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Excellence in Science

Monitoring of PFAS by LC-QTOF: Streamlined Workflow for the Non-research Environmental Laboratories

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Today’s presentation

1. HRMS for non-R&D labs
2. Experimental plan
3. Results
4. Take-home messages
5. Q&A

HRMS for the non-research labs

- High resolution mass spectrometry (HRMS) instruments are the best tool for analyzing unknown and suspected PFAS;
- Data processing required for extracting meaningful results has traditionally been the bottleneck of analytical workflows:

  Non-research laboratories:
  - quick turn-around times are required
  - HRMS experts may not be present

Easy workflows that enable screening and quantitation are essential

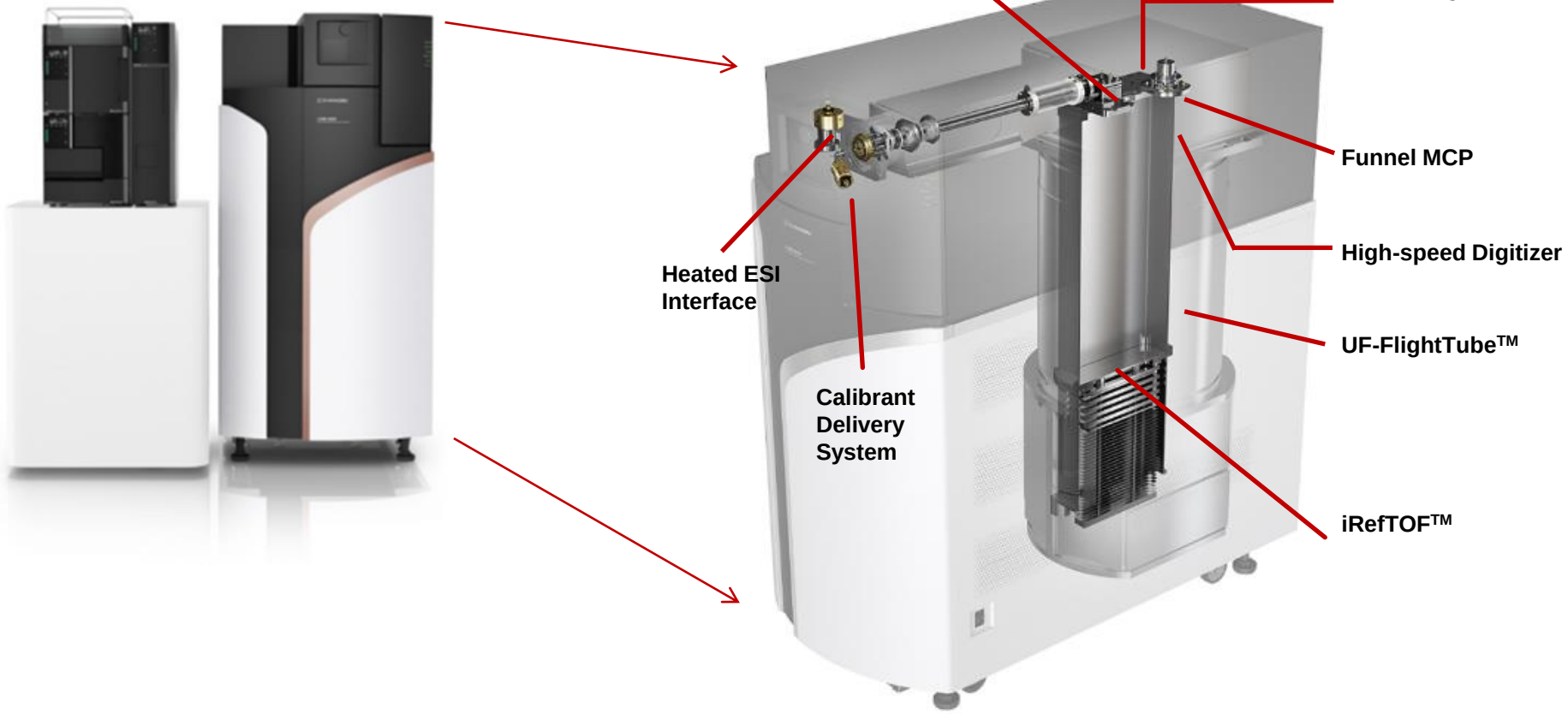
LCMS-9030 and LCMS-9050

LCMS IT-TOF
(2004)

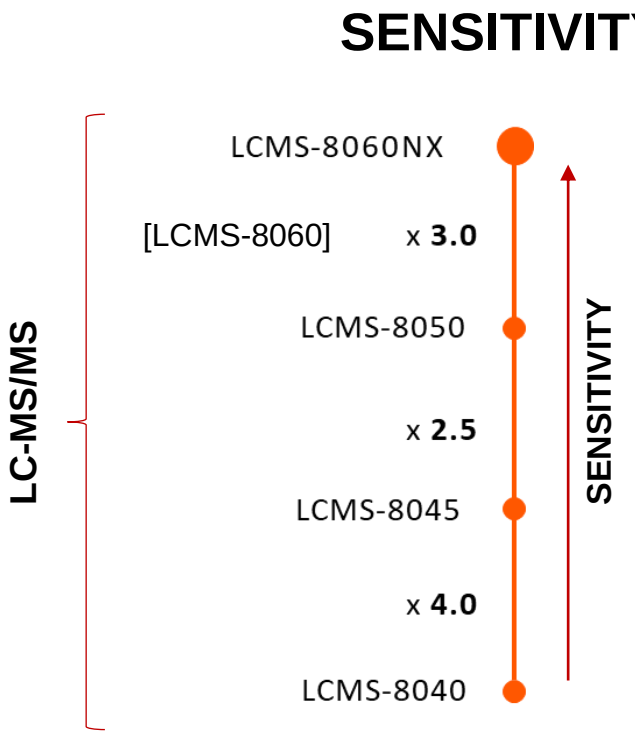
LCMS-9030
(QTOF)

LCMS-9030
(QTOF)

LCMS-9030 and LCMS-9050



PFAS and the quest for sensitivity

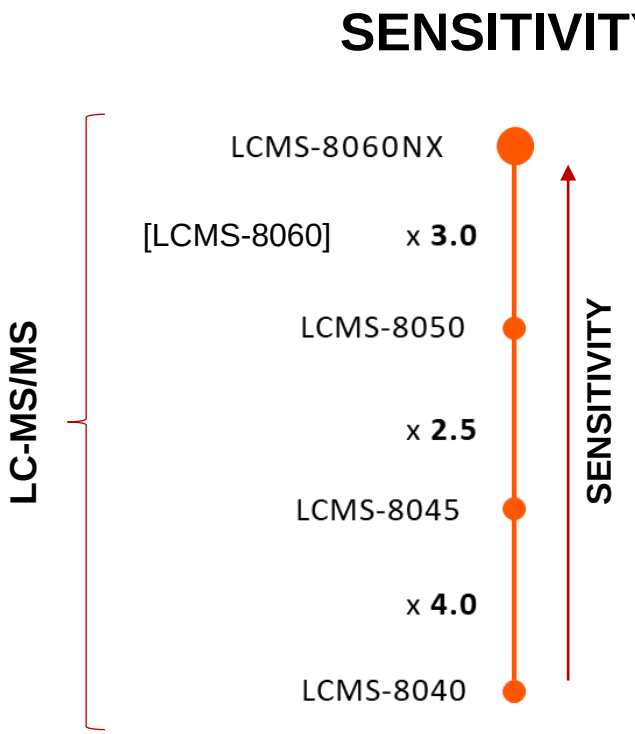


QTOF
9030 & 9050

Compound	Proposed MCLG	Proposed MCL (enforceable levels)
PFOA	Zero	4.0 parts per trillion (also expressed as ng/L)
PFOS	Zero	4.0 ppt
PFNA	1.0 (unitless) Hazard Index	1.0 (unitless) Hazard Index
PFHxS		
PFBS		
HFPO-DA (commonly referred to as GenX Chemicals)		

