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Extractable Leachable Analysis Workflow using MassHunter Explorer 2.0 with NIST and Sirius MS/MS Searching for Enhanced Compound Identification

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Introduction

New Software for Non-targeted HRMS Extractable and Leachable Data Analysis

One of the biggest challenges of doing extractable leachable (E&L) analysis is converting non-targeted high-mass resolution data into the confident identification of suspect, target and unknown compounds. Chromatographic deconvolution is one method utilized extensively for high-resolution GCMS data where fragment ions are aligned and searched against EI spectral libraries. Similar analysis for high-resolution LCMS data is more complicated since compounds can generate multiple adducts, charge states, and in-source fragment ions prior to generating any MSMS fragment ions.

Herein, we demonstrate the use of a new project-based software program, MassHunter Explorer 2.0, that extracts MS and data dependent acquisition (DDA) high-resolution MSMS data. MassHunter Explorer 2.0 uses a simple user-interface to import and data files and do chromatographic retention time (RT) correction to adjust for run-to-run variation. Unsupervised non-targeted data mining provided a means to generated chromatographically significant features with grouping of adducts (H^+, NH_4^+, Na^+, K^+), dimers, trimers, simple in-source fragments (e.g. H_2O, H_2, NH_3, C_4H_8, CO_2, etc) and isotopes. Non-targeted MSMS spectra are extracted along with the MS data by adduct and average collision energy. Statistical analysis such as pair-wise fold change, volcano plots, unique features and hierarchical clustering provides a means to quickly determine differences between groups.

Compound identification uses accurate mass and isotope peak abundances to search E&L application-focused commercial and user-generated databases and MS/MS libraries. For compounds not observed in these databases, MassHunter Explorer supports direct export of MSMS spectra for searching either NIST MS Search 3.0 or SIRIUS MSMS software.

Figure 1: Infinity II 1290 LC with Revident LC/Q-TOF.

Experimental

Extractables & Leachables from Catheters

Catheters from three suppliers were used in this study:

- Catheter A: Made from PVC, no DEHP, DINP, BPA or natural rubber latex
- Catheter B: Made with natural rubber latex base and silicone elastomer coating, no claim to be phthalate or BPA free.
- Catheter C: Made from silicone-coated natural rubber latex, no DEHP or BPA; contained in secondary plastic packaging bag

The catheters were extracted with 20 mL of pH 2.5 water, pH 10 water, or 50:50 ethanol: water at 37 °C for 24, 48 and 162 hours. The additional bag from catheter C was extracted using 50:50 ethanol: water to check for secondary migration. The extracts were all analyzed using a 1290 Infinity II HPLC system coupled to the Revident LC/Q-TOF (Figure 1, Table 1).

Non-targeted DDA MSMS data was collected using multiple collision energies (10, 20, 40 V); and both medium ion and narrow ion isolations mode. Triplicate Injections using iterative active exclusion was utilized to enhance the collection of MSMS over wide concentration range.

Table 1. Revident LC/Q-TOF Method Parameters

Parameter	Value
Column	Agilent Poroshell AQ-C18 2.1 x 100 mm
Column temperature	40 °C
Injection volume	1 µL
Flow rate	0.35 mL/min
Mobile Phase	A: Water, 2.5 mM NH_4Formate, 0.05% FA B: Methanol, 2.5 mM NH_4Formate, 0.05% FA
LC Gradient	2% B for 1.0 min; to 100% B at 16.0; hold 100% B to 28.0 min; to 2% at 28.1 min post time 5 min
MS Source	Dual AJS ESI
MS Mode	Auto MS/MS, positive polarity
Collison Energy	20; 40
MS Range	40-1700 *m/z*
Acquisition Rate	MS: 4 spectra/s, MSMS: 6 spectra/s
Drying Gas	250 °C, 11 L/min
Nebulizer Pressure	35 psi
Sheath Gas	300 °C, 11 L/min
Capillary Voltage	3500 V

Results and Discussion

MassHunter Explorer 2.0 Streamlines E&L Analysis Workflows for LC/Q-TOF

An overview of the LC/Q-TOF workflow for E&L analysis is shown in Figure 2. The LC/Q-TOF workflow for E&L analysis consists of untargeted analysis, identification using MassHunter Explorer 2.0 software, and optional exporting of remaining unknowns to either NIST MS Search 3.0 or SIRIUS for further identification (Figure 2).

