Theoretical and Practical Understanding of XICs (E*x*tracted Ion Chromatograms)

Video/Handout

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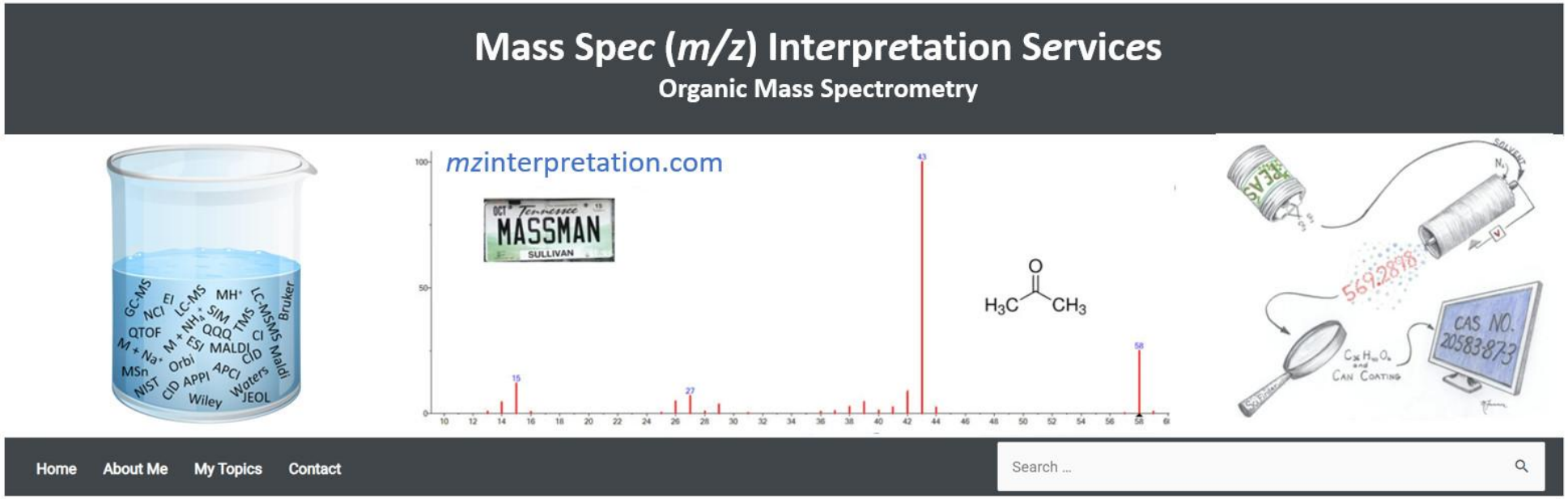

Topics in Video/Handout

Theoretical Understanding XICs (Extracted Ion Chromatograms)

An **extracted ion chromatogram (XIC)** is one of the most fundamental tools in LC-MS analysis. At its simplest, an XIC tracks the **intensity of a specific m/z value over time**, producing a chromatographic peak that represents the elution of a compound from the LC.

Traditionally, XICs have been used primarily for **quantitation**—the peak area or height reflects the abundance of a detected ion. In a typical workflow, an identification (from MS/MS) provides an m/z and retention time, and an XIC is then extracted to measure its signal.

In the NIST26 MSMS MS/MS Chromatogram windos software—the **XIC-centric approach described by Stein and co-workers**—reframes this role. Instead of treating XICs as a downstream product, the workflow becomes **bidirectional**:

- **Identification → XIC:**
- Use m/z and RT to extract the chromatogram
- **XIC → Identification validation:**
- **Full chromatographic peak (all MS1 scans)** to confirm or correct the identification with their associated MS/MS spectra

👉 This is a key conceptual shift: The XIC is not just a measurement—it is a **data-rich representation of the ion itself**.

More Detailed **Understanding XICs used in *New* Chromatogram Window in NIST Search**

In My Website Resources:

- Read Stein *et. al.* paper
- Review MSMS Workflow created by Chat GPT

Journal of proteome research

pubs.acs.org/jpr

Article

An XIC-Centric Strategy for Improved Identification and Quantification in Proteomic Data Analyses

Guanghui Wang,* Zheng Zhang, Yi Liu, Meghan C. Burke, Sergey L. Sheetlin, and Stephen E. Stein

Cite This: *J. Proteome Res.* 2024, 23, 1571−1582 Read Online

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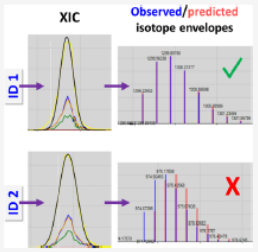
ABSTRACT: Reproducibility is a "proteomic dream" yet to be fully realized. A typical data analysis workflow utilizing extracted ion chromatograms (XICs) often treats the information path from identification to quantification as a one-way street. Here, we propose an XIC-centric approach in which the data flow is bidirectional: identifications are used to derive XICs whose information is in turn applied to validate the identifications. In this study, we employed liquid chromatography-mass spectrometry data from glycoprotein and human hair samples to illustrate the XIC-centric concept. At the core of this approach was XIC-based monoisotope repicking. Taking advantage of the intensity information for all detected isotopes across the whole range of an XIC peak significantly improved the accuracy and uncovered misidentifications originating from monoisotope assignment mistakes. It could also rescue non-top-ranked glycopeptide hits. Identification of glycopeptides is particularly susceptible to precursor mass errors for their low abundances, large masses, and glycans differing by 1 or 2 Da easily confused as isotopes. In addition, the XIC-centric strategy significantly reduced the problem of one XIC peak associated with multiple unique identifications, a source of quantitative irreproducibility. Taken together, the proposed approach can lead to improved identification and quantification accuracy and, ultimately, enhanced reproducibility in proteomic data analyses.