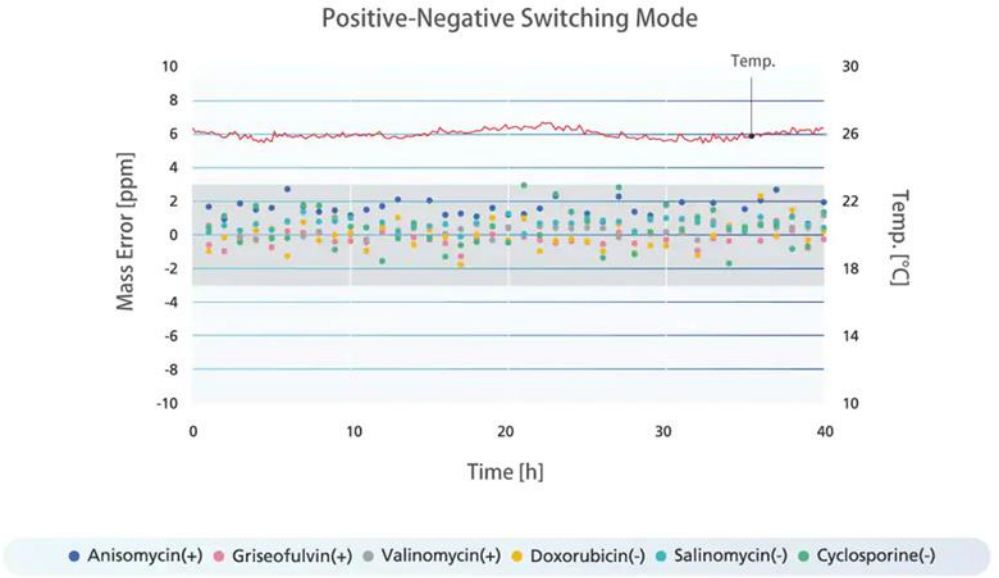
All LCMS manufacturers offer instruments designed to achieve different sensitivity

PFAS and other parameters of interest



QTOF
9030 & 9050(*)

- Polarity Switching (fast)
- Acquisition Rate (fast)
- Mass accuracy (stable long term)



(*)LCMS-9050: pol switching 800 ms; 200 Hz (200 MS/MS spectra per second)

All LCMS manufacturers offer instruments designed to achieve different sensitivity

Experimental plan

Goal: To validate an accessible workflow for the analysis of known and suspected PFAS, using targets included in EPA 1633 as reference.

Nexera LC		LCMS-9030	
Flow Rate:	0.25 mL/min	Nebulizing Gas:	3 L/min
Oven Temp.:	45 °C	Drying Gas:	5 L/min
Injection Vol.:	1 µL	Heating Gas:	15 L/min
Mobile Phase:	A: 5 mM Ammonium acetate in water B: Methanol	Desolvation Temp.:	160 °C
Delay Column:	Shim-pack Scepter C18-120 (2.1 × 50 mm; 3 µm)	DL Temp.:	150 °C
Analytical Column:	Shim-pack Scepter C18-120 (2.1 × 100 mm; 3 µm)	Heat Block Temp.:	250 °C
Gradient:	0 min %B = 5; 1 min %B = 40; 8 min %B = 95; 8.1 min %B = 100; 13 min %B = 100; 13.1 min %B = 5; 18 min %B = 5	Interface Temp:	100 °C
		Probe Position:	+1 mm
		Interface:	ESI

Experimental plan

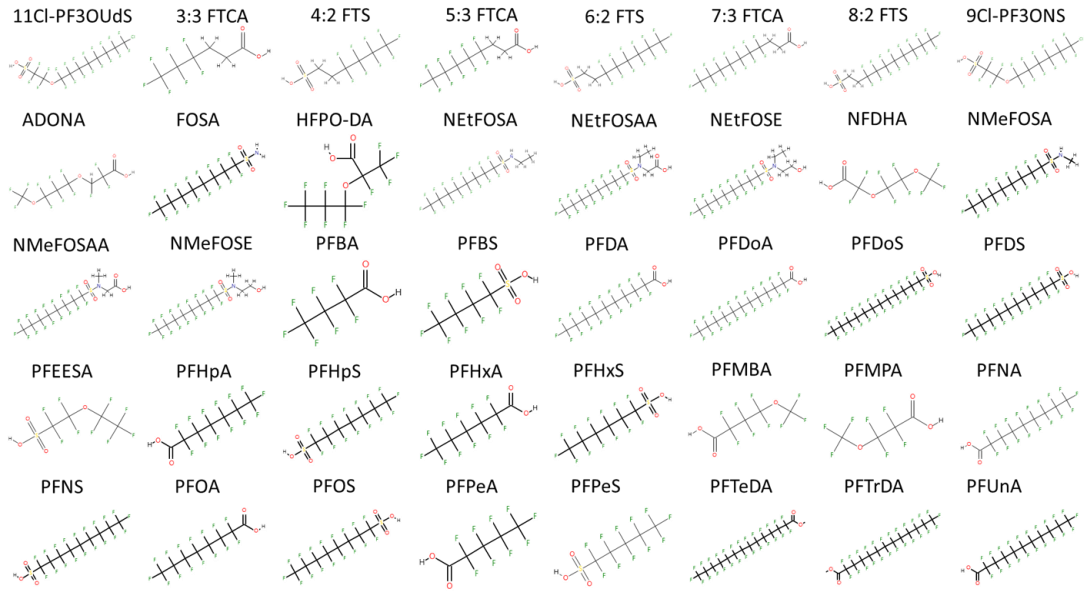
Goal: To validate an accessible workflow for the analysis of known and suspected PFAS, using targets included in EPA 1633 as reference.

Step 1 – Library creation and curation

Step 2 – Suspects screening

Step 3 – Quantitation

LabSolutions and LabSolutions Insight Explore software were used to acquire and process the data



Library Creation and Curation

1. Acquire MS1 scan of EPA 1633 Standard Mix to identify precursors and RT
2. Acquire MS/MS spectra to create library entries:
   - CE 5-55 V
   - CE 15-65 V
3. Library curation:
   1) With Assign:
      - confirm all fragments match theoretical fragmentation, and
      - realign fragmentation pattern

      Other open-source resources can be used for fragmentation matching
   2) With Explore: confirm m/z of fragments, precursor and RT

Suspects Screening

- EPA 1633 standard mix spiked into ground water, known to be contaminated with several classes of organic compounds
- Data acquired using Data Independent Acquisition (DIA) mode
- Results evaluated against the in-house curated library

Targets Quantification

- Based on EPA 537.1
- Comparison of LCMS-9030 and LCMS-8045

Table 1: LC parameters

LC System	Nexera-X2 UHPLC System
Analytical Column	Shim-pack ™ Velox , 150mm x 2.1mm x 2.7µm, Part No. 227-320094-04
Solvent Delay Column	Shim-pack XR-ODS 50mm x 2mm x 2.2µm, Part No. 228-41605-93
Column Temp.	40 °C
Injection Volume	5 µL
Mobile Phase	A: 20 mM Ammonium Acetate B: Methanol
Flow Rate	0.25 mL/min
Run Time	35 minutes

Table 2: LCMS parameters

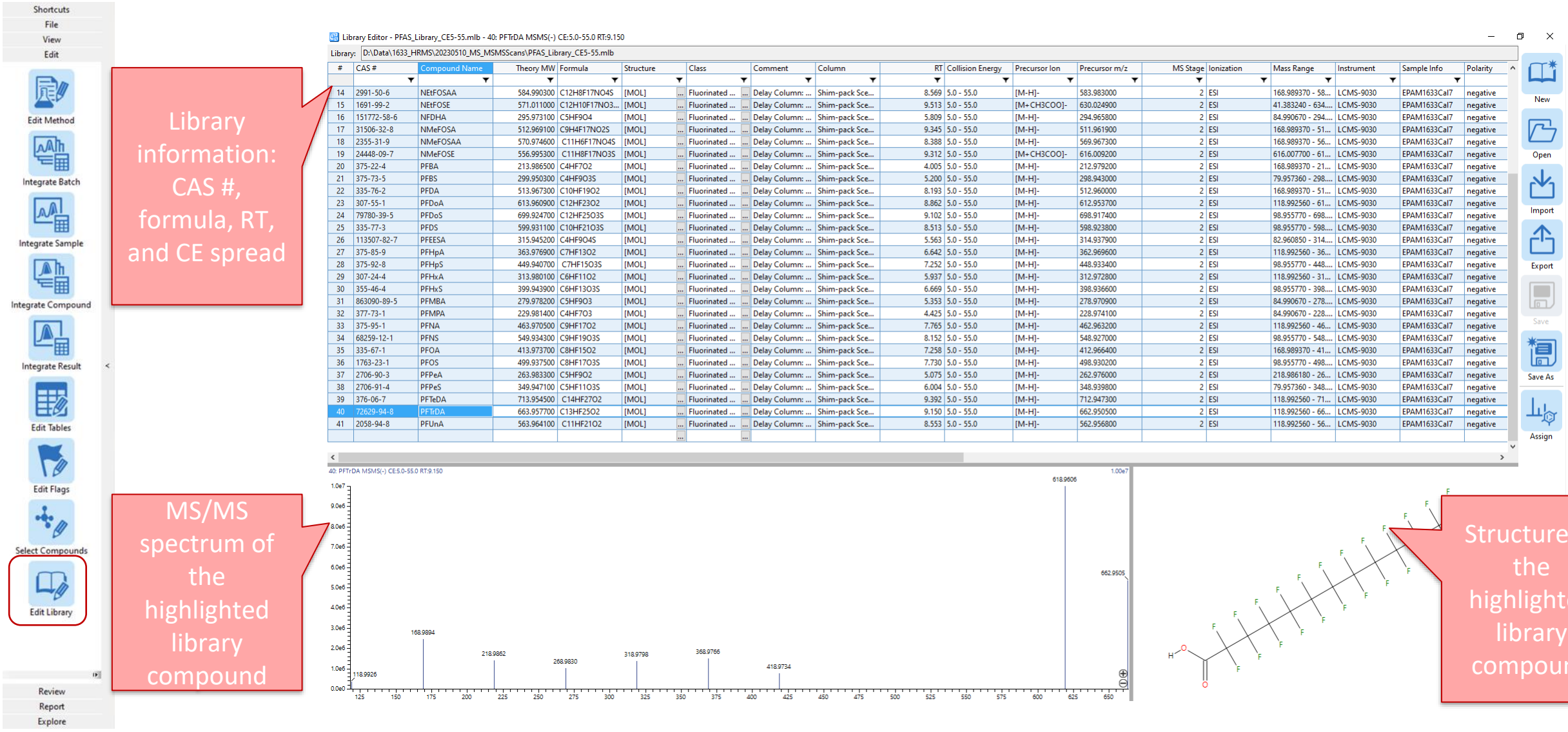
MS Instrument	LCMS-8045 and LCMS-9030
Interface	Electrospray Ionization (ESI) Negative mode
Interface Temp.	100 °C
Desolvation Line Temp.	100 °C
Heat Block Temp.	200 °C
Heating Gas Flow	15 L/min
Drying Gas Flow	5 L/min
Nebulizing Gas Flow	3 L/min
Total MRMs	48

