Manual and Automated Hybrid Searches in NIST26

Video/Handout

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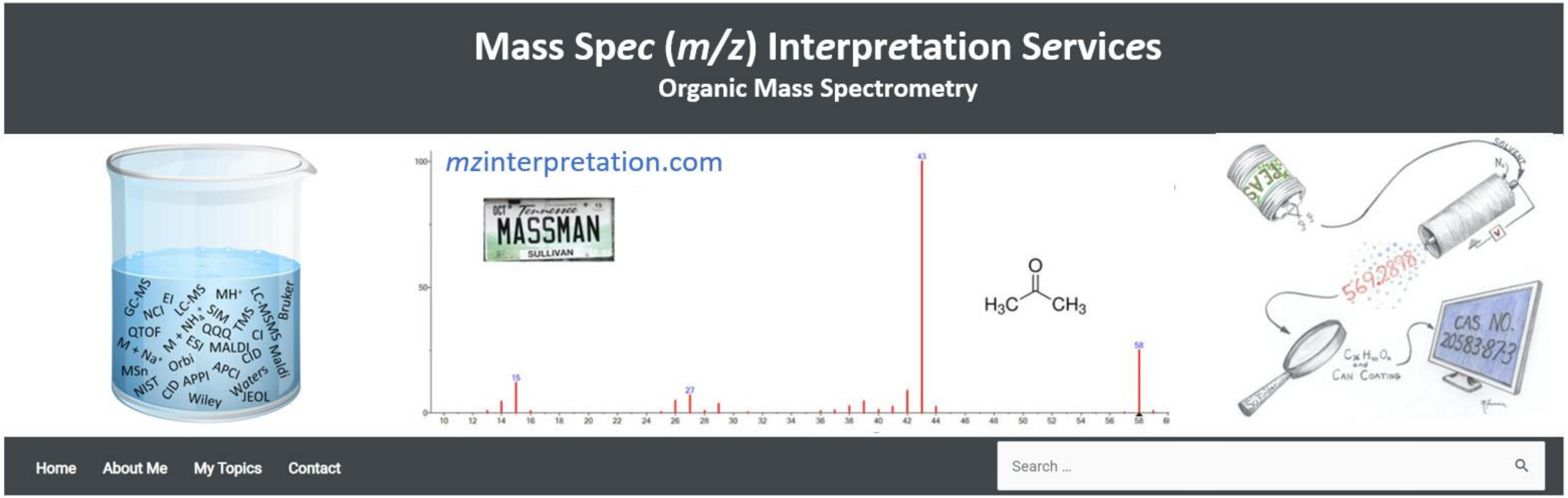
See *Full Course* on NIST26 with new *Integrated* Deconvolution/Library Searching for *EI GC-MS* and *LC-MS/MS*!



Mass Spec (m/z) Interpretation Services

Organic Mass Spectrometry

Hybrid MS/MS Search: Extending Library Coverage

Conventional MS/MS searching depends on exact spectral matches, limiting identification when compounds are absent from libraries. Hybrid MS/MS search overcomes this by recognizing ***structurally related compounds***, expanding effective library coverage.

In the NIST Mass Spectral Search Program, the hybrid search is available both ***manually*** in the Library Search window and ***automatically*** within the Chromatogram workflow.