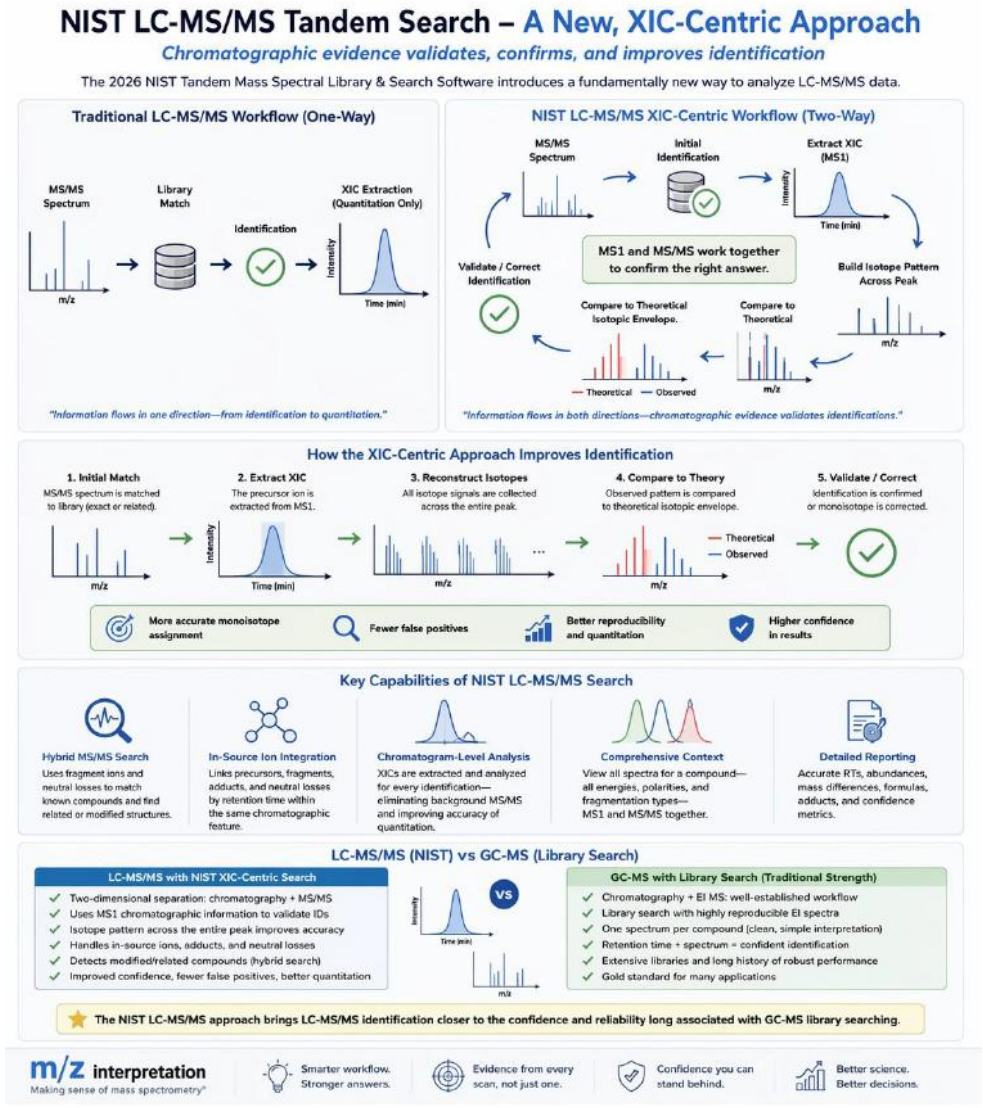


KEYWORDS: *XIC-centric, XIC, monoisotope, glycopeptide, validation, identification, quantification, reproducibility*

INTRODUCTION

Quantitative proteomics concerns discovering significant protein abundance changes at the proteome scale and plays an increasingly important role not only in basic research but also in

to represent the abundance of the identified peptide ion. Downstream analysis could include RT alignment, intensity normalization, protein abundance determination, and various statistical methods to find proteins that have significant changes



Practical Use of XICs used in *New* Chromatogram Window in NIST Search

Add some XIC Information to Properties Displayed

#	Scan	RT	Score	Score (Unfiltered)	Abund.Rel.	Prec. m/z	dPPM	Prec. Type	nSpec	Iso. Profile	Width	XIC Num.	Lib	Lib ID
1	834	2.8621	600	600	15.4	209.1287	-1.0	[M+H]+	7	1.0000	4.1	9	hr_msms_...	Aminocarb
2	1117	3.8618	318	318	14.5	202.0433	-0.0	[M+H]+	4	1.0000	3.9	7	hr_msms_...	Thiabendazole