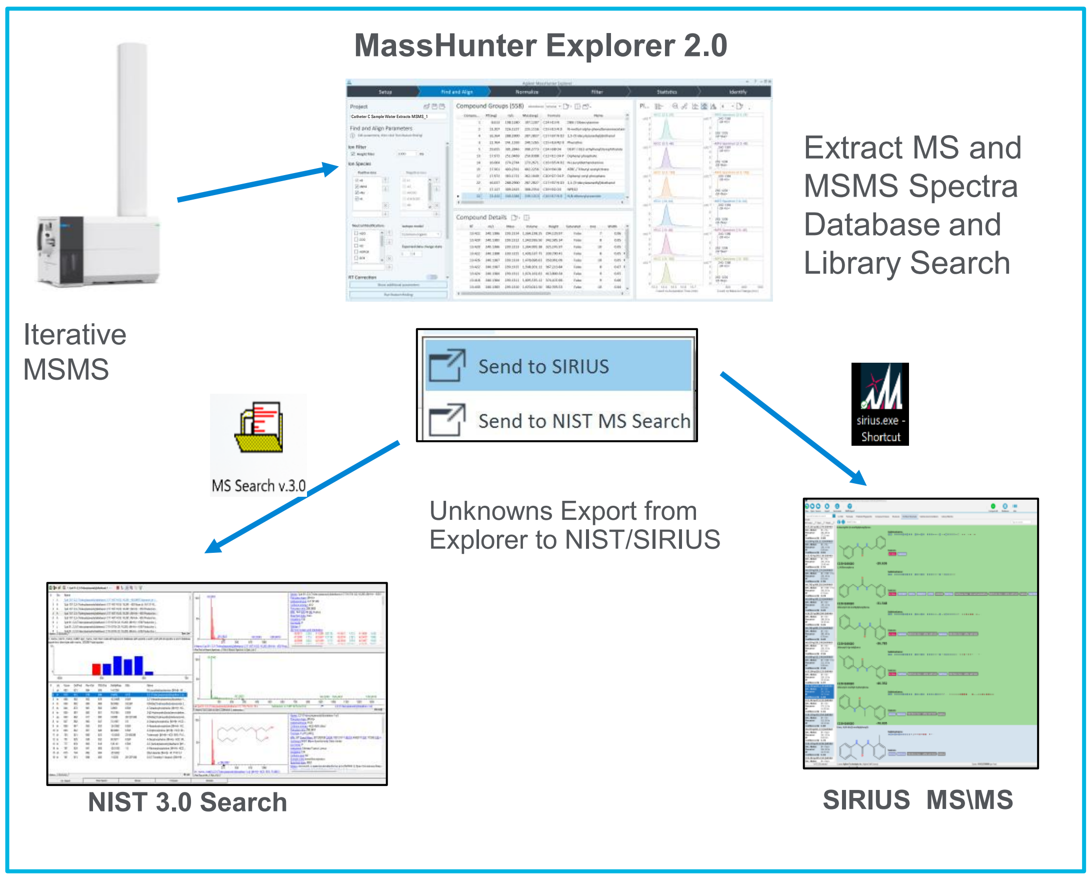
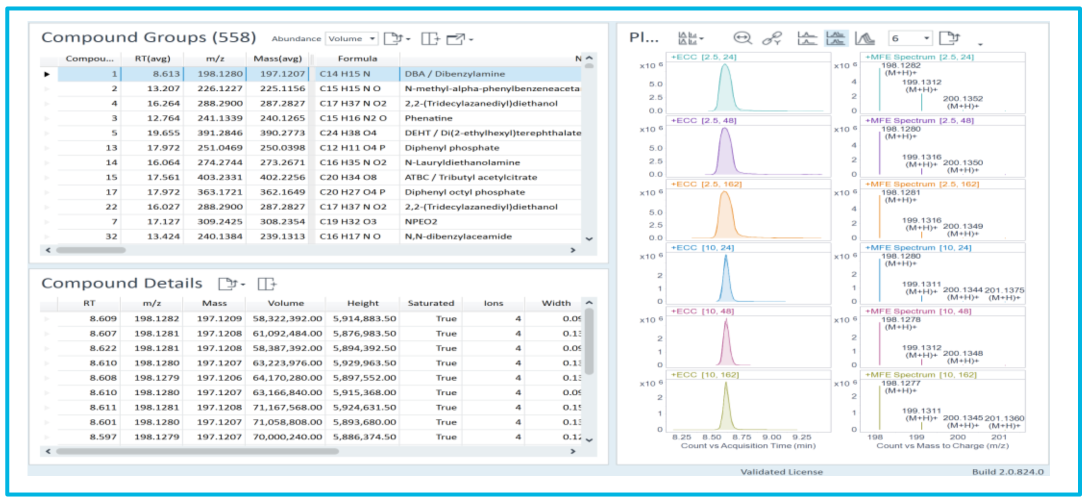


