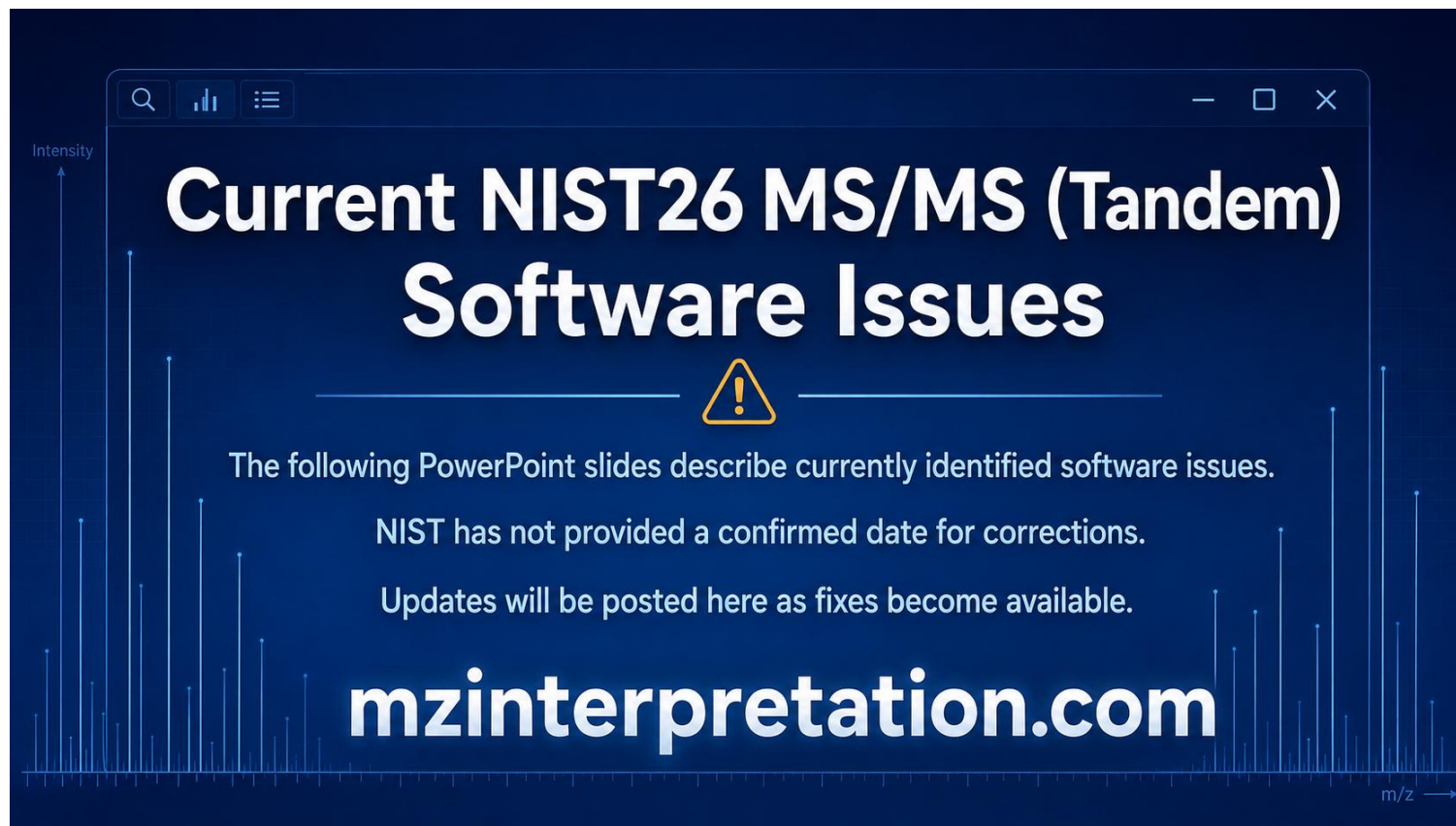


Current Bugs in NIST26 MS/MS (Tandem) Software

Video and Handout

J. L. Little



See **Full Course** on NIST26 with new **Integrated** Deconvolution/Library Searching for **LC-MS/MS!**

[link to course](#)

Current Status

64-bit Version

The NIST Mass Spectral Search Program
for the NIST/EPA/NIH EI and NIST Tandem Mass Spectral Library
Version 4.0, build Jun 11 2026

Prepared by NIST Mass Spectrometry Data Center.