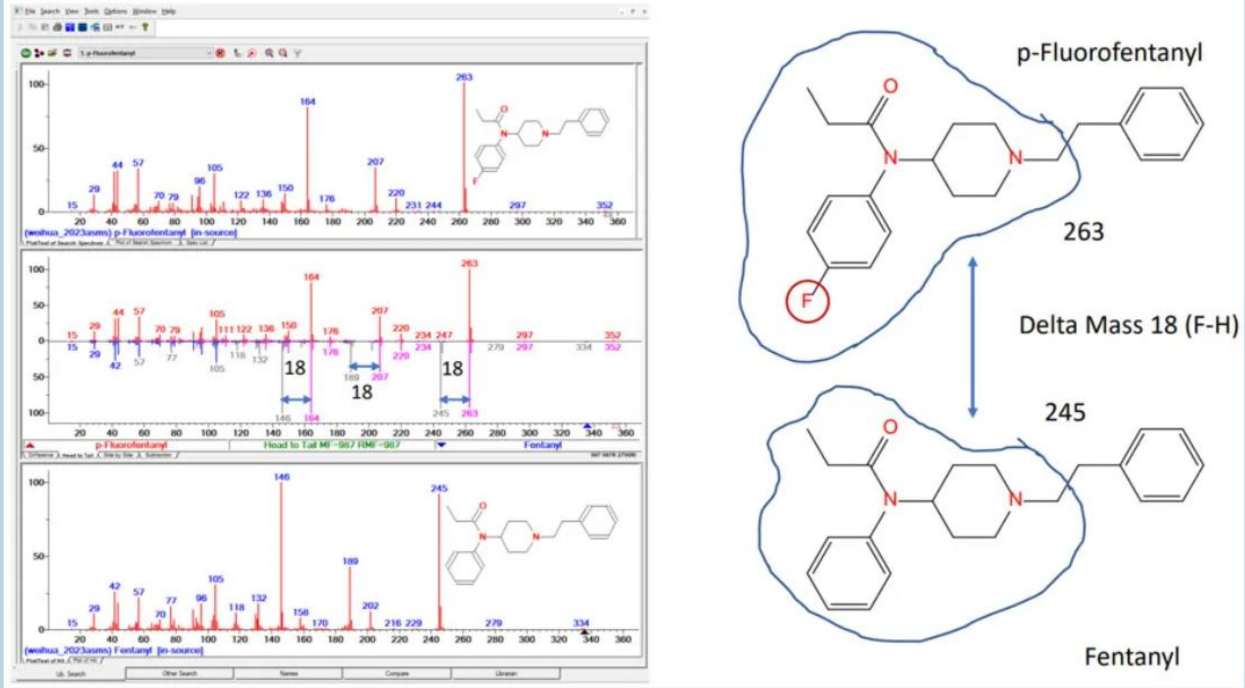
The use of **Δform (dform)** highlights mass differences corresponding to plausible structural modifications, guiding interpretation. These capabilities enable more confident exploration of unknowns beyond exact matches.

Additional Information

Novel NIST Hybrid Search for Unknown Identifications

See Link

Example Identifying Compound with NIST Hybrid Search



Excel DeltaMass Table
PDF DeltaMass Table

NIST initially developed the hybrid search and I have listed their publicatio

Videos

YouTube: Advanced NIST Hybrid Search of EI Spectra
YouTube: Advanced NIST Hybrid Search for MS/MS (Tandem) Spectra
YouTube: Application NIST Hybrid in EI in Seized Drug Analyses

Associated Handouts

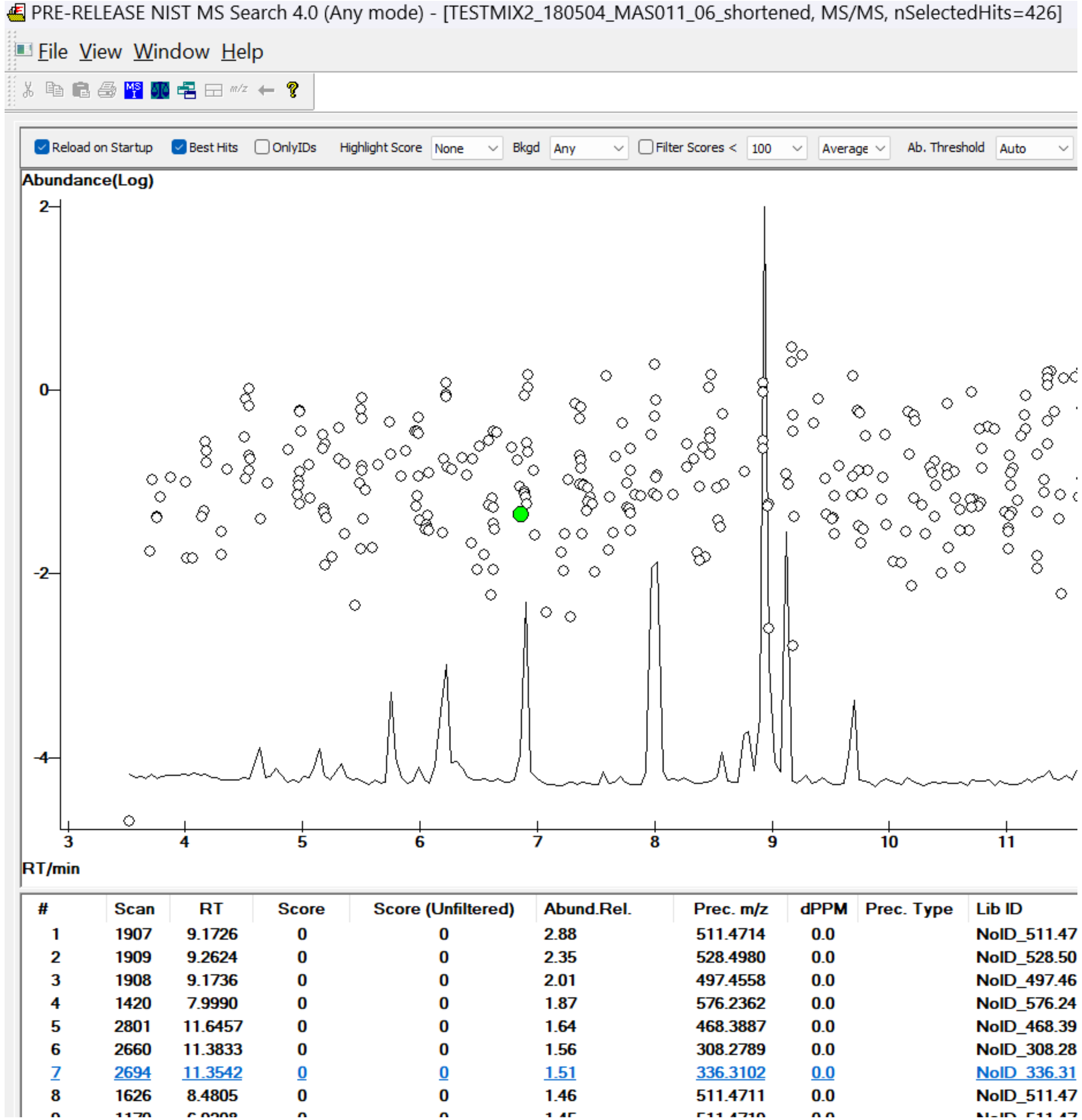
Handout: Advanced NIST Hybrid Search of EI Spectra
Handout: Advanced NIST Hybrid Search of MS/MS (Tandem) Spectra
Handout: Application NIST Hybrid in EI in Seized Drug Analyses