Figure 2 Data Analysis Workflow

LC/Q-TOF MS and MS/MS Data Processing in MassHunter Explorer 2.0

MassHunter Explorer 2.0 can now extract both MS and MS/MS high-resolution mass spectra by RT, accurate mass, and abundance. The MSMS spectra are extracted by precursor mass (adducts) as average of collision energies with the option for deisotoping to simplify database searching. Results from the "Find and Align" step is shown below in Figure 3.



Compound Groups (558)

Compou...	RT(avg)	m/z	Mass(avg)	Formula	N
1	8.613	198.1280	197.1207	C14 H15 N	DBA / Dibenzylamine
2	13.207	226.1227	225.1156	C15 H15 N O	N-methyl-alpha-phenylbenzeneaceta
4	16.264	288.2900	287.2827	C17 H37 N O2	2,2-(Tridecylazanediyl)diethanol
3	12.764	241.1339	240.1265	C15 H16 N2 O	Phenatine
5	19.655	391.2846	390.2773	C24 H38 O4	DEHT / Di(2-ethylhexyl)terephthalate
13	17.972	251.0469	250.0398	C12 H11 O4 P	Diphenyl phosphate
14	16.064	274.2744	273.2671	C16 H35 N O2	N-Lauryldiethanolamine
15	17.561	403.2331	402.2256	C20 H34 O8	ATBC / Tributyl acetylcitrate
17	17.972	363.1721	362.1649	C20 H27 O4 P	Diphenyl octyl phosphate
22	16.027	288.2900	287.2827	C17 H37 N O2	2,2-(Tridecylazanediyl)diethanol
7	17.127	309.2425	308.2354	C19 H32 O3	NPEO2
32	13.424	240.1384	239.1313	C16 H17 N O	N,N-dibenzylaceamide