- Software bugs listed in this handout
- If temporary solution, will be noted
- Hopefully sometime in future, NIST will offer a patch
- Probably when enough found to justify corrections

Index of Software Bugs in NIST26 Tandem (MS/MS) Software

Name	Status	Comment	Date	page no.
Use of XIC's with Background Can Remove Useful identifications	Just turn of background to see if any peaks with XIC=0 offer useful data	Probably will not be fixed, just need to remember to check if problem suspected	8/29/2026	4-5
Intensity Threshold Setting causes conversion of spectra to nominal mass	Needs to be fixed, hopefully revised version in future will fix this, for now just avoid using!	Possibly fixed in in-house version, but will not be released until more problems are noted	8/29/2026	6
Chromatogram Window decimal places are determined by Spectrum Import	Needs to be separate control of accurate mass decimal places displayed in chromatogram window, currently "steals" those values from the Spectrum Import	Possibly fixed in in-house version, but will not be released until more problems are noted	8/29/2026	7
Identity MS/MS and Hybrid Searches do not work for User library spectrum	When a user adds a spectrum to their library, it will only perform the MS/MS and NOT the MS/MS no Precursor or hybrid search; trying to use the hybrid will sometimes error 5 warning and crash the software; the library can be fixed by exporting with Lib2NIST and then importing the msp files and associated mol files to generate a library that works; the EI identity offers useful results in the interim and performs much like the MS/MS no precursot	hopefull fixed in a newer version; there is no Tool to fix the hybrid index files; that option is present for EI user libraries, but never has been offered for Tandem (MS/MS) user libraries. Don't know if NIST will find it high enough priority to fix?	8/29/2026	8
win.ini concern	when installing demo, can write extra stuff in Win.ini, probably should delete after using, be sure to make backup of win.ini before installing and then restore original	don't know if NIST will address this, only problem if for example, NIST23 or some other older version of software there originally	8/29/2026	9-11

Software Bug: Background Setting Removing XICs with Useful Identities (Slide 1 of 2)

Approach

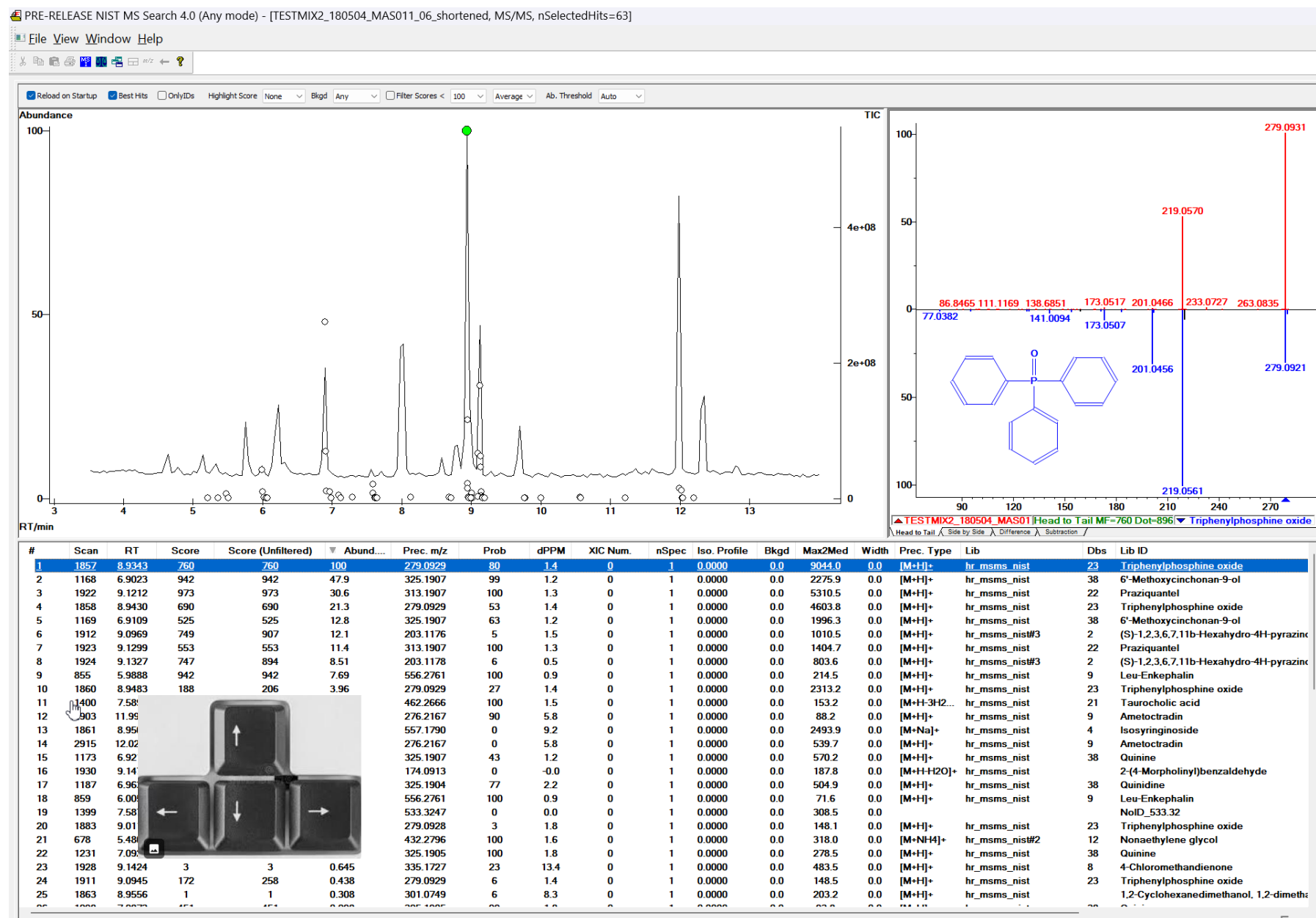
- For a few files, a background of a selected value, even 90, removes peaks of interest
- This was not the case when “any” was selected
- Suggest finding all with XICs = 0, then select, sort by abundance with highest at top
- Step through to review to see if anything of interest
- XIC analyzer is removing things with background= 0, nspec=1, XIC = 0, ion profile = 0. width = 0
- None of these peaks can be sent to XIC analyzer, that option is grayed out
- **However**, the peaks can have large abundance and high scores and should be considered
- If **background filter is selected**, they **will NOT** be included in abundance calculations!
- Pay careful attention to butterfly plot, other components in mix, sample history
- Can always process by vendor’s software for further confirmation and then import to NIST search