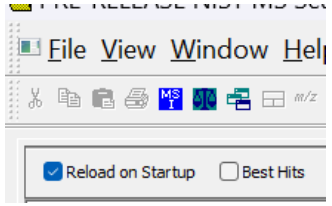
XIC Number – Sequence number of component XIC peak

nSpec – Number of MS2 spectra acquired for a component (XIC) peak

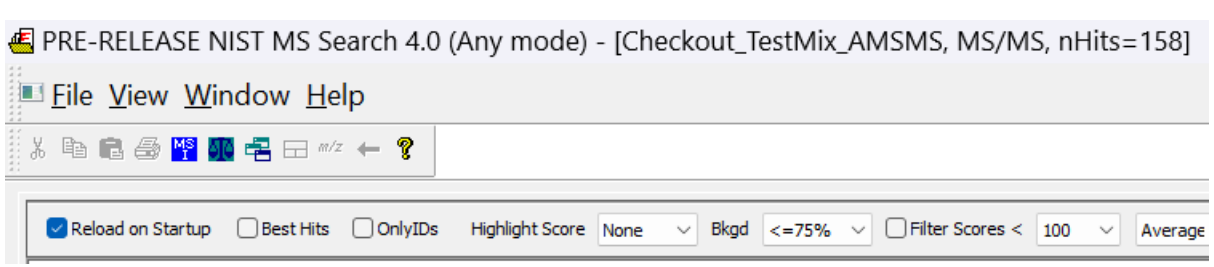
Iso.Profile – Degree of matching isotope peaks in a component peak (XIC)

Width – Width of component peak (XIC) in seconds

Turn off Best Hits on Top of Chromatogram Window

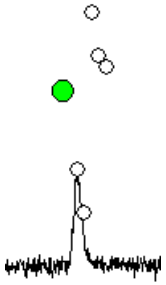


Now You Will See All the MS2's Library Searched, *158* in this case



If you look at Aminocarb for XICs, will see 7 spec (nSpec) within the *tolerance* User set for analysis and the Library ID for each from the search

#	Scan	RT	Score	Abund.Rel.	Prec. m/z	Prob	dPPM	XIC Num.	nSpec	Iso. Profile	Width	Prec. Type	Lib	Dbs	Lib ID
1	808	2.7668	1	31.3	209.1257	6	13.4	9	7	1.0000	4.1	[M+H]+	hr_msms_nist#2	17	Aminocarb
2	821	2.8166	389	53.0	209.1280	98	2.4	9	7	1.0000	4.1	[M+H]+	hr_msms_nist#2	17	Aminocarb
3	834	2.8618	600	22.1	209.1287	100	-1.0	9	7	1.0000	4.1	[M+H]+	hr_msms_nist#2	17	Aminocarb
4	848	2.9112	219	17.2	209.1289	76	-1.9	9	7	1.0000	4.1	[M+H]+	hr_msms_nist#2	17	Aminocarb
5	862	2.9644	74	40.5	209.1285	9	0.0	9	7	1.0000	4.1	[M+H]+	hr_msms_nist#2	17	Aminocarb
6	875	3.0141	43	35.4	209.1296	3	-5.3	9	7	1.0000	4.1	[M+H]+	hr_msms_nist#2	17	Aminocarb
7	888	3.0640	40	34.1	209.1289	6	-1.9	9	7	1.0000	4.1	[M+H]+	hr_msms_nist#2	17	Aminocarb



When you select best hits, ONLY, the spectrum with highest score in this "*XIC bin*"
***Thus*, gets rid of replicates in results!**

#	Scan	RT	Score	Abund.Rel.	Prec. m/z	Prob	dPPM	XIC Num.	nSpec	Iso. Profile	Width	Prec. Type	Lib	Dbs	Lib ID
1	834	2.8621	600	15.4	209.1287	100	-1.0	9	7	1.0000	4.1	[M+H]+	hr_msms_nist#2	17	Aminocarb
2	1117	3.8618	318	14.5	202.0433	54	-0.0	7	4	1.0000	3.9	[M+H]+	hr_msms_nist#2	36	Thiabendazole
3	1198	4.1932	10	0.0146	202.0432	4	0.5	8	2	0.9925	1.0	[M+H]+	hr_msms_nist#2	36	Thiabendazole
4	1344	4.6649	0	0.0130	261.1762	0	18.0	18	1	0.9906	1.2	[M+H]+	hr_msms_nist	28	Carisoprodol

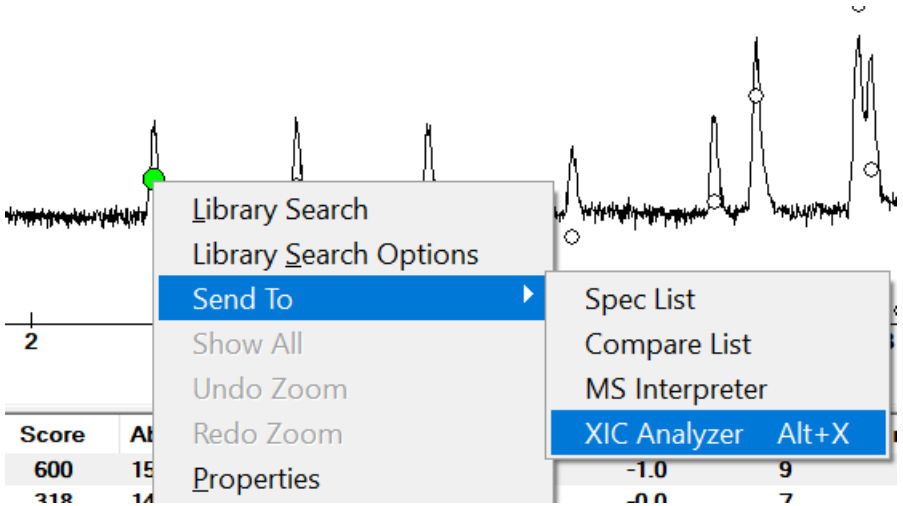
- Library Hit Not Necessarily Same for Every Spectrum in XIC bin
- When Best Hits checked, one with *highest Score Displayed* in List
- NIST Could have averaged the spectra and searched the averaged spectrum for searching then reporting
- However, their studies showed using one with *highest score superior approach for reporting and reporting*

