Jul 09, 2025 Version 2

Running a MSn Library V.2

DOI
https://dx.doi.org/10.17504/protocols.io.yxmvm5×6bg3p/v2

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Protocol Citation: Fede B 2025. Running a MSn Library. **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvm5x6bg3p/v2Version created by **Fede B**

Protocol status: Working
We use this protocol and it's working

Created: July 07, 2025

Last Modified: July 09, 2025

Protocol Integer ID: 221857

Keywords: msn library instruction, library, msn library

Abstract

Instructions to Run the MSn Library

Setting Up the Instrument

1 Clean the source (Follow Steps in My Protocol)

2 Calibrate the instrument (Follow Steps in My protocol)

3 Wash the columns before storing them.

3.1 Disconnect the tubbing that connects the instrument to the MS analyser

3.2 Set Flow Lines to B2 in both pumps

3.3 Set solvent composition to 100% B

3.4 Start the flow gradually to wash the columns (up to 0.3 mL/min) for 5 to 7 minutes. (The limit pressure is set manually when changing the columns). Note: if message "Compression Limit Reached" appears: 1) Purge the pump. 2) Wash the pump piston manually with the syringe. You can use both pumps to do this simultaneously, set top pump to A: A3 channel and B:B2 to wash HILIC column with water first.

4 Unscrew and remove columns carefully. Insert the protecting black plastic screws on both extremes and stow away in the columns drawer. Unscrew the top side first and close it with the plastic screw, next the bottom side and close it.

5 Insert the black rubber protection in the top side. It's in the drawer.

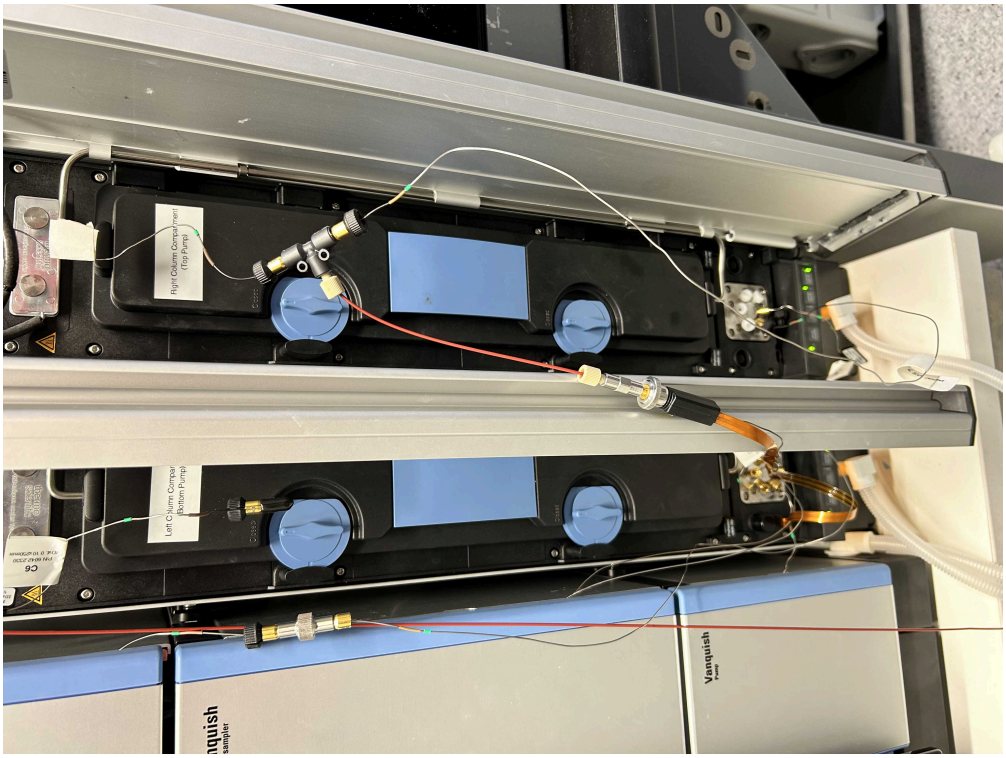
6 Purge Both Pumps with 50% B

Connecting the MSn library accessory

7 Disconnect the solvent heater chip at the bottom of the right column compartment and stow away in the dedicated "MS library bag" in the drawer.

