# In-Source Fragmentation Searches in NIST26 Chromatogram Window

## Video/Handout

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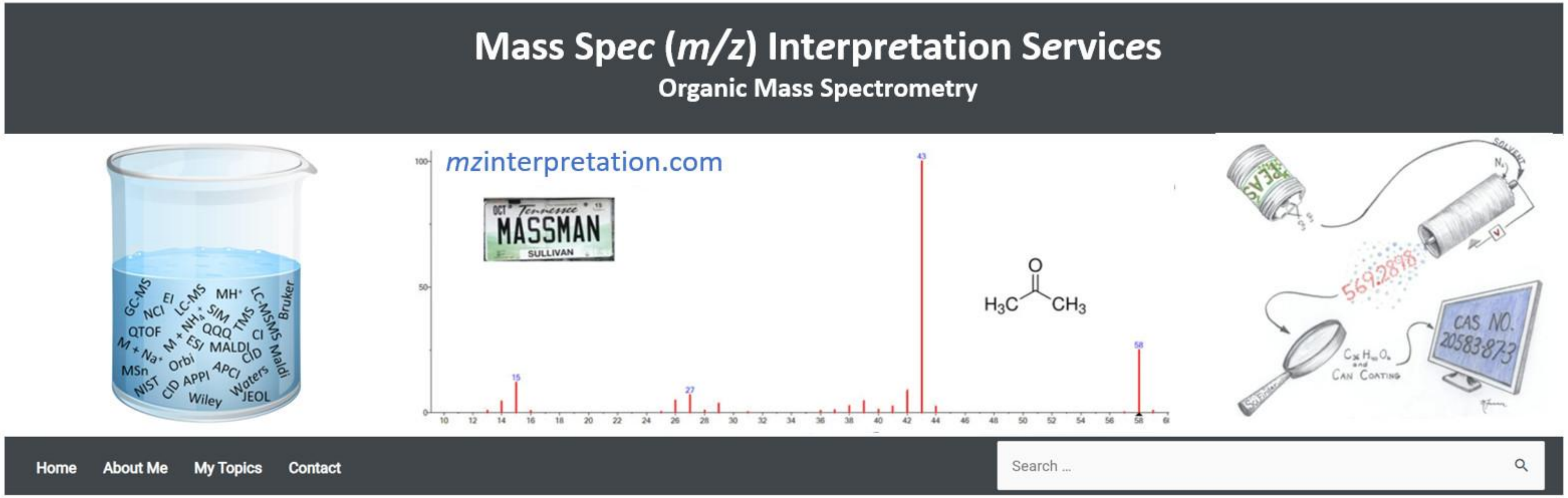
[mzinterpretation.com](mzinterpretation.com)

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



# “In-source MS/MS”, spectra like these are typical when fragmentation occurs in the source

1. **Mixed precursor + fragment ions**

- You usually see the **protonated molecule** (e.g., $[M+H]^+$) still present
- Alongside **multiple fragment ions** produced before any collision cell

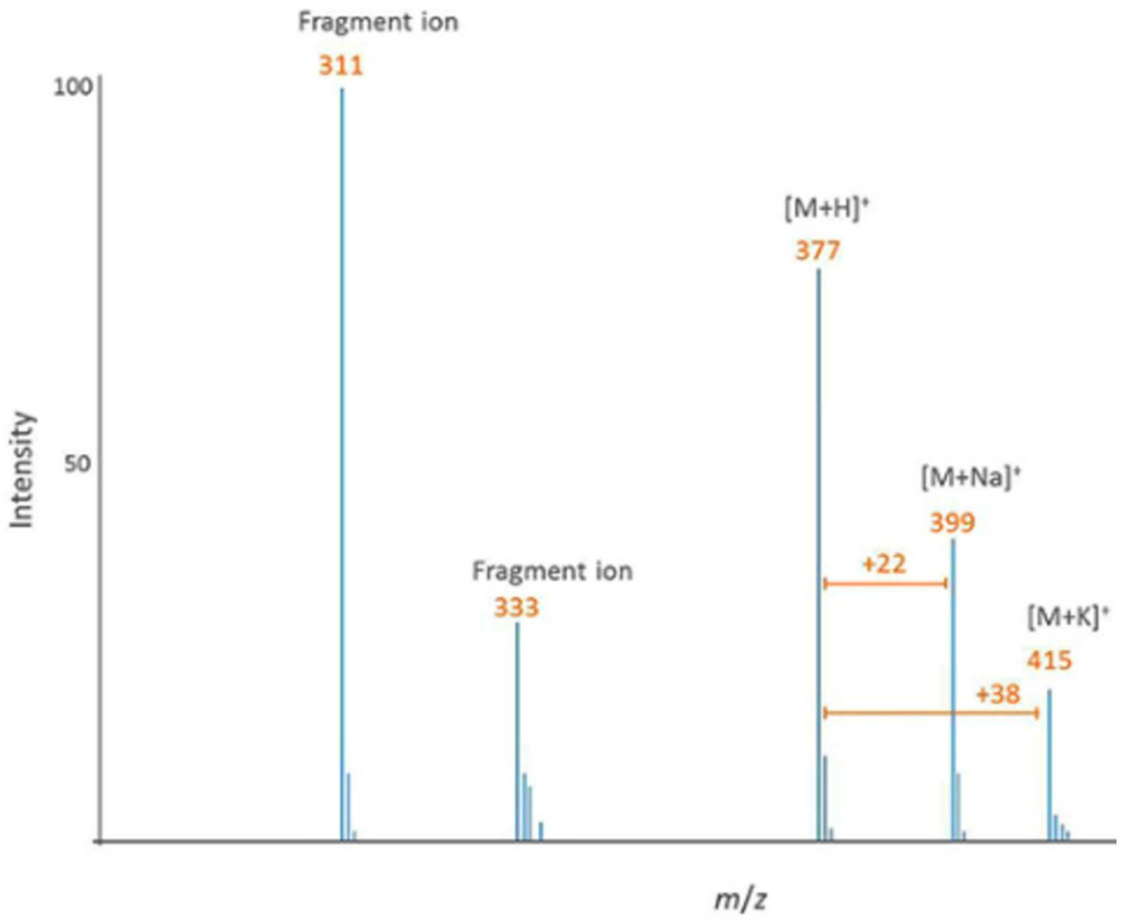
👉 That’s the key difference vs true MS/MS:

