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The Standard Operating Procedure from which information in this document has been extracted, is a version controlled document, managed within a Quality Management System. However, extracts that document the technical aspects can be made more widely available. Standard Operating Procedures are more than a set of detailed instructions; they also provide a necessary record of their origination, amendment and usage within the setting in which they are used. They are an important component of any Quality Assurance Framework, but in themselves are insufficient and need to be used and interpreted with care.

Alongside the extracts from our Standard Operating Procedures, we have also made available here an example Standard Operating Procedure and a word version of a Standard Operating Procedure template. Using the example and the Standard Operating Procedure template, institutions can generate their own Standard Operating Procedures and customise them, in line with their own institutions.

Simply offering a list of instructions to follow does not assure that the user is able to generate a value that is either accurate or precise so here in the BRC we require that Standard Operating Procedures are accompanied by face-to-face training. This is provided by someone with a qualification in the area or by someone with extensive experience in making the measurements. Training is followed by a short competency assessment and performance is monitored and maintained using annual refresher sessions. If you require any extra information, clarification or are interested in attending a training session, please contact Dr Kesta Durkin (k.i.durkin@soton.ac.uk).

This document has been prepared from Version 3 of the BRC Standard Operating Procedure for start-up, tuning and shut-down of the profile 3 SIFT/FA-MS analyser. It was last reviewed in July 2014 and the next review date is set for July 2016. The version number only changes if any amendments are made when the document is reviewed.

**NIHR Southampton Biomedical
Research Centre**

**Procedure for START-UP, TUNING
AND SHUT DOWN OF THE PROFILE
3 SIFT/FA-MS ANALYSER**

BACKGROUND

This procedure is for the start-up and tuning of the Profile 3 SIFT/FAMS-MS prior to use to measure volatile compounds in breath samples, either by direct breathing into the instrument, by sampling from breath collection bags or by sampling the headspace above a solution. This instrument samples gas mixtures such as breath, converts compounds present in the gas to ions, separates the ions according to their weight (m/z) and measures the number of each ion generated as a count rate (cps). The count rates can be used to calculate the concentration of designated compounds in the gas mixture.

Data acquisition modes

The instrument can either collect data on a continuous range of ion weights (Mass Spectrum mode: e.g. m/z 10 to m/z 150) or on a smaller number of specific values of m/z (Time profile or Multi ion monitoring mode).

Sampling Modes

For each data acquisition mode, breath may be sampled either by breathing directly into the instrument's sample head or by connecting the sample head to a breath bag containing a breath sample. In addition, measurements can be made on the vapour in the headspace above a liquid held in a glass sample bottle. In the latter case, a needle is connected to the instrument sample head and used to pierce a silicone septum that seals the sample bottle.

PURPOSE

To ensure correct and reproducible operating conditions of the SIFT/FA-MS analyser.

SCOPE

This procedure applies to any study that requires the operation of the Profile 3 SIFT/FA-MS analyser.

RESPONSIBILITIES

It is the responsibility of the operator to use this procedure when preparing the instrument for subsequent measurements of volatile compounds in breath or in the enclosed headspace above a solution. If the operator is not the person who will be making the subsequent measurements, it is the responsibility of the operator to ensure that the measurer has adequate knowledge and ability to do so.

PROCEDURE

Introduction

The Profile 3 SIFT/FA-MS Analyser is used in the standard mode for making measurements of volatile organic compounds in breath or in the headspace above liquids in a container. Additionally, an alternative mode is provided through the operating software for the specific measurement of the abundance of deuterated water (D₂O: deuterium oxide). This measurement is most usually made with the purpose of measuring the total body water volume by the isotope dilution technique. Make sure that you know which mode is to be used.

OPERATIONAL SEQUENCE

1. Check that the level of water in the reagent bowl located at the top left side of the instrument panel is on or between the two black marks. These approximately indicate a volume range between 10 and 15 ml. If volume is insufficient, make sure that the instrument is turned off (pumps not running), unscrew the bowl, top up to the top line and reassemble.

Check that both the Profile 3 SIFT/FA-MS and the computer are plugged into the mains supply and that the power is turned on at the wall switches.

Check that the grey Ethernet cable is plugged into the left side of the computer.

If the instrument is to be used for D₂O abundance measurements, the source partial pressure line that protrudes from the left side of the instrument body should be closed by fitting an appropriate screw into the block. If the instrument is to be used for

general SIFT measurements the line must be open to allow inflow of atmospheric oxygen and nitric oxide.