Supporting Software Courses for Utilizing Hybrid and Adaptive Search

NIST EI Search Software
NIST MSMS Search Software

Manually Sending One Spectrum to Lib Search from Chromatogram

- Perform standard search in Chromatogram
- Find, select, and display NoID's
- Sort by Abundance Relative
- Step through with keyboard arrows and butterfly plots
- Send ones of interest manually to Lib Search for Hybrid search to find candidates

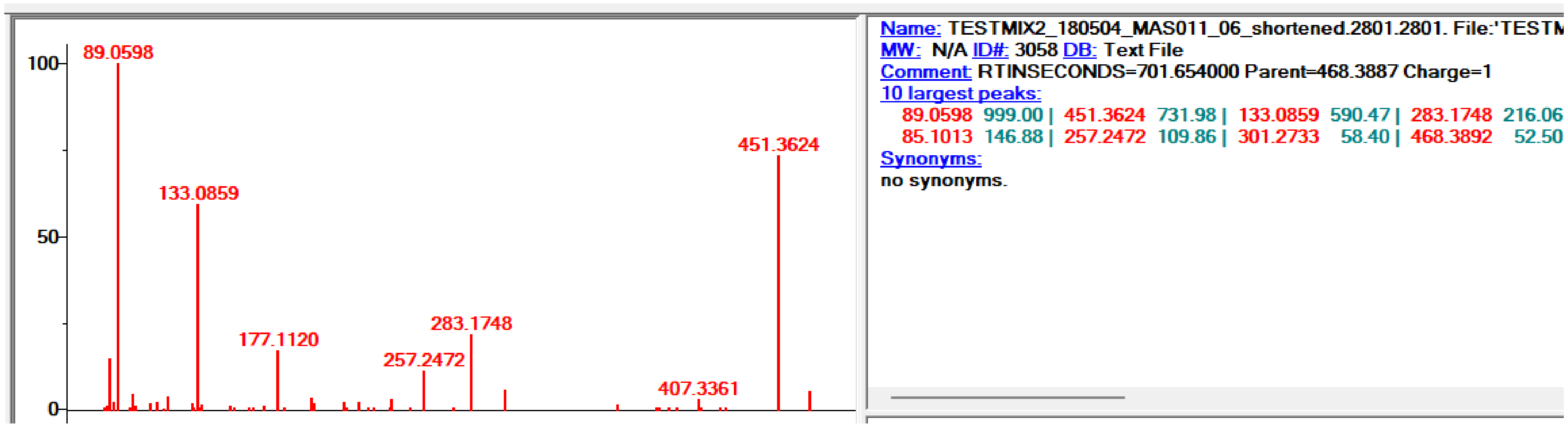


PRE-RELEASE NIST MS Search 4.0 (Any mode) - [TESTMIX2_180504_MAS011_06_shortened, MS/MS, nSelectedHits=426]