- **MS/MS:** isolated precursor → clean fragment spectrum
- **In-source:** everything fragments together → “mixed” spectrum

## Other Names

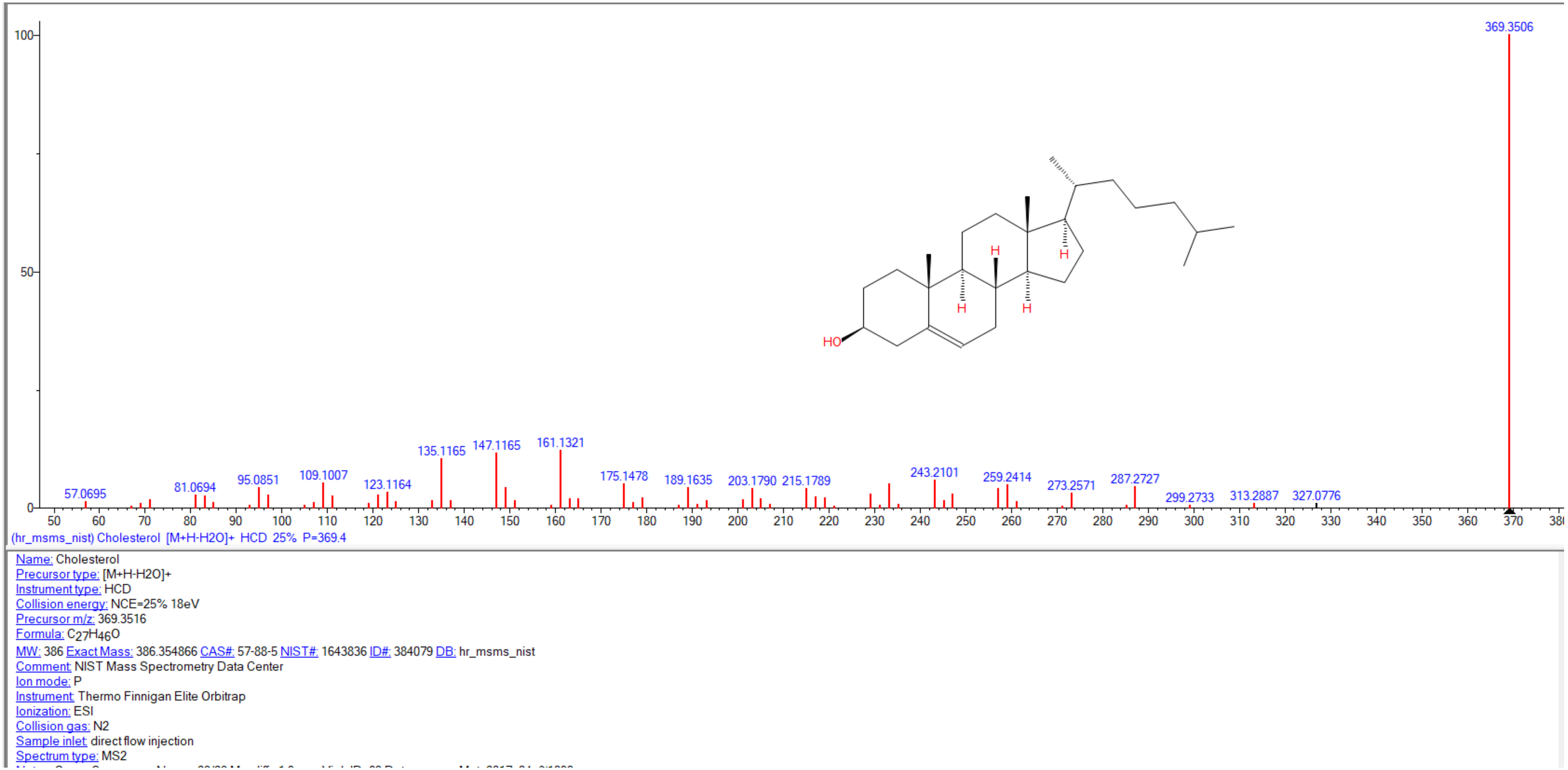
- In-source fragment ions *(best, most explicit)*
- Source-induced fragments
- In-source fragmentation products
- Source fragmentation ions
- In-source CID products