2. Turn on the computer by pressing the power on/off button. Go to the right side of the instrument. The arrow at the bottom of the side panel indicates where the mains power on/off switch can be located on the back panel of the instrument. Turn on the SIFT/FA-MS at the power on/off switch which is located beside the power input socket. There will be an immediate sound of the vacuum pumps operating.

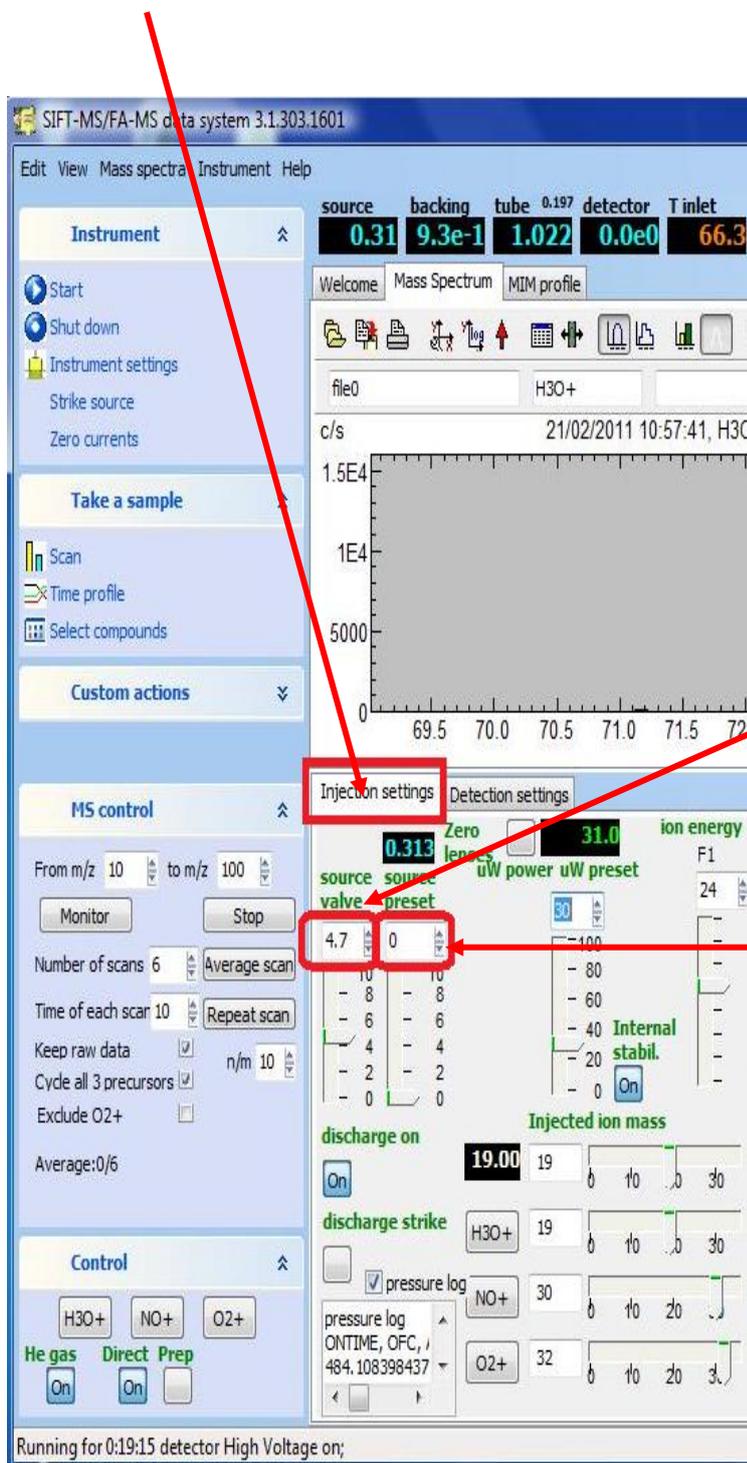
3. Observe the SIFT/FA-MS status indicator light at the top front left of the instrument. When it shows a solid green light, click on the SIFT-MS icon on the computer screen to load the SIFT/FA-MS operating software.

4. The SIFT/FA-MS operating software should automatically connect to the SIFT/FA-MS (via the View/Preferences/Automatically Connect setting). If this does not happen, the numbers in the black read-back boxes at the top of the screen will show 0 (zero). In this case, click on the CONNECT button. If connection again fails, check the grey Ethernet cable connection and /or reboot the computer before trying again. If still unsuccessful, refer to the Instrument Manual and then seek help.

