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Analytical Workflow for Extractable and Leachable Impurities

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WHAT are **EXTRACTABLES** and **LEACHABLES**?

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What is E&L testing for?

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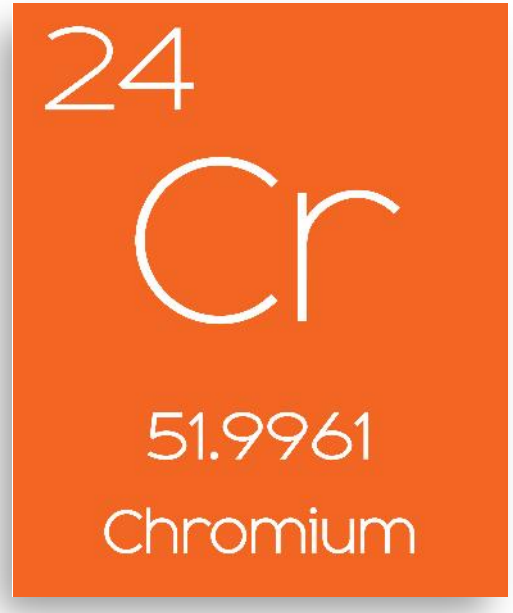
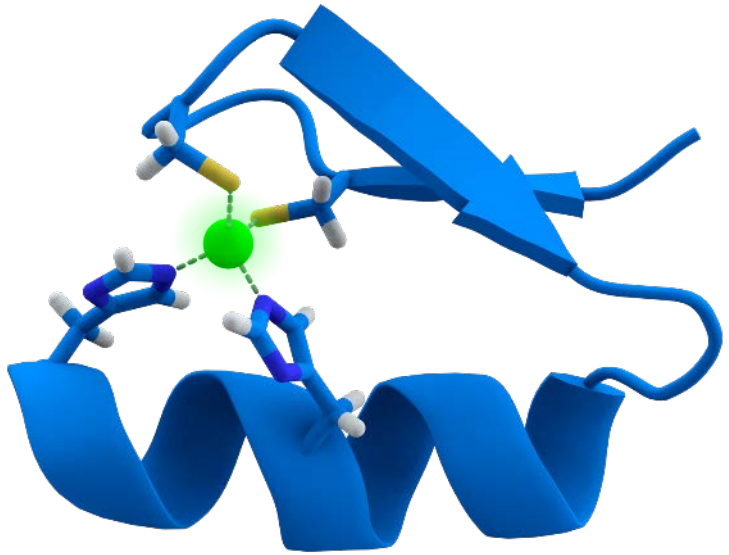
Areas of concern

CONTAMINANT TOXICITY



Is there risk of harm to the patient?

DRUG EFFICACY



Is there impact on drug potency?

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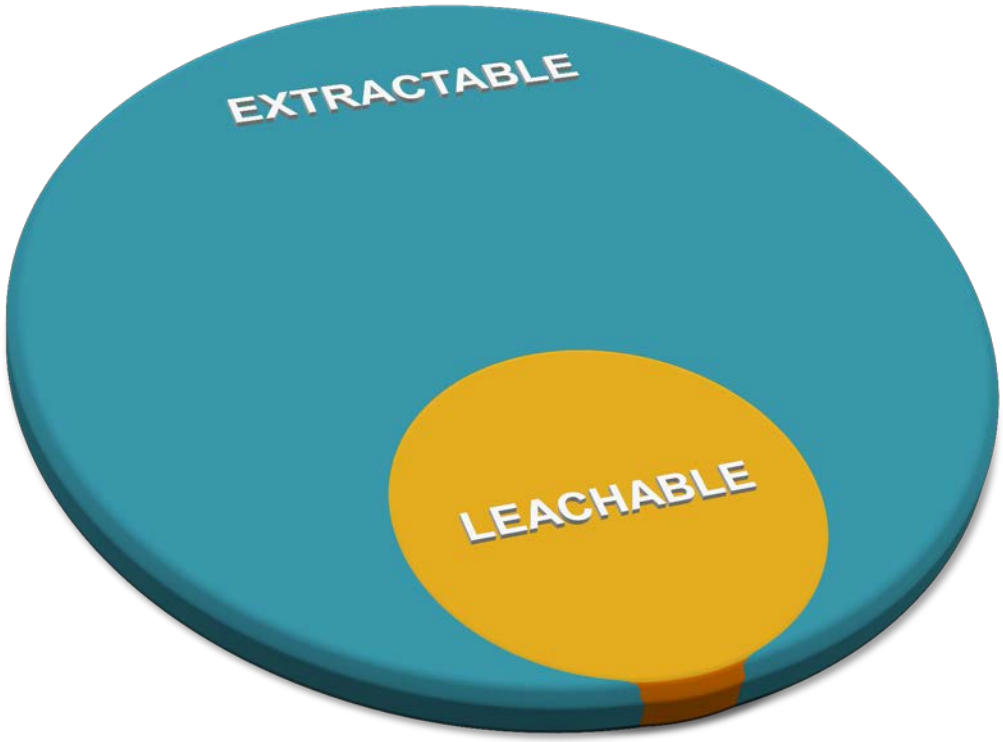
Extractables & Leachables

