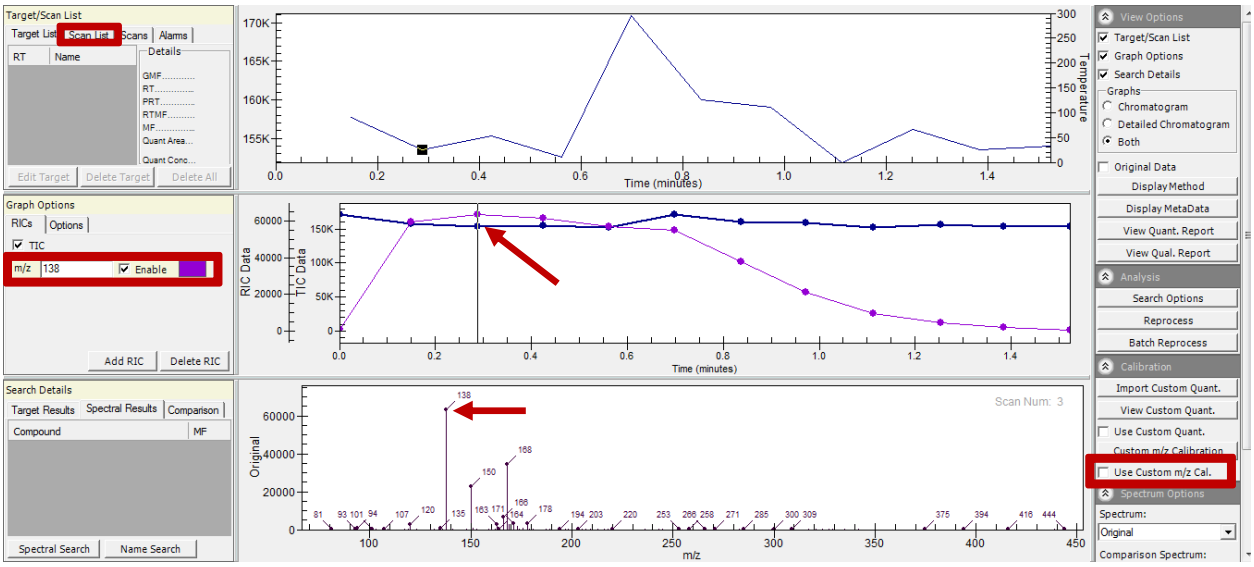

Figure 4.15 – Data file from frequency macro experiment

8. Determine the point in the chromatogram that results in the highest fragment intensity for the peak specified in step 7. The frequency at this point can be determined by clicking the "Scan List" tab and finding the specific scan number that correlates to the point of interest in the chromatogram in the list. Hovering the cursor over the corresponding "scan number" will result in the frequency being shown in a pop-up window (Figure 4.15). Record this frequency value.

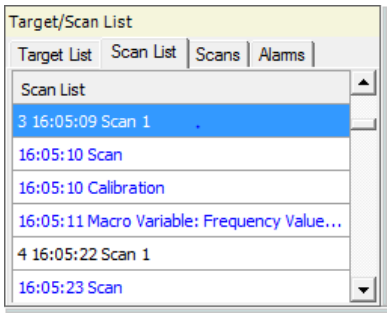

Figure 4.16 – Scan list

9. After recording all data values, close out of all windows except the original MS/MS scan from Step 1. Input the new frequency value in the window that results when clicking the bottom "Adjust" button in the MS/MS scan settings (Figure 4.13). Save this value to the instrument by pressing the "Download scan configurations to the instrument" button (Figure 4.7).

10. Note the value for the dissociation voltage (amplitude variable), as this will be needed for the next macro parameter (will be inserted for Instrument Value for the amplitude macro range in table 4.1). Run the MS/MS scan to insure that changing the frequency resulted in higher intensity MS/MS fragments of the precursor ion.

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11. After running the MS/MS scan with the new frequency value. Carryout Step 2, but for the file name use "voltage" instead of "frequency." Make sure that the file is saved in a known location.

12. Repeat Steps 3-6 by macroing the Amplitude (voltage) variable. Follow the start, end, and increment values as displayed for the Macro Values Ranges (i.e. instrument recommended voltage ± 0.05 with an increment of 0.005). Insure that the data file path is correct, and run the macro.

13. After the amplitude (voltage) macro has finished, open the data file and find the voltage that resulted in the highest intensity for the MS/MS fragments. Record this value. Close out of all windows except the original Level-2 MS/MS scan.

14. Input the new voltage (amplitude) value in the MS/MS scan settings (Figure 4.13). Save this value to the instrument by pressing the "Download scan configurations to the instrument" button (Figure 4.7).

15. Run the MS/MS scan to insure that changing the voltage resulted in higher intensity MS/MS fragments of the precursor ion.

16. After running the MS/MS scan with the new voltage (amplitude) value, make sure the scan is stopped. Carryout Step 2, but for the file name use "Duration" (time) instead of "Amplitude" (voltage). Make sure that the file is saved in a known location.

17. Repeat Steps 3-6 by macroing the "Duration" (time) variable. Follow the start, end, and increment values as displayed for the Macro Values Ranges (i.e. start = 10, stop = 18 with an increment of 0.5). Insure that the data file path is correct and run the macro.

18. After the duration (time) macro has finished, open the data file and find the time value that resulted in the highest intensity for the MS/MS fragments. Record this value. Close out of all windows except the original Level-2 MS/MS scan.

19. Input the new duration (time) value in the MS/MS scan settings (Figure 4.13). Save this value to the instrument by pressing the "Download scan configurations to the instrument" button (Figure 4.7).

20. Run the MS/MS scan to insure that changing the duration (time) resulted in high intensity MS/MS fragments of the precursor ion.

21. The resulting MS/MS spectra for the target analyte should now have optimized values for the frequency, voltage, and time parameters, giving rise to enhanced MS/MS signal intensity. Note all of these values as they will be needed in Section 4.3.

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SECTION 4.3 – METHOD SETUP FOR DATA DEPENDENT SCANNING (DDS)

A DDS method must be created for use in the automated Level 1 software.

4.3.1 Data Dependent Scanning Procedure

1. Open the "Griffin System Software" and select the "Method" button from the top of the toolbar (Figure 4.17).

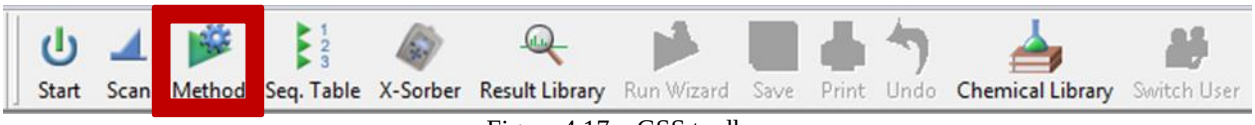

Figure 4.17 – GSS toolbar

2. Select "New" under the method option (Figure 4.14).

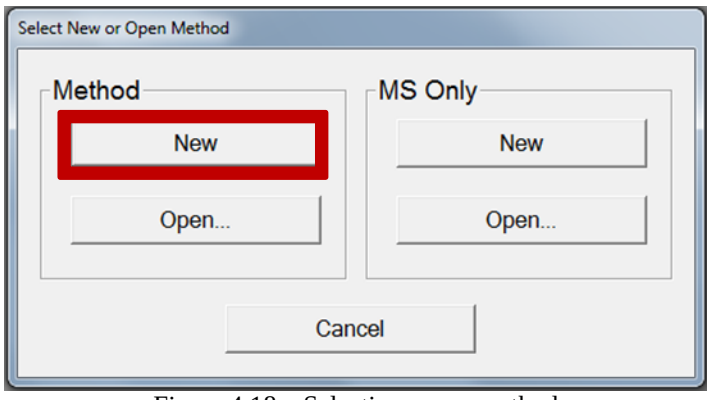

Figure 4.18 – Selecting a new method

The method setup for DDS (Data Dependent Scanning) will open (Figure 4.19).

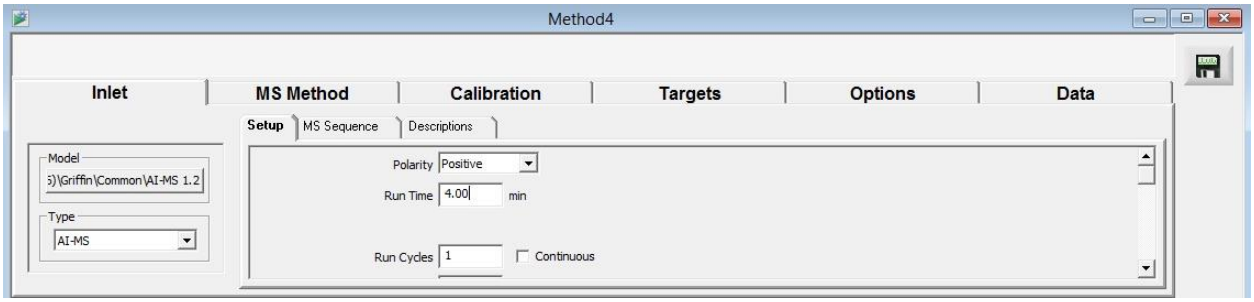

Figure 4.19 – Method set-up for DDS

3. Under the "Inlet" tab and "Setup" subtab, specify the polarity for the method being created with the drop down menu. Then specify a maximum running time for the method (Figure 4.19). The base MS scan will run for one half of the time entered, and any triggered MS/MS confirmatory scans will also run for one half of the time entered, starting from the time in which they first triggered. For example, entering a time of 4.00 minutes will set the method to collect data in the base scan for a maximum of 2.00 minutes, at which point the method will end unless a target chemical is detected and

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triggers confirmatory scanning. Also, if a chemical triggers a warning at t = 1.00 minutes, its specific confirmatory scan will run for two minutes, ending at t = 3.00 minutes, and a chemical that triggered at 2.00 minutes will end at the absolute maximum time of 4.00 minutes.

4. Under the "Inlet" and "MS Sequence" tab, make sure that a "start time" and "#Scans" is specified. Also confirm that the boxes are check marked as in Figure 4.20.