5. Click 'Instrument Settings' entry in the left side control panel. If it is not visible then click on the arrows at the side of the 'Instrument' heading to drop down the entries.



The screen should open in the 'Injection settings' mode but to make sure, click the 'Injection settings' tab in the middle of the screen.



On the computer screen enter a value of 4.7 in the white box under the heading 'Source valve'. This may easily be done by using the mouse left button to highlight the existing value and then typing in the required value.

Similarly in the white box to the right, marked 'Source preset', enter the value 0.24.

Note that a zero value will disable the preset and default source valve voltage value of 4.7 will apply until manually adjusted to give a working source pressure value of 0.24 following stabilisation of source conditions.

6. The software will control and monitor the pump-down process. During this time the pumps are reducing the pressure to a level that will sustain a stable microwave discharge
7. The green light on the SIFT/FA-MS will flash and the SIFT/FA-MS will continue to pump down for 5 minutes. The time from switch-on of the SIFT/FA-MS is shown at the bottom left side of the computer display.
8. After 5 minutes a caption box appears, prompting the user to turn on the helium supply and start the instrument. Keep the caption box open but do not click anything yet.



9. a) If the instrument is installed in SCBR Consulting Room 16 the helium supply is turned on and off using the hand wheel on the top of the cylinder and not the black knob on the regulator valve. The wheel is turned anti-clockwise to open.
- b) If the instrument is in the SCBR Mass Spec laboratory the supply is turned on and off at the wall supply using the blue lever control.

When ready to proceed, turn on the helium supply and check that the pressure in the supply line is 2 bar on the pressure gauge.

In the daily record sheet, enter the value shown under SOURCE in the panel of read-back boxes (box on extreme left side). It is important to do this.

In the caption box (Step 8 above), click 'Yes' to activate the source microwave discharge.

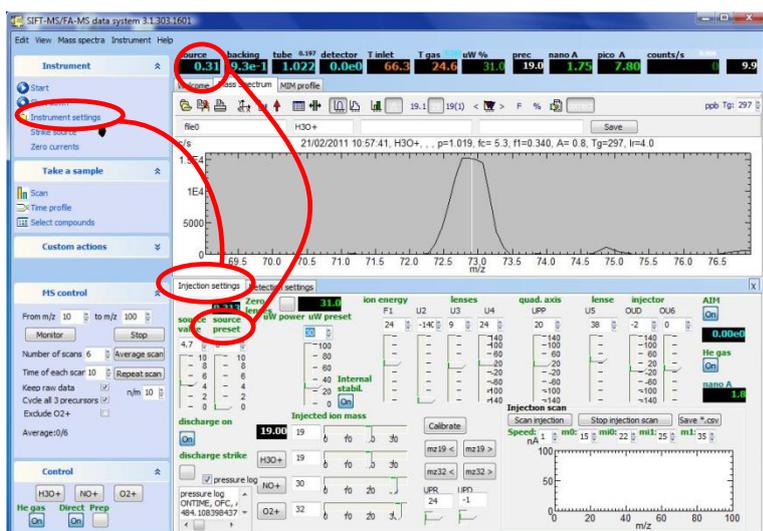
In some situations, e.g. when the software has not been turned on until after the instrument has been pumping down for five minutes or when the discharge has either failed to strike or has been lost during operation, there will not be a caption box. In this case simply ensure that the helium supply is turned on at the cylinder or wall tap and click the 'Start' button at the top of the left side control panel.



10. A script box will open on screen indicating the progress of the start-up. It will report the voltage ramp on the source and the attempt to strike the source discharge. This is not always successful. If successful, a pink glow from the source can be seen through the grill at the top of the left side panel of the SIFT/FA-MS. In the read-backs, pico A will start to rise.

11. If start-up is not successful, the pico A will remain about 0. Wait until the script box has closed and the SIFT-MS indicator light shows solid green. Repeat the start-up using the 'Start' button on the upper left side of the screen.

12. When start-up is successful, click 'Instrument settings' in the left side control panel. The instrument settings window opens. It has two tabs located in the middle of the screen.