- **EXTRACTABLE**
  - Chemical released from process equipment, packaging or delivery system; **under laboratory extraction conditions**.
  - Process must not degrade or deform material.

- **LEACHABLE**
  - Chemical that **migrates** from process equipment, packaging or delivery system; into drug formulation **under normal usage conditions**.



Leachables are typically a subset of extractables.

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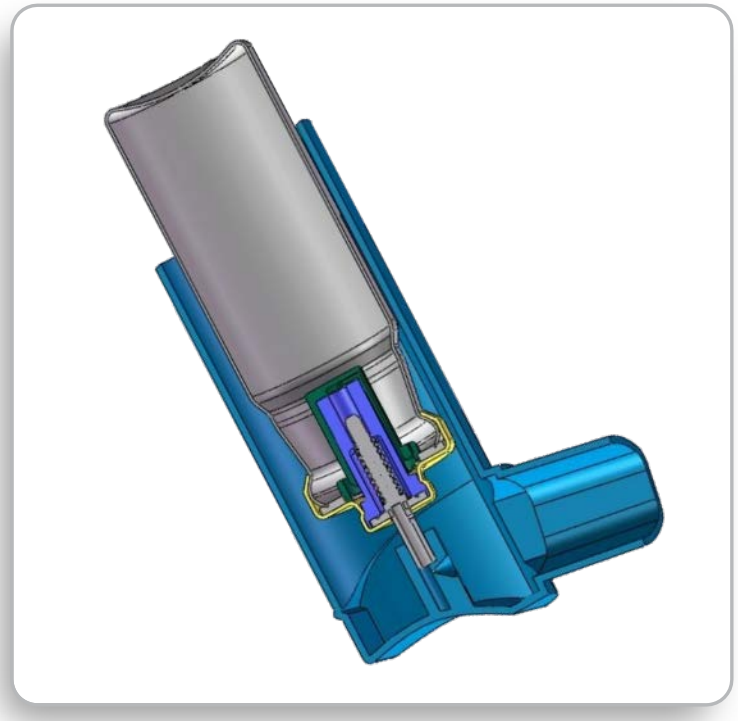
Extractables versus Leachables testing

EXTRACTABLE



Test the materials

LEACHABLE

Test the product

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Regulations

There are as yet **no single specific standards or guidance** for extractables and leachables testing.