☑ Reload on Startup ☑ Best Hits ☐ OnlyIDs Highlight Score None Bkgd Any ☐ Filter Scores < 100 Average Ab. Threshold Auto

#	Scan	RT	Score	Score (Unfiltered)	Abund.Rel.	Prec. m/z	dPPM	Prec. Type	Lib ID
1	1907	9.1726	0	0	2.88	511.4714	0.0		NoID_511.47
2	1909	9.2624	0	0	2.35	528.4980	0.0		NoID_528.50
3	1908	9.1736	0	0	2.01	497.4558	0.0		NoID_497.46
4	1420	7.9990	0	0	1.87	576.2362	0.0		NoID_576.24
5	2801	11.6457	0	0	1.64	468.3887	0.0		NoID_468.39
6	2660	11.3833	0	0	1.56	308.2789	0.0		NoID_308.28
7	2694	11.3542	0	0	1.51	336.3102	0.0		NoID_336.31
8	1626	8.4805	0	0	1.46	511.4711	0.0		NoID_511.47

Many related to PEG type Materials, Note Precursor for $(M+NH_4)^+$ and loss of NH_3, MH^+ -17



Name: TESTMIX2_180504_MAS011_06_shortened.2801.2801. File:'TESTM

MW: N/A **ID#:** 3058 **DB:** Text File

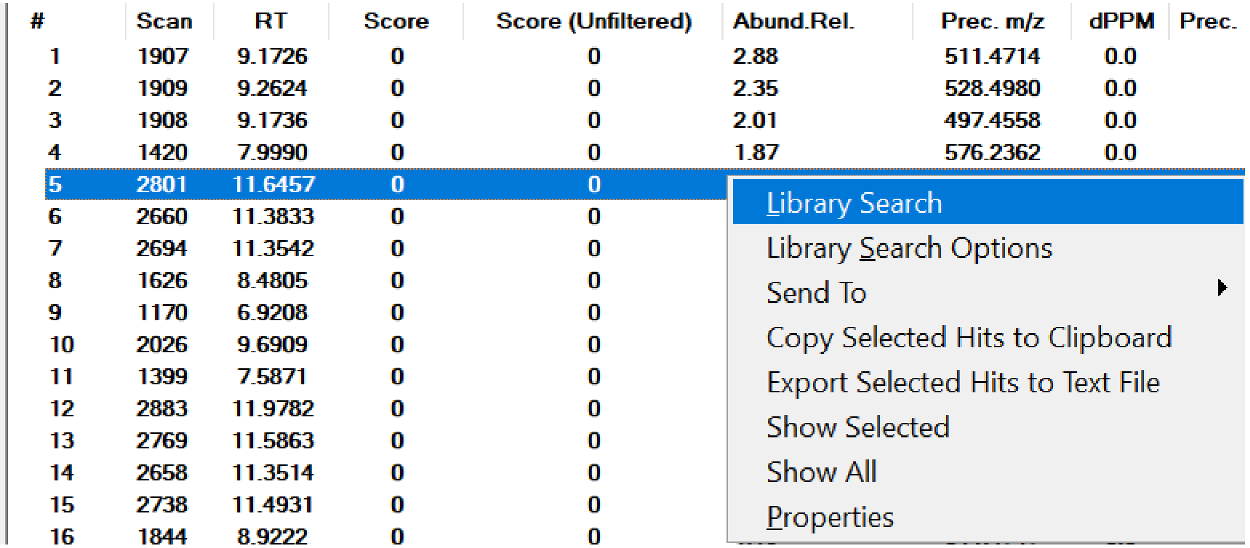
Comment: RTINSECONDS=701.654000 Parent=468.3887 Charge=1

10 largest peaks:

89.0598 999.00 | 451.3624 731.98 | 133.0859 590.47 | 283.1748 216.06
85.1013 146.88 | 257.2472 109.86 | 301.2733 58.40 | 468.3892 52.50

Synonyms:

no synonyms.