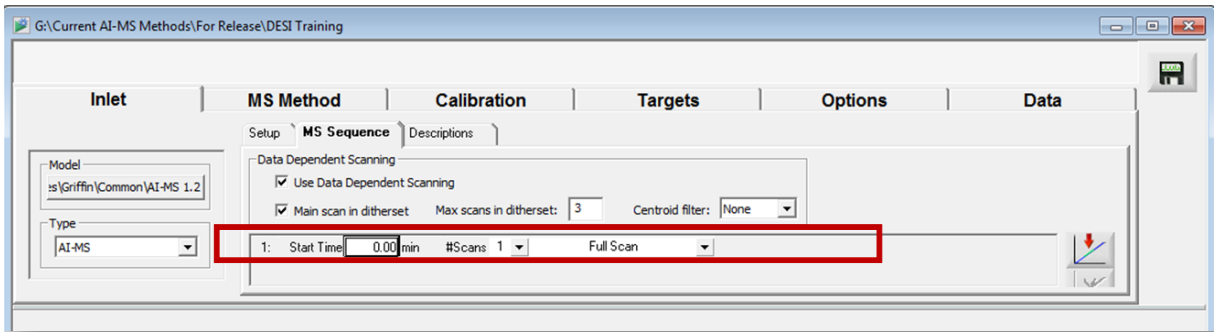

Figure 4.20 – "Inlet" "Setup" window

A ditherset is the max number of "dithers" that will be run at a given time. This number is determined by the user. The main scan in the ditherset will always include a "MS scan" as one of the dithers in the set. The main scan is utilized by the DDS to look for triggers that will initiate compound-specific MS/MS analyses. This will continue to be used while the software performs said MS/MS scans. Compound-specific protonated ion signatures are typically used as the triggers in question. The main scan is highlighted above (Figure 4.20).

5. Proceed to the "MS Method" tab (Figure 4.21).

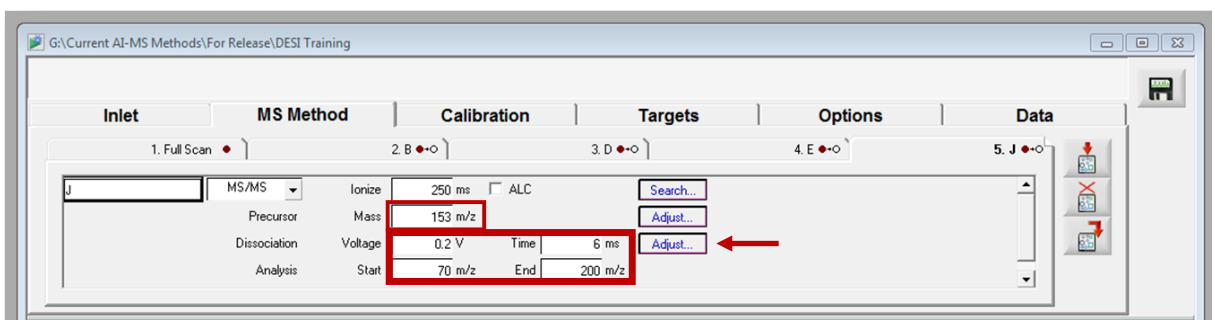

Figure 4.21 – MS Method tab

The "MS Method" tab is where the main scan (MS full scan) and MS/MS scans are created through the input of compound-specific settings. Settings that are often altered for specific compounds are the mass, voltage, time, mass range (start/end), and frequency (obtained by the compound specific macro, see Section 4.2). The frequency can be accessed under the bottom "Adjust" button (Figure 4.21). Other MS/MS scans be added or removed by clicking the "Insert New Scan" or "Delete Scan" buttons. If inserting a new MS/MS scan, "MS/MS" must also be selected on the left of the screen (Figure 4.22). There can be a total of 30 scans for each method (1 main MS scan + 29 target MS/MS scans).

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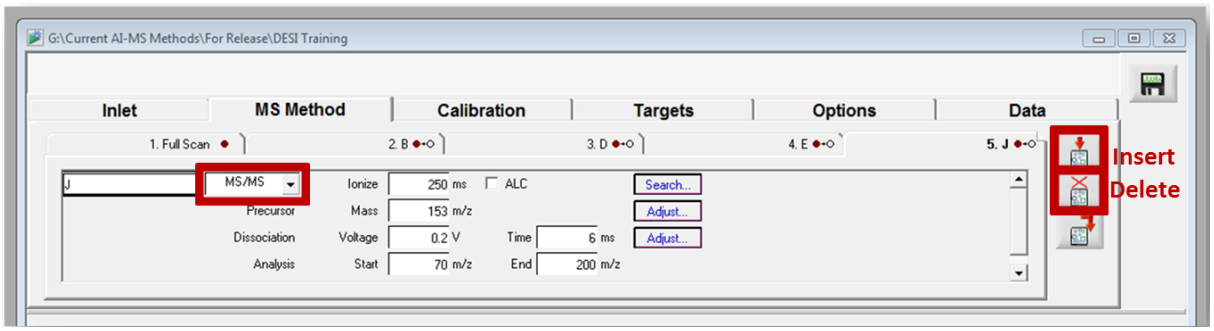

Figure 4.22 – Selecting, inserting, or deleting an MS/MS scan.

6. Proceed to the "Targets" tab (Figure 4.23).

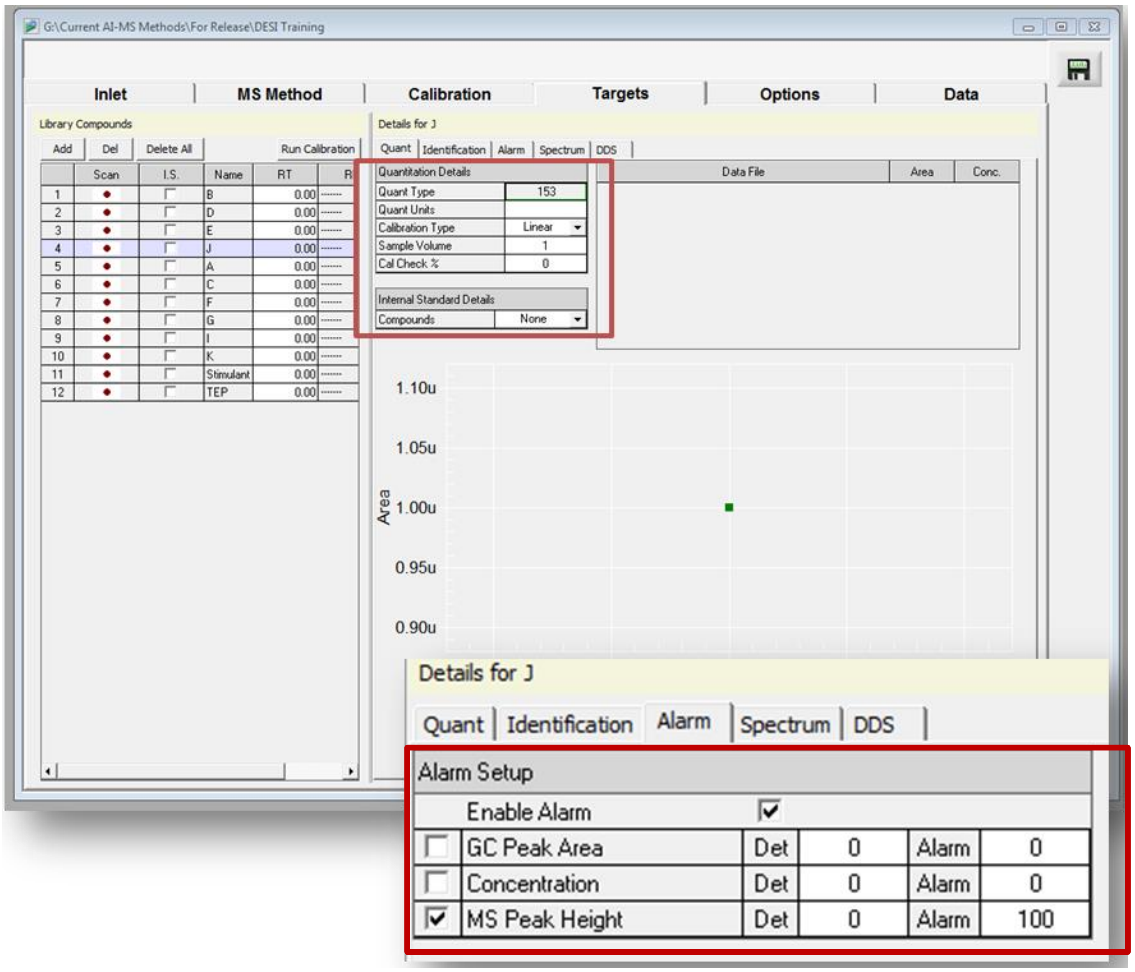

Figure 4.23 – Targets tab

The "Targets" tab is where the specific DDS triggers are configured. To add compounds, a library containing the compound of interest must be present. To create a library, select "Chemical Library" under View in the main menu of the Level 2 software to bring up the library dialog box. Next, click on the "Create New Library" box. Provide a name for the library and select MS Only Identification under the search type field before clicking the

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"Create Library" button (Figure 4.24). The same dialog box can also be used to import preexisting libraries created on other computers by checking the Import from file field, selecting the file type used when the library was created, and providing the file path.

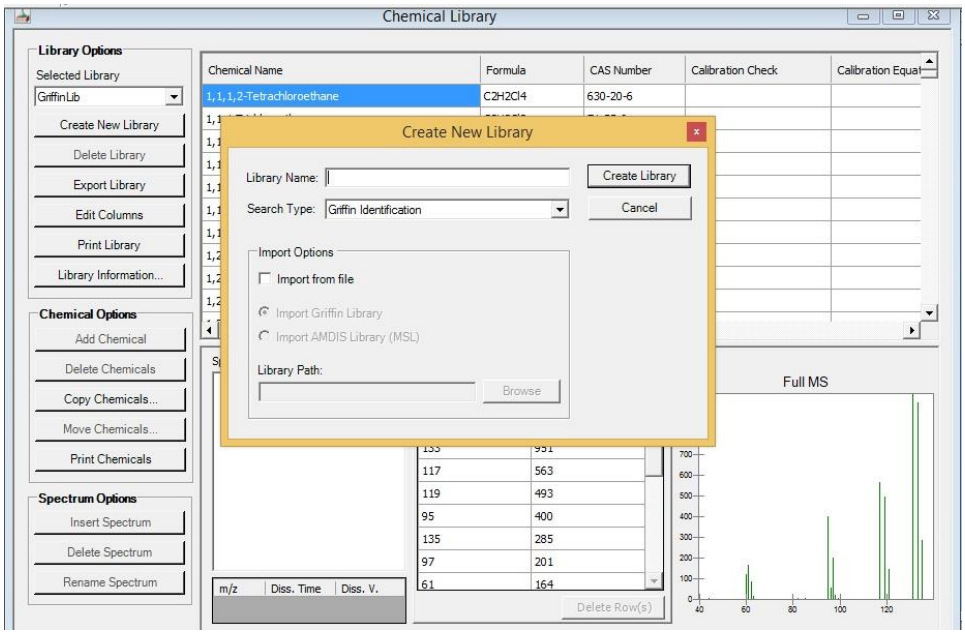

Figure 4.24 – "Create Library" button

To populate a library, select the library of interest under the drop down menu in the library options section of the main library dialog. Under the chemical options section, click the "Add Chemical" button to create a blank entry for the library in the top half of the dialog box labeled as [new]. The name field should already be highlighted to enter the name of the chemical.