Why is this occurring?

- The peaks are usually single scans, and characterized as zero width
- This does not meet the criteria for XIC analyzer software to process
- Could indicate some parameter used in file acquisition was not set properly
- The program assigns **all such peaks** an XIC of 0
- They are grouped together **even if they have different precursor ions**

Software Bug: Background Setting Removing XICs with Useful Identities (Slide 2 of 2)

- To the right, is data with XIC = 0 ready to be reviewed, Note Critical Properties Selected for Display!
- Step through with up and down arrows on keyboard and review the butterfly plot, can also send to Lib Search if necessary to see other possibilities
- If none of interest, can use a non-zero background setting to remove **ALL** of them from results
- **NOTE** this will also remove their values for abundance calculations
- In this case, one of the peaks was 100 Relative abundance and was a good score!



Software Bug: Intensity Threshold in Spectrum Import Options Window

- When any Intensity Threshold is used, the imported spectra from Vendor's Software are nominal
- Should be top spectrum with 4 digits to right of decimal
- Instead, nominal as shown on bottom spectrum
- Would be useful if worked properly to remove electronic noise noted in Agilent MS/MS spectra imported into NIST26 Lib Search

Spectrum Import Options

EI Spectrum m/z Rounding
Multiply m/z in imported spectra by and round to the nearest integer
Example: (CH₂)_n correction is 0.99888
Optional
Add this term to all m/z before rounding

Tandem Spectrum Accuracy
Precursor ion m/z decimal places
Product ion m/z decimal places
HiRes No Precursor ion m/z decimal places

Adding spectra to Spec List
☒ Prepend (add to the top)
☐ Overwrite (replace)
☐ Ask

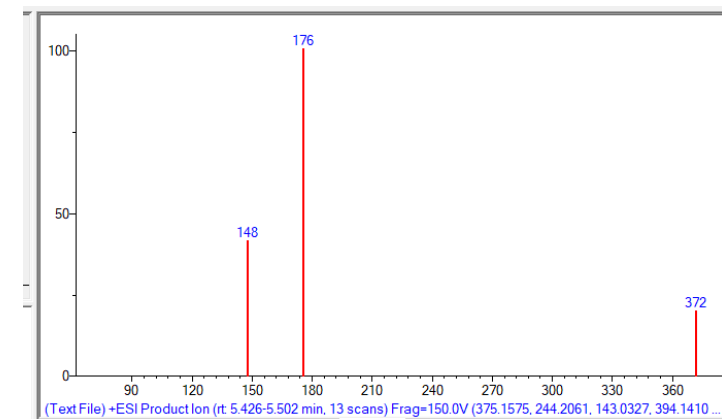
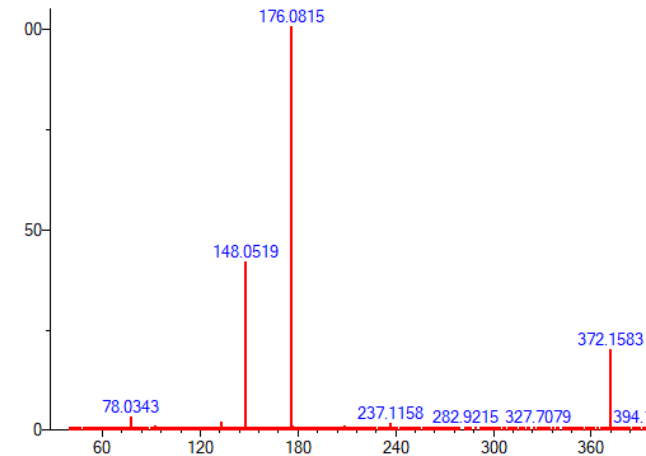
Intensity threshold
☒ % of max.
☐ absolute

Spectra without precursor ion m/z value are
☒ EI (nominal m/z) ☐ In-source ☐ Accurate m/z