**If NIST saw these ions when infusing a reference standard, they would obtain MS2 spectra for the molecular ion adducts *and* fragment ions, of course *at high resolution***



# Molecules that primarily lose water from their $[M+H]^+$ in Positive Ion Electrospray

- For certain compounds such as cholesterol, there would be ***no*** MS/MS (MS2) Spectrum in Library if the $[M+H]^+$ ion was necessary
- Essentially ***no significant ion intensity*** is noted for $[M+H]^+$ ion adduct for cholesterol
- ***Thus***, the major MS/MS spectra is for the $[M+H-H_2O]^+$ peak at *m/z* 369.3506 in NIST MS/MS library

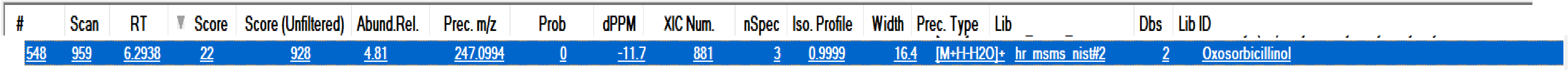


(hr_msms_nist) Cholesterol [M+H-H2O]+ HCD 25% P=369.4

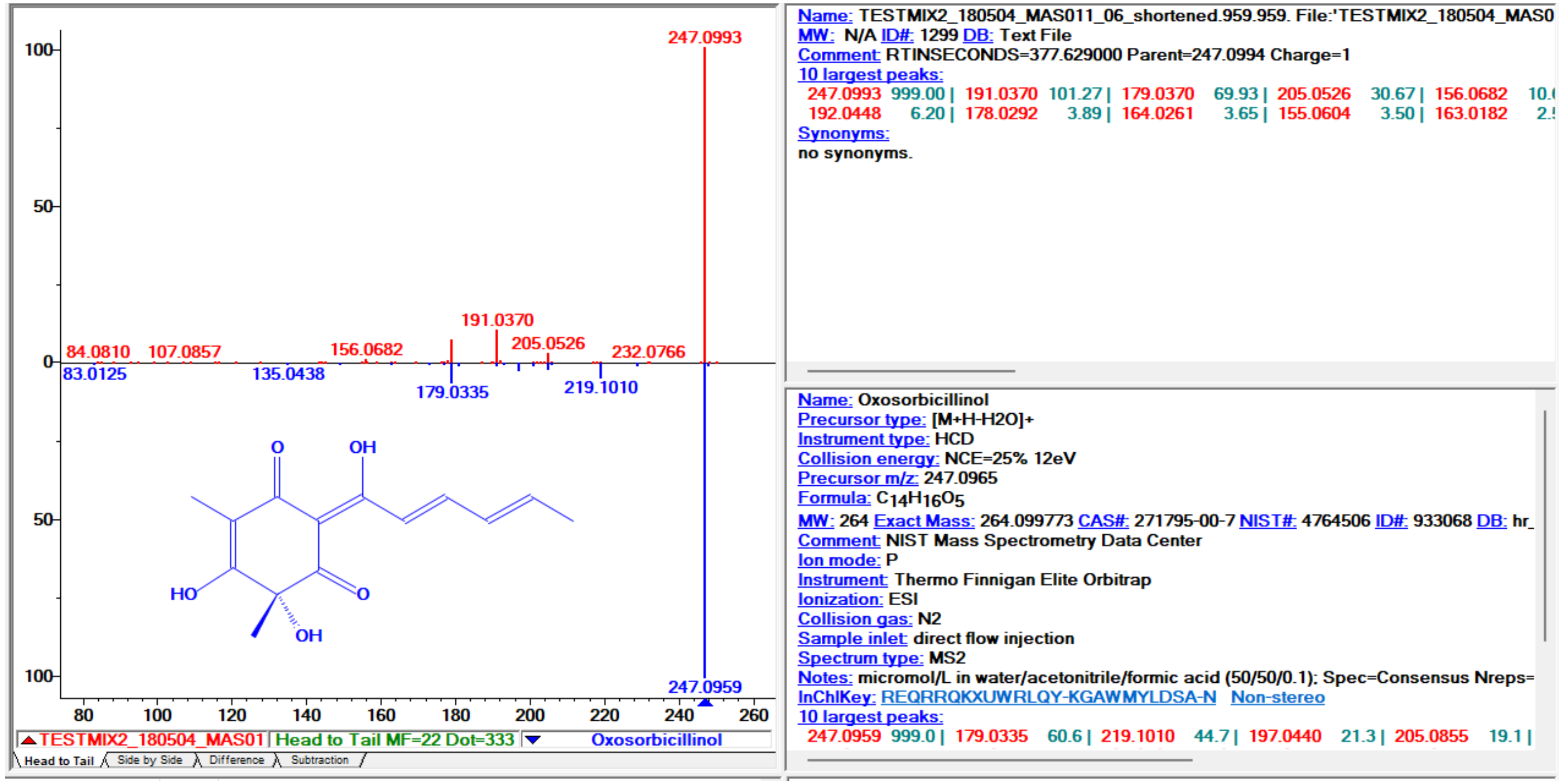
Name: Cholesterol
Precursor type: [M+H-H2O]+
Instrument type: HCD
Collision energy: NCE=25% 18eV
Precursor m/z: 369.3516
Formula: $C_{27}H_{46}O$
MW: 386 Exact Mass: 386.354866 CAS#: 57-88-5 NIST#: 1643836 ID#: 384079 DB: hr_msms_nist
Comment: NIST Mass Spectrometry Data Center
Ion mode: P
Instrument: Thermo Finnigan Elite Orbitrap
Ionization: ESI
Collision gas: N2
Sample inlet: direct flow injection
Spectrum type: MS2