Compound Details

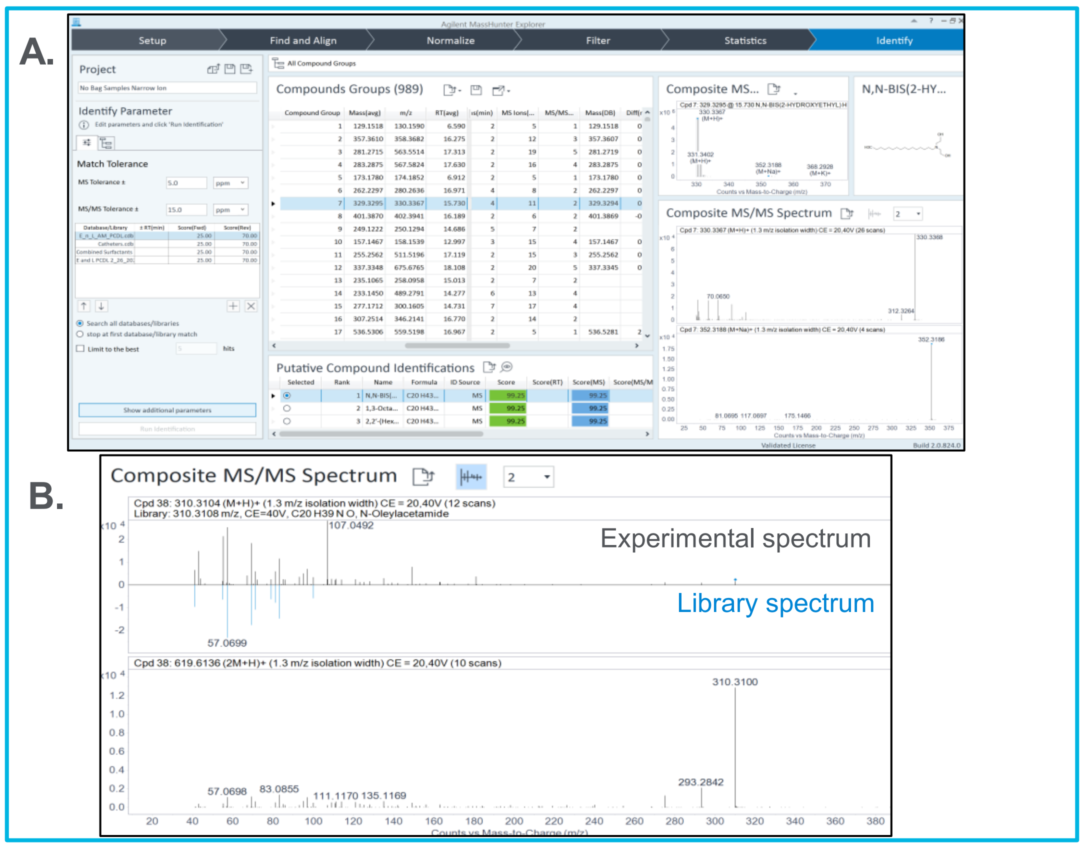
RT	m/z	Mass	Volume	Height	Saturated	Ions	Width
8.609	198.1282	197.1209	58,322,392.00	5,914,883.50	True	4	0.0
8.607	198.1281	197.1208	61,092,484.00	5,876,983.50	True	4	0.1
8.622	198.1281	197.1208	58,387,392.00	5,894,392.50	True	4	0.0
8.610	198.1280	197.1207	63,223,976.00	5,929,963.50	True	4	0.1
8.608	198.1279	197.1206	64,170,280.00	5,897,552.00	True	4	0.1
8.610	198.1280	197.1207	63,166,840.00	5,915,368.00	True	4	0.0
8.611	198.1281	197.1208	71,167,568.00	5,924,631.50	True	4	0.1
8.601	198.1280	197.1207	71,058,808.00	5,893,680.00	True	4	0.1
8.597	198.1279	197.1207	70,000,240.00	5,886,374.50	True	4	0.1

Validated License Build 2.0.824.0

Figure 3. Results from "Find and Align" Function in MassHunter Explorer 2.0.

Compound Identification in MassHunter Explorer 2.0 Utilizes MS and MSMS Data

Batch compound identification in MassHunter Explorer 2.0 leverages both the MS and MSMS spectra, when available, to match MS-level results based on accurate mass, isotope fidelity, and optional retention time. For compounds with MSMS data, the extracted MSMS data is then searched against the MSMS library spectra based on MSMS tolerance, and forward and reverse scoring, with example library search results shown in Figure 4.



Cpd 38: 310.3104 (M+H)+ (1.3 m/z isolation width) CE = 20.40V (12 scans)
Library: 310.3108 m/z, CE=40V, C20 H39 N O, N-Oleylacetamide

Cpd 38: 619.6136 (2M+H)+ (1.3 m/z isolation width) CE = 20.40V (10 scans)

Figure 4A. MassHunter Explorer 2.0 Identification lists library search results, composite MS spectrum, proposed structure, putative compound ID, and the composite MSMS spectra. B) For the MSMS spectra with a library match, mirror plots comparing experimental and library spectra are shown.

Development of E&L LC/Q-TOF Focused Database

Compound identification in E&L is hindered by the lack of commercial E&L focused databases. Agilent released the first commercial E&L based database that contained 1000+ compounds including over 360 with corresponding MS/MS spectra in 2017. Even large LC-MSMS search libraries like NIST, only contain a very small number of E&L focused compounds compared to metabolomics and environmental application space (Figure 5).