Accurate m/z spectrum type

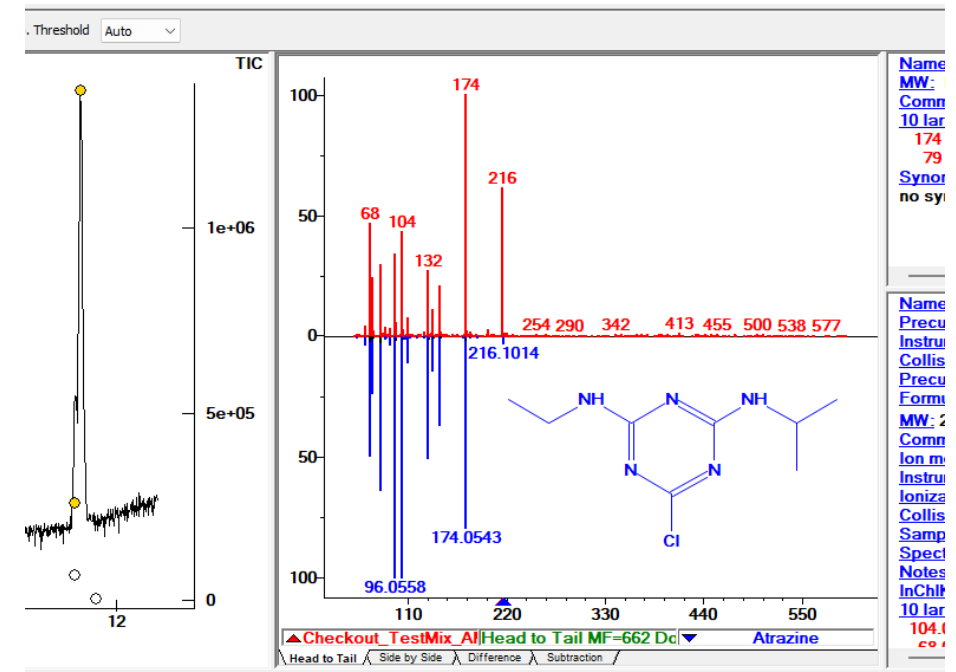
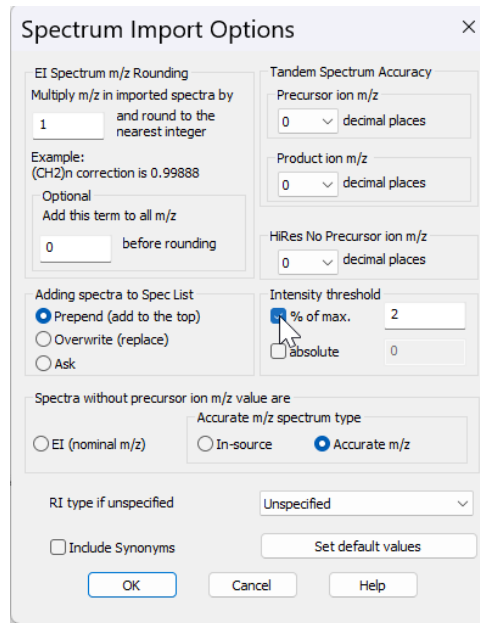
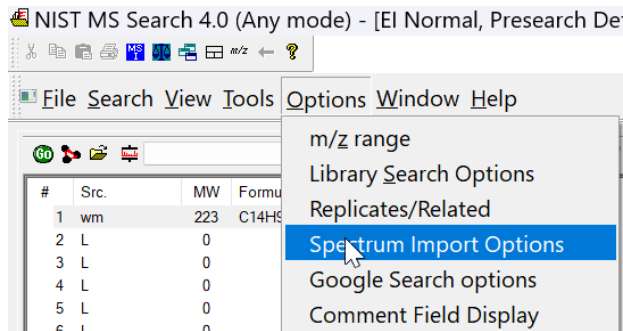
RI type if unspecified

☒ Include Synonyms



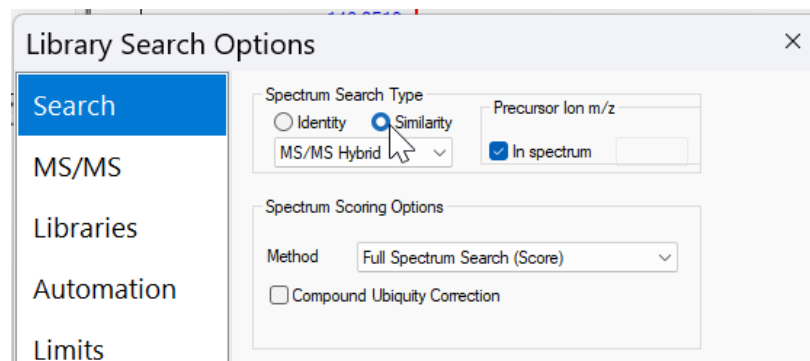
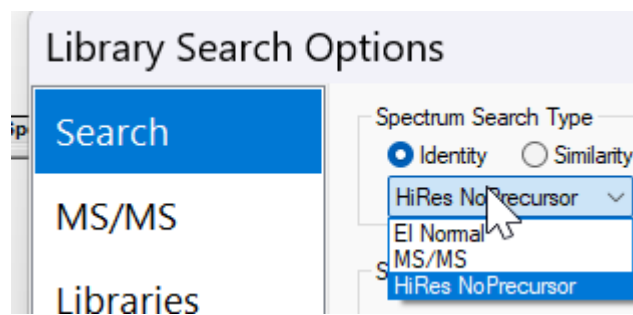
Software Bug: Chromatogram Window Software