# Chromatogram Results for Small Losses

- Thus, for such molecules as cholesterol, the chromatogram window search results are shown for $[M+H-H_2O]^+$ ***with the correct structure***
- This is ***only done*** for small losses such as $NH_3$, $H_2O$, *etc.*
- *Or sodium adducts, $[M+Na]^+$*
- Another example is shown below for Oxosorbicillinol



| # | Scan | RT | Score | Score (Unfiltered) | Abund.Rel. | Prec. m/z | Prob | dPPM | XIC Num. | nSpec | Iso. Profile | Width | Prec. Type | Lib | Dbs | Lib ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 548 | 959 | 6.2938 | 22 | 928 | 4.81 | 247.0994 | 0 | -11.7 | 881 | 3 | 0.9999 | 16.4 | [M+H-H2O]+ | hr_msms_nist#2 | 2 | Oxosorbicillinol |

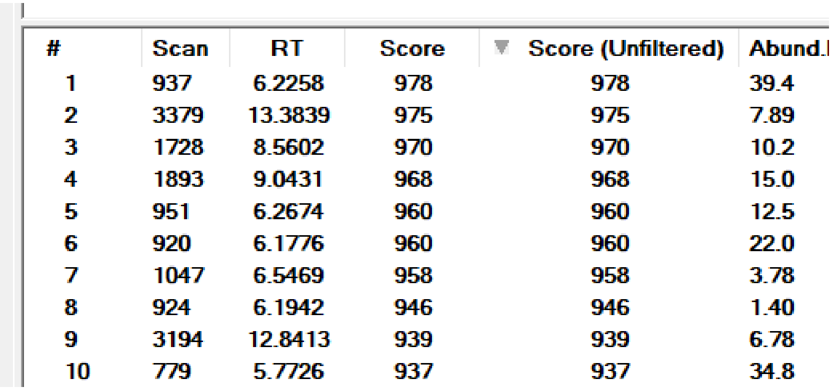


Name: TESTMIX2_180504_MAS011_06_shortened.959.959. File:'TESTMIX2_180504_MAS0
MW: N/A ID#: 1299 DB: Text File
Comment: RTINSECONDS=377.629000 Parent=247.0994 Charge=1
10 largest peaks:
247.0993 999.00 | 191.0370 101.27 | 179.0370 69.93 | 205.0526 30.67 | 156.0682 10.
192.0448 6.20 | 178.0292 3.89 | 164.0261 3.65 | 155.0604 3.50 | 163.0182 2.
Synonyms:
no synonyms.

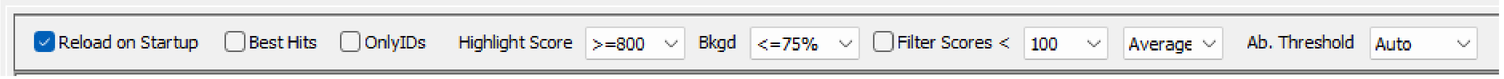
Name: Oxosorbicillinol
Precursor type: [M+H-H2O]+
Instrument type: HCD
Collision energy: NCE=25% 12eV
Precursor m/z: 247.0965
Formula: $C_{14}H_{16}O_5$
MW: 264 Exact Mass: 264.099773 CAS#: 271795-00-7 NIST#: 4764506 ID#: 933068 DB: hr_
Comment: NIST Mass Spectrometry Data Center
Ion mode: P
Instrument: Thermo Finnigan Elite Orbitrap
Ionization: ESI
Collision gas: N2
Sample inlet: direct flow injection
Spectrum type: MS2
Notes: micromol/L in water/acetonitrile/formic acid (50/50/0.1); Spec=Consensus Nreps=
InChIKey: REQRRQKXUWRLQY-KGAWMYLDSA-N Non-stereo
10 largest peaks:
247.0959 999.0 | 179.0335 60.6 | 219.1010 44.7 | 197.0440 21.3 | 205.0855 19.1 |