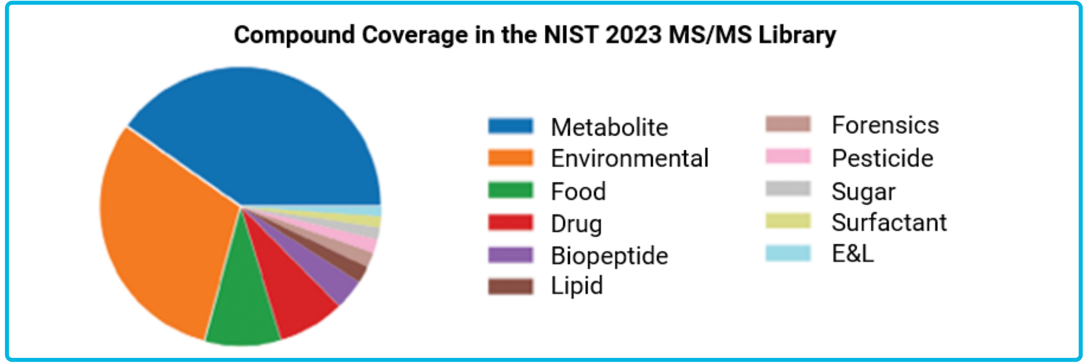
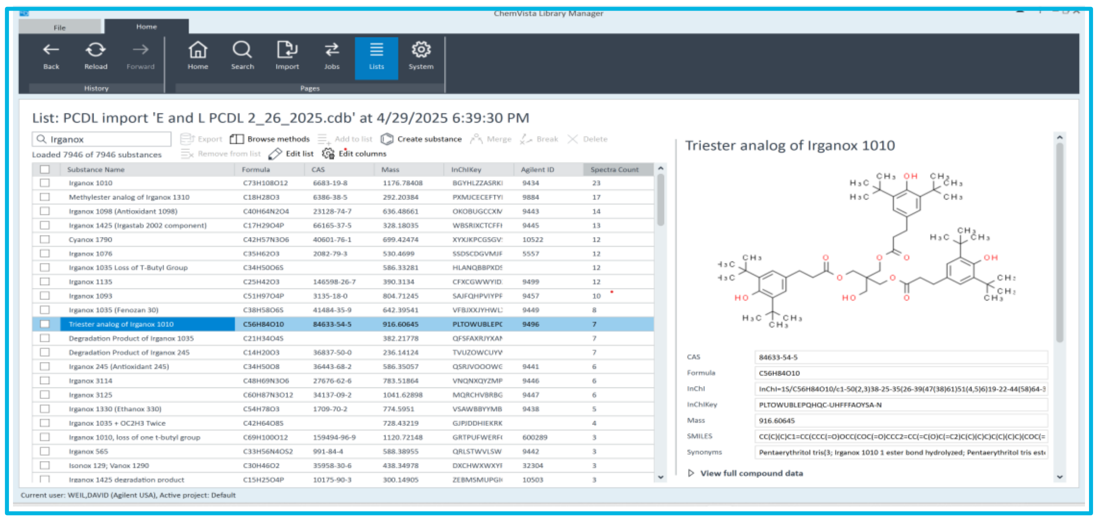


Figure 5. Application Coverage of NIST 2023 Search

Results and Discussion

Agilent ChemVista Combines Existing 3rd Party Data for Easy Library Building

An alternative approach is to build E&L focused database using information available from resources such as the EPA Comptox dashboard, ELSIE, SciFinder™ and the MassBank of North America (MONA). The Agilent ChemVista database and library management software provides a means to easily import, organize and export databases with chemical identifiers (CAS, SMILES, InChI), MSMS spectra, and RT (Figure 5). Compound lists with MSMS spectra can be exported as *.SDF files and then converted and used as custom database in NIST MS Search 3.0 software. One can also export a list of compounds with SMILES structures in *.SDF format that can be used by the SIRIUS MSMS search software.