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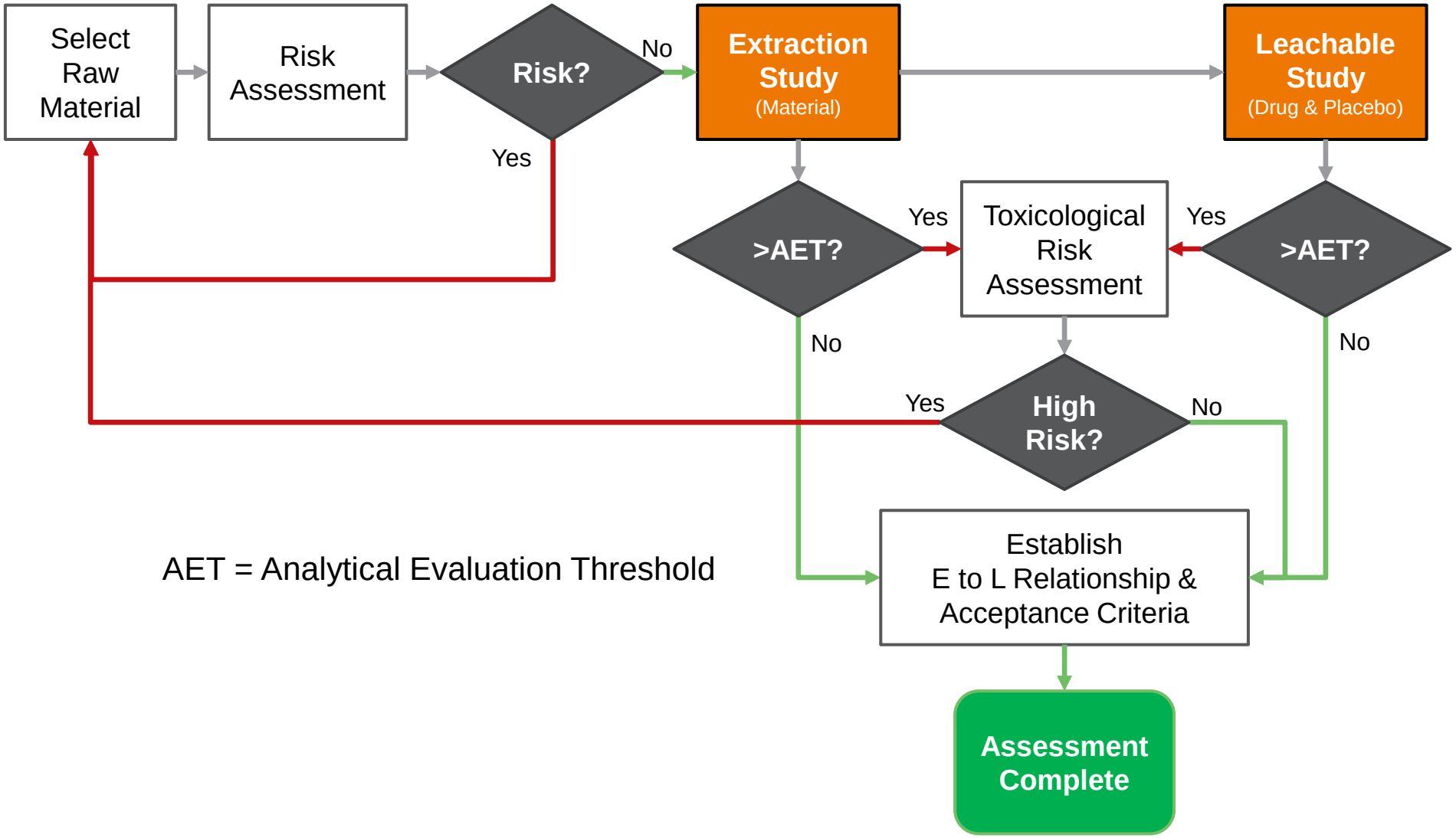
Extractables & Leachables Assessment Workflow