# Chromatogram Results for Small Losses

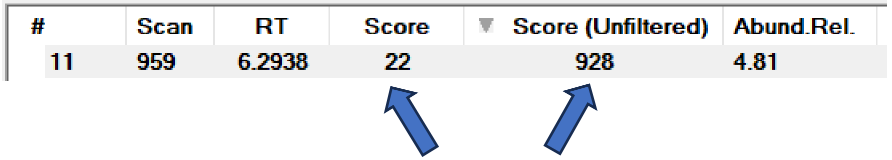
- For large fragment losses, the correct structure ***is not noted*** for the entry in the list
- The list only shows structures for M+H, M+Na, etc. ions and for small molecule loss as mentioned previously
- ***Thus, to see*** the proposed structure or structures, one ***must send*** the spectrum to NIST Lib Search Window
- Add Score(Unfiltered) as a property in the lower window
- Pick a reasonable value of >800 in top bar filter settings
- Also useful to sort by Score (unfiltered) with highest value at top of list
- Look for Entries whose Score is ***MUCH*** lower than Score (unfiltered)



| # | Scan | RT | Score | ▼ Score (Unfiltered) | Abund. |
|---|---|---|---|---|---|
| 1 | 937 | 6.2258 | 978 | 978 | 39.4 |
| 2 | 3379 | 13.3839 | 975 | 975 | 7.89 |
| 3 | 1728 | 8.5602 | 970 | 970 | 10.2 |
| 4 | 1893 | 9.0431 | 968 | 968 | 15.0 |
| 5 | 951 | 6.2674 | 960 | 960 | 12.5 |
| 6 | 920 | 6.1776 | 960 | 960 | 22.0 |
| 7 | 1047 | 6.5469 | 958 | 958 | 3.78 |
| 8 | 924 | 6.1942 | 946 | 946 | 1.40 |
| 9 | 3194 | 12.8413 | 939 | 939 | 6.78 |
| 10 | 779 | 5.7726 | 937 | 937 | 34.8 |



☑ Reload on Startup ☐ Best Hits ☐ OnlyIDs Highlight Score >=800 Bkgd <=75% ☐ Filter Scores < 100 Average Ab. Threshold Auto