List: PCDL import 'E and L PCDL 2_26_2025.cdb' at 4/29/2025 6:39:30 PM

Irganox

Loaded 7946 of 7946 substances

☐	Substance Name	Formula	CAS	Mass	InChIKey	Agilent ID	Spectra Count
☐	Irganox 1010	C73H108O12	6683-19-8	1176.78408	BGYHLZZASRKI	9434	23
☐	Methylester analog of Irganox 1310	C18H28O3	6386-38-5	292.20384	PXMJCECEFTYI	9884	17
☐	Irganox 1098 (Antioxidant 1098)	C40H64N2O4	23128-74-7	636.48661	OKOBUGCCXN	9443	14
☐	Irganox 1425 (Irgastab 2002 component)	C17H29O4P	66165-37-5	328.18035	WBSRIXCTCFFI	9445	13
☐	Cyanox 1790	C42H57N3O6	40601-76-1	699.42474	XYXJKPCGSGV:	10522	12
☐	Irganox 1076	C35H62O3	2082-79-3	530.4699	SSDSCDGVMJF	5557	12
☐	Irganox 1035 Loss of T-Butyl Group	C34H50O6S		586.33281	HLANQBBPXDS		12
☐	Irganox 1135	C25H42O3	146598-26-7	390.3134	CFXCGWWYID:	9499	12
☐	Irganox 1093	C51H97O4P	3135-18-0	804.71245	SAJFQHPVIYPF	9457	10
☐	Irganox 1035 (Fenozan 30)	C38H58O6S	41484-35-9	642.39541	VFBJXXJYHWL:	9449	8
☐	Triester analog of Irganox 1010	C56H84O10	84633-54-5	916.60645	PLTOWUBLEPC	9496	7
☐	Degradation Product of Irganox 1035	C21H34O4S		382.21778	QFSFAXRJYXAN		7
☐	Degradation Product of Irganox 245	C14H20O3	36837-50-0	236.14124	TVUZOWCUYV		7
☐	Irganox 245 (Antioxidant 245)	C34H50O8	36443-68-2	586.35057	QSRJVOOOWG	9441	6
☐	Irganox 3114	C48H69N3O6	27676-62-6	783.51864	VNQNXQYZMP	9446	6
☐	Irganox 3125	C60H87N3O12	34137-09-2	1041.62898	MQRCHVBRBG	9447	6
☐	Irganox 1330 (Ethanox 330)	C54H78O3	1709-70-2	774.5951	VSAWBBYYMB	9438	5
☐	Irganox 1035 + OC2H3 Twice	C42H64O8S		728.43219	GJPJDDHIEKRK		4
☐	Irganox 1010, loss of one t-butyl group	C69H100O12	159494-96-9	1120.72148	GRTPUFWERF(	600289	3
☐	Irganox 565	C33H56N4OS2	991-84-4	588.38955	QRLSTWVLSW	9442	3
☐	Isonox 129; Vanox 1290	C30H46O2	35958-30-6	438.34978	DXCHWXWXYI	32304	3
☐	Irganox 1425 degradation product	C15H25O4P	10175-90-3	300.14905	ZEBMSMUPGI	10503	3

Triester analog of Irganox 1010

CAS: 84633-54-5
Formula: C56H84O10
InChI: InChI=1S/C56H84O10/c1-50(2,3)38-25-35(26-39(47(38)61)51(4,5)6)19-22-44(58)64-3
InChIKey: PLTOWUBLEPQHQC-UHFFFAOYSA-N
Mass: 916.60645
SMILES: CC(C)(C)C1=CC(CCC(=O)OCC(COC(=O)CCC2=CC(=C(O)C(=C2)C(C)(C)C)C(C)(C)C)(COC(=
Synonyms: Pentaerythritol tris(3; Irganox 1010 1 ester bond hydrolyzed; Pentaerythritol tris est

Current user: WEIL,DAVID (Agilent USA), Active project: Default

Figure 6. ChemVista database and library software.

Streamlined Unknowns Identification with Direct Export from Explorer 2.0 to NIST MS Search 3.0

Exporting of compounds directly from Explorer 2.0 into NIST 2023 program provides a quick means to identify compounds using Hybrid MSMS Impurity Tolerant or Partial Spectrum searching. In Figure 7A, we show the MSMS search of *m/z* 280.2636 correctly identifies adduct as $[M+H]^+$ corresponding Linoleamide $C_{18}H_{33}NO$. Second example, in Figure 7B, has *m/z* 310.2378 corresponding to $[M+NH_4]^+$ of $C_{18}H_{28}O_3$ Methyl 3-(3,5-di-tert-butyl4-hydroxylphenyl) propanoate, a possible breakdown product.

A.