Results – PFAS Library Creation and Curation

Library information: CAS #, formula, RT, and CE spread

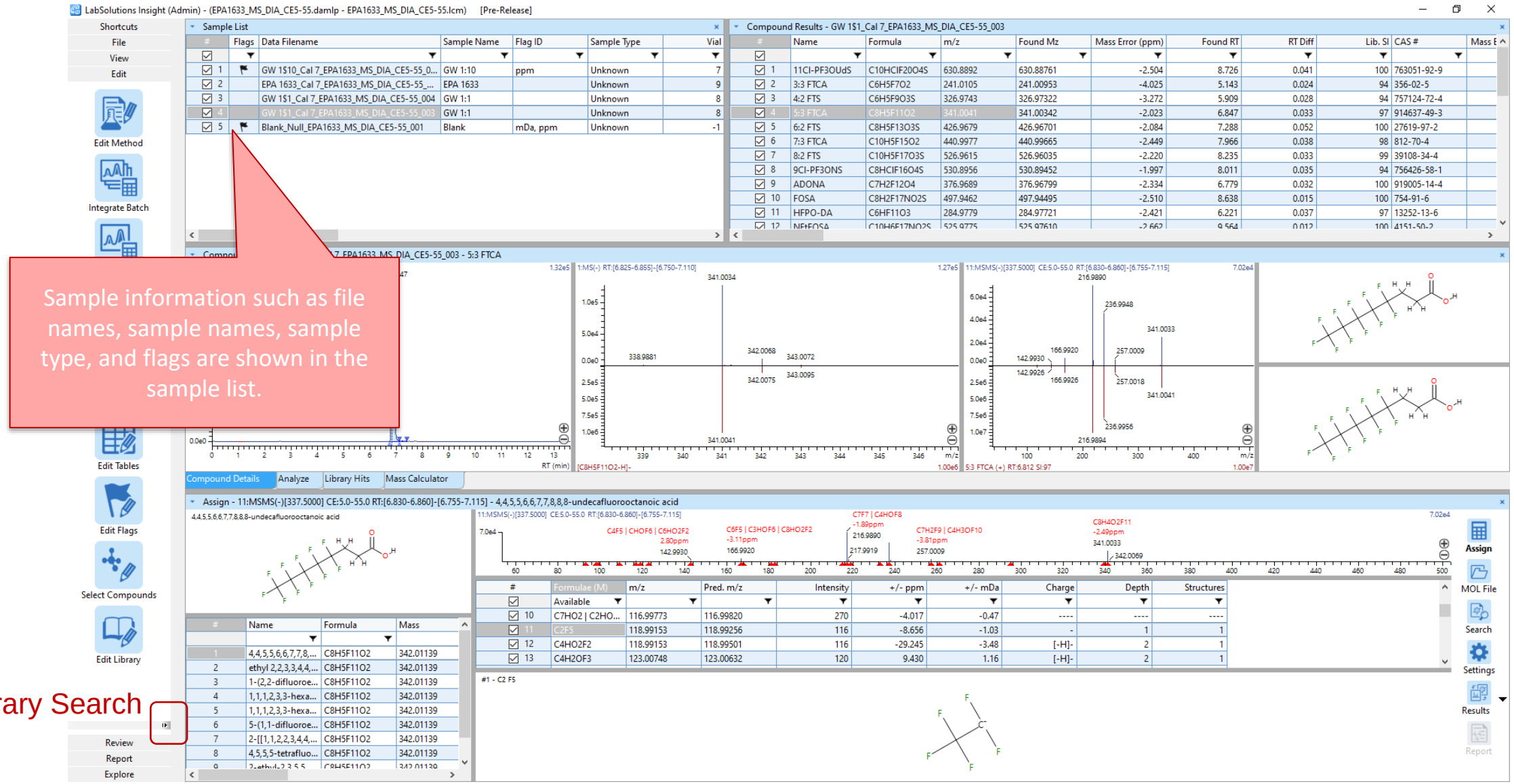
MS/MS spectrum of the highlighted library compound