Spectral features are entered in the lower half of the screen. Ensure the correct chemical is selected, and then click on "Full MS" and "Library Spectrum". The m/z of the parent peaks visible in the base scan can then be entered in the library with their relative intensities. The Full MS diagram will then update to reflect the peaks entered. DDS operation only requires a library entry with base scan spectral information; however, additional reference information can be included in a library entry. Right clicking upon the Full MS heading or clicking the insert spectrum button under spectral options provides the option to create an MS/MS subheading. MS/MS spectral data can then be added into the entry as for the base scan, by clicking and selecting the new subheading.

Additional columns of reference information can be added to the right of the chemical's name in the library table using the "Edit Columns" button on the left side of the dialog box. Options include but are not limited to chemical formula, molecular weight, parent ion, and fragment ions. Custom columns can be created under the same Edit Columns dialog box. When finished, the Export Library button can be utilized to create a backup file of the library for transfer to another computer.

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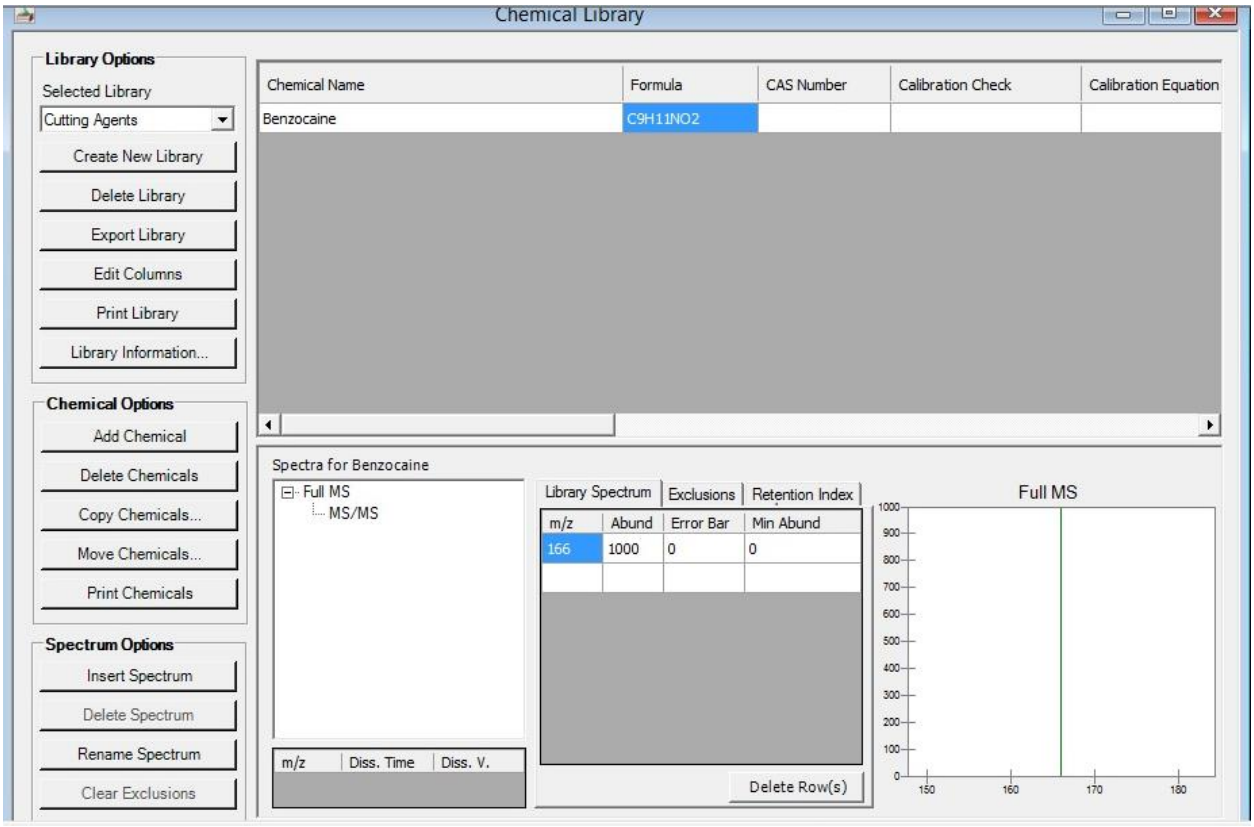

Figure 4.25 – "Chemical Library" window

To enter a compound in the target list, click the "Add" button. A new dialog box opens with a drop down menu to select the library in which the chemical of interest is located (Figure 4.25). Ensure that the full scan library spectrum for the chemical and not the MS/MS spectrum is highlighted, then click "Select" to add the chemical to the targets list for the method.

Ensure the appropriate chemical is selected in the target list and open the "Quant" tab on the right portion of the window. Check that the "Quant type" field lists the m/z of the parent ion utilized in MS/MS scan setting and the alarm plugin. Under the "Alarm" tab, check the "Enable Alarm" box to enable detection of the chemical in the method and check the MS peak height option (Figure 4.23). Minimum values for detection/ warning and for alarm can be entered into the same row. Typical values of 500 are entered for the "Detection" and "Alarm" fields.

7. Under the "Targets" tab, select "DDS" (Figure 4.26).

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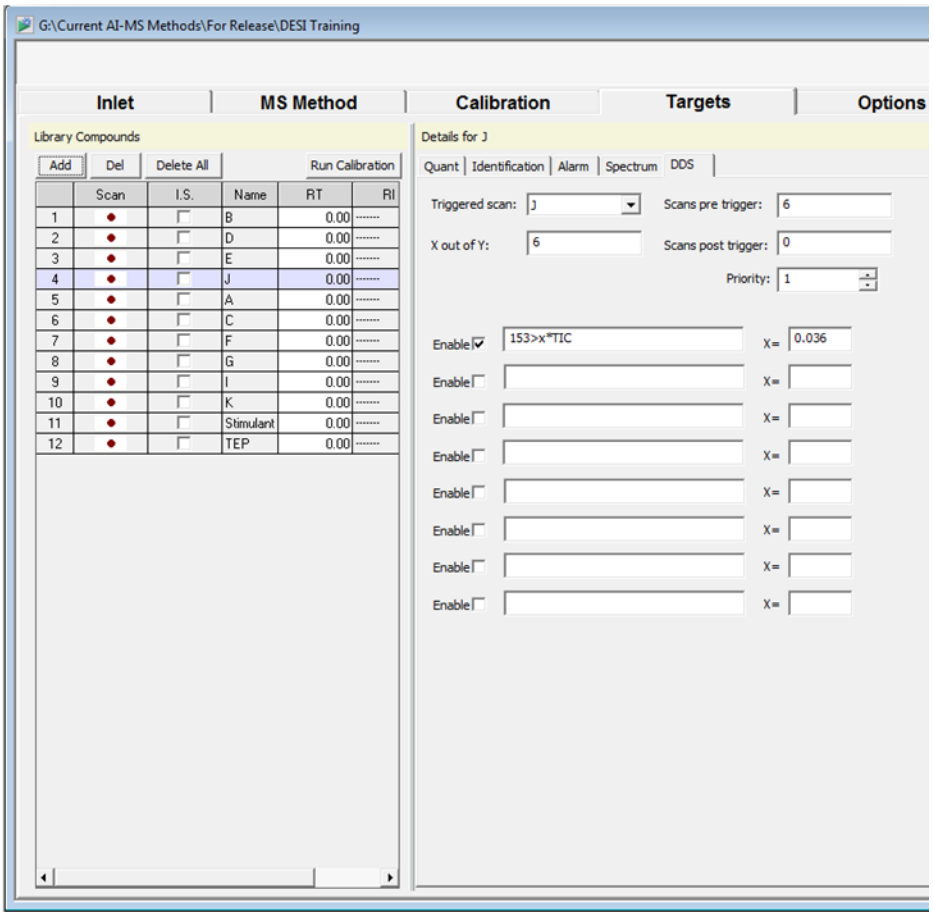

Figure 4.26 – "DDS" tab

The "DDS" tab is where settings are entered for triggering a configured MS/MS scan. The "Triggered scan" entry corresponds to the MS/MS scan that will be run for the target and must match the name and number for the MS/MS scan set under the MS Method tab, not the number of the analyte in the targets list. "Scans pre trigger" is characterized as the number of mass spectra searched before a trigger occurs. Equally important, "X out of Y" is classified as how many pre trigger scans must meet the requirements for a trigger. "Scans post trigger" specifies a maximum number of MS/MS scans collected once a target triggers and is generally set to 0 to allow unlimited scans up to the set time limit. The "Priority" designates the importance of the specific target. A value of zero is assigned maximum priority, with priority decreasing with increasing number. Should the target(s) assigned the highest priority in the method trigger and then fully alarm, the method will immediately stop. If desired, this behavior can be avoided by creating a blank target entry assigned highest priority and assigning chemical targets a priority one higher. The pre-loaded method delivered on this instrumentation is configured to have equal priority for all targets and disable automatic method. This allows the user to screen for other targets after a compound of interest had already alarmed.

Eight rules can be used in order to initiate a triggering event during MS scanning. Generally, the pre-loaded method focuses on the total ion count, "TIC," as shown above (Figure 4.26). The number entered into the rule slot is the m/z of the precursor ion, which is then followed

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by a greater than with a value of X multiplied by the TIC. The appropriate X value is determined by subtracting the upper mass limit from the lower mass limit for the target/mass range of the base scan, inversing the value, and multiplying by 10.

For example:
Mass range: 50 – 450 m/z

(1/(450 – 50)) * 10 = 0.025

Additional rules can be entered based on the relative intensities between peaks of different m/z value present in collected spectra. The rules follow a general form of A > (or <) X*B, where A and B are the m/z values of the peaks of interest and X is the scaling factor as before. The "Enable" box must be checked to apply its corresponding rule during scanning (Figure 4.26).