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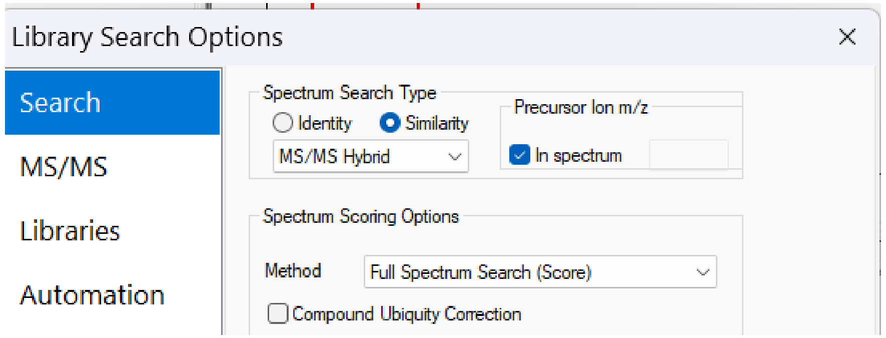
1	1907	9.1726	0	0	2.88	511.4714	0.0	
2	1909	9.2624	0	0	2.35	528.4980	0.0	
3	1908	9.1736	0	0	2.01	497.4558	0.0	
4	1420	7.9990	0	0	1.87	576.2362	0.0	
5	2801	11.6457	0	0				
6	2660	11.3833	0	0				
7	2694	11.3542	0	0				
8	1626	8.4805	0	0				
9	1170	6.9208	0	0				
10	2026	9.6909	0	0				
11	1399	7.5871	0	0				
12	2883	11.9782	0	0				
13	2769	11.5863	0	0				
14	2658	11.3514	0	0				
15	2738	11.4931	0	0				
16	1844	8.9222	0	0				



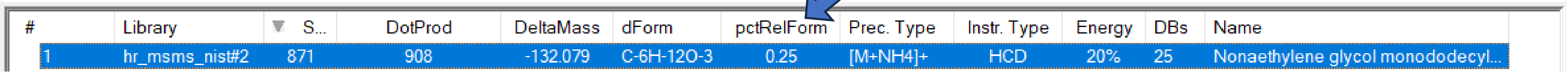
Spectrum Search Type
○ Identity ● Similarity
MS/MS Hybrid

Precursor Ion m/z
☑ In spectrum

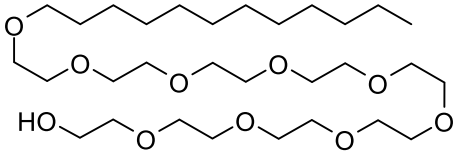
Spectrum Scoring Options
Method: Full Spectrum Search (Score)
☐ Compound Ubiquity Correction

- Most reasonable candidate is a dodecyl ether of PEG (polyethylene glycol)
- Sent Hit 1 to MS interpreter to understand fragmentation
- dForm showing loss of three PEG groups for Delta Mass of 3 x EG group
- Observed dForm 132.079, theoretical 132.079
- Sent proposed structure to drawing program and removed 3 EG repeat groups
- Sent to modified structure to MS Interpreter for confirmation

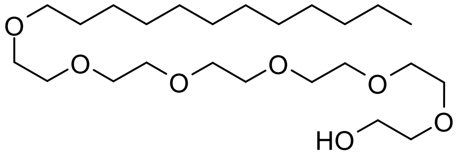
Definition Below*



#	Library	▼ S...	DotProd	DeltaMass	dForm	pctRelForm	Prec. Type	Instr. Type	Energy	DBs	Name
1	hr_msms_nist#2	871	908	-132.079	C-6H-12O-3	0.25	[M+NH4]+	HCD	20%	25	Nonaethylene glycol monododecyl...