1. Cpd Grp 6, M = 262.2297, RT = 16.971, m/z

#	Src.	Name
1.	A	Cpd Grp 6, M = 262.2297, RT = 16.971, m/z = 263.237, (M+H)+, No Bag Samples Narrow Ion
1.	A	Cpd Grp 6, M = 262.2297, RT = 16.971, m/z = 280.2636, (M+NH4)+, No Bag Samples Narr...
1.	A	Cpd Grp 7, M = 329.3295, RT = 15.73, m/z = 330.3367, (M+H)+, No Bag Samples Narrow Ion
1.	A	Cpd Grp 7, M = 329.3295, RT = 15.73, m/z = 352.3188, (M+Na)+, No Bag Samples Narrow...
1.	A	Cpd Grp 8, M = 401.387, RT = 16.189, m/z = 402.3941, (M+H)+, No Bag Samples Narrow Ion
2.	A	Cpd Grp 8, M = 401.387, RT = 16.189, m/z = 424.3759, (M+Na)+, No Bag Samples Narrow...

hr_msms_nist; apci_msms_nist; apci_msms_nist; hr_msms_nist#2; lr_msms_nist; apci_msms_nist; applied_lcqtof_chemvista_output; chem vista sdf original enl database with spectra; 2411692 total spectra

Lib.	Score	DotProd	Rev-Dot	PSS-Dot	DeltaMass	Name
hr	962	983	974	951	0.0001	Linoleamide [M+H]+ HCD 60% P=280.3
ap	954	957	968	949	-2.0155	Oleamide [M+H]+ QTOF 25V P=282.3
hr	923	927	960	916	27.0474	ω-6-Hexadecenlactone [M+H]+ HCD 44% P=253.2
hr	909	916	939	908	0.9954	9,10-Methyleneoctadecanoic acid [M+H-H2O]+ HCD 44...
ap	909	917	960	907	15.0110	Elaidic Acid [M+H-H2O]+ QTOF 30V P=265.3
hr	897	909	958	906	43.0423	cis-9-Hexadecenoic acid [M+H-H2O]+ HCD 65% P=237.2
ap	908	916	951	906	15.0110	Oleic acid [M+H-H2O]+ QTOF 25V P=265.3
hr	911	916	950	906	17.0267	6-Oxooctadecanoic acid [M+H-2H2O]+ HCD 54% P=263.2
hr	910	916	968	905	15.0110	cis-Vaccenic acid [M+H-H2O]+ HCD 60% P=265.3
hr	908	915	967	905	15.0110	trans-Vaccenic acid [M+H-H2O]+ HCD 60% P=265.3
hr	908	914	948	905	0.9954	cis-10-Nonadecenoic acid [M+H-H2O]+ HCD 60% P=27...
hr	896	905	938	903	15.0110	(Z)-6-Octadecenoic acid [M+H-H2O]+ HCD 60% P=265.3
hr	904	911	949	902	15.0110	Oleic acid methyl ester [M+H-CH4O]+ HCD 60% P=265.3

B.

1. Cpd Grp 29, M = 292.2039, RT = 17.478, m/

#	Src.	Name
69	A	Cpd Grp 29, M = 292.2039, RT = 17.478, m/z = 310.2378, (M+NH4)+, No Bag Samples Na...
70	A	Cpd Grp 29, M = 292.2039, RT = 17.478, m/z = 315.1932, (M+Na)+, No Bag Samples Narr...
71	A	Cpd Grp 29, M = 292.2039, RT = 17.478, m/z = 331.1669, (M+K)+, No Bag Samples Narro...
72	A	Cpd Grp 30, M = 358.3236, RT = 17.365, m/z = 359.3309, (M+H)+, No Bag Samples Narro...

hr_msms_nist; apci_msms_nist; apci_msms_nist; apci_msms_nist; hr_msms_nist#2; lr_msms_nist; 2385492 total spectra