Structure of the highlighted library compound



#	CAS #	Compound Name	Theory MW	Formula	Structure	Class	Comment	Column	RT	Collision Energy	Precursor Ion	Precursor m/z	MS Stage	Ionization	Mass Range	Instrument	Sample Info	Polarity
14	2991-50-6	NEtFOSAA	584.990300	C12H8F17NO4S	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	8.569	5.0 - 55.0	[M-H]-	583.983000	2	ESI	168.989370 - 58...	LCMS-9030	EPAM1633Cal7	negative
15	1691-99-2	NEtFOSE	571.011000	C12H10F17NO3...	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	9.513	5.0 - 55.0	[M+CH3COO]-	630.024900	2	ESI	41.383240 - 634....	LCMS-9030	EPAM1633Cal7	negative
16	151772-58-6	NFDHA	295.973100	C5HF9O4	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	5.809	5.0 - 55.0	[M-H]-	294.965800	2	ESI	84.990670 - 294....	LCMS-9030	EPAM1633Cal7	negative
17	31506-32-8	NMeFOSA	512.969100	C9H4F17NO2S	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	9.345	5.0 - 55.0	[M-H]-	511.961900	2	ESI	168.989370 - 51...	LCMS-9030	EPAM1633Cal7	negative
18	2355-31-9	NMeFOSAA	570.974600	C11H6F17NO4S	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	8.388	5.0 - 55.0	[M-H]-	569.967300	2	ESI	168.989370 - 56...	LCMS-9030	EPAM1633Cal7	negative
19	24448-09-7	NMeFOSE	556.995300	C11H8F17NO3S	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	9.312	5.0 - 55.0	[M+CH3COO]-	616.009200	2	ESI	616.007700 - 61...	LCMS-9030	EPAM1633Cal7	negative
20	375-22-4	PFBA	213.986500	C4HF7O2	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	4.005	5.0 - 55.0	[M-H]-	212.979200	2	ESI	168.989370 - 21...	LCMS-9030	EPAM1633Cal7	negative
21	375-73-5	PFBS	299.950300	C4HF9O3S	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	5.200	5.0 - 55.0	[M-H]-	298.943000	2	ESI	79.957360 - 298....	LCMS-9030	EPAM1633Cal7	negative
22	335-76-2	PFDA	513.967300	C10HF19O2	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	8.193	5.0 - 55.0	[M-H]-	512.960000	2	ESI	168.989370 - 51...	LCMS-9030	EPAM1633Cal7	negative
23	307-55-1	PFDoA	613.960900	C12HF23O2	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	8.862	5.0 - 55.0	[M-H]-	612.953700	2	ESI	118.992560 - 61...	LCMS-9030	EPAM1633Cal7	negative
24	79780-39-5	PFDoS	699.924700	C12HF25O3S	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	9.102	5.0 - 55.0	[M-H]-	698.917400	2	ESI	98.955770 - 698....	LCMS-9030	EPAM1633Cal7	negative
25	335-77-3	PFDS	599.931100	C10HF21O3S	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	8.513	5.0 - 55.0	[M-H]-	598.923800	2	ESI	98.955770 - 598....	LCMS-9030	EPAM1633Cal7	negative
26	113507-82-7	PFEESA	315.945200	C4HF9O4S	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	5.563	5.0 - 55.0	[M-H]-	314.937900	2	ESI	82.960850 - 314....	LCMS-9030	EPAM1633Cal7	negative
27	375-85-9	PFHpA	363.976900	C7HF13O2	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	6.642	5.0 - 55.0	[M-H]-	362.969600	2	ESI	118.992560 - 36...	LCMS-9030	EPAM1633Cal7	negative
28	375-92-8	PFHpS	449.940700	C7HF15O3S	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	7.252	5.0 - 55.0	[M-H]-	448.933400	2	ESI	98.955770 - 448....	LCMS-9030	EPAM1633Cal7	negative
29	307-24-4	PFHxA	313.980100	C6HF11O2	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	5.937	5.0 - 55.0	[M-H]-	312.972800	2	ESI	118.992560 - 31...	LCMS-9030	EPAM1633Cal7	negative
30	355-46-4	PFHxS	399.943900	C6HF13O3S	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	6.669	5.0 - 55.0	[M-H]-	398.936600	2	ESI	98.955770 - 398....	LCMS-9030	EPAM1633Cal7	negative
31	863090-89-5	PFMBA	279.978200	C5HF9O3	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	5.353	5.0 - 55.0	[M-H]-	278.970900	2	ESI	84.990670 - 278....	LCMS-9030	EPAM1633Cal7	negative
32	377-73-1	PFMPA	229.981400	C4HF7O3	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	4.425	5.0 - 55.0	[M-H]-	228.974100	2	ESI	84.990670 - 228....	LCMS-9030	EPAM1633Cal7	negative
33	375-95-1	PFNA	463.970500	C9HF17O2	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	7.765	5.0 - 55.0	[M-H]-	462.963200	2	ESI	118.992560 - 46...	LCMS-9030	EPAM1633Cal7	negative
34	68259-12-1	PFNS	549.934300	C9HF19O3S	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	8.152	5.0 - 55.0	[M-H]-	548.927000	2	ESI	98.955770 - 548....	LCMS-9030	EPAM1633Cal7	negative
35	335-67-1	PFOA	413.973700	C8HF15O2	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	7.258	5.0 - 55.0	[M-H]-	412.966400	2	ESI	168.989370 - 41...	LCMS-9030	EPAM1633Cal7	negative
36	1763-23-1	PFOS	499.937500	C8HF17O3S	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	7.730	5.0 - 55.0	[M-H]-	498.930200	2	ESI	98.955770 - 498....	LCMS-9030	EPAM1633Cal7	negative
37	2706-90-3	PFPeA	263.983300	C5HF9O2	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	5.075	5.0 - 55.0	[M-H]-	262.976000	2	ESI	218.986180 - 26...	LCMS-9030	EPAM1633Cal7	negative
38	2706-91-4	PFPeS	349.947100	C5HF11O3S	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	6.004	5.0 - 55.0	[M-H]-	348.939800	2	ESI	79.957360 - 348....	LCMS-9030	EPAM1633Cal7	negative
39	376-06-7	PFTeDA	713.954500	C14HF27O2	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	9.392	5.0 - 55.0	[M-H]-	712.947300	2	ESI	118.992560 - 71...	LCMS-9030	EPAM1633Cal7	negative
40	72629-94-8	PFTrDA	663.957700	C13HF25O2	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	9.150	5.0 - 55.0	[M-H]-	662.950500	2	ESI	118.992560 - 66...	LCMS-9030	EPAM1633Cal7	negative
41	2058-94-8	PFUnA	563.964100	C11HF21O2	[MOL]	Fluorinated ...	Delay Column: ...	Shim-pack Sce...	8.553	5.0 - 55.0	[M-H]-	562.956800	2	ESI	118.992560 - 56...	LCMS-9030	EPAM1633Cal7	negative

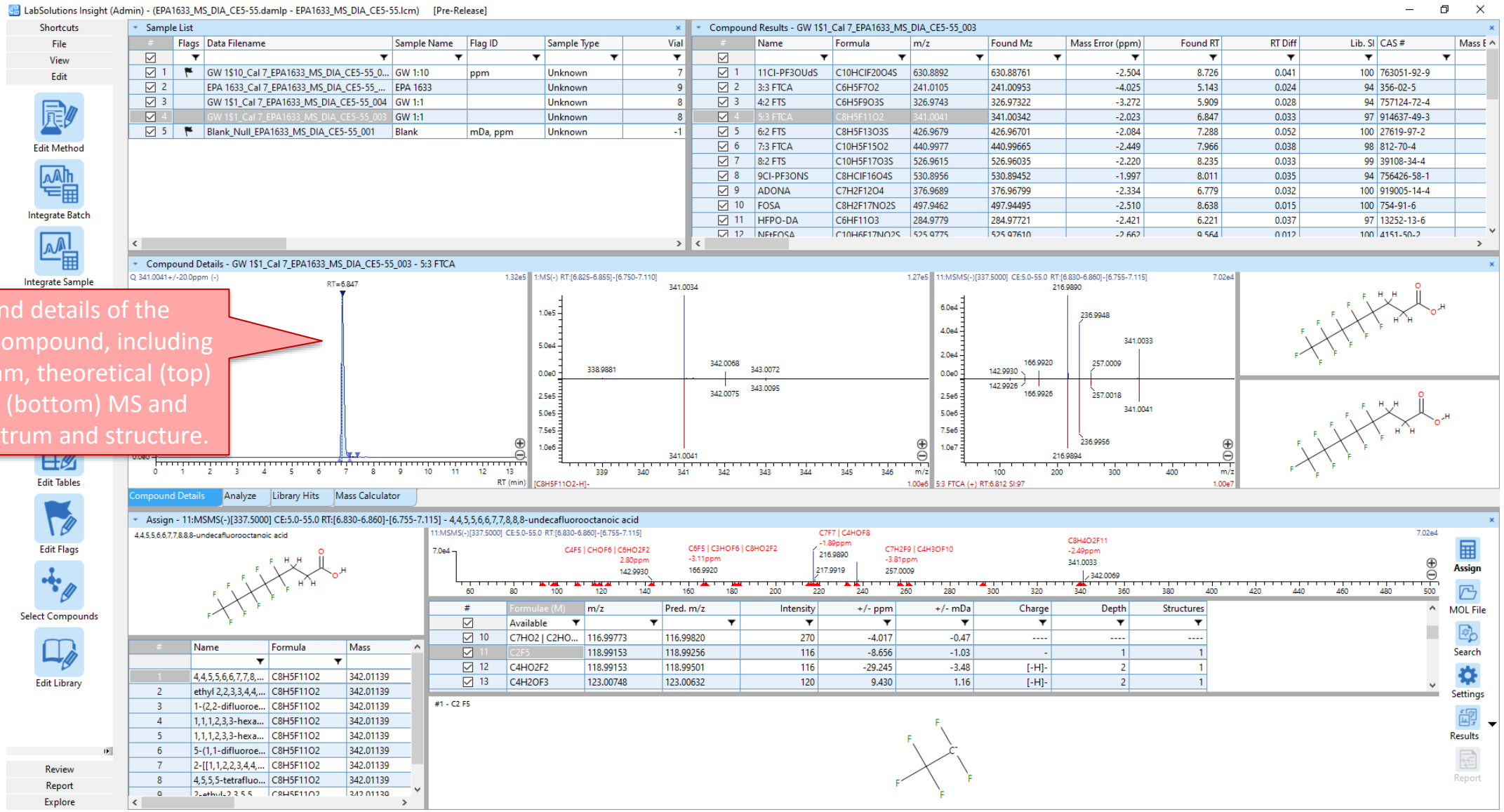
Results – Suspects Screening *After running Library Search*



Sample information such as file names, sample names, sample type, and flags are shown in the sample list.