8. After entering the desired rules, select the "Options" tab (Figure 4.27).

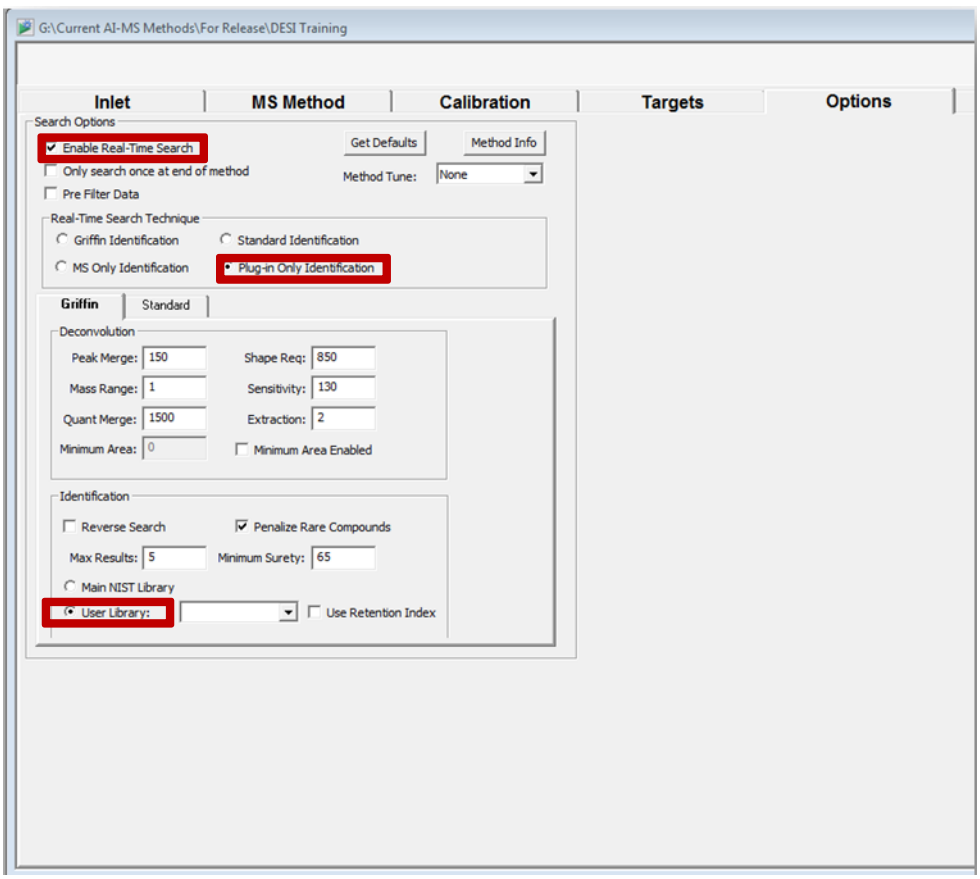

Figure 4.27 – Options tab

9. Enable "Real-Time Search," check "Plug-in Only Identification", and select the library used to set targets in the previous steps under "User Library." Also, ensure that all other settings match the above figure.
10. Select the "Data" tab (Figure 4.28).

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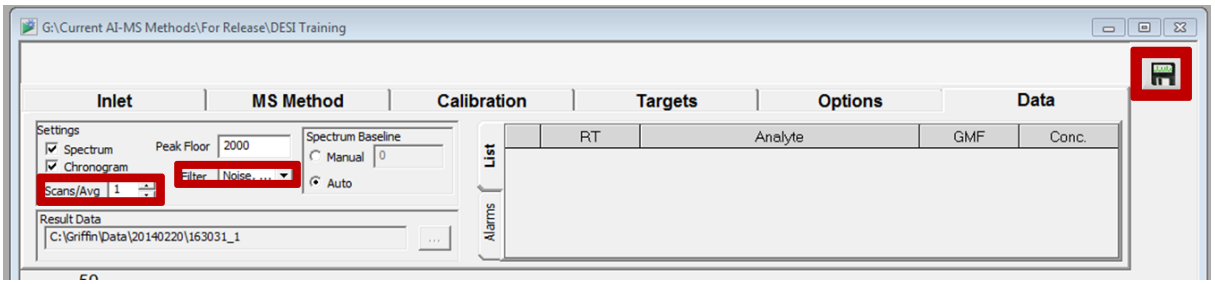

Figure 4.28 – Data tab

11. Use single scan averaging and turn on the noise filter.
12. Save the method using a descriptive title by pressing the save button in the top right corner of the screen. Note the name of the file, as it will be needed in the next steps when developing a MACOI plug-in file.
13. To enable the method for use in Level 1 software, click "Operator Options" from "Setup" in the main menu. In the dialog box, click "Add," then provide the name and location of the method file. Save the updated options and exit the Operator Options window

SECTION 4.4 – MACOI PLUGIN SYNTAX

Developing a Macoi Plugin

All MS/MS operation or triggering parameters are expressed using a "MACOI" text file. Each separate target will need to have its own MACOI file.

```
1  <?xml version="1.0" encoding="UTF-8" ?>
2  <Targets method="DESI training.gfnM" log="false">
3      <Target name="A" quant="193" scanId="8" consecutive="2">
4          <BKGND mz="70-120" />
5          <TRIC mz="137"  factor="1" min="100"/>
6          <TRIC mz="165" factor="1" min="100"/>
7      </Target>
8  </Targets>
```

Lines in MACOI

Line 1: Standard opening for XML based plugin file

Line 2: This line is where the name of the Level 1 method that this plugin is tied to goes, Section 4.3, Step 11. Each MACOI can be tied to multiple methods, but each name should be separated by a comma ("methodA.gfnM, methodB.gfnM"). Log="false" means that logging is not enabled for this MACOI (if set to true, some amount of logging occurs). Logging is not necessary for this application.

Line 3: This line is where the information for the specified target is setup. The **name="A"** should correspond to how the user wants the target to be identified when alarm occurs. This should also match how the target is setup in the targets in the method. The **quant="number"** is

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the quant ion specified when setting up the method in GSS. The **scanId="number"** is the scan index number corresponding to the MS/MS scan in the method as specified in the "MS Method Tab," see figure 4.17. This number will be one less than the number of the scan in the method (i.e. if MS/MS scan for "A" was #2 in the method, the scanId will be 1). The **consecutive="number"** is the number of times the subsequent rules must be met before an alarm will occur.

Line 4: This line allows the operator to select the range of background ions (**mz="numbers"**) to compare the generated fragment ions to. Multiple ranges can be used, but should be separated by a comma (i.e. "70-90, 170-190"). This background calculation calculates an average of the ions set in the range.

Line 5: This is where the fragment ions of the compound being analyzed are specified. A separate line can be used for every individual ion, or ions can be combined into one line. The **mz="number"** is the ion (ions) of interest from the precursor. The **factor="number"** is the factor which the ion needs to be above the background ions (i.e. if it was 5, then the ion must be 5x the calculated background level). The **min="number"** is the minimum abundance for that ion. In our methods, the "factor" and "min" typically have values of 3 and 100.

Save all MACOI files to the "Griffin Common", "Internal Search Plugins" sub-folder.

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CH. 5 – TROUBLESHOOTING

SECTION 5.1 – DIAGNOSTIC MESSAGES

5.1.1: Common Error Codes

Error Code	Procedure
Harvard Pump Error	1. Refill syringe
MB Gain Tune Error Low Mass Tune Error Quant Tune Error RF Tune Error MS/MS Tune Error	1. Rerun system tune 2. If tune passes, confirm Error has cleared 3. If tune fails, follow troubleshooting procedures
Trap Pressure Error Vacuum Error Drag Pump Error Turbo Pump Error	1. Power down the AI-MS 2. Once the instrument is powered down a. Confirm capillary is present and installed b. Confirm capillary nut is finger tight c. Confirm Helium pressure is 25-30 psi d. Confirm ambient environmental conditions are within operating specifications 3. Once checks are satisfied, power up the AI-MS Confirm Error has cleared

Figure 5.1 – Vacuum and pump errors

Note: In most cases a reduction in vacuum pressure can be heard by the user (i.e. slow whining noise). There are cases where this has been observed when replacing the inlet capillary due to a malfunctioning ball valve. For information on inspecting the ball valve, see Section 5.3 (Advanced Troubleshooting).

Connection Error Laptop Launch Utility *Single-Laptop Configuration*	Procedure
Waiting for the AI-MS to be turned on...	1. Confirm the power indicator is illuminated a. Confirm the generator is operating, and the circuit breaker is on b. Confirm the power strip is on c. Confirm AI-MS power switch is on 2. Confirm Ethernet cable connections
Setting the laptop to run in Single laptop mode...	1. Restart laptop and cycle power to AI-MS
Verifying settings...	1. Restart laptop and cycle power to AI-MS
Launching software...	1. Restart laptop and cycle power to AI-MS

Figure 5.2 – Laptop connection errors

5.1.2: Harvard Pump Error

A Harvard Pump error will occur if the syringe is empty (Figure 5.3). The user should re-fill the syringe according to the steps in section 1.2.

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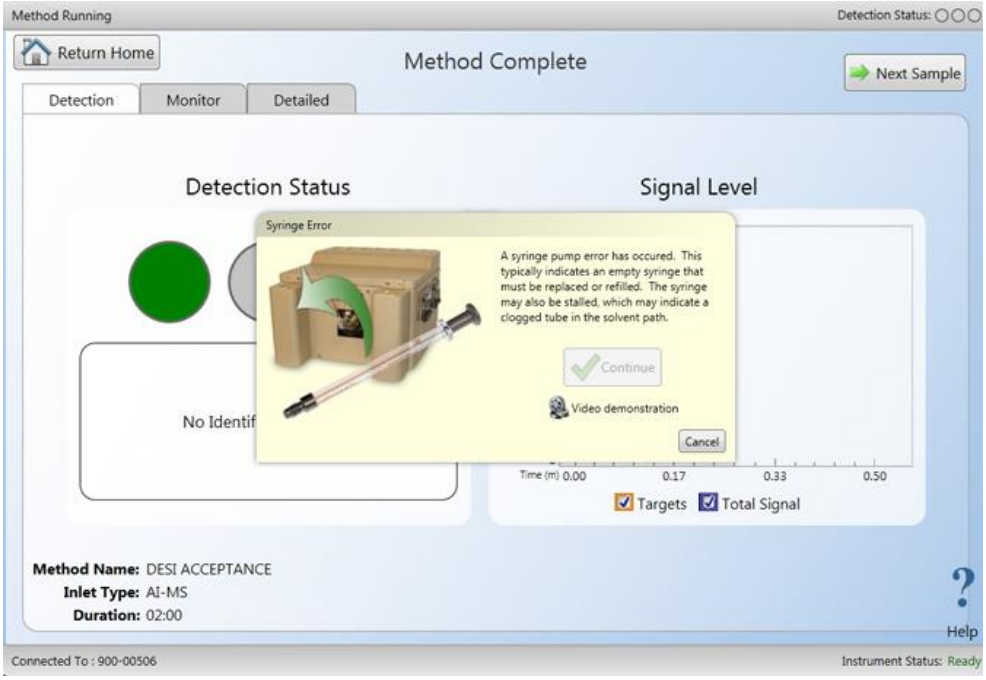

Figure 5.3 – Syringe pump error during scan

After the syringe has been refilled and replaced, the "continue" button will be available (Figure 5.4).

Figure 5.4 – Error screen after replacing syringe

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5.1.3: Tune Error

If a tune error is detected, an error message will occur (Figure 5.5). Diagnostic error(s) that can result from a tune error are “MG Gain Tune,” “Low Mass Tune,” “RF Tune,” “MSMS Tune,” or “Quant Tune”. The software may display one or more of these errors at a time. It is also important to note that these errors can occur in both level-1 and level-2, but the troubleshooting procedure will be the same.