#	Scan	RT	Score	Abund.Rel.	Prec. m/z	Prob	dPPM	XIC Num.	nSpec	Iso. Profile	Width	Prec. Type	Lib	Dbs	Lib ID
15	1350	4.7090	8	30.1	262.1238	4	-19.8	12	9	1.0000	3.5	[M+H]+	hr_msms_nist	18	Imazapyr
16	1357	4.7387	233	46.8	262.1173	83	5.0	12	9	1.0000	3.5	[M+H]+	hr_msms_nist	18	Imazapyr
17	1370	4.7839	522	21.7	262.1194	99	-3.1	12	9	1.0000	3.5	[M+H]+	hr_msms_nist	18	Imazapyr
18	1384	4.8338	177	20.7	262.1192	52	-2.3	12	9	1.0000	3.5	[M+H]+	hr_msms_nist	18	Imazapyr
19	1398	4.8870	45	31.8	262.1187	8	-0.4	12	9	1.0000	3.5	[M+H]+	hr_msms_nist	18	Imazapyr
20	1411	4.9368	1	31.6	262.1211	4	10.3	12	9	0.9957	3.5	[M+NH4]+	hr_msms_nist#2	2	2-(2'-Fluoro[1,1'-biphenyl]-4-yl)propanoic acid
21	1419	4.9699	1	30.9	262.1158	6	10.7	12	9	1.0000	3.5	[M+H]+	hr_msms_nist	5	Gly-Trp
22	1435	5.0360	0	28.6	262.1182	0	16.8	12	9	0.9983	3.5	[M+H]+	hr_msms_nist		4-([1,1'-Biphenyl]-4-yloxy)aniline

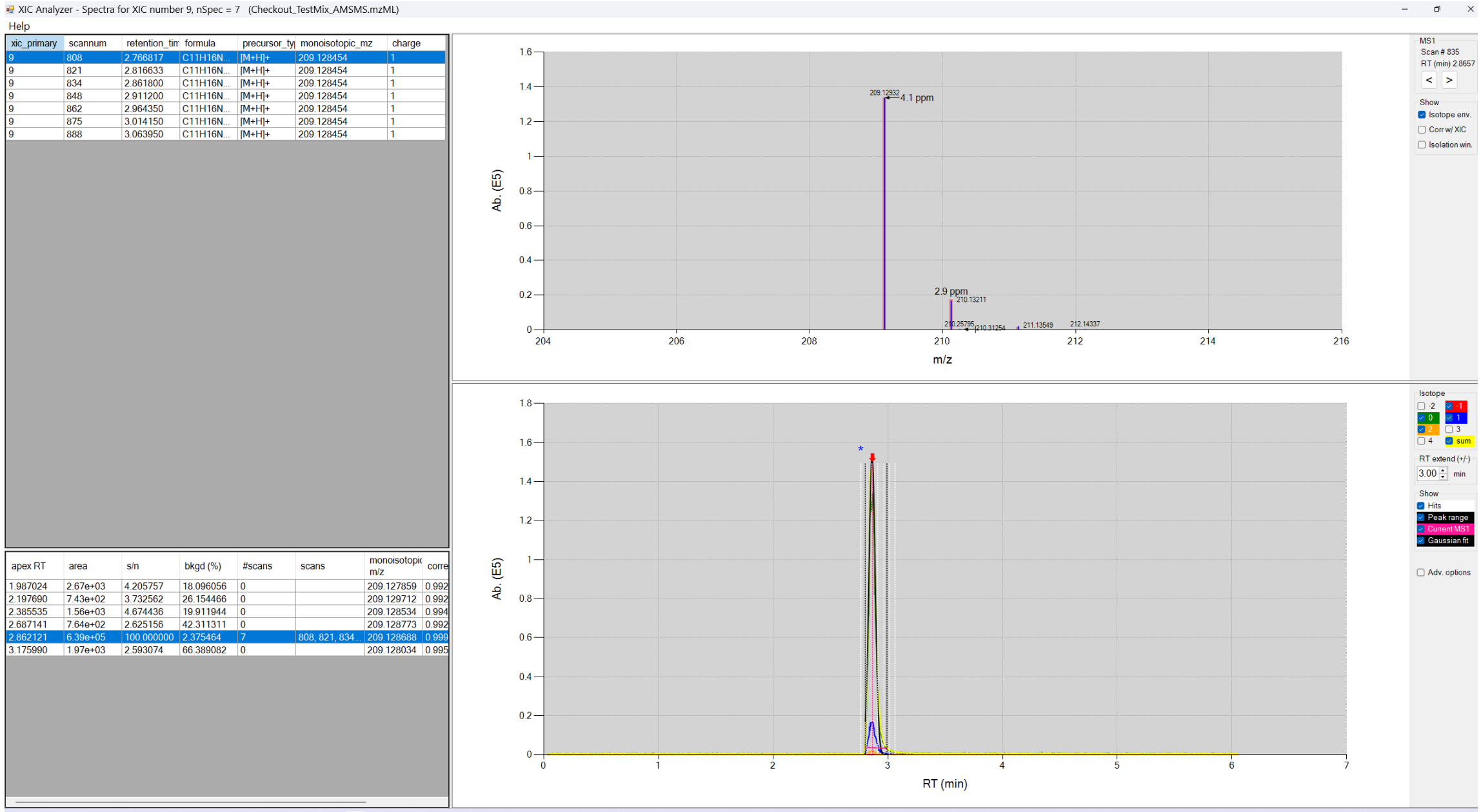
#	Scan	RT	Score	Abund.Rel.	Prec. m/z	Prob	dPPM	XIC Num.	nSpec	Iso. Profile	Width	Prec. Type	Lib	Dbs	Lib ID
1	1117	3.8618	318	14.5	202.0433	54	-0.0	7	4	1.0000	3.9	[M+H]+	hr_msms_nist#2	36	Thiabendazole
2	1198	4.1932	10	0.0146	202.0432	4	0.5	8	2	0.9925	1.0	[M+H]+	hr_msms_nist#2	36	Thiabendazole
3	834	2.8621	600	15.4	209.1287	100	-1.0	9	7	1.0000	4.1	[M+H]+	hr_msms_nist#2	17	Aminocarb
4	1370	4.7813	522	14.0	262.1194	99	-3.1	12	9	1.0000	3.5	[M+H]+	hr_msms_nist	18	Imazapyr