Library Search

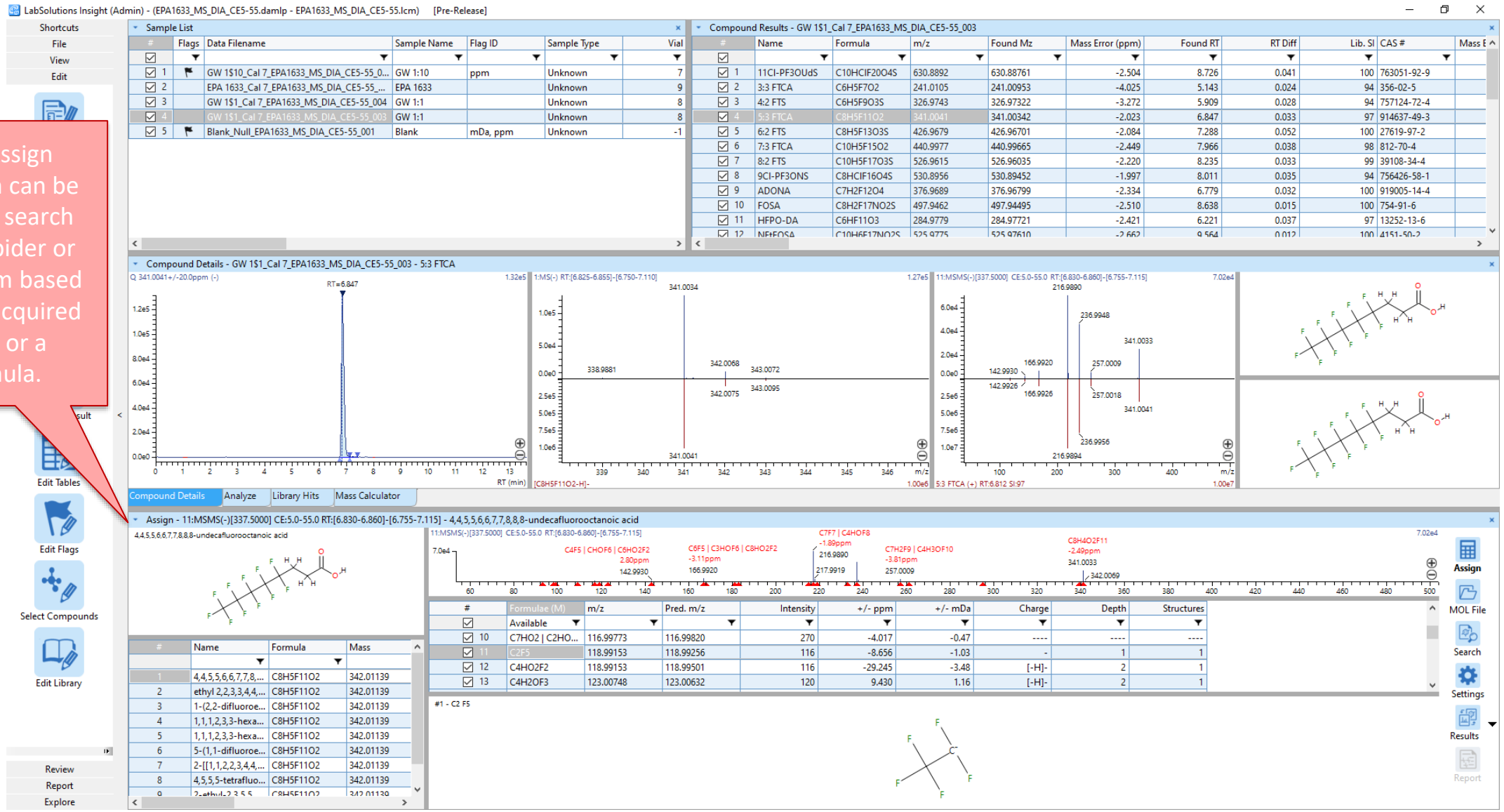
Results – Suspects Screening



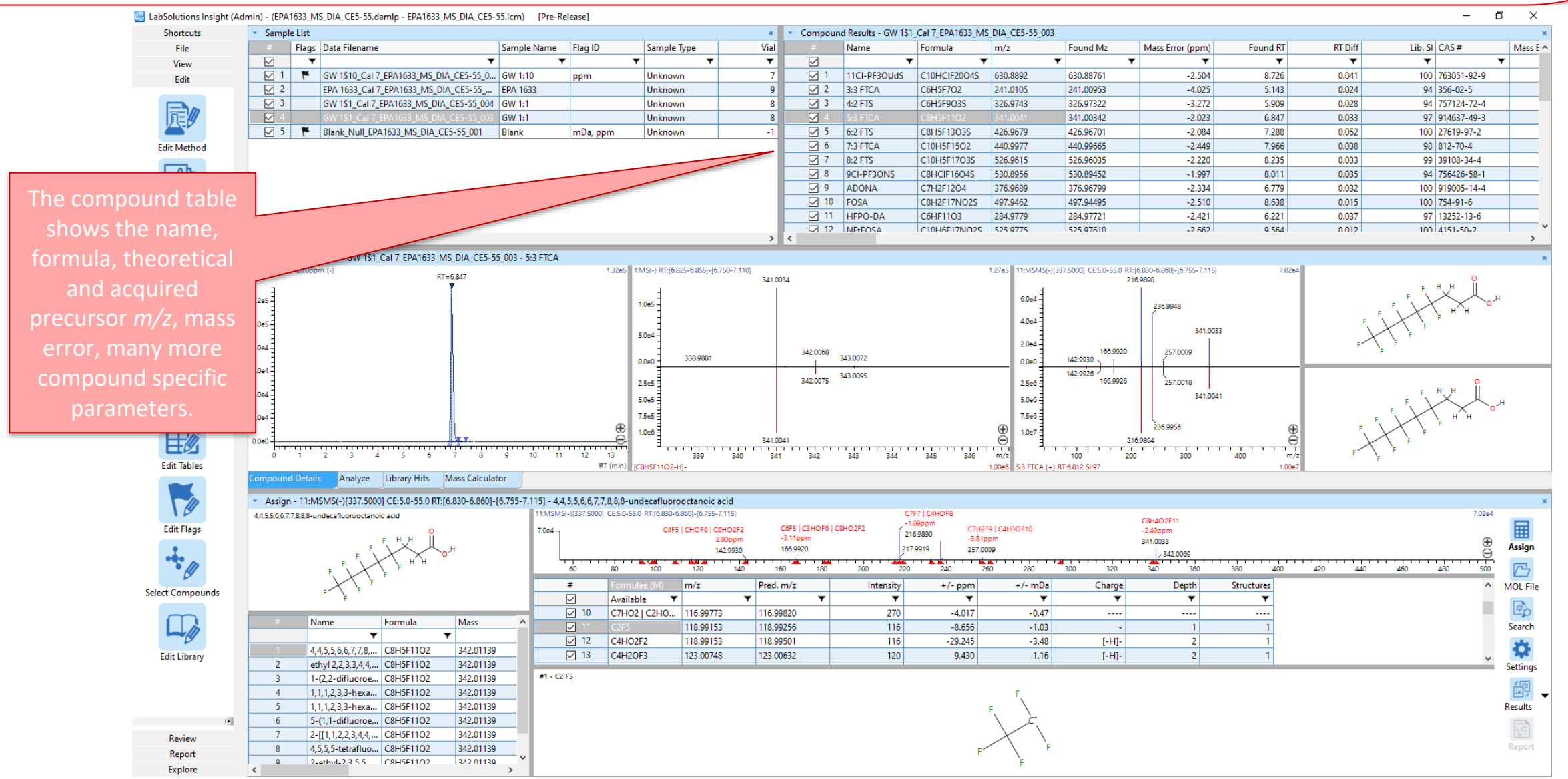
Compound details of the highlighted compound, including chromatogram, theoretical (top) and actual (bottom) MS and MS/MS spectrum and structure.

Results – Suspects Screening

The Assign function can be used to search ChemSpider or PubChem based off the acquired mass or a formula.