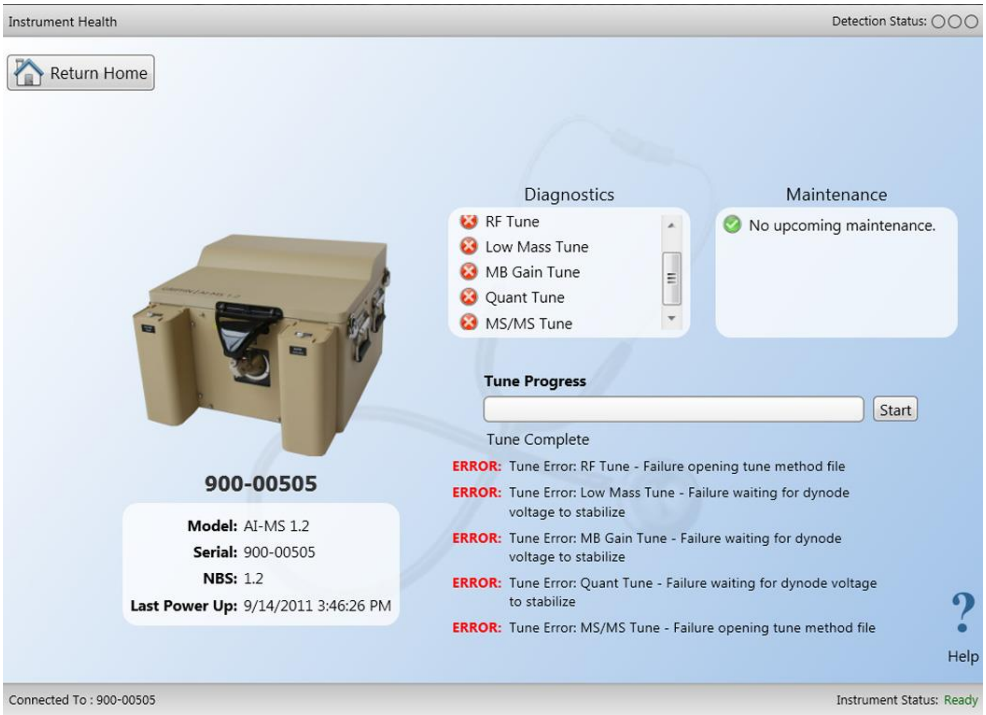

Figure 5.5 – Calibration tune error

The following steps should be followed if a tune error occurs.

1. If any of the tune errors are displayed, rerun the entire system tune
2. If any of the tune errors occur again, refer and follow the troubleshooting flowcharts found in Appendix A. Proceed through Troubleshooting Flowchart 1 (Figure A5) followed by troubleshooting Flowchart 2 (Figure A6) and troubleshooting Flowchart 3 (Figure A7) if necessary.

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5.1.4: Trap Pressure Error

An error message will result if the trap does not meet or sustain the required pressure (Figure 5.6).

Figure 5.6 – Trap error

If this error occurs, the following steps should be followed.

1. Power-down the AI-MS.
2. Confirm that the inlet capillary is present and installed correctly.
3. Confirm that the capillary nut is tight.
4. Confirm that the helium pressure is 25-30 psi.
5. Confirm that the ambient environmental conditions are within operating specifications
6. After completing steps 2 – 5, power-up the AI-MS and confirm that the error has cleared

5.1.5: Turbo or Drag Pump Error

An error message will result if the turbo or drag pump does not meet or sustain the required pressure (Figure 5.7). For this case, the troubleshooting procedure is the same as the trap pressure error.

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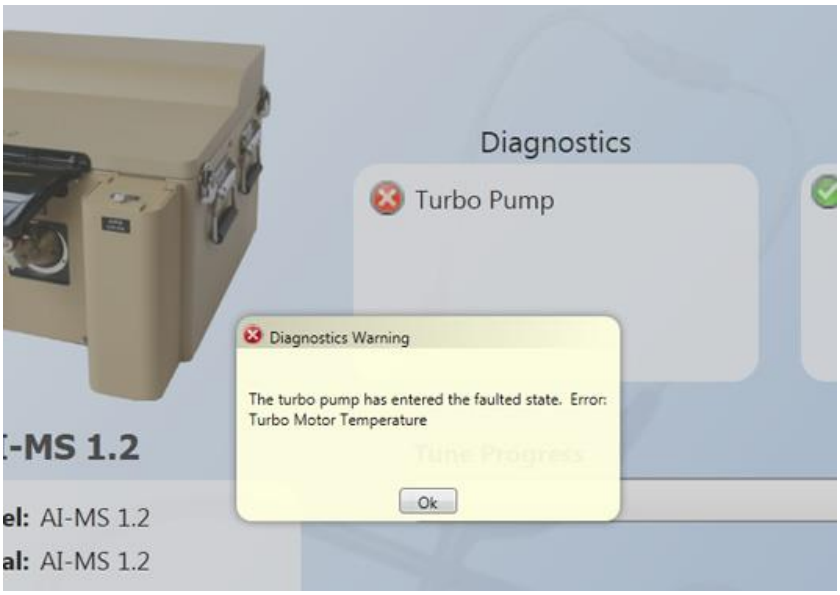

Figure 5.7 – Turbo or drag pump error

Note: In most cases a reduction in vacuum pressure can be heard by the user (i.e. slow whining noise). There are cases where this has been observed when replacing the inlet capillary due to a malfunctioning ball valve. For information on inspecting the ball valve, see Section 5.3 (Advanced Troubleshooting).

5.1.6: Connection Error

If the software is unable to connect to the instrument, a "Connection Error" message will result (Figure 5.8).

Figure 5.8 – Connection Error

For this type of error, the user should perform the following steps.

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1. Confirm that the AI-MS is powered on and not already connected in another instance of the Level 1, Level 2, or Toolbox software.
2. Confirm that the wall power is operational.
3. Confirm that the AI-MS power cable is connected and power switch is in the on position.
4. Confirm that all cables are connected to the laptop correctly.
5. Restart the laptop.

SECTION 5.2 – SOFTWARE MALFUNCTIONS AND LIMITATIONS

5.2.1 Level 1 Missing Results Files

Datafiles may only be accessed in one instance of the Griffin System Software at once. Because of this, files currently opened in a different instance of the software or opened for advanced viewing in Level 2 are removed from the Level 1 software results list. To use Level 1 to open files missing from the results list, the file must be manually opened using the browse button and located in the results directory.

5.2.2 Level 2 Axis Display Error

When first opening the software, switching from a centroid view to detailed view display may cause the x axis of the mass spectrum to be labeled in scientific notation. The labeling can be reset by changing the lower bound of the mass range to a different value, writing to the instrument, changing the bound to the correct value, writing to the instrument, and then pressing "Run".

Additionally, the software can potentially crash after changing to detailed view. The software can be reopened without requiring any additional adjustment.

5.2.3 Level 2 Tandem Mass Spectrometry Frequency

When adjusting fragmentation settings for MS/MS scans in MS Only methods, changing the mass to charge ratio being isolated resets the frequency value to the default 173.5 value.

5.2.4 Level 2 Software Slowdown

During an analysis session in Level 2, the instrument keeps a temporary cache of the entire chromatogram and all mass spectra for the session in the computer's memory. This may result in the software slowing down or briefly locking up. The cache can be cleared using the "Clear Contents of the Chromatogram" button, located directly under the "Stop" button.

5.2.5 Macoi Non-Specificity

When writing MACOI files, the current version of the Level 1 and Level 2 software does not correctly associate MACOI files to only the methods specified in line 2 of the file. Currently, methods will apply any MACOI file possessing the same "scanId" number in line 3 of the file. A future software update from Flir will correct this issue.

5.2.6 Harvard Pump Settings

The software discards any changes made to the Harvard Pump flow rate setting in Level 2 whenever scanning is stopped. When scanning is resumed, the value will reset to the values specified in the instrument configuration file and must be manually changed.

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5.2.7 Comment Inaccessibility in Level 2

Comments about samples entered using the Level 1 software are not accessible when viewing the result file in the Level 2 software.

5.2.8 Inability to Average or Change Scans in Level 2 Result Viewer

When using Level 2 to view result files for data that was collected with multiple simultaneous scans being run at once, mouse focus must be in the top chromatogram to change the scan being displayed in the detailed chromatogram. Clicking anywhere on the lower, detailed chromatogram disables the ability to use the arrow keys to change the scan settings until focus is restored to the top chromatogram. Selecting a region of the chromatogram to average must also be performed using the top chromatogram by dragging the selection box over the region on interest then releasing the mouse button and clicking "Add Average" before selecting "Average" in the "Spectrum" drop down menu in the "Spectrum Options" tab.

SECTION 5.3 – AI-MS QUICK START-UP GUIDE

1. Power-up the AI-MS according to the protocol in Section 1.3
2. While in Level 2, purge the solvent transfer lines with the positive-ion mode calibration mixture, see Section 1.5.
3. After successfully purging the solvent transfer lines, perform the positive-ion calibration and tune (Section 1.5).
4. Once all five steps of the positive-ion calibration tune have passed, an ionization source can be chosen.
5. If ESI (Section 2.1) or DESI (Section 2.2) is chosen as an ionization source, the solvent transfer lines must be purged with the analyte of interest ESI or positive-ion mode spray solvent (DESI). See Section 1.5 for purging. The Configurator may also need to be accessed to alter flow rates for the desired ionization source (ESI: 10µL/min and DESI: 3µL/min), see Section 3.3.
6. If PSI (Section 2.3) or APCI (Section 2.4) is chosen as an ionization source, purging the solvent transfer lines is not required, as both ionization sources do not utilize the solvent system.
7. After the ionization source has been chosen and set up according to its protocol in CH.2, a Level 1 (CH. 3) or Level 2 (CH. 4) analysis can be chosen. In the case that APCI is chosen as the ionization source, only Level 2 can be performed.