Click the tab marked injection settings. At the top of the screen, watch the source pressure reading in the black box on the extreme left of the row of read-backs. Wait for the source pressure to stabilise. This should initially fall rapidly and the pink glow will darken slightly as the pressure changes. It will then decrease more slowly and stabilise at a value of 0.24. On some occasions, the discharge may go out before stable conditions have been reached. If

this happens, reset the source voltage to a value of about 4.6 and set the Source Preset to 0 (disabled). Re-ignite the source discharge by clicking the Start button. When the source pressure in the read-back box has become relatively stable, note this value and enter it in the Source Preset box that was earlier set to zero. Now, manually decrease this value towards 0.24, waiting for the value in the Source Pressure box to stabilise at each new Source preset value. It may take a bit of practice to find how quickly you can reduce this setting! If it is reduced too quickly the discharge may be lost again and it will be necessary to repeat the process.

13. House keeping checks: check the read-backs on the computer as follows-

Source pressure: 0.24

Flow tube pressure: about 1 (This will be about 0 if helium supply is off).

T-inlet at set value: currently 70°C

uW%: 26

Sample T: initially between 20-30°C

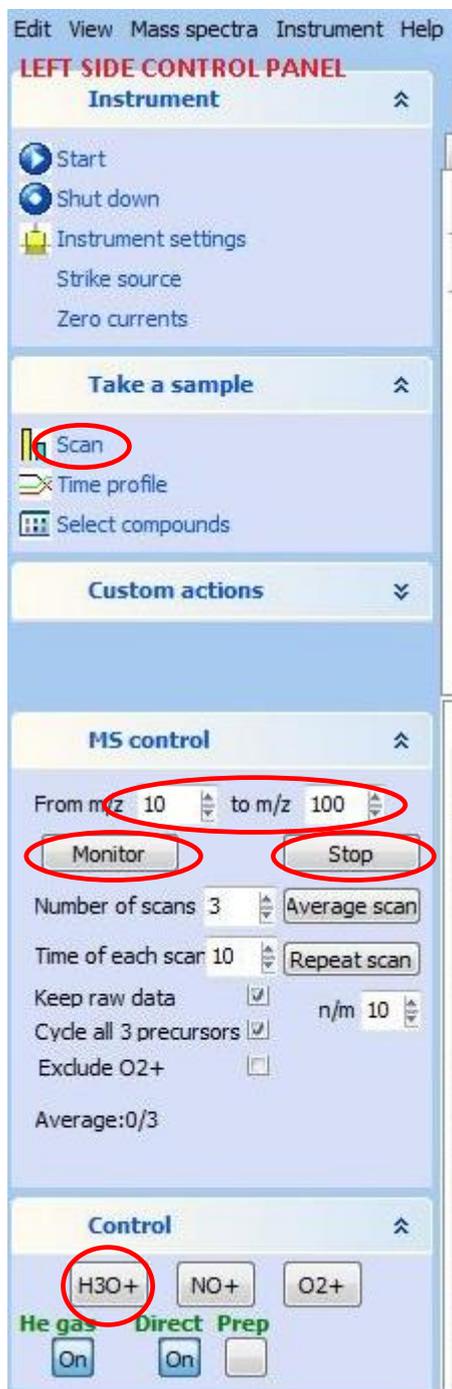
Nano A and Pico A: These should be about 12 and 25, but the actual values depend on the sample flow rate. If the source fails to ignite, this will stay at 0.

If necessary, adjust the flow tube pressure to read 1.0 using the black wheel on the helium cylinder regulator if in Consulting Room 16 (clockwise to increase) or the large regulator wheel at the wall tap if in the Mass Spectrometry laboratory.