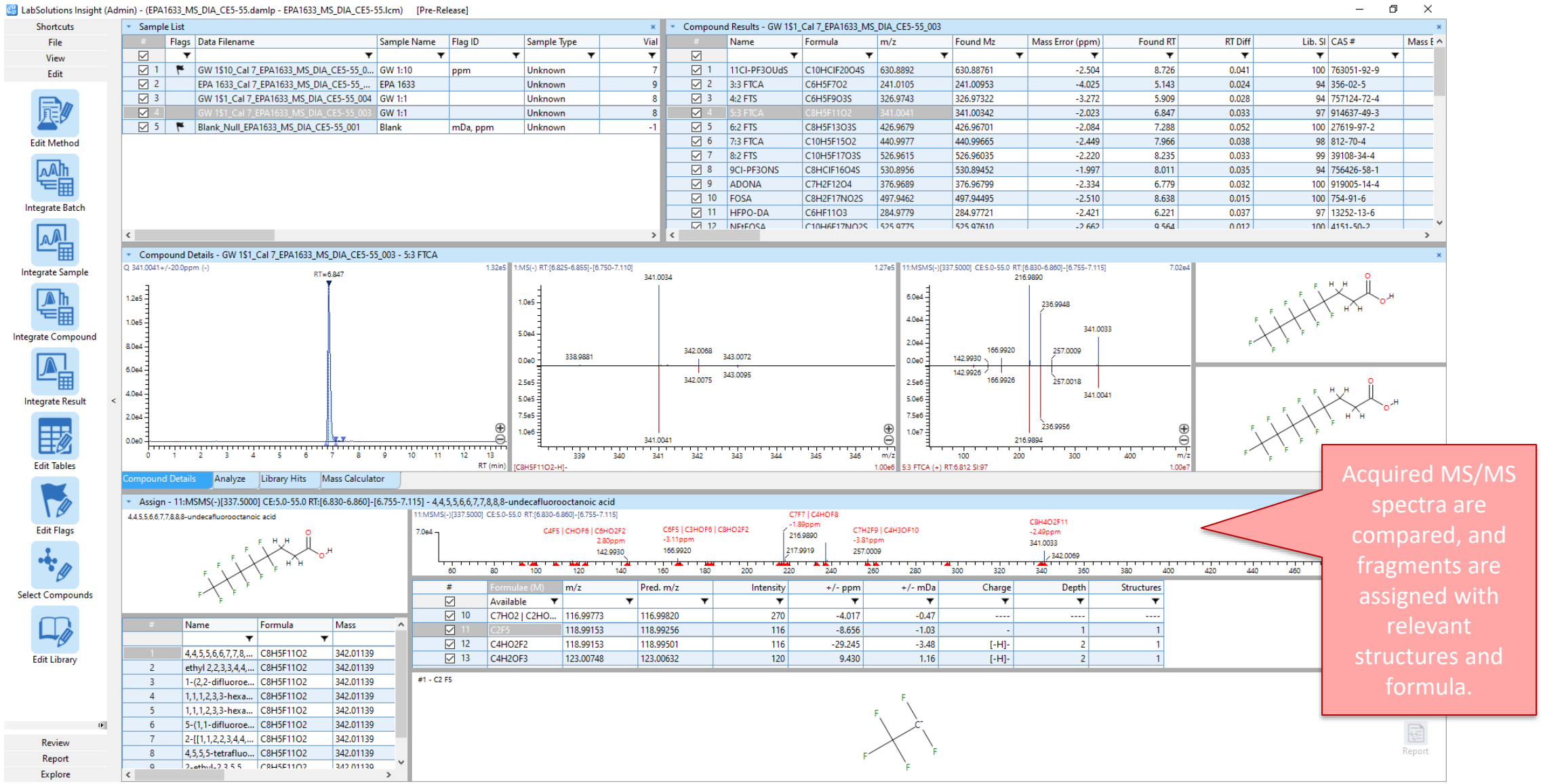


Results – Suspects Screening

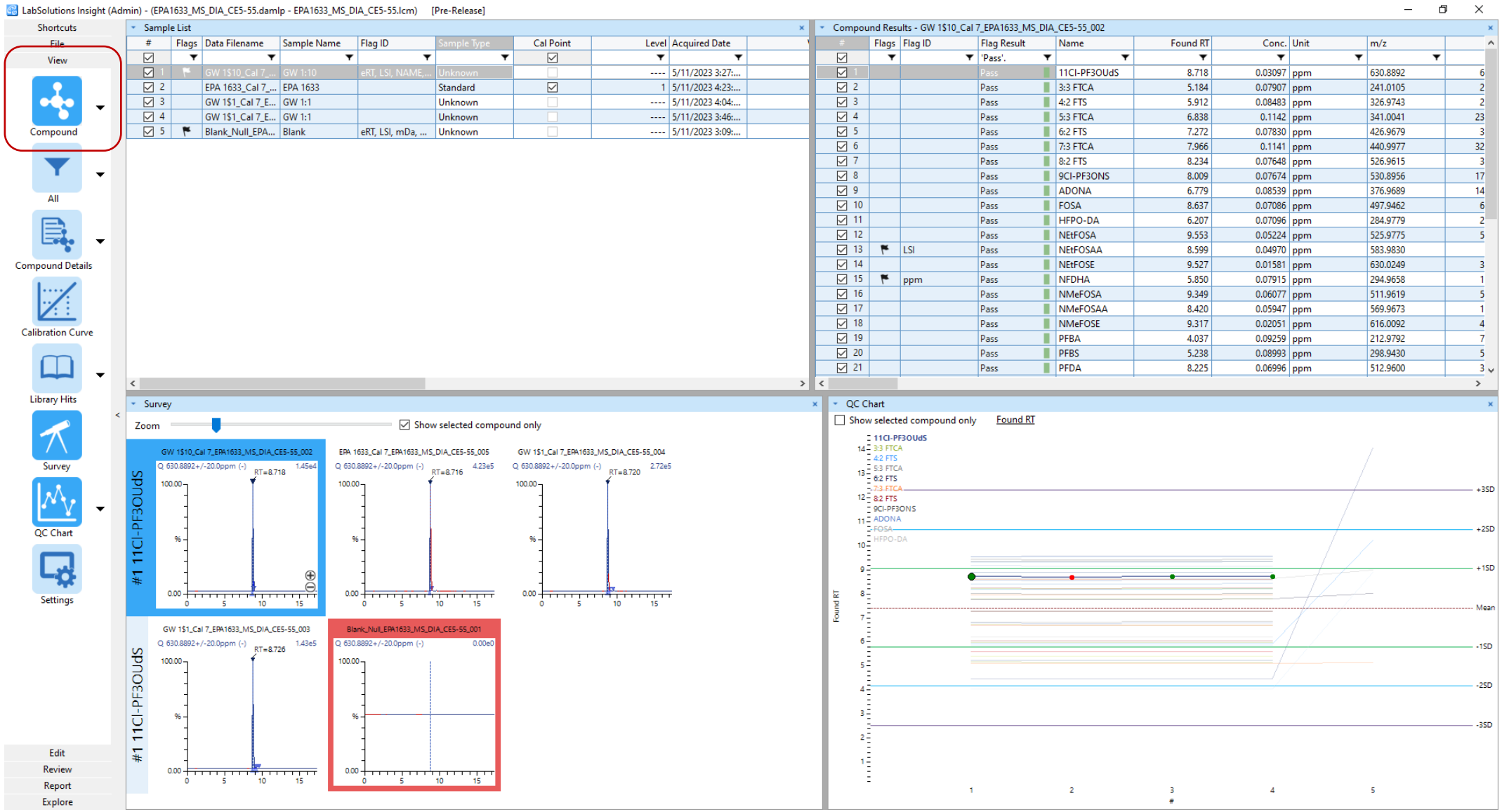


The compound table shows the name, formula, theoretical and acquired precursor *m/z*, mass error, many more compound specific parameters.