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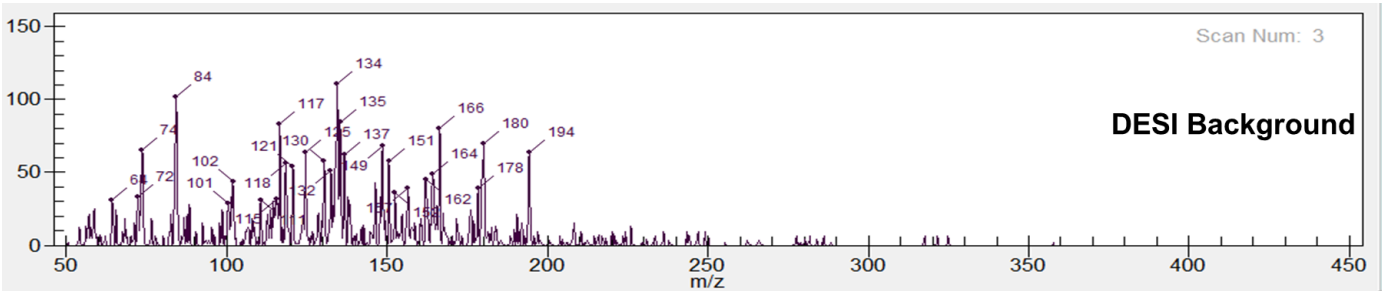
SECTION 5.4 – OPERATIONAL CHEAT SHEET FOR IONIZATION SOURCES

5.4.1 ELECTROSPRAY IONIZATION (ESI) – SECTION 2.1

1. Ensure Source is in ESI position (lever up) – Section 2.1
2. Purging is required prior to analysis of sample – Section 3.3
3. Configurator Solvent flow rate: 10 µL/min – Section 3.3
4. Operational modes – Section 2.1
    a. Dissolved solid or liquid samples

5.4.2 DESORPTION ELECTROSPRAY IONIZATION (DESI)



1. Ensure source is in DESI position (lever down) – Section 2.2
2. Purging is required prior to analysis of sample – Section 3.3
3. Configurator Solvent flow rate: 3 µL/min – Section 3.3
4. Operational modes
    a. Residue deposited on glass slides – Section 2.2.1
    b. Physical transfer swab (swab guide required) – Section 2.2.2

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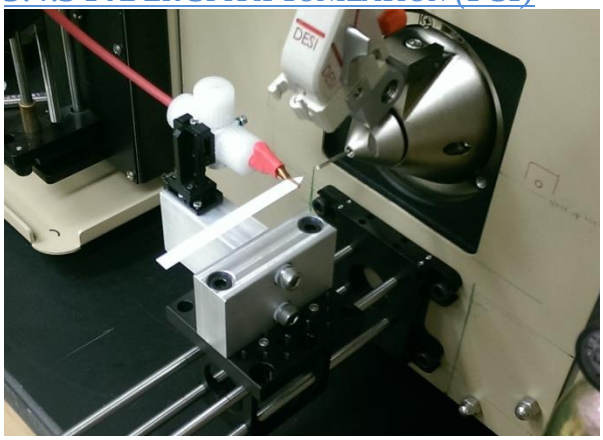
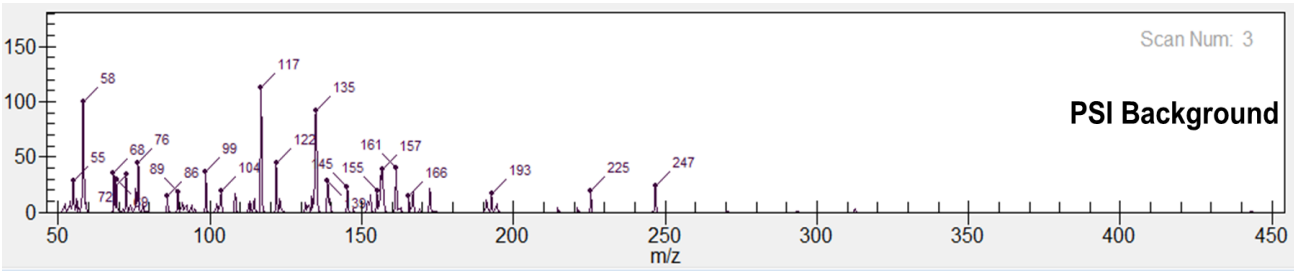
5.4.3 Paper Spray Ionization (PSI)





1. Ensure source is correctly attached to front plate mounting rig and the ESI/DESI source is in the "up" position – Section 2.3
2. The paper substrate should be placed slightly above capillary and ~2 mm away from the entrance of the capillary – Section 2.4
3. Purge not required
4. Configurator Solvent flow rate: empty syringe or 0.5 µL/min flow rate – Section 3.3
5. Operational modes – Sections 2.3.2 and 2.3.3
    a. Residue deposited onto paper substrate – Section 2.3.2
    b. Physical transfer swab – Section 2.3.3

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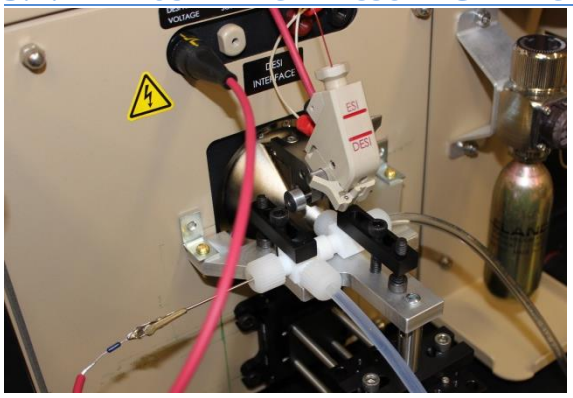
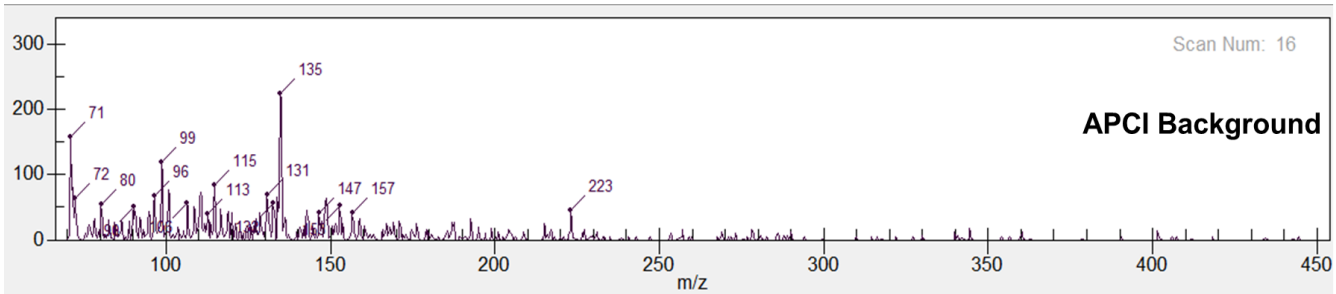
5.4.4 Atmospheric Pressure Chemical Ionization (APCI)





1. Ensure APCI source is correctly attached to front plate mounting rig and ESI/DESI source is in the "up" position – Section 2.4
2. Purge not required
3. Configurator Solvent flow rate: empty syringe or 0.5 µL/min flow rate – Section 3.3
4. Operational modes – Section 2.4
    a. Ambient air sampling via auxiliary pumping system
    b. Direct headspace injection

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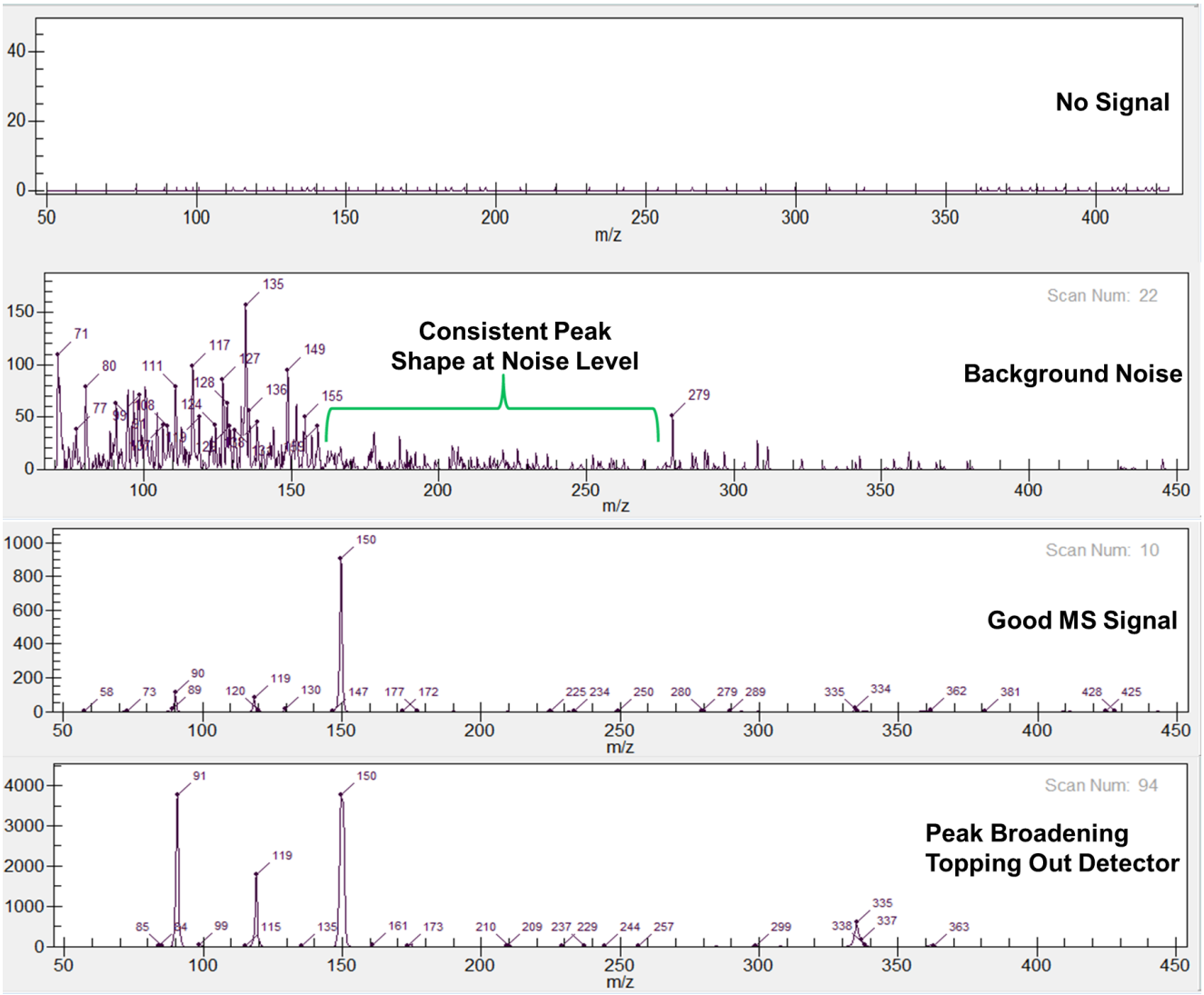
SECTION 5.5 – COMPARISON OF COMMONLY ENCOUNTERED MASS SPECTRA



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SECTION 5.6 – CLEANING THE INLET CAPILLARY

After a dirty inlet capillary has been replaced with a clean inlet capillary, see Section 1.9.2, the dirty capillary can be cleaned according to the following protocol.

1. Follow the procedure for removing the dirty capillary (Section 1.9.2)
2. After the dirty capillary has been removed, completely submerge it in a beaker of methanol.
3. Place the capillary in a sonicator for 15 min.
4. After sonication, rinse again with clean methanol
5. Place clean capillary in a drying oven or in a clean beaker until all methanol has evaporated from inside the capillary.

In the event that a sonicator is not available, a methanol wash bottle can be used to rinse methanol though the inlet capillary multiple times, followed by drying at room temperature or with compressed air.