8

9 Get the accessory from the MS library bag and connect it to the instrument as indicated in the picture. This accessory "bridges" the column compartments as it isn't necessary to obtain LC data for a compound library. The metallic connector is in the drawer.



Final setting of the MSn library accessory. Attention, it could leak! adjust carefully! check viper tubings.

10 Use the special screwdriver accessory to test if metallic fittings are correctly inserted. Use it also to unscrew the golden screw in position 5 right column. You will hear a repeated click after the maximum force has been applied. This will prevent overscrewing. The accessory has a wedge that goes inserted into the metallic tubbing and the tip of the accessory fits the size of the metallic tubbing connector.

11 Test the system by starting the flow to 0.3 mL (valve bottom pump position to waste). The pressure will be very low since the column was removed.

Checking the Instrument

12 Start a flow of 0.15 mL/min in both pumps for 5 minutes. the valve position is set to waste. 1→6. Check for any leaks in the installed accessory. Set to A1 B1 in bottom pump.

13 Start a flow of 0.3 mL/min in the MS window and click "Get Defaults".

14 Start the MS analyser and set valve position to 1→2.

15 Check if any signals are observed in the MS.

Running the Blank and SST samples

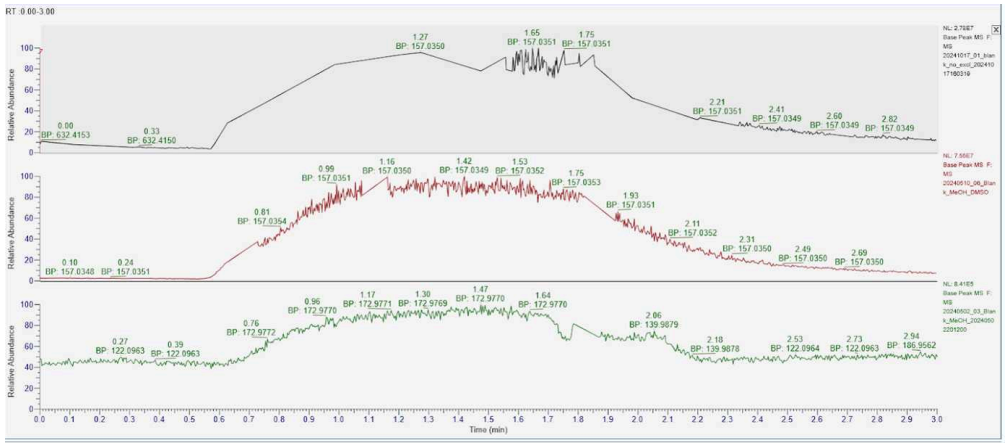
16 Set MS parameters for 200-300 uL/min flow rate, and flow to waste 1-6. check for leaks, and pressure.

17 Set the flows to 0.045 mL/min in top pump and 0.01 mL/min in bottom pump. pressure should be around 3-6 (bottom) and 11-13 (top) bar in the beginning,

18 Set the MS flow to 55 uL/min and click Get Defaults.

19 Let the system condition for 5 minutes. Check background signals

20 Prepare the corresponding blank samples with the appropriate solvent mixture. Adding 2% DMSO to the blank solvent mixture can help get the following profiles: (normally, the solvents used for mixing don't contain DMSO. So keep in mind to keep DMSO on the exclusion list if you create a new one)

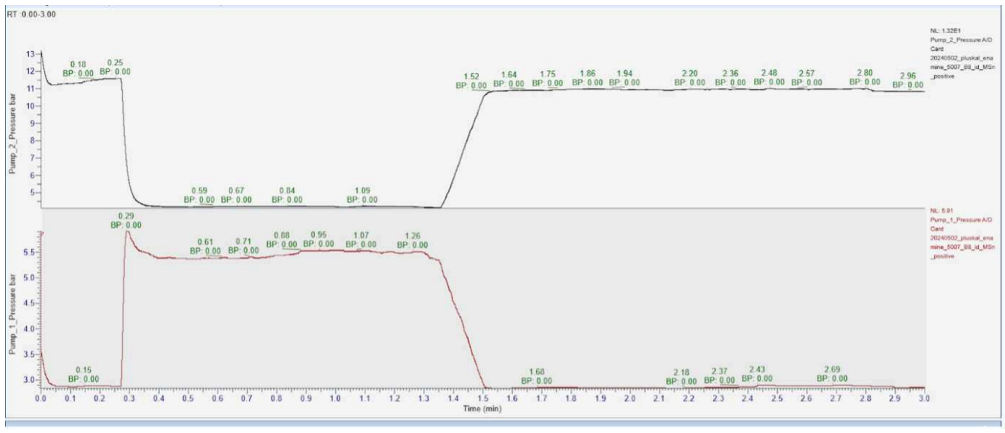


- Top: no exclusion list, MeOH/H2O with DMSO
- Middle: exclusion list, MeOH/H2O with DMSO
- Bottom: no exclusion list, MeOH