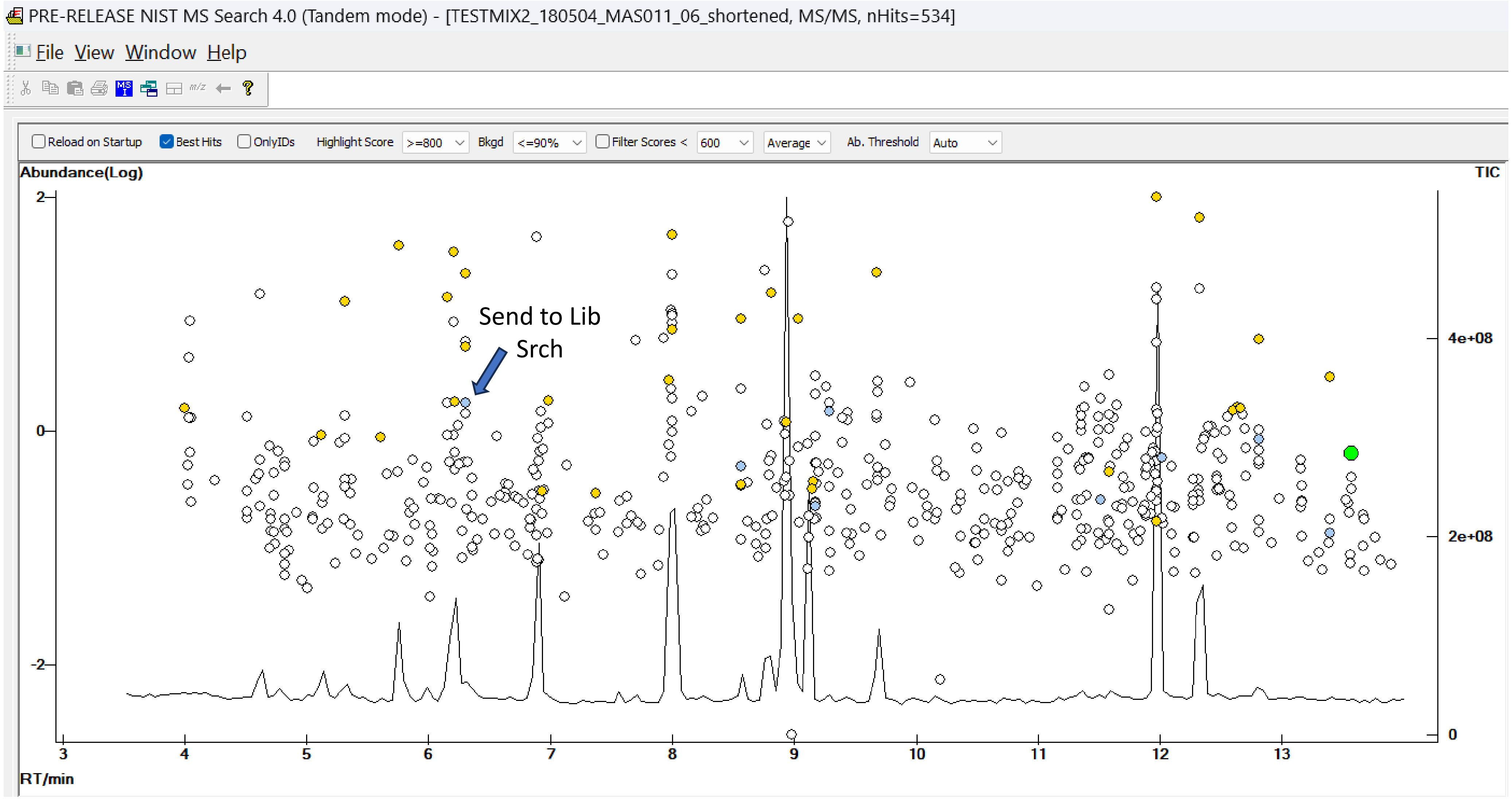


| # | Scan | RT | Score | ▼ Score (Unfiltered) | Abund.Rel. |
|---|---|---|---|---|---|
| 11 | 959 | 6.2938 | 22 | 928 | 4.81 |

**Example in list, Big Difference!**

# In-Source Candidates for Sending to Lib Search for Identity

- Possible In-source spectra are noted with blue dot in Chromatogram TIC plot
- Reasonable structures ***ONLY found*** by sending spectrum to Lib search



PRE-RELEASE NIST MS Search 4.0 (Tandem mode) - [TESTMIX2_180504_MAS011_06_shortened, MS/MS, nHits=534]

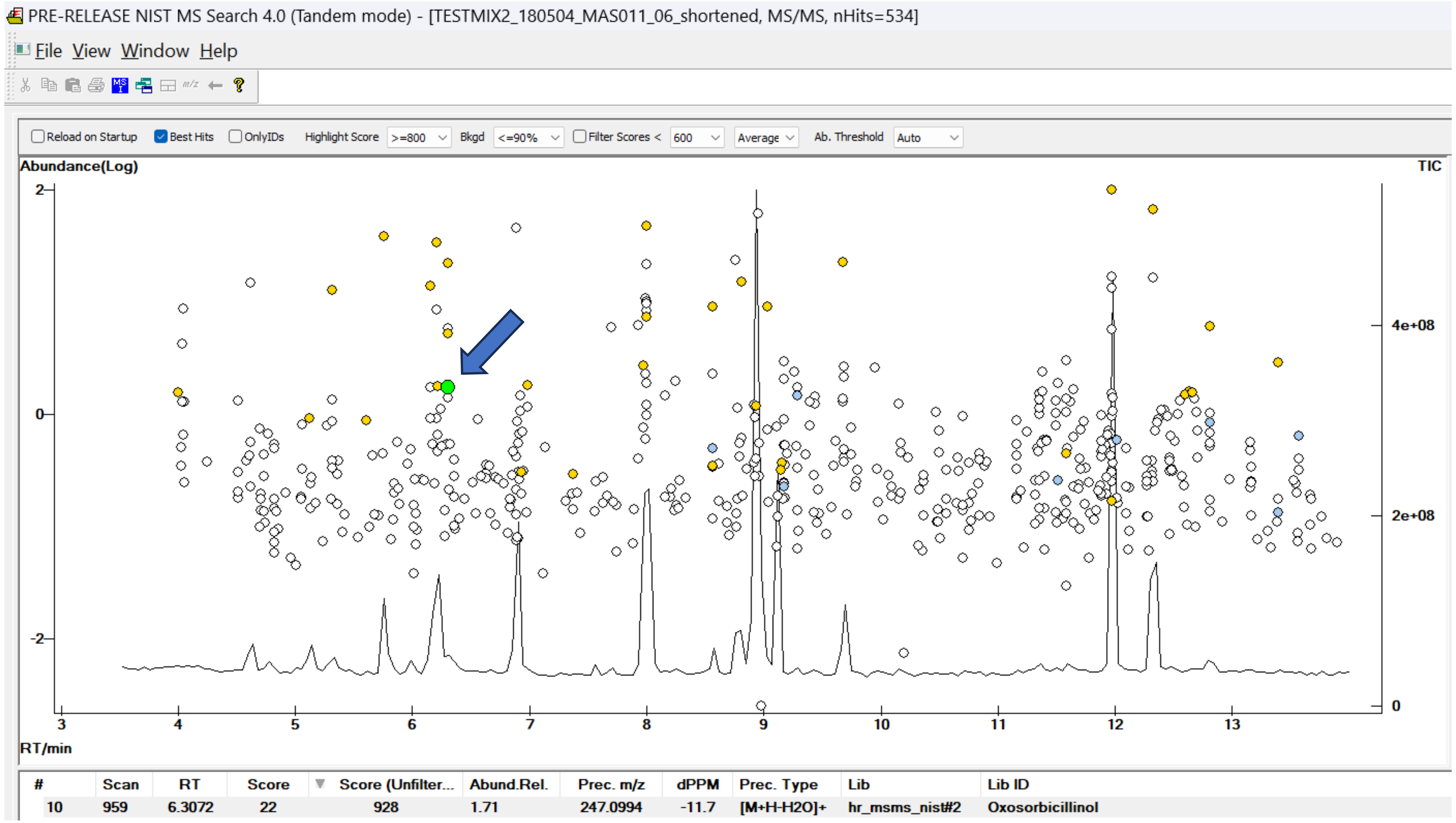
☐ Reload on Startup ☑ Best Hits ☐ OnlyIDs Highlight Score >=800 Bkgd <=90% ☐ Filter Scores < 600 Average Ab. Threshold Auto

