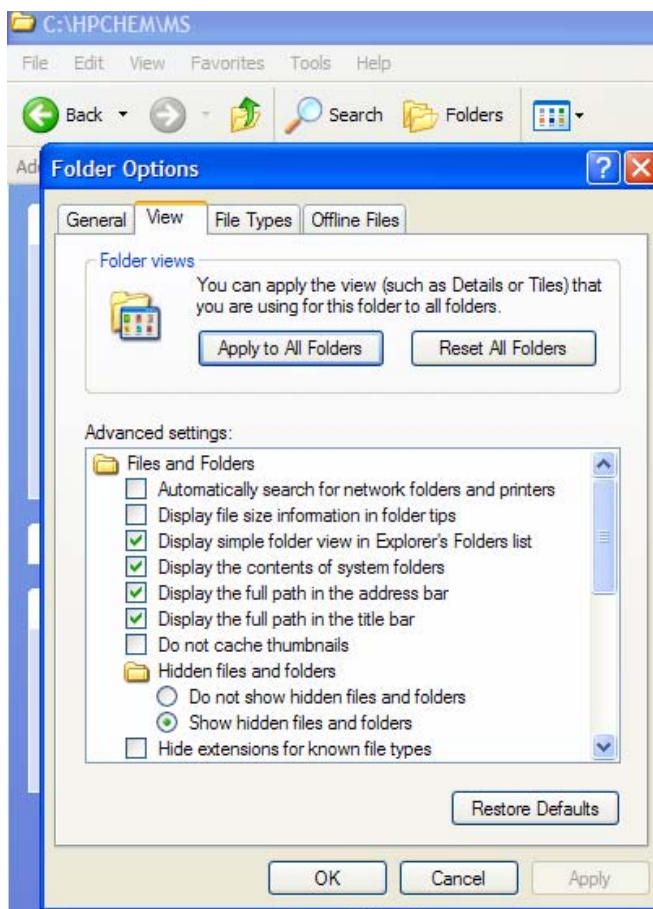


## Initializing NIST Search on HP LC MSD Software

**NIST Search and HP LC MSD Software:** The NIST library is very useful approach for searching either MS/MS spectra or in-source CID spectra. The spectra can be stored with structures and the resulting database searched by structure and by spectrum. Also, newer test versions of MS Interpreter can be used to understand fragmentation patterns of protonated species in addition to standard odd electron ions formed in electron ionization mode. We store both positive and negative MS/MS and in-source CID data in our corporate database.

Using the LC/MSD ChemStation Rev. A.09.03 [1417] version of the Agilent software with the NIST Version 2.0 search software required some minor changes in software. David Sparkman showed us how to perform the changes.

The file nistms.ini must be installed in the c:\hpcchem\ms folder. The file is actually a text file, but its extension must be .ini and NOT .txt. Some computers do not show the file extension in the default mode. To see the file extensions, open the C drive icon and select Tools\folder options from the menubar to get the following menu:



Then deselect the “Hide extensions for known file types.”

The file, nistms.ini, should contain the following information:

```
[general]
autoparms= /instrument /par=2
nistdirectory=c:\nist98
program=nistms$.exe
manualparms= \instrument
nominalmass=Sum
clipboard=Append
```

The nistdirectory must contain the location of the NIST library executable. This location varies depending on the installation program defaults or the location selected by the user. The NIST library executable is nistms\$.exe for Version 2. The older versions of the NIST library search executables (Version 1.6 and 1.7) were named nist\$.exe. Thus the program variable in the above ini file would be either nistms\$.exe or nist\$.exe depending on the version of software being utilized.

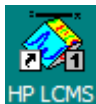
We initially thought the program nistint.mac needed to be installed in the c:\HPCHEM or C:\HPCHEM\MS folder, but its absence had no effect on the use of the LC MSD software with the NIST Version 2.0 search program. It is unknown whether it is needed for the Versions 1.6 or 1.7 NIST search programs.