- The values used in the Spectrum Import Options “echoed” in the Chromatogram Tab
- Normally these values are shown to 4 significant figures to right of decimal
- When set as zero to import low-resolution spectra, truncated to values selected
- DOES NOT affect the results in accurate mass deconvolution/library searching table!
- Just the “head-to-head” display at top of Chromatogram Tab
- Does affect the spectrum sent to library search from chromatogram window



Software Bug: Identity MS/MS No Precursor and Similarity Hybrid MS/MS

- After adding high-resolution spectra, the HiRes NoPrecursor and Similarity Hybrid MS/MS Searches do not work
- This can be fixed by exporting in Lib2NIST to msp files ASCII files with associated mol structure files
- Then importing back with Lib2NIST in NIST library format
- **Do not** attempt to run the MS/MS Hybrid Search, it does not work and will crash the program giving 0005 error code
- The EI Normal *mimics the results of HiRes No Precursor* and does work after initial addition and **works very well** when both high-resolution and low-resolutions libraries selected together
- The HiRes NoPrecursor will work with presearch turned off, but the search time is **VERY** long



Software Bug: Win.ini Concern (Slide 1 of 3)

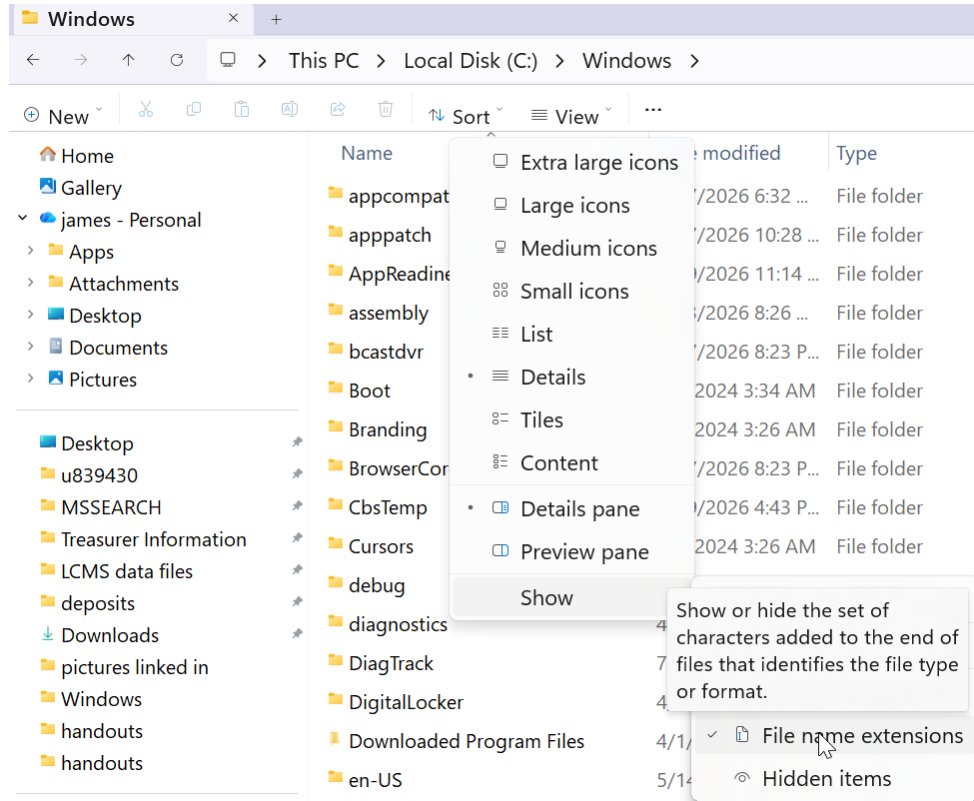
Possible Problems Installing/Uninstalling with Win.ini, especially if previous version of NIST Present (1 of 2)

- **ALWAYS** make backup of C:WINDOWS/win.ini file **BEFORE** installation!
- When downloading the TANDEM demo when previous version of NIST 23, the win.ini is changed
- See sequence on **NEXT SLIDE 3 of 3** before and after installation, and after uninstallation
- Can possibly cause issues with the original version, e.g., NIST23, but could be even earlier version
- Thus, be sure to return to original state when uninstalling Tandem demo when finished
- Can overwrite win.ini after uninstallation with win_backup.ini you created
- Or edit after finishing to remove the Tande, Demo changes
- Whichever, c:/windows/win.ini is a protected folder
- Requires some administrative privileges
- Might need to take outside that folder and edit
- Then drop it back in C:/windows folder to overwrite it
- **VERY** important to make sure file type (extension, .ini) is correct!
- Cannot be save as text, see **Slide 2 of 3**

Software Bug: (Slide 2 of 3)

Possible Problems Installing/Uninstalling with Win.ini, especially if previous version of NIST Present (2 of 2)

- Make sure after editing, it is still an .ini file extension (Configuration Settings), NOT, .txt, Text Document
- Show file configurations while doing operations!