Very Useful to Send to XIC Analyzer via “Right Clicking” the table entry or by selecting in TIC

#	Scan	RT	Score	Abund.Rel.	Prec. m/z	Prob	dPPM	XIC Num.	nSpec	Iso. Profile	Width	Prec. Type	Lib	Dbs	Lib ID
1	834	2.8621	600	15.4	209.1287	100	-1.0	9	7	1.0000	4.1				Aminocarb
2	1117	3.8618	318	14.5	202.0433	54	-0.0	7	4	1.0000	3.9				Thiabendazole
3	1198	4.1932	10	0.0146	202.0432	4	0.5	8	2	0.9925	1.0				Thiabendazole
4	1344	4.6649	0	0.0130	261.1762	0	18.0	18	1	0.9906	1.2				
5	1370	4.7813	522	14.0	262.1194	99	-3.1	12	9	1.0000	3.5				
6	1649	5.7934	304	8.66	230.0069	94	0.0	21	11	1.0000	3.9				
7	1652	5.7967	0	0.413	251.9891	0	18.3	13	1	0.9961	2.7				
8	1923	6.7850	194	12.7	229.0740	56	-0.9	26	7	1.0000	4.1				
9	2001	7.0795	394	25.1	297.0556	98	-0.0	37	8	0.9999	3.9				
10	2017	7.1496	2	2.16	311.0698	7	2.3	40	2	0.8740	4.0				7-tert-Butyl-5,6,7,8-tetrahydrc
11	2028	7.1758	54	0.607	202.0856	4	-1.0	51	1	0.9992	4.7				Simazine
12	2075	7.3610	0	0.00579	295.9967	0	1.7	28	1	0.5502	1.4				7-Nitronaphtho[1,2-d][1,2,3]o



XIC Analyzer for Aminocarb



Information

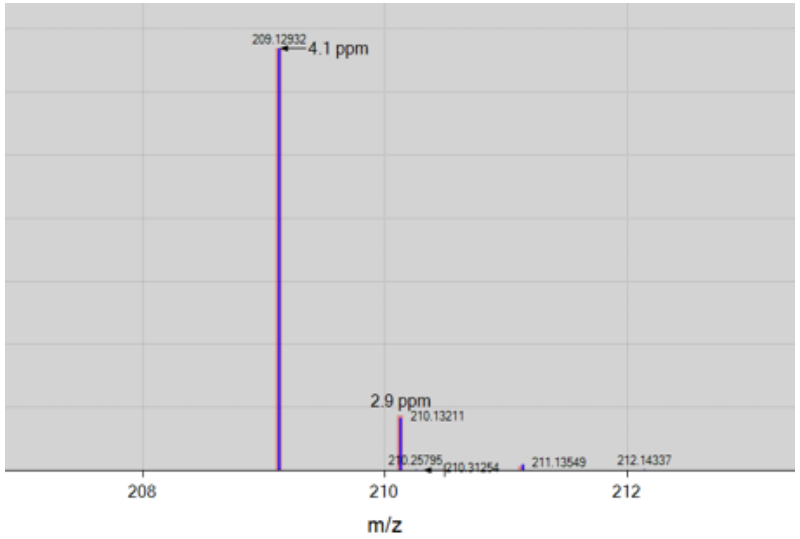
Scan Numbers included in XIC bin specified by User Tolerance

xic_primary	scannum	retention_tim	formula	precursor_ty	monoisotopic_mz	charge
9	808	2.766817	C11H16N...	[M+H]+	209.128454	1
9	821	2.816633	C11H16N...	[M+H]+	209.128454	1
9	834	2.861800	C11H16N...	[M+H]+	209.128454	1
9	848	2.911200	C11H16N...	[M+H]+	209.128454	1
9	862	2.964350	C11H16N...	[M+H]+	209.128454	1
9	875	3.014150	C11H16N...	[M+H]+	209.128454	1
9	888	3.063950	C11H16N...	[M+H]+	209.128454	1