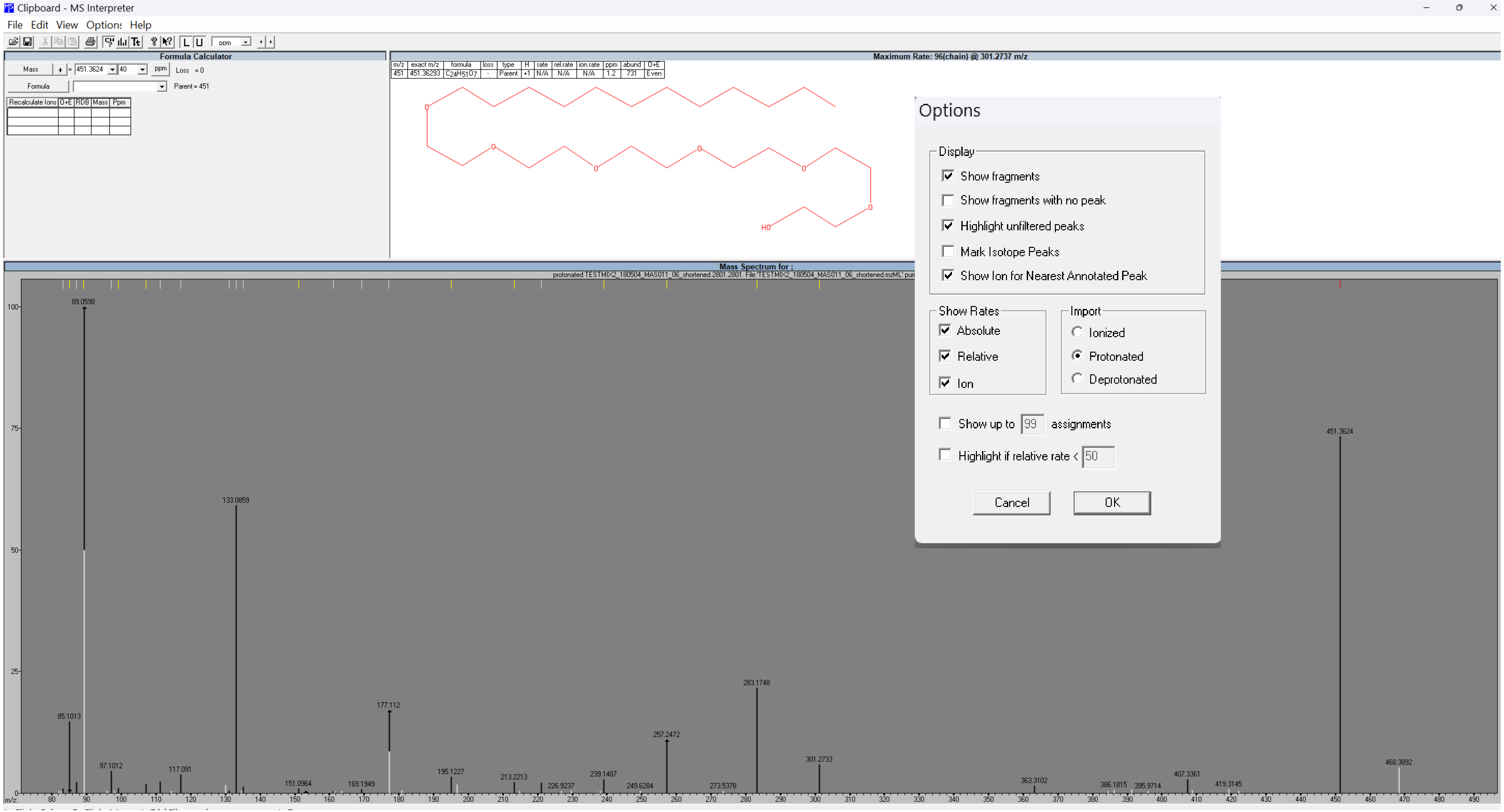
Best Candidate



Reasonable Structure

*pctRefForm Percent: Probability that a dForm(ula) is correct. Based on statistics of occurrences of each dForm relative to the most common (CH2 which is assigned 100%) – for high scoring hits (>900). The higher, the most likely correct – values like 0.01 are so rare, they are very likely accidental and wrong for example

Proposed Structure, Changed Imported Structure to Protonated, Default is Ionized, Loss of ammonia *1.2 ppm difference*



Clipboard - MS Interpreter

Formula Calculator

Mass + = 451.3624 40 ppm Loss = 0

Formula Parent = 451

Recalculate Ions	O+E	RDB	Mass	Ppm

Maximum Rate: 96(chain) @ 301.2737 m/z

m/z	exact m/z	formula	loss	type	H	rate	rel.rate	ion.rate	ppm	abund	O+E
451	451.36293	$C_{24}H_{51}O_7$	-	Parent	+1	N/A	N/A	N/A	1.2	731	Even

Mass Spectrum for ;

protonated TESTMIX2_180504_MAS011_06_shortened.2801.2801. File:'TESTMIX2_180504_MAS011_06_shortened.mzML' pur

Options

Display
- ☑ Show fragments
- ☐ Show fragments with no peak
- ☑ Highlight unfiltered peaks
- ☐ Mark Isotope Peaks
- ☑ Show Ion for Nearest Annotated Peak