Send to Lib Srch

# Sending In-Source Spectrum to Lib Search

- When selected with mouse, turns from blue to green to show active spectrum linked to list
- Note peak detected has very large difference in Score of 22 and Score Unfiltered of 928!

- Right click on entry and select library search

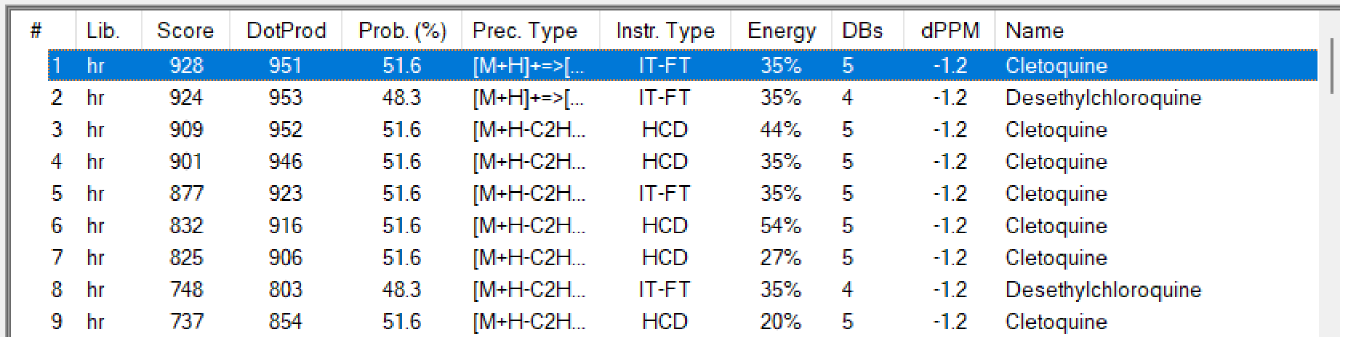


PRE-RELEASE NIST MS Search 4.0 (Tandem mode) - [TESTMIX2_180504_MAS011_06_shortened, MS/MS, nHits=534]

☐ Reload on Startup ☑ Best Hits ☐ OnlyIDs Highlight Score >=800 Bkgd <=90% ☐ Filter Scores < 600 Average Ab. Threshold Auto

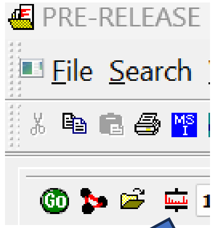
| # | Scan | RT | Score | Score (Unfilter... | Abund.Rel. | Prec. m/z | dPPM | Prec. Type | Lib | Lib ID |
|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 959 | 6.3072 | 22 | 928 | 1.71 | 247.0994 | -11.7 | [M+H-H2O]+ | hr_msms_nist#2 | Oxosorbicillinol |

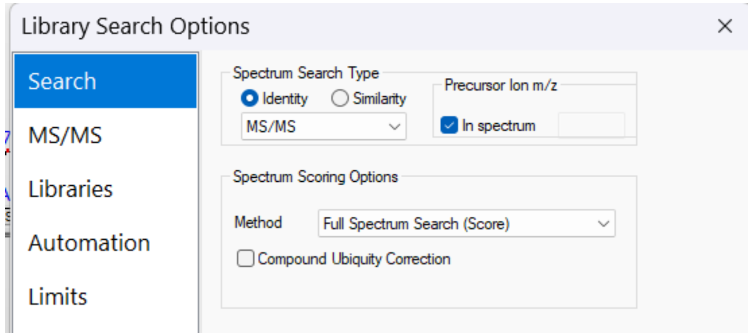
# Several *Candidates* Found in Lib Search Results from In-Source Spectrum with High Scores