AET = Analytical Evaluation Threshold

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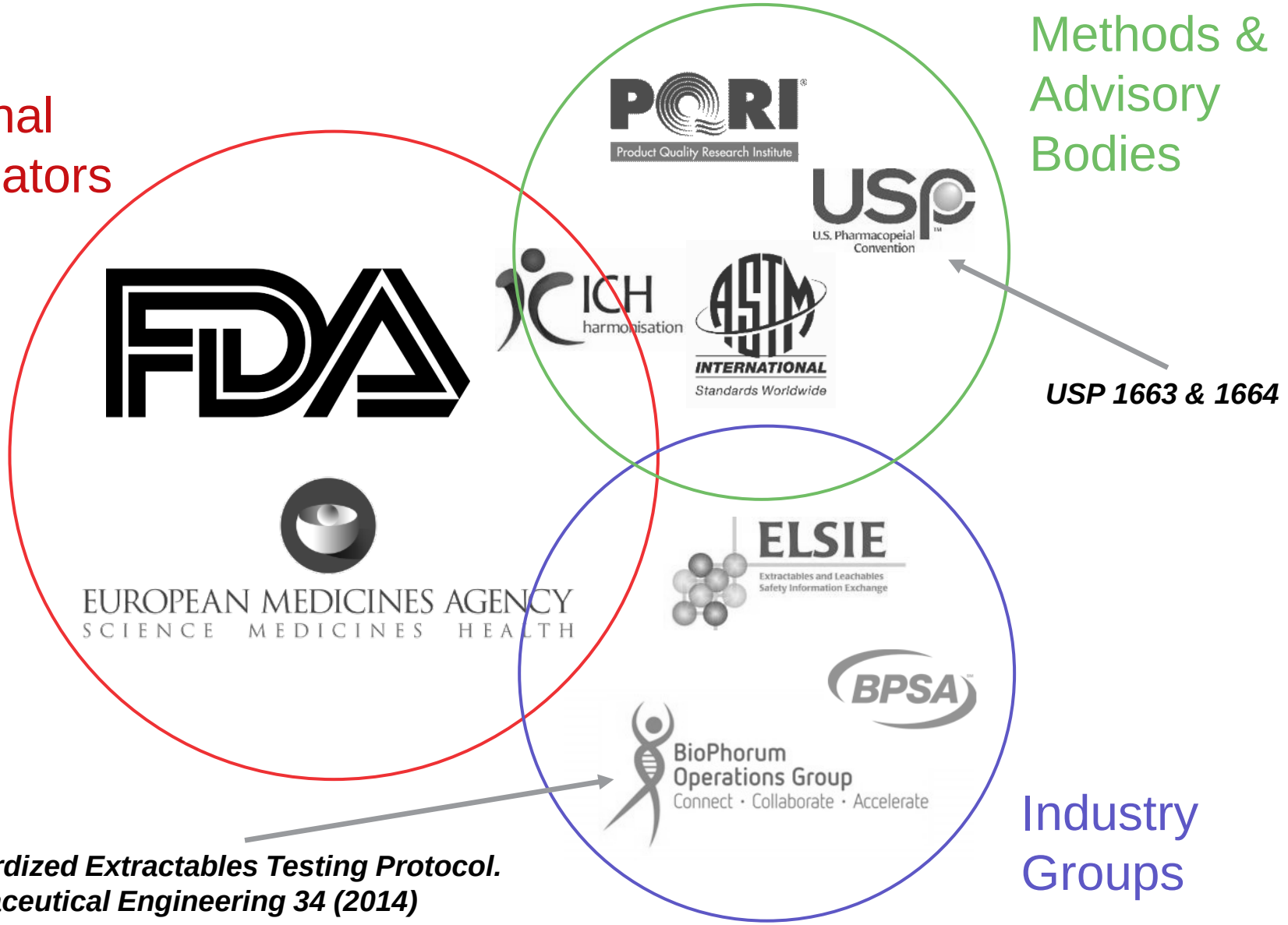
E&L Regulatory and method landscape



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BPOG Single-use System – Extractables Test Guidelines