Show Rates
- ☑ Absolute
- ☑ Relative
- ☑ Ion

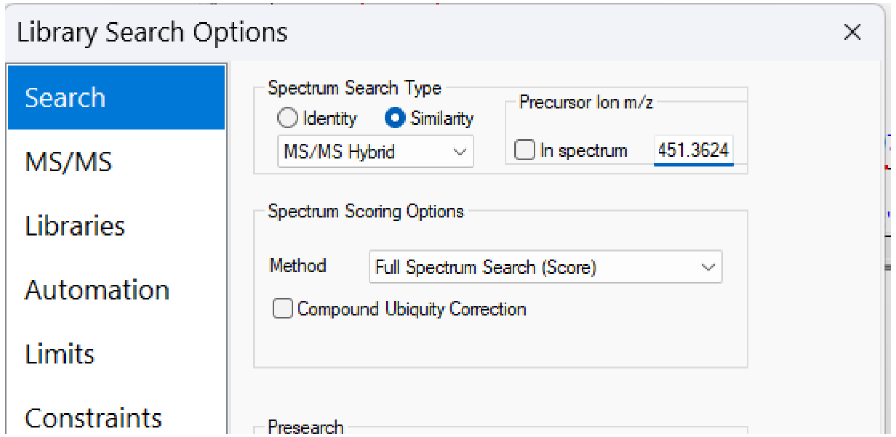
Import
- ○ Ionized
- ● Protonated
- ○ Deprotonated

☐ Show up to 99 assignments

☐ Highlight if relative rate < 50

General Comments for Ammonium in MSMS

- ***More $(M+H)^+$ than $(M+NH_4)^+$ adducts for species in NIST MS/MS Library***
- NIST ***does not purposely add*** ammonium when acquiring reference spectra
- Often better when user sees loss of 17 for NH_3
- Change specified ion to the $(M+H)^+$ value noted for fragmentation
- Thus, for the previous example, unclick “In spectrum” and insert value for $(M+H)^+$ noted in spectrum
- Top two hits still monodecyl ethers
- ***The major fragments in MS/MS of $(M+NH_4)^+$ and $(M+H)^+$ are the same***



Spectrum Search Type: ○ Identity ● Similarity

MS/MS Hybrid

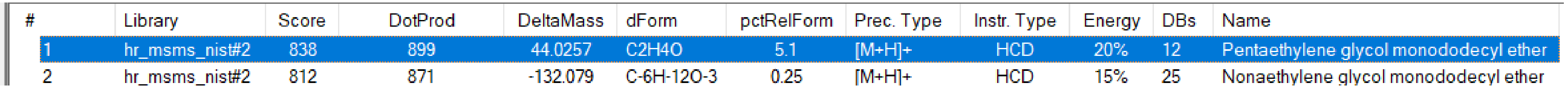
Precursor Ion m/z: ☐ In spectrum 451.3624

Spectrum Scoring Options

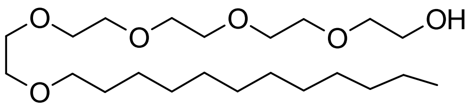
Method: Full Spectrum Search (Score)

☐ Compound Ubiquity Correction

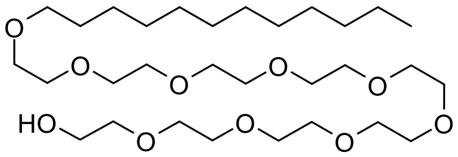
Presearch



#	Library	Score	DotProd	DeltaMass	dForm	pctRelForm	Prec. Type	Instr. Type	Energy	DBs	Name
1	hr_msms_nist#2	838	899	44.0257	C2H4O	5.1	[M+H]+	HCD	20%	12	Pentaethylene glycol monododecyl ether
2	hr_msms_nist#2	812	871	-132.079	C-6H-12O-3	0.25	[M+H]+	HCD	15%	25	Nonaethylene glycol monododecyl ether