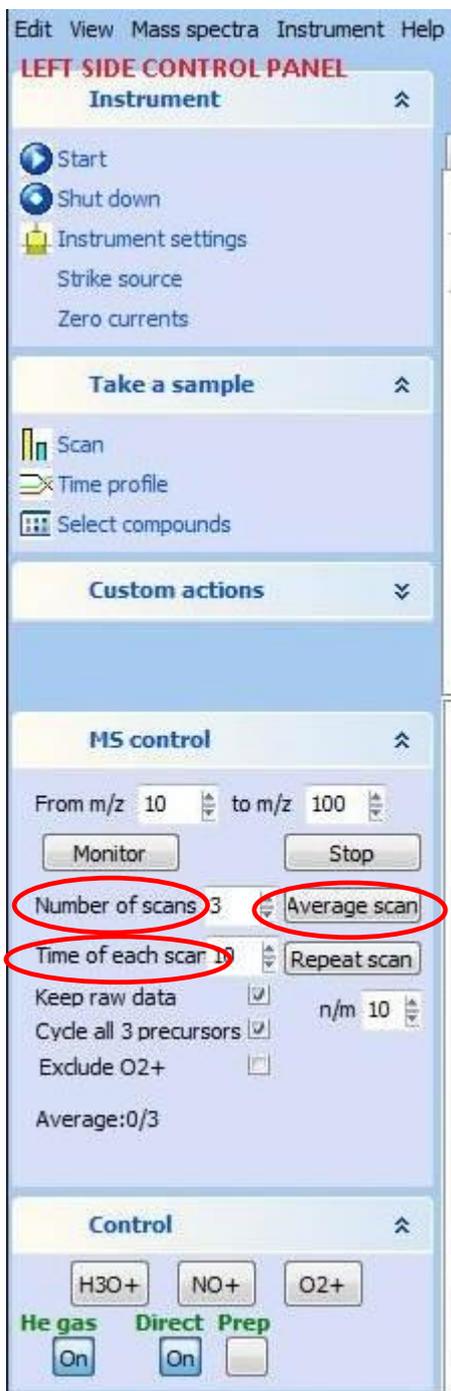
Make sure that you record the readings above in the daily settings log-kept adjacent to the SIFT/FA-MS instrument.

TUNING

- Click 'SCAN' mode from the items under 'Take A Sample' in the left side control panel. If the entry is not visible then click on the arrows at the right of the 'Take A Sample' heading.



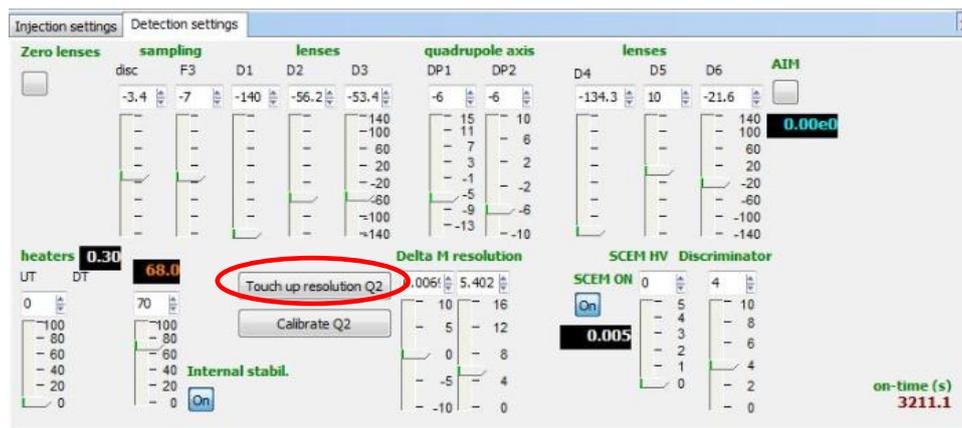
- Set the Scan range from m/z 10 to m/z 100.
- Click on the H₃O⁺ precursor button to select this precursor. The value 19 should show in the 'prec.' read-back box.
- Click 'Monitor' from under the MS Control heading. If the entry is not visible then click on the arrows at the right of the 'MS Control' heading. Use the 'Log' button from the toolbar to select the log scale for the Y-axis. When in the log scale, many peaks can be seen, in the linear scale only 3 or 4 peaks are clearly apparent.
- Briefly check that peaks can be seen at m/z 19 and 73 (37 and 55 should also be seen). The m/z 19 peak should be 1E+6 or greater. If not, check that the H₃O⁺ precursor has been selected and that 19 is showing in the prec. read-back box. If still not showing the required peaks then check the m/z calibration settings (see below) against the last recorded good values in the daily instrument log.
- Click 'STOP',



Set 'Number of scans' to 3 (use up/down arrows or type into box) and set 'Time of each scan' to 10 sec. Click 'Average Scan'. When the scan is complete, expand the window to Full Screen mode using the rectangular Windows button second from right in the extreme top right of the active software window.

Note: it may be helpful to breathe into the mouthpiece of the sample head to obtain a large m/z 73 peak. This can be done by watching the scan and breathing just before and during the scanning of the m/z 73 region.