Scans grouped together to calculate area (used for relative abundance)

apex RT	area	s/n	bkgd (%)	#scans	scans	monoisotopic m/z	correlation	group
1.987024	2.67e+03	4.205757	18.096056	0		209.127859	0.992140	1
2.197690	7.43e+02	3.732562	26.154466	0		209.129712	0.992140	1
2.385535	1.56e+03	4.674436	19.911944	0		209.128534	0.994648	1
2.687141	7.64e+02	2.625156	42.311311	0		209.128773	0.992140	1
2.862121	6.39e+05	100.000000	2.375464	7	808, 821, 834...	209.128688	0.999987	1
3.175990	1.97e+03	2.593074	66.389082	0		209.128034	0.995514	1

Isotope pattern observed versus theoretical and error



Help for XIC Browser Window

Can access by Keyboard F1 or Help on XIC Analyzer window

XIC Analyzer - Spectra for XIC number 9, nSpec = 7 (Checkout_TestMix_AMSMS.mzML)

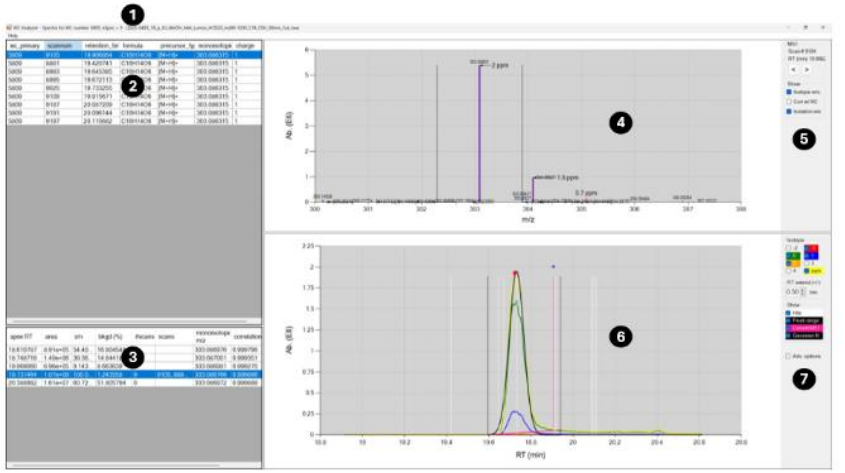
Help

xic_primary	scannum	retention_tim	formula	precursor_ty	monoisotopic_mz	charge
9	808	2.766817	C11H16N...	[M+H]+	209.128454	1
9	821	2.816633	C11H16N...	[M+H]+	209.128454	1

XIC Analyzer

XIC Analyzer

MS1 information underlying MS2 spectra



1. Title

XIC Analyzer - Spectra for XIC number 5809, nSpec = 9 (2025-0405_18_p_OJ_MeOH_Met_Lumos_HCD20_mz80-1200_C18_CSH_30min_5uL.raw)

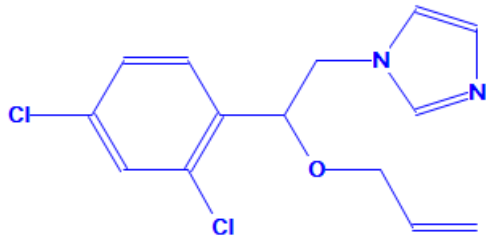
Summary: XIC#, number of MS2 spectra and file name