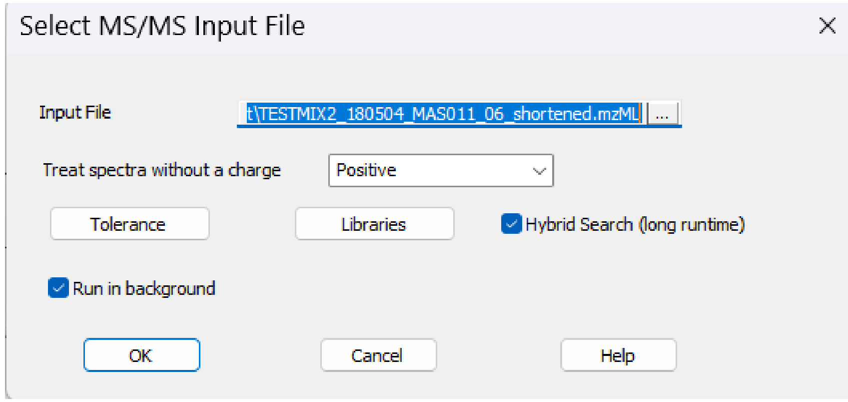
1



2

Running Hybrid Search in Automated Chromatogram Approach

- Select Input File with Hybrid Search checked
- Select “***Run in Background***”
- Note will take ***significantly more time*** than standard method
- Note user alert shows “***Running Background Task***”
- Alerted when finished
- ***Properties*** in menu ***automatically displayed***
- See results sorted by NoID, selected, sorted by abundance
- ***Same hit shown*** for component done manually for comparison
- Very useful to ***send to Lib Search*** Window for manual search ***for structures***



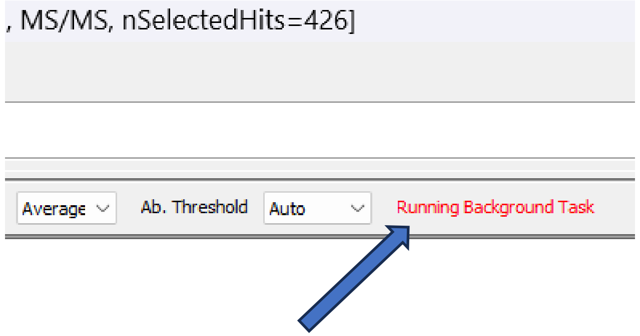
Select MS/MS Input File

Input File: t\TESTMIX2_180504_MAS011_06_shortened.mzML

Treat spectra without a charge: Positive

☑ Hybrid Search (long runtime)

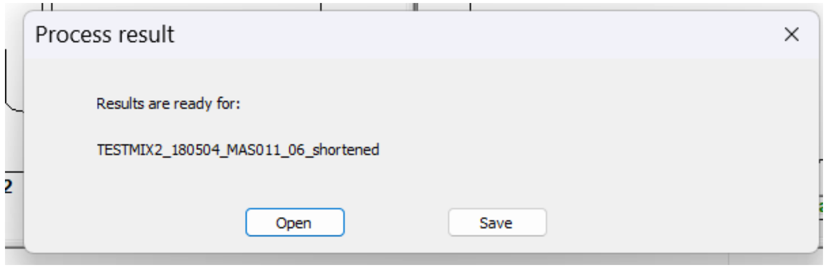
☑ Run in background



, MS/MS, nSelectedHits=426]

Ab. Threshold: Auto

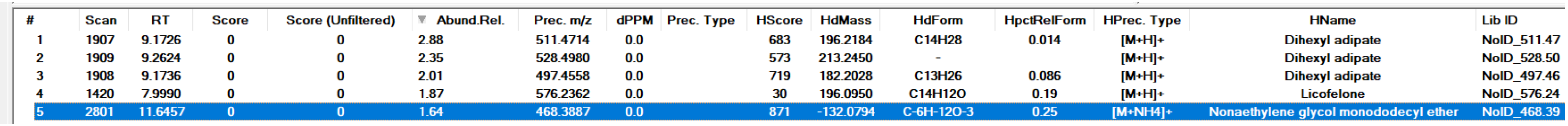
Running Background Task



Process result

Results are ready for:

TESTMIX2_180504_MAS011_06_shortened



#	Scan	RT	Score	Score (Unfiltered)	▼ Abund.Rel.	Prec. m/z	dPPM	Prec. Type	HScore	HdMass	HdForm	HpctRelForm	HPrec. Type	HName	Lib ID
1	1907	9.1726	0	0	2.88	511.4714	0.0		683	196.2184	C14H28	0.014	[M+H]+	Dihexyl adipate	NoID_511.47
2	1909	9.2624	0	0	2.35	528.4980	0.0		573	213.2450	-		[M+H]+	Dihexyl adipate	NoID_528.50
3	1908	9.1736	0	0	2.01	497.4558	0.0		719	182.2028	C13H26	0.086	[M+H]+	Dihexyl adipate	NoID_497.46
4	1420	7.9990	0	0	1.87	576.2362	0.0		30	196.0950	C14H12O	0.19	[M+H]+	Licofelone	NoID_576.24
5	2801	11.6457	0	0	1.64	468.3887	0.0		871	-132.0794	C-6H-12O-3	0.25	[M+NH4]+	Nonaethylene glycol monododecyl ether	NoID_468.39