21 Create a sequence in the corresponding folder

22 Run 10 blank samples in positive mode with the MS1 method for the specific number of compounds of the mixture (10 or 20) and positive mode. The method is in the shared methods folder.

23 Check the pressure plots after running some samples. They should look like this (Picture)



o Pressure profile should look like this, sometimes little pressure pumps can happen, no worries (Top: profile top pump Bottom: profile bottom pump)

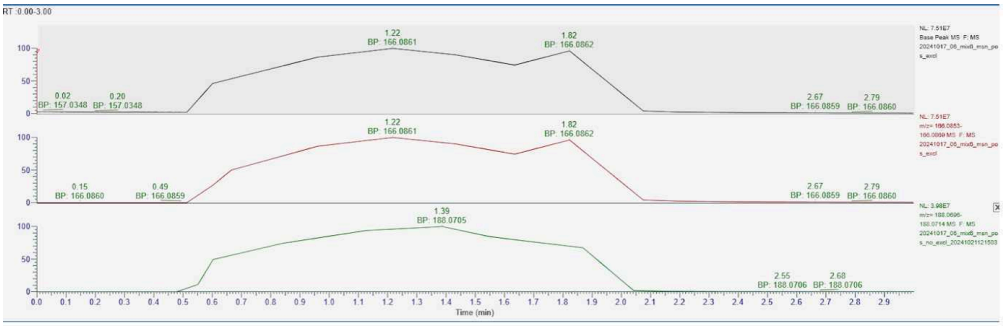
24 Check the MS profile

25 Use a mixture for real profile checking, best is QC, vion mix, or mix6, mix8 or mix10. These vials are in Freezer -80 door R18-D1.

26 Add 2 different SST samples below blanks in the sequence. They contain a mix of standard compounds.

27 Run SST samples with the same method according to the number of compounds. Click "Standby" or "ON" if you want the pump to stop or continue working after analysis

28 Check SST samples MS profile. They should look like this



29 Repeat steps 19-24 but blanks and SST samples in negative mode.

Creating an Exclusion List and running the library samples

30 Open mzmine. Start batch mode with Ctrl + B

31 Load the batch file for positive mode (extension is .mzbatch)

32 Drag and drop the best spectra (looking like the picture in step 13) (.raw) to the Data Import step.

33 Set the mass detection to the factor of lowest signal 2

34 Keep only signals that were in 50% of the samples in feature row list filter

35 Run the batch mode, the batch file is already saved in the computer with extension .mzbatch. Check parameters in the batch file just in case

36 Go to feature lists menu

36.1 Sort the obtained feature by m/z ascending values

36.2 Copy the values and paste them in a new excel file. The file will contain two columns: One named "Compound" which will be empty and one named m/z where the values will be pasted.

36.3 Save the file in the directory of the project in new folder named "pos" as "pos.csv".

37 Modify the existing method to run the library samples

37.1 Open the method file

37.2 Go to the Scan parameters menu

37.3 Click targeted mass exclusion properties from the top block of the workflow

37.4 Click Import at the right side of the screen

37.5 Load the .csv file with the masses.

38 Save the method in the directory folder. This will be a MSn method (not MS1 method). Include the legend "excl_list_pos" or similar to identify the polarity and the exclusion list in the method.

38.1 Check the box Include Intensity Threshold. Add a third column in the .csv file and paste the Height column values from m/z mine results. Multiply the whole column with a factor

of at least 10 to only trigger if the signals are highly abundant. Set it manually it to E9 or higher to not trigger for signals that look really bad after manual check in mzmine.

39 Import the method to the sequence file

40 Run the samples.

41 Repeat steps 23 to 33 but for polarity: negative. In steps 32.3 and 34, change "pos" for "neg"

Creating the MSn library

42 Open mzmine and load the batch file for positive ionisation mode .mzbatch

43 Run the batch file and save the generated files to local folder

44