Also in the nistdirectory (folder containing nistms\$.exe or nist\$.exe) the autoimp.msd file must exist and contain the following text:

```
C:\hpchem\HPNIST.txt
```

If this exact text is not present (used notepad.exe or wordpad.exe to edit), the NIST program will open and/or come into focus, but the mass spectrum from the Agilent LC/MSD spectrum will not be imported!

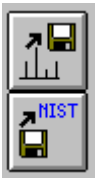
After the nistms.ini file is installed and the contents of autoimp.msd file are properly edited, select the HP LCMS program icon:



Go to the menu bar under file/load signal and open the demo file MS3FRAG.d in the c:\hpchem\msdemo folder. Obtain a spectrum for one of the peaks in the API-ES, Pos, Scan, Frag: "high fragmentor" TIC, e.g. the peak around 4.5 minutes. Then select the "Load a Library" icon



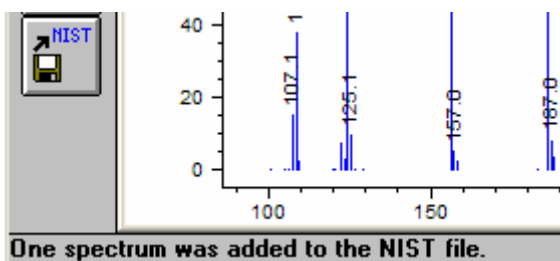
and open the demodad.uvl library. You must select the UV library even though you do not want to search it. This is necessary to display the following NIST search icons:



Click on the top NIST icon



which will show that the displayed spectrum was added to the NIST file by the message at the bottom of the HP screen:



Then click on the bottom NIST icon:

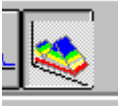


which will open the NIST software and search the displayed spectrum (actually temporarily stored as a .msp text file) against the libraries specified in the NIST search settings.

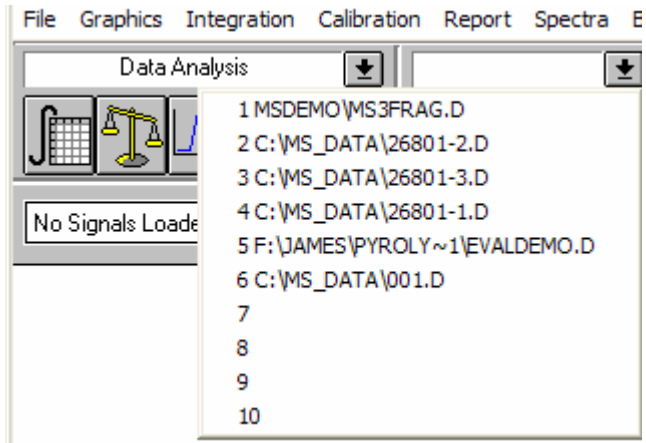
You can also search a library spectrum by going to the Spectra/Library/MS Create/Append NIST file on the top HPCHEM menubar then Spectra/Library/MS Close File and Go to NIST program option. However, the first approach is much simpler when multiple spectra are to be searched.

The spectrum to be searched must be in centroid mode (bar graph) and not in continuum mode (profile). If a continuum spectrum is chosen, the HPCHEM software will close immediately and will not reopen properly until the computer is shutdown and restarted.

**General Comments on Using HP LCMSD Software:** First open the HP LCMS software. Then switch to Spectral task mode with the following ICON:



Then go to file/load signal or to the pull-down menu (second white box from the left)



and pick a file that was previously processed. A good file is HPCHEM/1/Data/msdemo/MS3Frag.d.

To get a spectrum, select the following icon:



A MS or UV spectrum window will open depending which one you select.

To get background subtraction, select the following icon:



This will open the toolbar on the side. Close the current spectrum window and select the following icon from the toolbar on the ride side of the spectrum:



and go to the chromatogram to select a spectrum.

Then select the



icon and go back to the spectrum to select the background. You must close the spectrum window and deselect both of the above two icons and repeat the process. If you don't deselect them, the buffer will not be cleared so one can enter new foreground and background spectra.