Lib.	S...	Dot...	R...	PSS-...	D...	Name
hr	8...	912	992	890	14.01...	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propanoic acid [M+NH4]+ HCD 40% P=296.2
hr	8...	899	978	877	14.01...	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propanoic acid [M+NH4]+ HCD 45% P=296.2
hr	8...	889	967	867	14.01...	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propanoic acid [M+NH4]+ HCD 35% P=296.2
hr	7...	832	905	812	14.01...	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propanoic acid [M+NH4]+ HCD 30% P=296.2
hr	7...	874	985	852	14.01...	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propanoic acid [M+NH4]+ HCD 50% P=296.2
hr	6...	733	797	715	14.01...	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propanoic acid [M+NH4]+ HCD 25% P=296.2
hr	6...	844	951	823	14.01...	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propanoic acid [M+NH4]+ HCD 60% P=296.2
hr	5...	636	691	620	14.01...	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propanoic acid [M+NH4]+ HCD 20% P=296.2
hr	4...	613	716	601	14.01...	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propanoic acid [M+NH4]+ IT-FT 35% P=296.2
hr	6...	867	969	846	0.0001	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propanoate [M+NH4]+ HCD 60% P=310.
hr	6...	879	969	857	0.0001	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propanoate [M+NH4]+ HCD 40% P=310.
hr	6...	891	982	869	0.0001	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propanoate [M+NH4]+ HCD 45% P=310.
hr	6...	823	908	803	0.0001	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propanoate [M+NH4]+ HCD 35% P=310.
hr	6...	868	980	847	0.0001	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propanoate [M+NH4]+ HCD 50% P=310.

Figure 7. Results from NIST MSMS Search

https://www.agilent.com/en/promotions/asms

Unknowns Identification using Sirius MSMS Search

A major problem in E&L compound identification based on formulas alone is that results are possibly biased towards compounds present in a database but not confirmed with RT or MSMS library matching. An issue compounded by the limited number of MSMS spectra, including very few from structural isomers, available in commercial databases.

A major advantage of SIRIUS over NIST searching is that SIRIUS will recalculate molecular formulas using MS and MSMS data, correlate MSMS data with predicted molecular fingerprints, generate fragmentation trees, and propose structures from online and custom databases or generate De Novo structures. Using SIRIUS, the presence of several sulfur containing compounds were identified in the EtOH:Water 7-day extract (Catheter C) that were not detected using the NIST 2023 search. Elemental compositions and tentative structures (some de novo generated) are shown below in Figure 8.

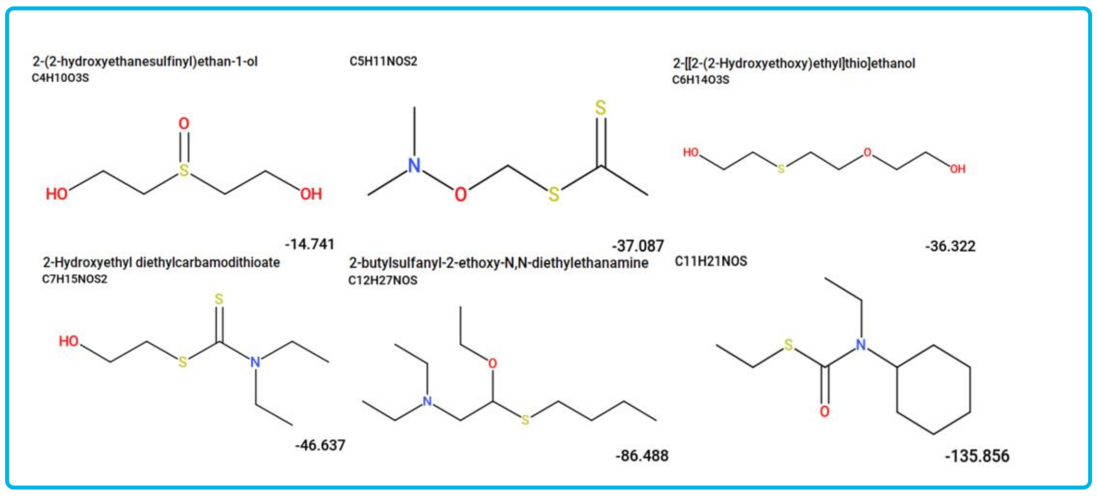


Figure 8. Proposed structures for sulfur compounds in Catheter C EtOH:Water extract

Conclusions

MassHunter Explorer 2.0 provides a streamlined workflow that improves productivity by:

- Processing of both MS and MSMS mass spectral data
- MSMS searching using custom databases/libraries
- Streamlining export of MSMS spectra to both NIST2023 MSMS Search and SIRIUS MSMS
- Importing results using ChemVista Software

References

1. Nature BioTechnology, 40, 411-421 (2022)
2. Bright Giant, Sirius Version 6.1.1 (Jan. 2025)

COI: David A. Weil, Sierra D. Durham, James Pyke, and Matt Curtis are employees of Agilent Technologies.

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