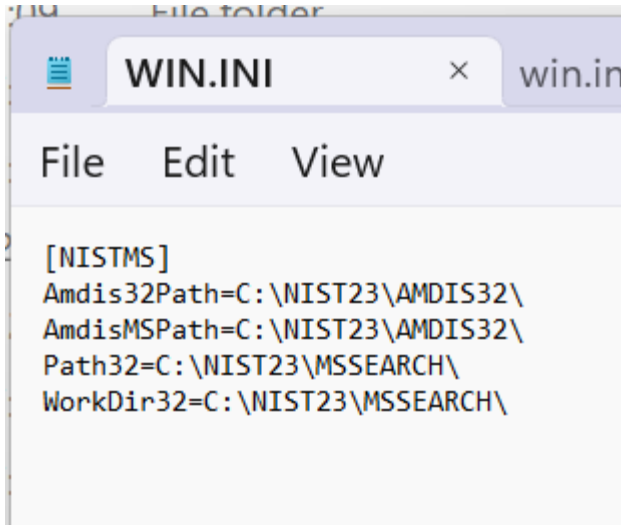


notepad	8/27/2026 1:24 P...	Application	32 KB
PFR0	8/29/2026 4:43 P...	Text Document	146 KB
regedit	8/27/2026 7:52 P...	Application	564 KB
setupact	8/27/2026 9:24 P...	Text Document	40 KB
setuperr	4/1/2024 2:43 AM	Text Document	0 KB
splwow64	8/27/2026 7:52 P...	Application	216 KB
system	4/1/2024 3:24 AM	Configuration settings	1 KB
twain_32.dll	8/27/2026 7:53 P...	Application extension	94 KB
win - Copy	8/27/2026 12:44 ...	Configuration settings	1 KB
WIN	8/29/2026 4:46 P...	Configuration settings	1 KB

Win.ini Sequence in Tandem Mode (Slide 3 of 3)

MassHunter Import

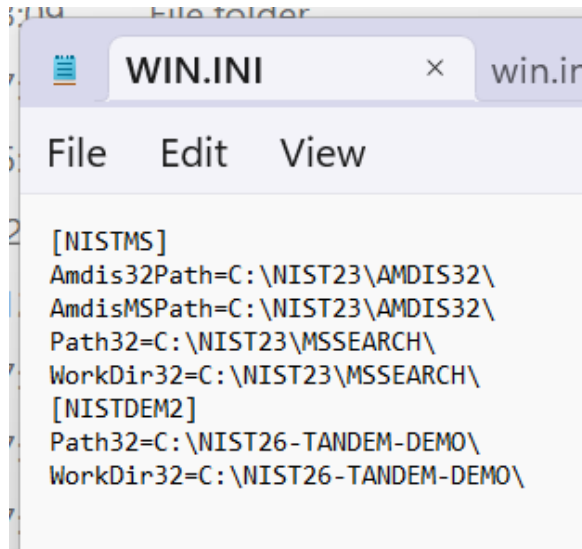
NIST23 Installed EI and MSMS Version



```
WIN.INI
File Edit View

[NISTMS]
Amdis32Path=C:\NIST23\AMDIS32\
AmdisMSPath=C:\NIST23\AMDIS32\
Path32=C:\NIST23\MSSEARCH\
WorkDir32=C:\NIST23\MSSEARCH\
```

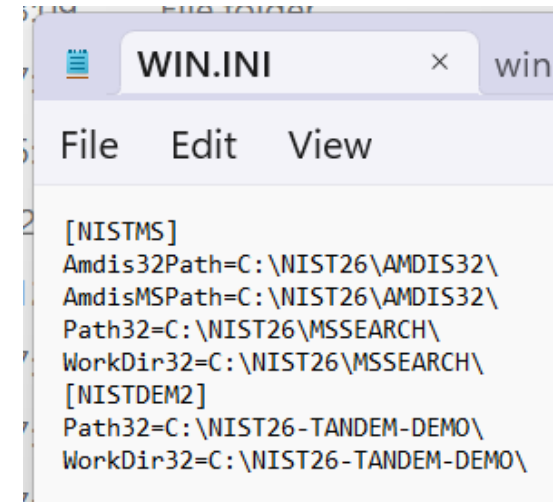
NIST23 Installed NIST Tandem Demo Installed



```
WIN.INI
File Edit View

[NISTMS]
Amdis32Path=C:\NIST23\AMDIS32\
AmdisMSPath=C:\NIST23\AMDIS32\
Path32=C:\NIST23\MSSEARCH\
WorkDir32=C:\NIST23\MSSEARCH\
[NISTDEM2]
Path32=C:\NIST26-TANDEM-DEMO\
WorkDir32=C:\NIST26-TANDEM-DEMO\
```