	Solvents						Time				
	50% Ethanol	1% PS-80	5M NaCl	0.5N NaOH	0.1 M Phosphoric acid	WFI[a]	Time 0 (≤ 30 min)	24 hours	7 days	21 days	70 days
							Temperature				
							Ambient (25°C)	40°C			
Storage, Mixing, and Bioreactor Bags	X	X	X	X	X	X	X	X		X	X[b]
Tubing	X	X	X	X	X	X	X	X		X	X[b,c]
Tubing Connectors and Disconnectors	X	X	X	X	X	X	X	X		X	
Aseptic Connectors and Disconnectors	X	X	X	X	X	X	X	X	X		
Sterilizing-grade Filters/Process Filters	X	X	X	X	X	X	X	X	X		
Tangential-flow Filtration Cassettes	X	X	X	X	X	X	X	X		X	
Sensors and Valves	X	X	X	X	X	X	X	X		X[d]	
Chromatography Columns; Elastomeric Parts (gaskets, O-rings, diaphragms, and septum); Wetted Polymeric Surfaces of Positive Displacement Pumps	X	X	X	X	X	X	X	X			
Molded Parts of Mixers	X	X	X	X	X	X	X	X		X	
Filling Needles	X	X	X	X	X	X	X	X			

Standardized Extractables Testing Protocol. Pharmaceutical Engineering 34 (2014)

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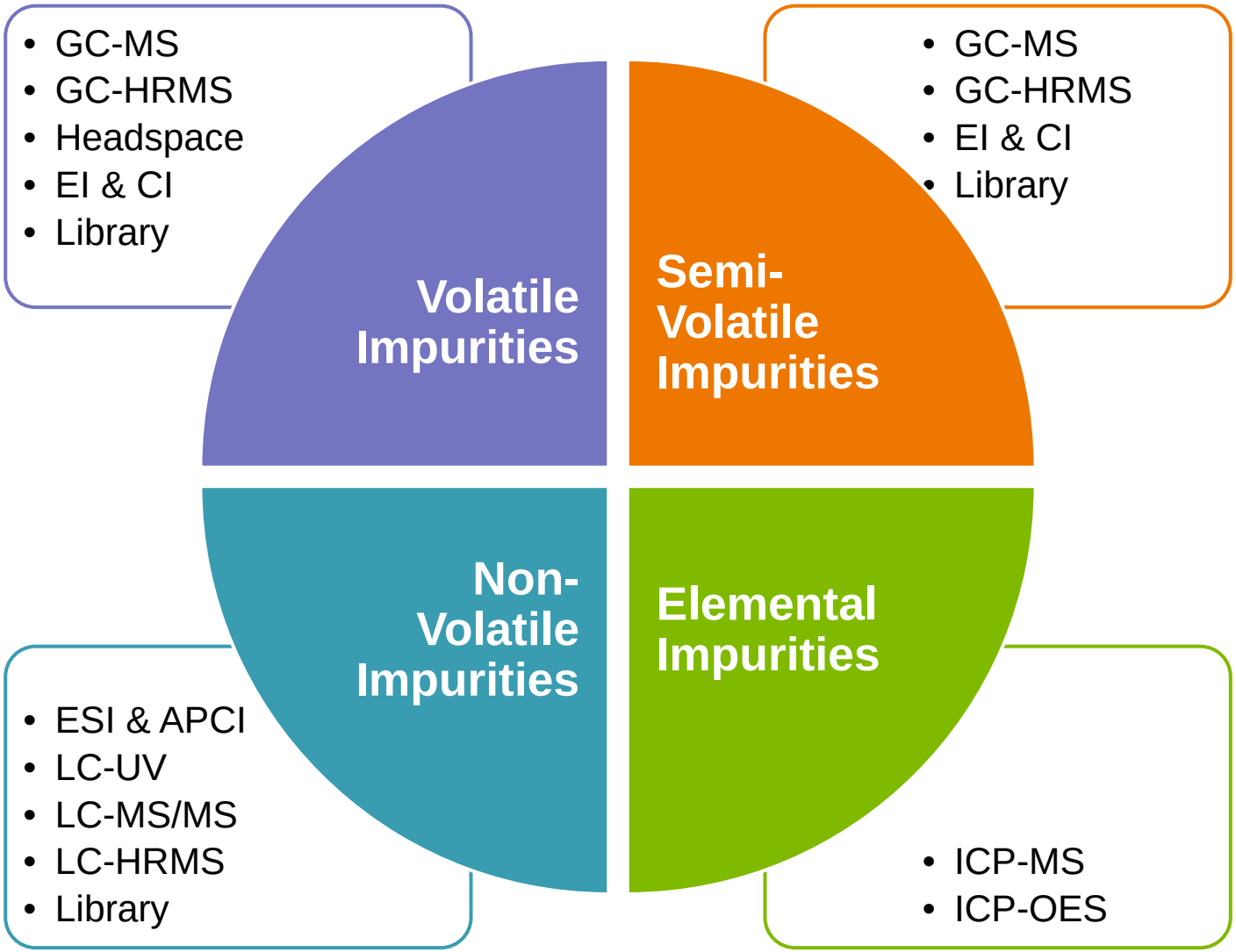
Analysis of Extractables & Leachables