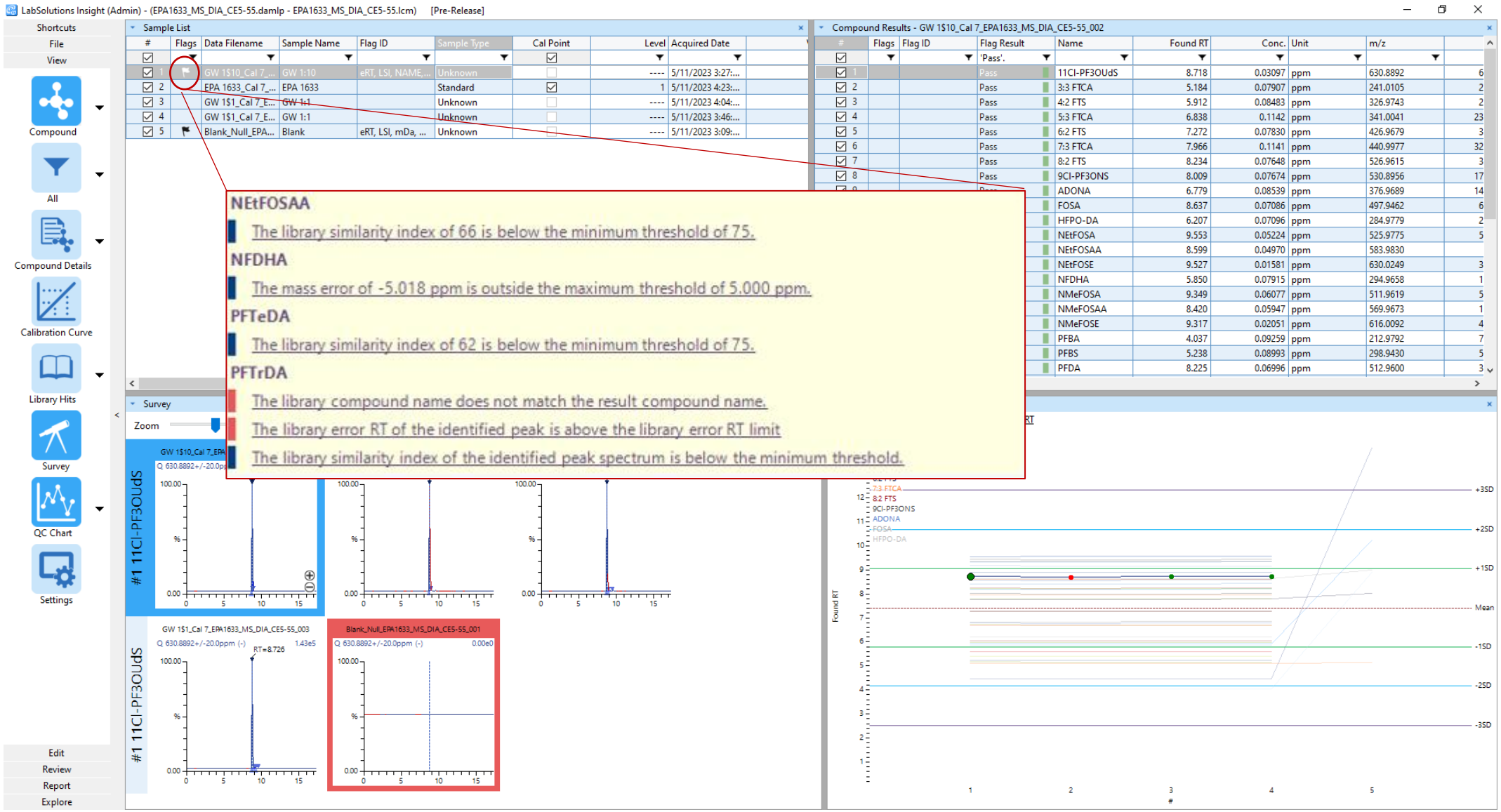
Results – Suspects Screening



Results – Suspects Screening

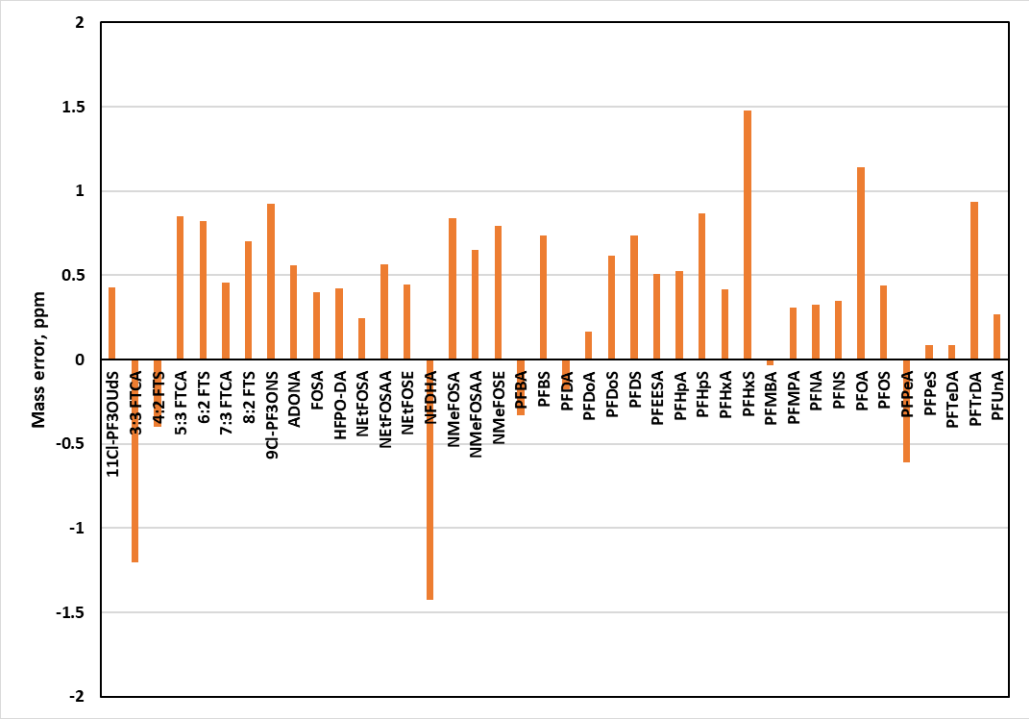


Results – Suspects Screening

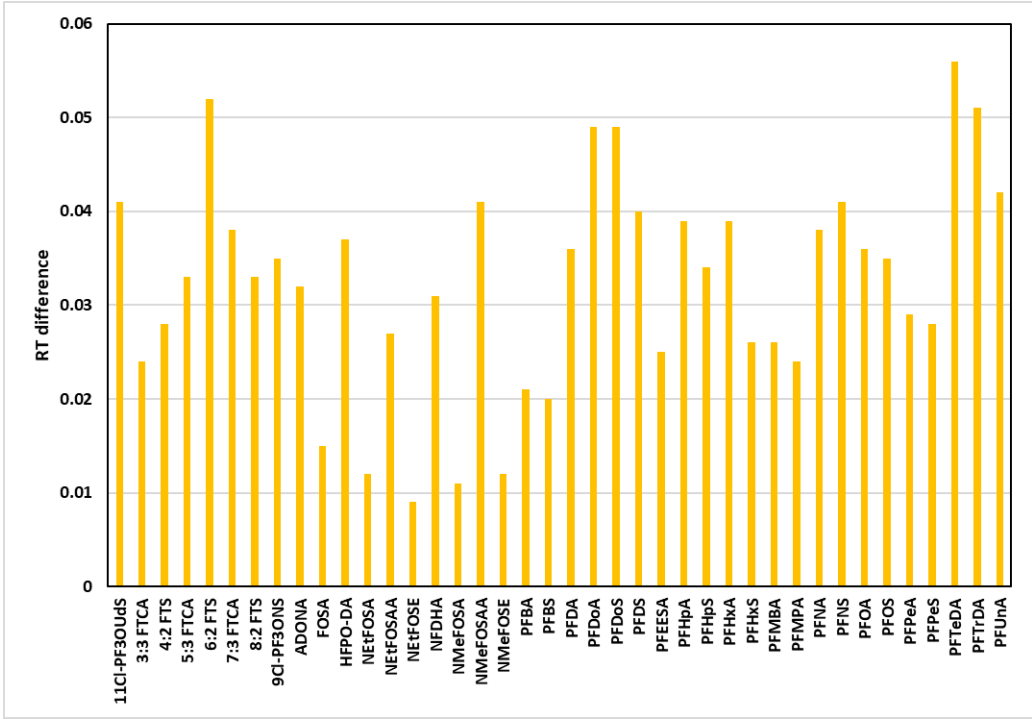


Results – Suspects Screening *After reviewing Library hits*

All PFAS listed in EPA 1633 were identified by comparing results against the in-house developed library, and when reprocessing samples in an untargeted analysis workflow