SECTION 5.7 – AI-MS QUICK REFERENCE GUIDE

- SOFTWARE NAMES AND DESCRIPTIONS
  - GSS Level 1 – Griffin System Software Level 1 – Used for screening with preloaded methods.
  - Griffin System Software – Griffin System Software Level 2 – Used for advanced operation with customizable scanning options, creation Level 1 methods, and for detailed result file viewing
  - Toolbox – Contains instrument health and usage logs. Also contains instrument configurator software for modifying default solvent flow rates.
    CAUTION: Configurator contains additional instrumental settings vital to proper performance
- FILETYPES
  - Name.gfnScan – Macro variable scan settings
  - Name.gfnResx – General result datafile
  - Name.gfnInst – Instrument configuration file
  - Name.gfnModel – Instrument configuration Model File
  - Name.gfnM – Full method file
  - Name.gfnMS – MS only method file
  - Name.gfnMacoiU – Full method MS/MS identification plugin
  - Name.gfnChemLib – Chemical library archive
- IONIZATION SOURCE SPECIFIC SAMPLE FLOW RATES
  - ESI – 10µL per minute
  - DESI – 3µL per minute
  - PSI – 0.5µL per minute, remove syringe
  - APCI – 0.5µL per minute, remove syringe.

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- FLIR CONTACT INFORMATION
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    Email: leonard.rorrer@flir.com | www.flir.com
- COMPOUNDS IN PROVIDED METHODS
  - Drugs of Abuse General

Chemical	Parent Ion	Main Fragment Ions
Alprazolam	309	281, 274
Amphetamine	136	119, 91
Cathinone	150	132
Cocaine	304	182
Cocaethylene	318	196
Codeine	300	215, 243
Desomorphine	272	215, 197
Diazepam	285	222
Dextromethorphan	272	215, 147
Fentanyl	337	188
Heroin	370	268, 328
Hydrocodone	300	199
Hydromorphone	286	185
Ketamine	238	220, 207
LSD	324	281, 223
MDMA	194	163
MDPV	276	205
Mephedrone	178	160
Mescaline	212	195
Methadone	310	265
Methamphetamine	150	119, 91
Methylone	208	190, 160
Morphine	286	201
Oxycodone	316	298
PCP	244	159
Pentedrone	192	174
Phenylephrine	168	150
Phenylpropanolamine	152	134
Pseudoephedrine	166	148
Triazolam	343	308

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- White Powder and Cutting Agents

Chemical	Parent Ion	Main Fragment Ions
Benzocaine	166	138
Caffeine	195	138
Cocaine	304	182
Diphenhydramine	256	167
Hydroxyzine	375	201
Levamisole	205	178
Lidocaine	235	86
Methamphetamine	150	119, 91
Phenacetin	180	138
Phenylephrine	168	150
Pseudoephedrine	166	148

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APPENDIX A – DESI ACCEPTANCE TEST AND FLOW CHARTS

A.1: General Screening Flowchart

After analyzing the first sample, the flowchart below should be followed to eliminate the possibility of carryover when analyzing the following sample (Figure A1).

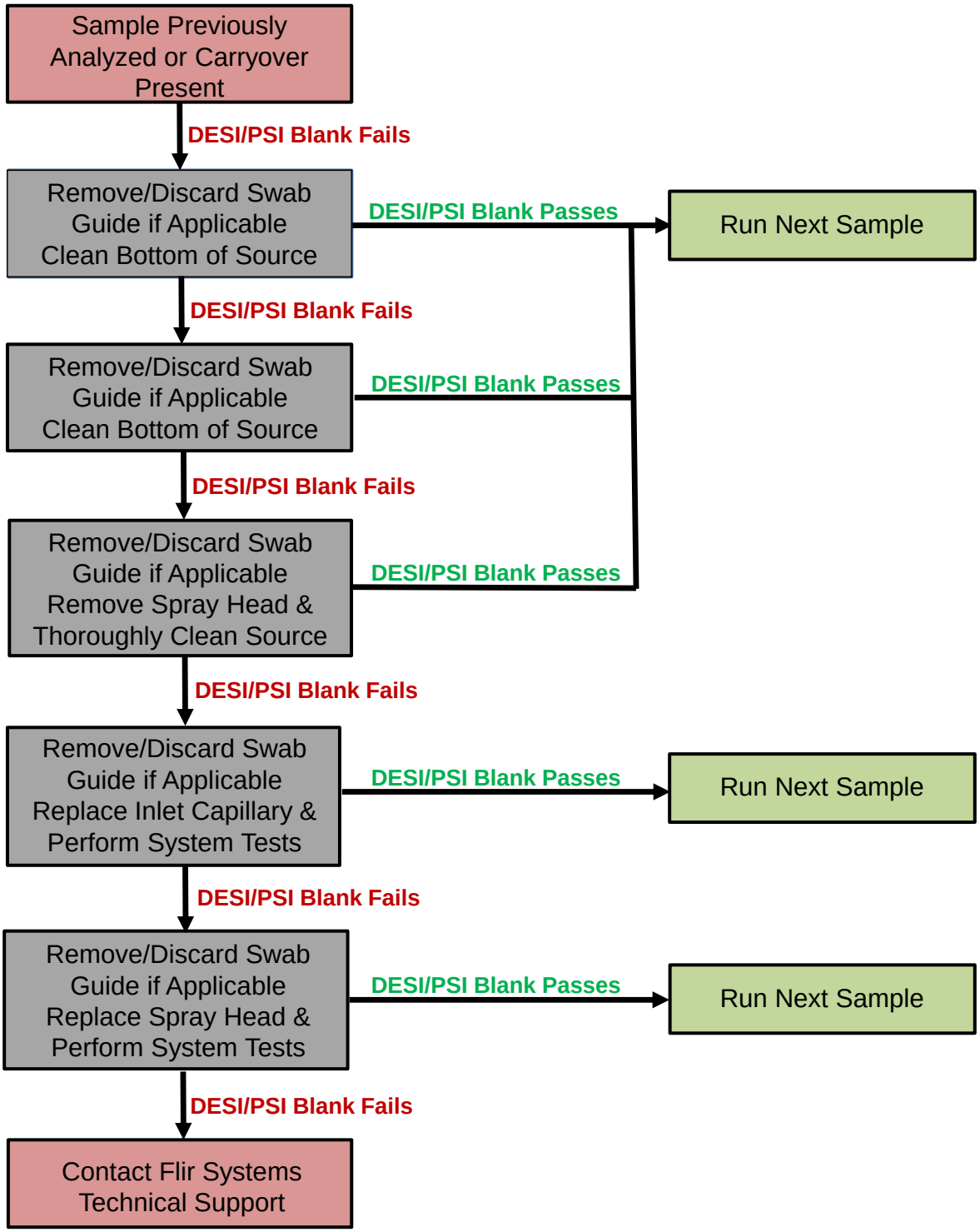

Figure A1 – Cleaning/carryover prevention flowchart

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A.2: DESI Acceptance Test

If a DESI acceptance test needs to be performed, follow the procedure below. The DESI acceptance test will need to be performed according to the Level 1 protocol, as seen in CH.4.

1. Setup and operate the DESI source according to the protocols in CH.2 and CH.4 (Level 1). The DESI acceptance test will need to be selected from the method's list once the "Methods" button has been pressed in Level 1 (Figure A2). The method is named "DESI Acceptance DDS."

Figure A2 – Methods button

2. Apply one drop of the DESI acceptance test solution (i.e. 50 ppm triethyl phosphate in water) to the top edge of a notecard using the provided dropper bottle (Figure A3).

Figure A3 – DESI acceptance dropper bottle

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3. Introduce the notecard under the DESI source once the instrument has instructed for samples to be introduced. If an ion at m/z 183 is witnessed, the DESI acceptance test has passed. This will be confirmed by a triggered alarm in Level 1 (i.e. screen will turn RED) (Figure A4). If the DESI acceptance test does not pass (no alarm), following the troubleshooting flowcharts (Figures A5, A6, and A7)