NIST23 Un-Installed NIST Tandem Demo Un-Installed NIST26 Installed



```
WIN.INI
File Edit View

[NISTMS]
Amdis32Path=C:\NIST26\AMDIS32\
AmdisMSPath=C:\NIST26\AMDIS32\
Path32=C:\NIST26\MSSEARCH\
WorkDir32=C:\NIST26\MSSEARCH\
[NISTDEM2]
Path32=C:\NIST26-TANDEM-DEMO\
WorkDir32=C:\NIST26-TANDEM-DEMO\
```

- MassHunter MS/MS Import Works

- MassHunter MS/MS Import Works
- Searches Version 3, not 4
- Reasonable result
- Standalone Demo works well with chromatogram

- MassHunter MS/MS Import Works
- *“Residue”* left in win.ini about tandem demo
- Does it matter??

Software Bug: MS Interpreter (Slide 1 of 7)

- Probably not really a bug
- Sometimes you might be having trouble with the program, and it will not open
- Possibly an earlier version might be present on the system, and you want to change to a newer version?
- Some instructions from different sources on the next few pages
- You need to be the *TRUE Administrator of your PC*
- Might require help from a IT corporate or university administrator to access Admin Rights on your computer
- Discussion of OLE and MS Interpreter by ChatGPT on next slide

Software Bug: MS Interpreter (Slide 2 of 7)

➤ Chat GPT Explanation

MS Interpreter uses **OLE registration** because **NIST MS Search** treats it as an external software component that must be located, started and controlled programmatically.

When you select **Send To → MS Interpreter** or press **F9**, NIST MS Search needs to:

- Locate the installed MSPCALC.EXE.
- Start the correct copy of MS Interpreter.
- Transfer the selected mass spectrum.
- Transfer the chemical structure, when available.
- Instruct MS Interpreter to display and analyze the information.

OLE registration places MS Interpreter's COM/OLE identity and executable location in the Windows Registry. NIST MS Search can then request MS Interpreter through Windows without knowing its folder directly.

Administrator access is required

Registering, unregistering or repairing MS Interpreter normally requires **Windows administrator privileges** because the necessary information is written to protected areas of the Windows Registry.

Therefore, to correct an OLE-registration problem, you generally must either:

- Sign in using an account with administrator rights; or
- Right-click MSINTREG.BAT, MSINTUNR.BAT or MSPCALC.EXE and select **Run as administrator**.

Within MS Interpreter, the corresponding commands are:

Options → OLE Registration → RegServer

and, when removing an obsolete registration:

Options → OLE Registration → UnregServer

Simply running these commands from a standard user account may appear to work but fail to update the protected registry information. In a company-controlled environment, such as Eastman, the user may need the IT department or a system administrator to perform the repair.

Moving, renaming or replacing MSPCALC.EXE may leave Windows pointing to an old program location. The old registration should then be removed and the correct copy registered again with administrator privileges.

Most other NIST programs do not require OLE registration because they communicate through command-line instructions, temporary files, shared folders or direct program calls rather than acting as registered Windows automation servers.

Although NIST calls it **OLE registration**, its primary purpose here is Windows **COM automation and program discovery**, not embedding an object in a document. NIST's instructions specifically require running the registration utilities or MSPCALC.EXE **as Administrator**. [NIST MS Interpreter documentation](#)

Program arrangement	Communication method
NIST MS Search → MS Interpreter	Registered OLE/COM connection
Vendor software → NIST MS Search	Files, macros, command lines or nistms\$.exe
Other NIST utilities	Direct execution or file exchange

Software Bug: MS Interpreter (Slide 3 of 7)