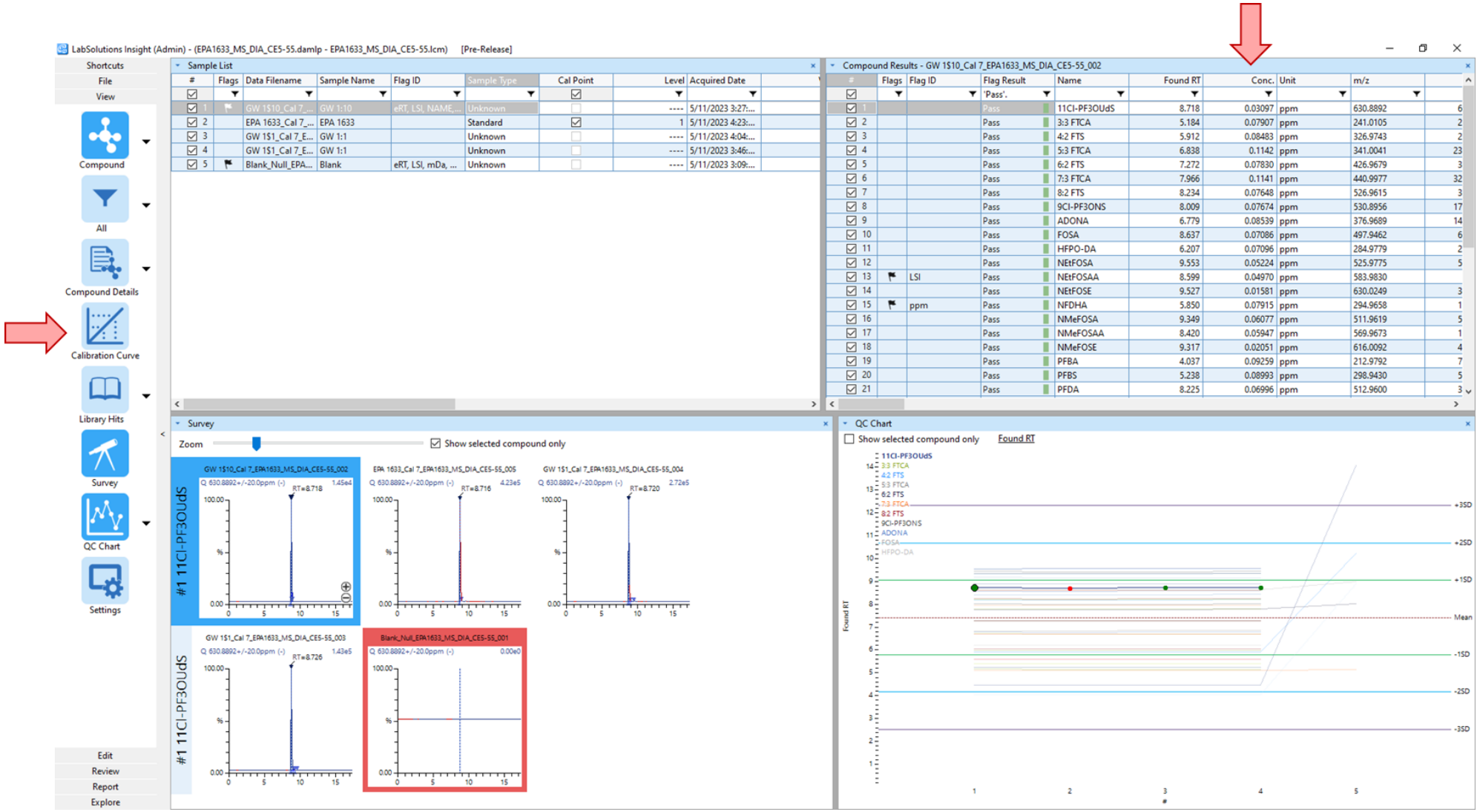
Mass accuracy ±2 ppm



RT diff < 0.06

Library Similarity Index: >75% (88-100%), and alignment of fragmentation pattern

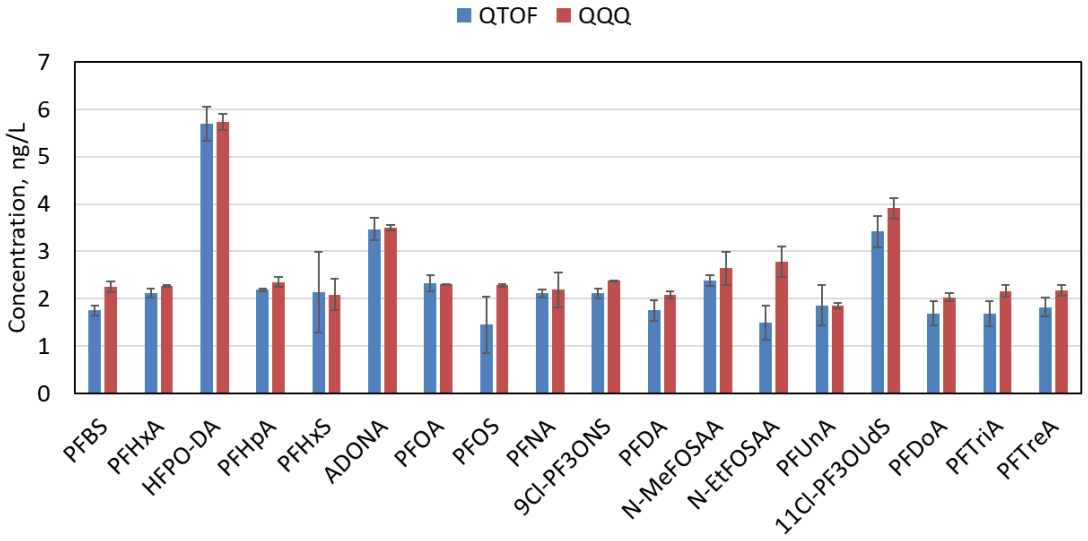
Results - Quantitation



Results - Quantitation

Comparable LOQs and Precision and Accuracy between QTOF and Triple Quadrupole

LOQ



	LCMS-9030				LCMS-8045			
25 ppt P&A	Avg	Std Dev	%REC	%RSD	Avg	Std Dev	%REC	%RSD
PFBS	23.7	1.80416	107	7.6	25.6	0.62923	115	2.5
PFHxA	29	1.38519	116	4.8	25.6	1.009	103	3.9
HFPO-DA	80.4	5.89465	129	7.3	67.2	2.45917	108	3.7
PFHpA	27.6	2.16336	110	7.8	26.4	0.95392	106	3.6
PFHxS	25.9	3.72591	113	14.4	26.4	1.09444	80	4.1
ADONA	46.2	2.80041	185	6.1	39.7	1.28787	159	3.2
PFOA	28.7	1.65379	115	5.8	25.1	0.61854	100	2.5
PFOS	21.7	4.99594	94	23.1	25.8	0.7969	112	3.1
PFNA	28.2	1.78411	113	6.3	25.7	0.93568	103	3.6
9Cl-PF3ONS	28	1.68409	121	6	27.7	0.80942	119	2.9
PFDA	25	1.56208	100	6.3	23.4	0.68554	94	2.9
N-MeFOSAA	29.2	3.31232	117	11.3	31.4	1.74514	126	5.6
N-EtFOSAA	25.9	6.11145	104	23.6	34.8	1.90454	139	5.5
PFUnA	24.4	1.88443	98	7.7	24.1	0.87477	96	3.6
11Cl-PF3OUdS	44.5	2.93683	189	6.6	46	1.8548	196	4
PFDoA	22.1	1.43221	88	6.5	23.5	0.66533	94	2.8
PFTriA	22.8	1.44296	91	6.3	24.4	0.91626	98	3.8
PFTreA	22.8	1.54577	91	6.8	25.2	0.72595	101	2.9

Take-Home Messages

- Highly reliable workflow for the targeted and suspect screening of 40 common PFAS was demonstrated in clean and environmental matrices, including:
  - High mass accuracy and
  - Low retention time differences
- The presented workflow for suspect and target screening workflows, including quantitation, is easily manageable and transferrable between platforms (LC-MS/MS and LC-QTOF) in non-R&D laboratories.

Acknowledgments

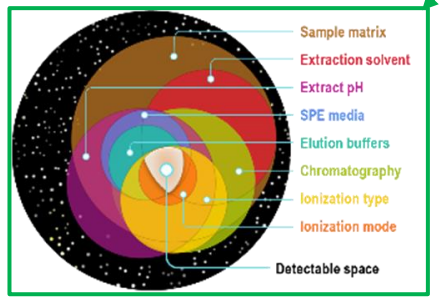
Ethan Hain, PhD
Product Specialist, LCMS

Christopher Gilles
General Manager – Product Managers

https://nontargetedanalysis.org/

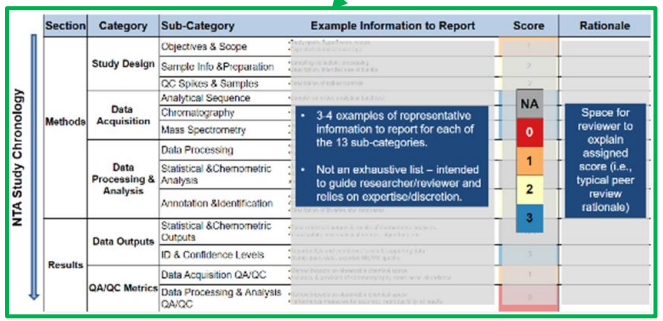
BP4NTA
BEST PRACTICES FOR NON-TARGETED ANALYSIS

About | Become A Member | NTA Forum | News | Jobs | NTA Study Reporting Tool (SRT) | ChemSpaceTool | Reference Content | Additional Resources



REFERENCE CONTENT
Study design
Data acquisition
Data processing and analysis
Data outputs
QA/QC metrics
Glossary

ADDITIONAL RESOURCES
Literature library
NTA Software Tools
Online Databases and Libraries
Events and organizations
Mass Spec vendors



Study Planning Tool – in preparation

Forum	Topics	Posts	Last Post
NTA Forum This is the BP4NTA forum to discuss all topics related to non-targeted analysis including instruments, software, workflows, and more! Instruments (3, 5), Software (3, 6), Workflows (1, 7), General (2, 3)	9	30	1 week ago gpblack

BP4NTA
BEST PRACTICES FOR NON-TARGETED ANALYSIS

Access the website
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(3rd Tuesday of the month, 12 pm EST)

For more information, reach out to the current BP4NTA Chairs at:

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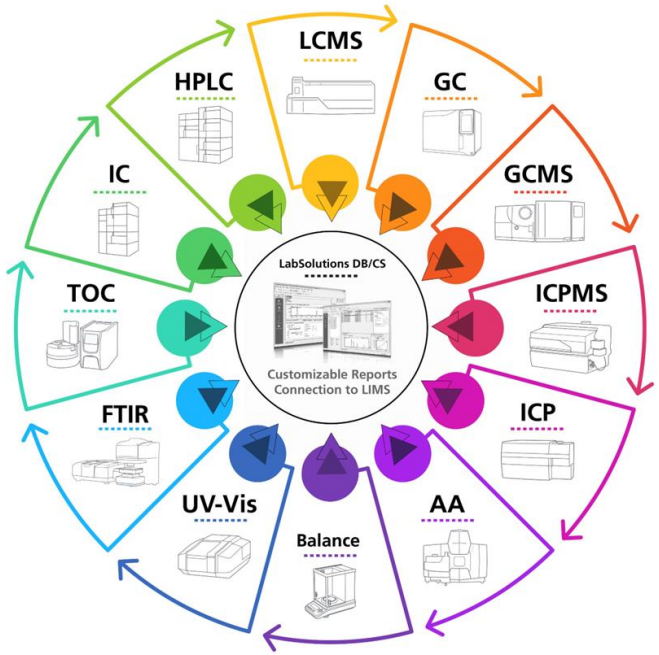

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