- Set the Y-axis scale to LINEAR mode by clicking the Log button appropriately. Only 4 or 5 peaks should now be seen.
- Check the resolution of peaks m/z 19 and m/z 73. Use the mouse to highlight the area around m/z 19 (position the mouse pointer to the left of the peak at m/z 19, press and hold the left mouse button and drag the pointer to the right of the peak and then release the mouse button). The highlighted area will appear with a white background. Expand this area by pressing the 'Expand' button and then in the same way exactly highlight and expand the area m/z 18 to m/z 20.
- Read the peak maximum height against the Y axis and calculate half this value (half-height). Place the transparent acetate sheet horizontally across the computer screen with the calibrated edge uppermost and level with the Y-axis value of the peak half-height. The acetate is marked at 0.1 m/z units. Position the acetate horizontally so that the width of the peak at the half-height can be estimated from the scale. For m/z 19, this should be about 0.55 mass units (mu) but 0.5 to 0.6mu is acceptable.
- Repeat as above for the peak at m/z 73, expanding the range m/z 72 to 74 mu. W1/2 for m/z 73 should be in the range 0.55 to 0.6 mu.
- If the resolution needs to be altered, then go to Instrument Settings/Detector settings tab and click 'Touch up Resolution'.



This will open a pop-up which will allow the resolution to be improved by selecting 'narrower' or 'wider' for the peaks at 19 m/z and 73 m/z. Adjust as necessary and obtain a new spectrum. Reassess the peak width in this new spectrum and adjust it again until you are satisfied.



Record the values under Delta M Resolution in the daily record log.

Next, check and adjust the accuracy of the peak mass alignment. This should be done after any adjustment to the peak resolution which may affect the peak alignment.

- Set the No of scans to 3 and click AVERAGE SCAN. When scan is complete, ensure the Y-axis is in the linear mode.
- Expand the area around m/z 19 so that the peak maximum can be clearly identified.
- The curve of the peak is not continuous, so identify and note the m/z value corresponding to where the top of the peak appears to be (new m/z 19). If it is more than 0.1 mass units different from 19.0, it will need to be reset.
- Repeat the process for m/z 73.
- In the Instrument Settings/Detector settings tab, click the Calibrate Q2 button and enter any required m/z re-calibration values noted above (new m/z 19, new m/z 73) into the right hand boxes of the calibration function, for example, if the peak value of m/z 73 appeared to be at 72.85 mu, then the entry should be – Read m/z 73 as m/z 72.85
- When complete, click OK. A box will open showing the new calibration parameters. **Record them in the daily setting log** and click OK to exit.

- Re-run the spectrum scan to ensure that the new settings remain suitable. If not, repeat the calibration.

In turn, select the H₃O⁺, NO⁺ and O₂⁺ precursors and carry out average scans (X3). Record the precursor peak heights in the daily record sheet (m/z 19, 30 and 32 respectively).

Check that the rear blanking plug is not fitted to the sample head and fit a new disposable mouthpiece to the front of the head. Click on MIM Profile Tab, click on Select Compounds in the Take a Sample panel on the left hand side and select Water from the list of compounds available in the drop-down menu.

Sample alternate periods of room air, breath, room air, breath and room air. Make sure that the 'Correct' function is operative (correction values displayed). Select the 'ppb' display mode and highlight the breath sample peaks. Check that the measured water concentrations fall in the range 5.5 to 6%. If not, adjust the flow rate in the configuration file (See instrument manual) and repeat until a within-range value is obtained. Record the altered setting in the daily settings record.

Turn off the 'ppb' display to show the individual and total ion traces. Highlight to alternately select breath and room air samples and record the Total counts of each sample in the daily settings record.

The instrument is now set-up and tuned and ready for use to measure samples in the standard SIFT mode.

Measurements should be carried out as described in the SOP appropriate to the specific study being undertaken.

If measurements of D₂O abundance measurements are required, the mode should be set up by clicking on the 'MIM Profile' tab, clicking the D/H in the upper middle of the screen and then clicking the 'Set D/H' button under the 'MS Control' heading on the left side of the screen. Measurements should be carried out as described in the SOP appropriate to the specific study being undertaken.

ROUTINE SWITCH OFF

1. Click the Shut down button on the left side dashboard and wait for a solid green light to show on the SIFT/FA-MS. The plasma should no longer glow.
2. Exit the SIFT/FA-MS software, switch off the helium supply at the wall tap or cylinder as appropriate to the location and wait for a solid green light on the SIFT/FA-MS.
3. Turn off the SIFT/FA-MS power switch and turn off the computer.

EMERGENCY SWITCH OFF

In an emergency, switch off the power supply to the SIFT/FA-MS and turn off the helium gas supply at the wall tap or cylinder tap.