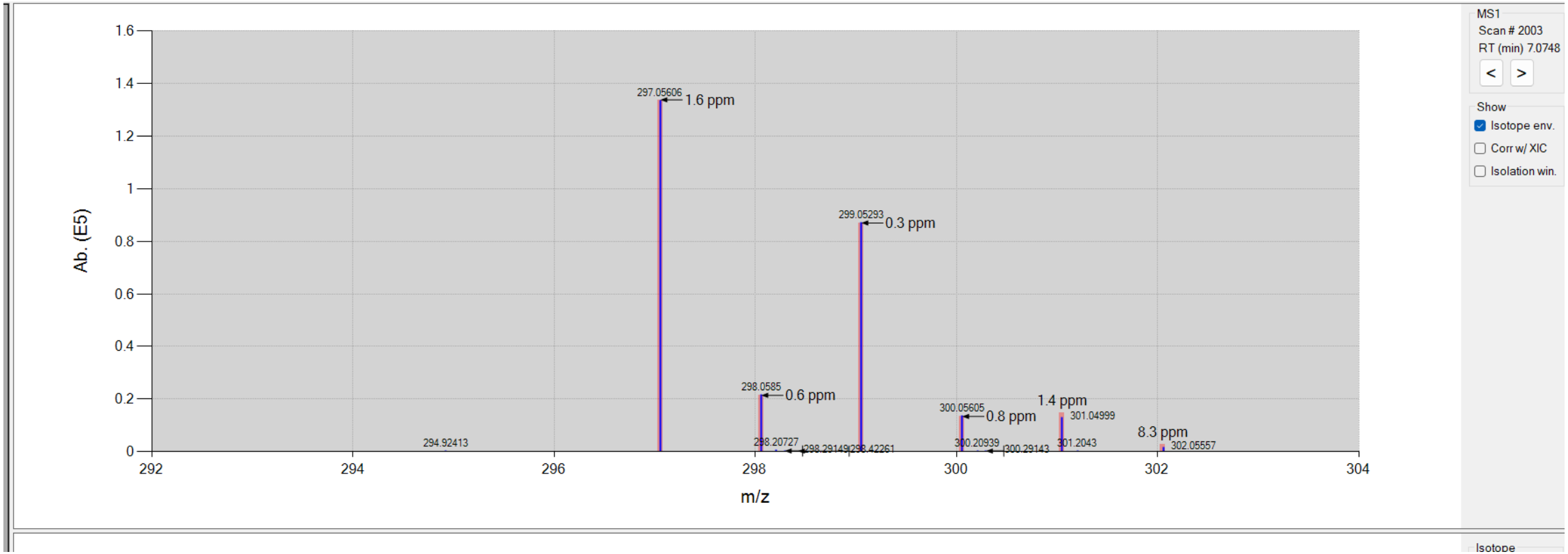
2. MS2 Spectra for current XIC

xic_primary	scannum	retention_tim	formula	precursor_type	monoisotopic	charge
5809	9105	19.906864	C16H14O6	[M+H]+	303.086315	1
5809	8881	19.420741	C16H14O6	[M+H]+	303.086315	1
5809	8983	19.645385	C16H14O6	[M+H]+	303.086315	1
5809	8995	19.672113	C16H14O6	[M+H]+	303.086315	1

XIC Analyzer especially good for confirming presence of compounds with significant isotopes such as chlorine, bromine, sulfur, etc.

#	Scan	RT	Score	Abund.Rel.	Prec. m/z	Prob	dPPM	XIC Num.	nSpec	Iso. Profile	Width	Prec. Type	Lib	Dbs	Lib ID
9	2001	7.0795	394	25.1	297.0556	98	-0.0	37	8	0.9999	3.9	[M+H]+	hr_msms_nist	26	Imazalil





Background Setting Removing XICs with Useful Identities

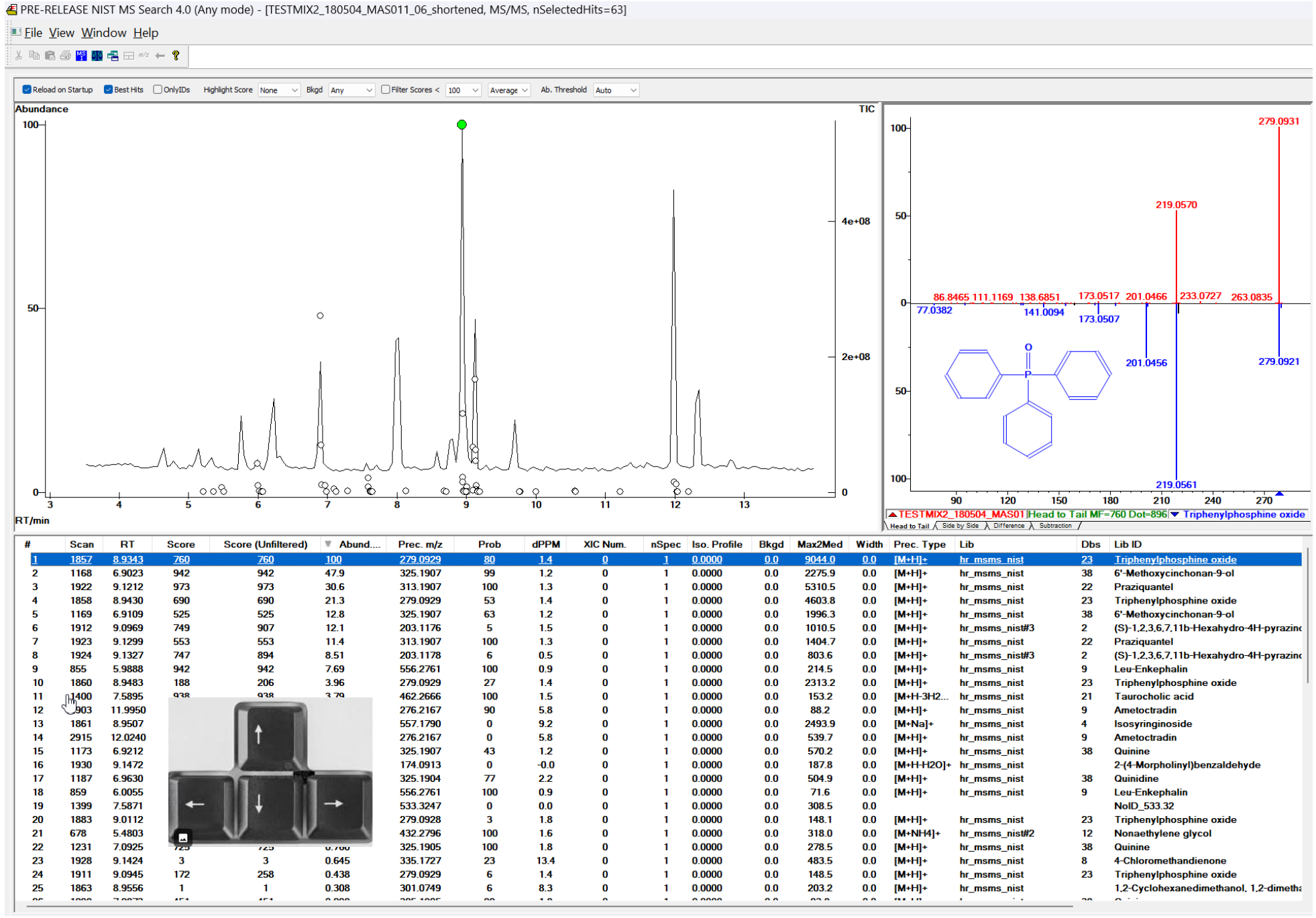
Approach

- 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***

- 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!