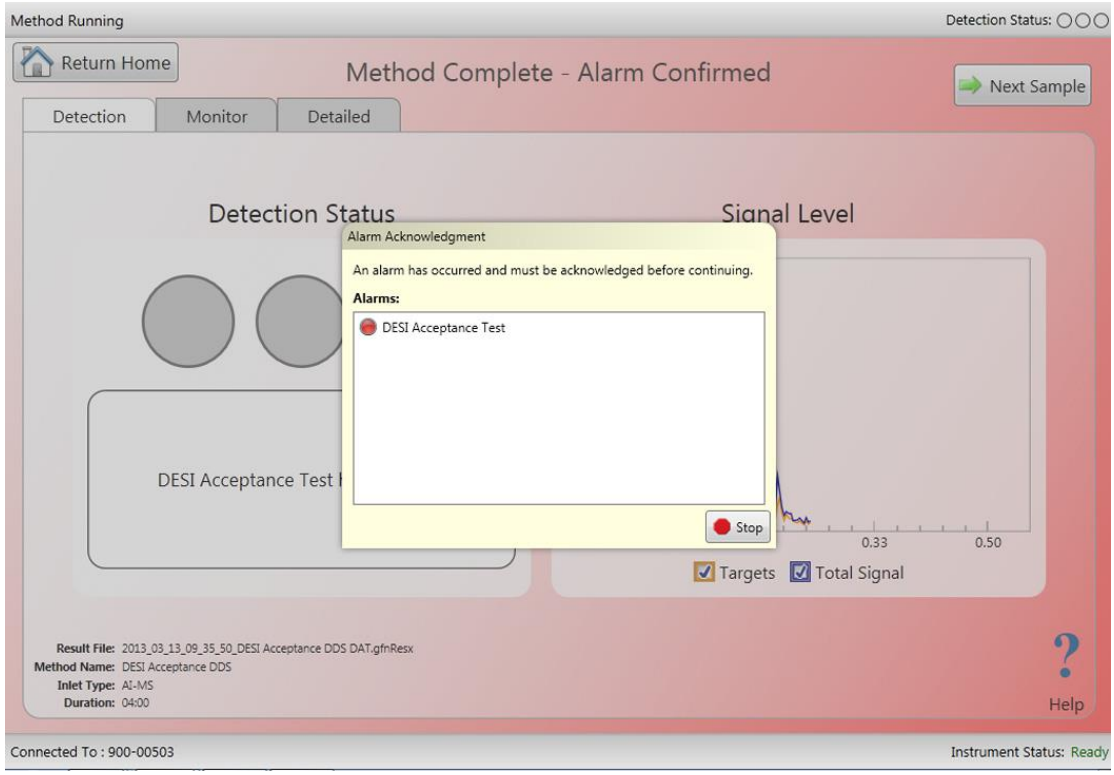

Figure A4 – DESI acceptance test passed

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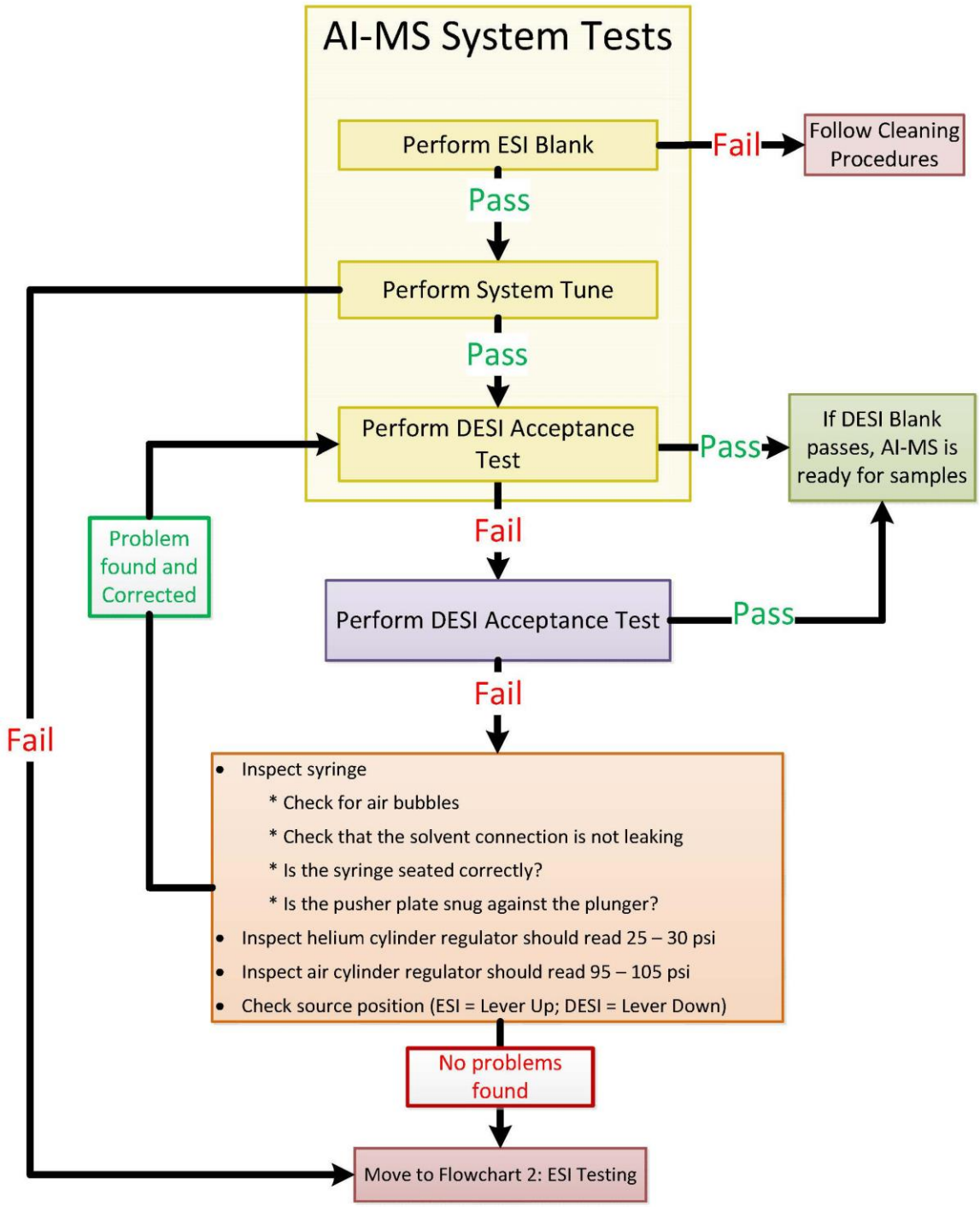

Figure A5 – Troubleshooting flowchart 1

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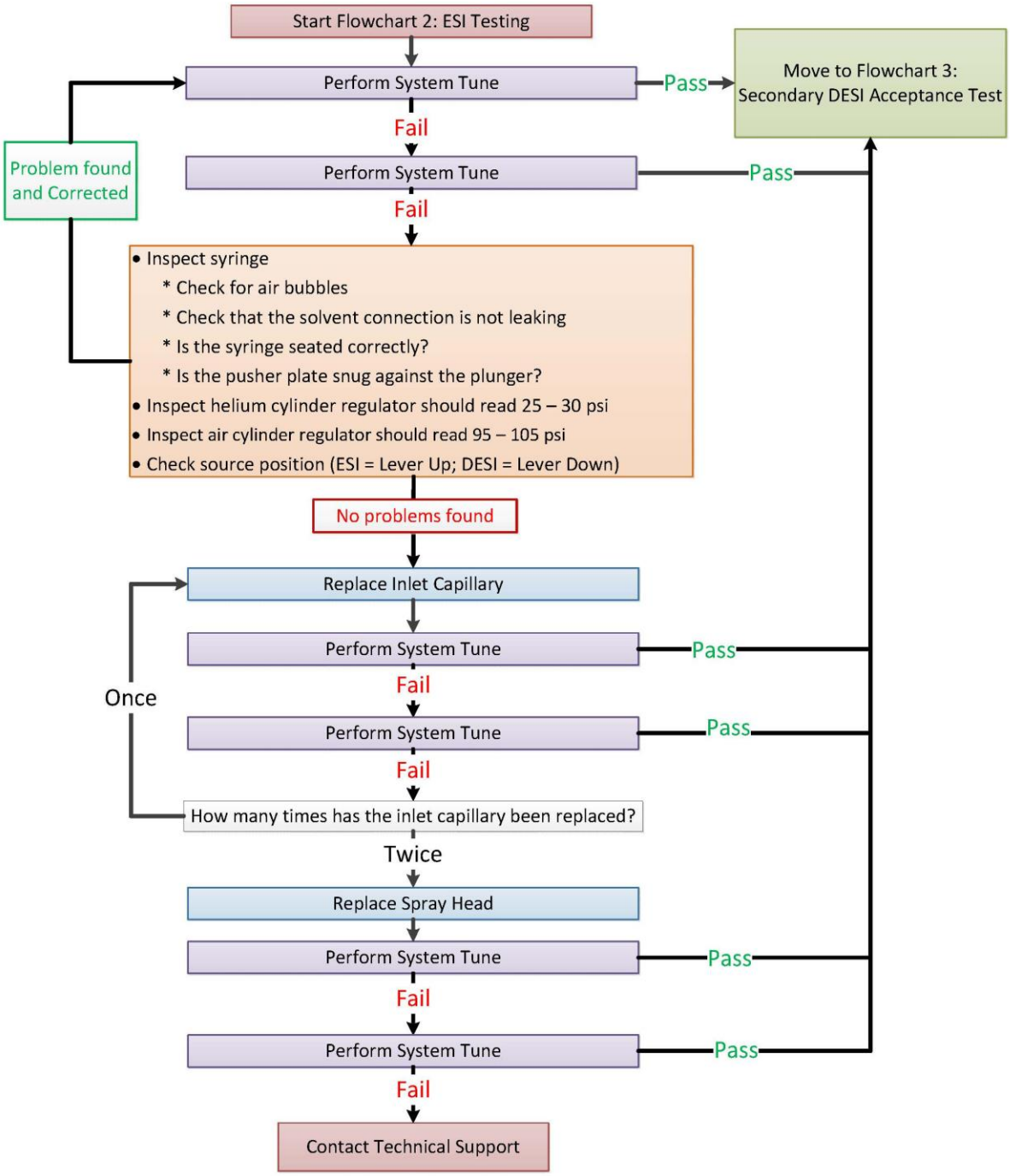

Figure A6 – Troubleshooting flowchart 2

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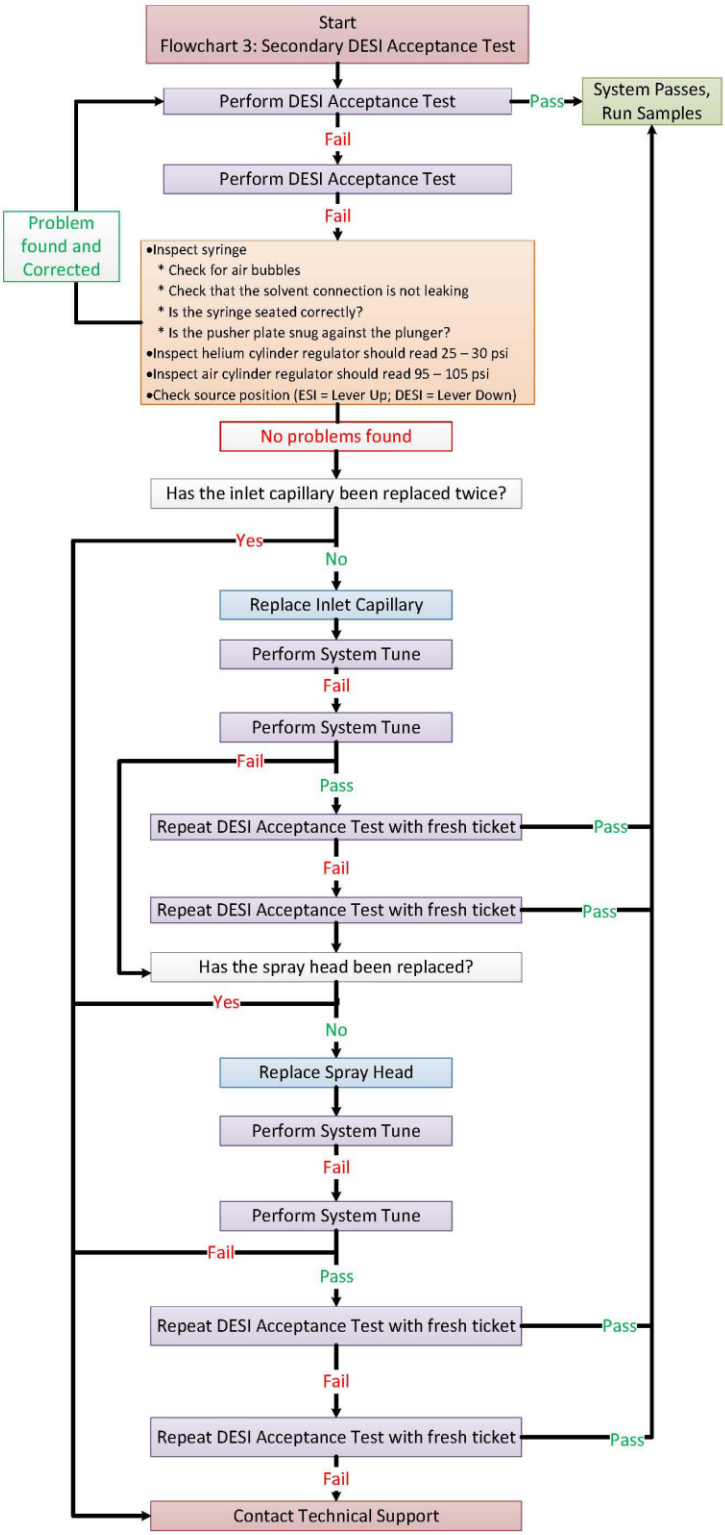

Figure A7 – Troubleshooting flowchart 3

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