- Instructions from NIST
- Need to be *TRUE administrator* for the system to perform operations for MS Interpreter
 - Download the archive with MS Interpreter Registration program MSINTREG.ZIP
 - and unpack the msintreg.exe file to the folder with the NIST MS Interpreter software (MSPCALC.EXE, Frag32.dll, Qual.dll, etc.).
 - Purpose:
 - The program MSINTREG.EXE is supposed to completely replace instructions on registering NIST MS Interpreter in case all MS Interpreter files are in a single folder on user's PC.
 - How to use instructions:
 - (1) The purpose of Windows 32-bit console application msintreg.exe is to connect NIST MS Interpreter to NIST MS Search. It is designed to work under Win XP, 7, 8, 8.1, 10, 11.
 - (2) Shutdown NIST MS Search if it is running.
 - (3) Copy file msintreg.exe to the folder containing MS Interpreter files.
 - The minimal set of MS Interpreter files is: MSPCALC.EXE, Frag32.dll, Qual.dll.
 - (4) Run msintreg.exe in that folder.
 - Normally, Windows would request Administrative credentials to run msintreg.exe.
 - (5) In case of an error, a system-modal dialog entitled MSIntReg is displayed. In this case:
 - Before clicking OK, take a look at the console output to find out what caused the error.
 - To resolve the problem, you may need to close MS Search, remove read-only attribute, update MS Interpreter files and/or reboot Windows and repeat (4).
 - (6) To restore MSPCALC.INI removed by MSIntReg, shutdown MS Search and MS Interpreter, then copy MSPCALC.INI.BAK to MSPCALC.INI.
 - Details:
 - A successful run of msintreg.exe may look like this
 - ==begin=====
 - MSIntReg.exe: Connect MS Interpreter to NIST MS Search (ver. 1.0.2 build Thu Aug 1 11:51:28 2024)
 - Searching for the minimal set of MS Interpreter components
 - Searching for MSPCALC.EXE... - success
 - Searching for Frag32.dll... - success
 - Searching for Qual.dll... - success
 - Searching for running NIST MS Search
 - Running NIST MS Search not found.
 - Trying to kill running NIST MS Interpreter process/task "mspcalc.exe"
 - SUCCESS: The process "Mspcalc.exe" with PID 57572 has been terminated.
 - INFO: Found NIST MS Interpreter file MSPCALC.INI
 - Deleted file MSPCALC.INI.BAK
 - Renamed MSPCALC.INI to MSPCALC.INI.BAK
 - Unregistering NIST MS Interpreter... - success
 - Registering NIST MS Interpreter... - success
 - ==end=====

Software Bug: MS Interpreter (Slide 4 of 7)

- MS Interpreter from NIST Website Approach
- <https://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:interpreter>

Introduction

New Version – March 2023 (v. 3.4.5). Updated mechanisms and their rates for all fragmentation types (EI, Tandem positive and negative mode). This program connects mass spectral peaks to their probable chemical structure origin and can be quickly invoked from the NISTMS program with the F9 key or right mouse button followed by send to MS Interpreter. It also enables the display of isotopic envelopes, chemical formula calculations and other related calculations and user interface control.

Here is the link to download MS Interpreter:

 [Download MS Interpreter Ver.3.4.5 \(2023 Release\)](#)

Version 3.4.* replaces all earlier versions under 32-bit and 64-bit Windows.

MS Interpreter was described in a poster in the meeting of the International Mass Spectrometry Society, Edinburgh, UK, 2003, “An Automated Method for Verifying Structure-Spectral Consistency Based on Ion Thermochemistry”

MS Interpreter ASMS presentations (PDF) are also available:  [ASMS 2017](#),  [ASMS 2018](#),  [ASMS 2019](#)

No documentation is available except built-in help system. Select Help/What's New from MS Interpreter menu to get a list of newly added features, or open file MSPCALC.chm

Software Bug: MS Interpreter (Slide 5 of 7)

Installation

How to install MS Interpreter ver. 3.4

The following instructions assume that you do not have a earlier version of MS Interpreter installed on your PC.

1. Extract files from this package to a directory on your computer.
2. Run MSINTREG.BAT as Administrator. This will register the new MS Interpreter. This process lasts 1-3 sec., no new application window will be open. Or you can run newly extracted file MSPCALC.EXE as Administrator; select from its menu Options/OLE Registration/RegServer.
3. Optionally create a shortcut to MSPACALC.EXE and name it “MS Interpreter”.

[How to replace an earlier version of the MS Interpreter](#) ▶



How to verify that MS Interpreter is properly installed

1. Run NIST MS Search (your current version).
2. Open a mass spectrum in any of the MS Search windows
3. Right-click the window and select “Send To”, “MS Interpreter” from the floating menu.

The MS Interpreter window should pop up and display the selected spectrum and (if present) the chemical structure. The Help/About box should display “MS Interpreter Version 3.4” or later.

Software Bug: MS Interpreter (Slide 6 of 7)

How to Reattach MS Interpreter to the NIST MS search program

Download the archive with MS Interpreter Registration program:



Download MS Interpreter Registration program
archive

1. Unpack the ***msintreg.exe*** file to the folder with the NIST MS Interpreter software (mspcalc.exe, frag32.dll, Qual.dll).
2. Close the NIST MS Search program
3. Run this program (msintreg.exe)
4. Restart the NIST MS Search program.

More detailed instruction is in the Readme.txt file included in the msintreg.zip archive.

➤ Software Bug: MS Interpreter (Slide 7 of 7)

- Sometimes I have had success
- UnRegServer, then RegServer
- You need to be the *TRUE Administrator of your PC*
- Might require help from a IT corporate or university administrator to access Admin Rights on your computer
- MS Interpreter can be opened from program group
- MS Interpreter program is really named Mspcalc