#	Scan	RT	Score	Score (Unfiltered)	Abund....	Prec. m/z	Prob	dPPM	XIC Num.	nSpec	Iso. Profile	Bkgd	Max2Med	Width	Prec. Type	Lib	Dbs	Lib ID
1	1857	8.9343	760	760	100	279.0929	80	1.4	0	1	0.0000	0.0	9044.0	0.0	[M+H]+	hr_msms_nist	23	Triphenylphosphine oxide
2	1168	6.9023	942	942	47.9	325.1907	99	1.2	0	1	0.0000	0.0	2275.9	0.0	[M+H]+	hr_msms_nist	38	6'-Methoxycinchonan-9-ol
3	1922	9.1212	973	973	30.6	313.1907	100	1.3	0	1	0.0000	0.0	5310.5	0.0	[M+H]+	hr_msms_nist	22	Praziquantel
4	1858	8.9430	690	690	21.3	279.0929	53	1.4	0	1	0.0000	0.0	4603.8	0.0	[M+H]+	hr_msms_nist	23	Triphenylphosphine oxide
5	1169	6.9109	525	525	12.8	325.1907	63	1.2	0	1	0.0000	0.0	1996.3	0.0	[M+H]+	hr_msms_nist	38	6'-Methoxycinchonan-9-ol
6	1912	9.0969	749	907	12.1	203.1176	5	1.5	0	1	0.0000	0.0	1010.5	0.0	[M+H]+	hr_msms_nist#3	2	(S)-1,2,3,6,7,11b-Hexahydro-4H-pyrazinc
7	1923	9.1299	553	553	11.4	313.1907	100	1.3	0	1	0.0000	0.0	1404.7	0.0	[M+H]+	hr_msms_nist	22	Praziquantel
8	1924	9.1327	747	894	8.51	203.1178	6	0.5	0	1	0.0000	0.0	803.6	0.0	[M+H]+	hr_msms_nist#3	2	(S)-1,2,3,6,7,11b-Hexahydro-4H-pyrazinc
9	855	5.9888	942	942	7.69	556.2761	100	0.9	0	1	0.0000	0.0	214.5	0.0	[M+H]+	hr_msms_nist	9	Leu-Enkephalin
10	1860	8.9483	188	206	3.96	279.0929	27	1.4	0	1	0.0000	0.0	2313.2	0.0	[M+H]+	hr_msms_nist	23	Triphenylphosphine oxide
11	1400	7.5895	938	938	3.79	462.2666	100	1.5	0	1	0.0000	0.0	153.2	0.0	[M+H-3H2...	hr_msms_nist	21	Taurocholic acid
12	903	11.9950				276.2167	90	5.8	0	1	0.0000	0.0	88.2	0.0	[M+H]+	hr_msms_nist	9	Ametoctradin
13	1861	8.9507				557.1790	0	9.2	0	1	0.0000	0.0	2493.9	0.0	[M+Na]+	hr_msms_nist	4	Isosyringinoside
14	2915	12.0240				276.2167	0	5.8	0	1	0.0000	0.0	539.7	0.0	[M+H]+	hr_msms_nist	9	Ametoctradin
15	1173	6.9212				325.1907	43	1.2	0	1	0.0000	0.0	570.2	0.0	[M+H]+	hr_msms_nist	38	Quinine
16	1930	9.1472				174.0913	0	-0.0	0	1	0.0000	0.0	187.8	0.0	[M+H-H2O]+	hr_msms_nist		2-(4-Morpholinyl)benzaldehyde
17	1187	6.9630				325.1904	77	2.2	0	1	0.0000	0.0	504.9	0.0	[M+H]+	hr_msms_nist	38	Quinidine
18	859	6.0055				556.2761	100	0.9	0	1	0.0000	0.0	71.6	0.0	[M+H]+	hr_msms_nist	9	Leu-Enkephalin
19	1399	7.5871				533.3247	0	0.0	0	1	0.0000	0.0	308.5	0.0				NoID_533.32
20	1883	9.0112				279.0928	3	1.8	0	1	0.0000	0.0	148.1	0.0	[M+H]+	hr_msms_nist	23	Triphenylphosphine oxide
21	678	5.4803				432.2796	100	1.6	0	1	0.0000	0.0	318.0	0.0	[M+NH4]+	hr_msms_nist#2	12	Nonaethylene glycol
22	1231	7.0925				325.1905	100	1.8	0	1	0.0000	0.0	278.5	0.0	[M+H]+	hr_msms_nist	38	Quinine
23	1928	9.1424	3	3	0.645	335.1727	23	13.4	0	1	0.0000	0.0	483.5	0.0	[M+H]+	hr_msms_nist	8	4-Chloromethandienone
24	1911	9.0945	172	258	0.438	279.0929	6	1.4	0	1	0.0000	0.0	148.5	0.0	[M+H]+	hr_msms_nist	23	Triphenylphosphine oxide
25	1863	8.9556	1	1	0.308	301.0749	6	8.3	0	1	0.0000	0.0	203.2	0.0	[M+H]+	hr_msms_nist		1,2-Cyclohexanedimethanol, 1,2-dimetha