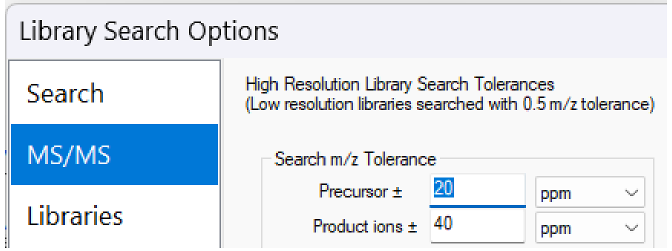
| # | Lib. | Score | DotProd | Prob. (%) | Prec. Type | Instr. Type | Energy | DBs | dPPM | Name |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | hr | 928 | 951 | 51.6 | [M+H]+=>[... | IT-FT | 35% | 5 | -1.2 | Cletoquine |
| 2 | hr | 924 | 953 | 48.3 | [M+H]+=>[... | IT-FT | 35% | 4 | -1.2 | Desethylchloroquine |
| 3 | hr | 909 | 952 | 51.6 | [M+H-C2H... | HCD | 44% | 5 | -1.2 | Cletoquine |
| 4 | hr | 901 | 946 | 51.6 | [M+H-C2H... | HCD | 35% | 5 | -1.2 | Cletoquine |
| 5 | hr | 877 | 923 | 51.6 | [M+H-C2H... | IT-FT | 35% | 5 | -1.2 | Cletoquine |
| 6 | hr | 832 | 916 | 51.6 | [M+H-C2H... | HCD | 54% | 5 | -1.2 | Cletoquine |
| 7 | hr | 825 | 906 | 51.6 | [M+H-C2H... | HCD | 27% | 5 | -1.2 | Cletoquine |
| 8 | hr | 748 | 803 | 48.3 | [M+H-C2H... | IT-FT | 35% | 4 | -1.2 | Desethylchloroquine |
| 9 | hr | 737 | 854 | 51.6 | [M+H-C2H... | HCD | 20% | 5 | -1.2 | Cletoquine |

## Make Sure Search Options Correct

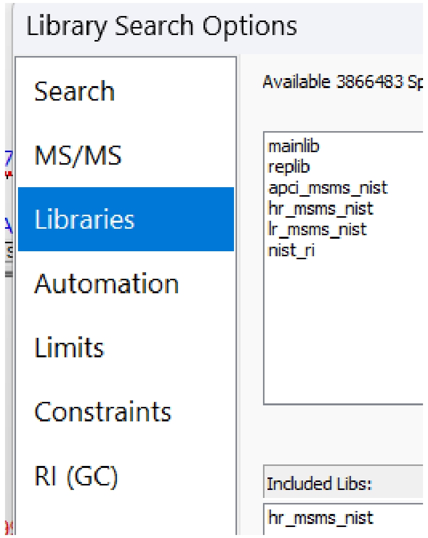




Spectrum Search Type
● Identity ○ Similarity
MS/MS
Precursor Ion m/z
☑ In spectrum
Spectrum Scoring Options
Method: Full Spectrum Search (Score)
☐ Compound Ubiquity Correction



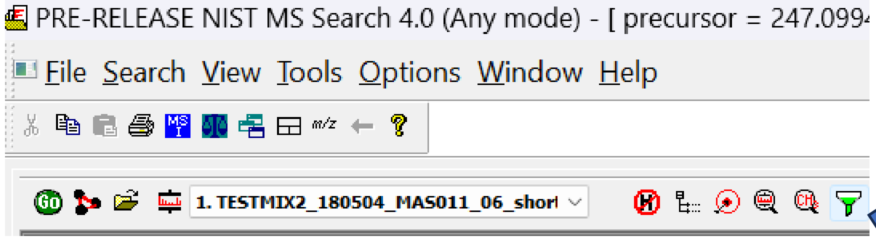
High Resolution Library Search Tolerances
(Low resolution libraries searched with 0.5 m/z tolerance)
Search m/z Tolerance
Precursor ± 20 ppm
Product ions ± 40 ppm



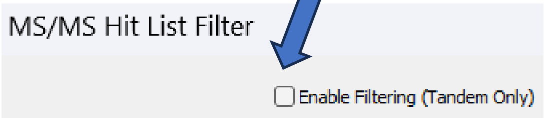
Available 3866483 Sp

mainlib
replib
apci_msms_nist
hr_msms_nist
lr_msms_nist
nist_ri

Included Libs:
hr_msms_nist



**Unclicked. off!**



☐ Enable Filtering (Tandem Only)

MS/MS Hit Filter Settings