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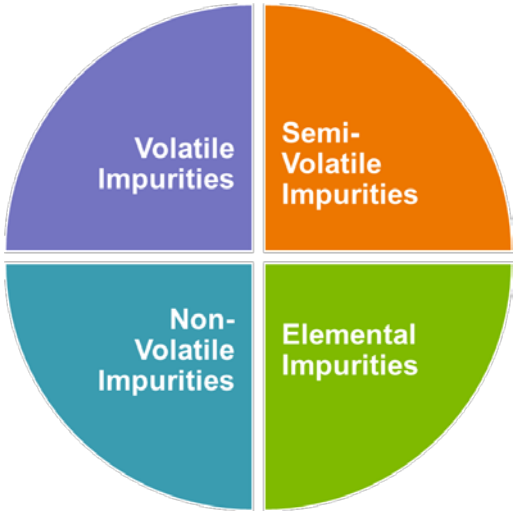
Our solution is **complete** for Extractables & Leachables



Preparation

ASE 350
Chromeleon

Volatiles

Trace 1300 GC
TriPlus 300
ISQ GC-MS
Chromeleon

Semi-volatiles

Q Exactive GC GC-MS/MS
Trace 1300 GC
TraceFinder

Non-Volatiles

Q Exactive LC-MS/MS
Vanquish UHPLC
ICS-5000 HPIC
Compound Discoverer
TraceFinder
MZ Cloud

Elemental

iCAP Q ICP-MS
iCAP 7000+ ICP-OES
Qtegra ISDS

Consumables

MS Certified Vials
Columns
Ultra-pure GC Resolv & Optima LCMS Solvents
Virtuoso

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Experimental – O-Ring Extractables Study

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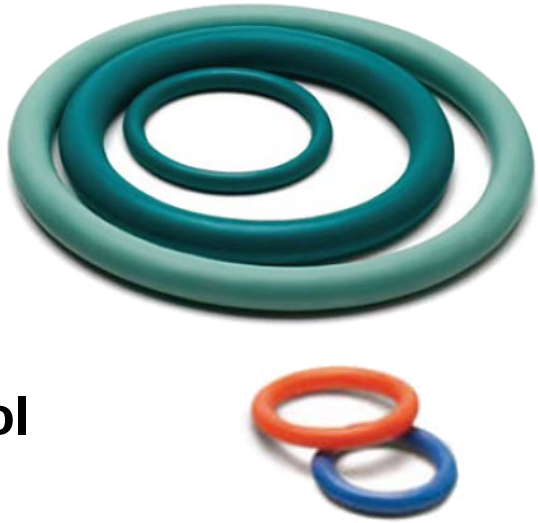
Pharmaceutical O-Rings extractables study

- 4 O-ring samples
  - **A** Red
  - **B** Brown
  - **C** White
  - **D** Black
  - **Blank** (control)

- Solvents
  - **Water**
  - **5M NaCl**
  - **50% Ethanol**
  - **100% Ethanol**
  - 1% PS-80
  - 0.5N NaOH
  - 0.1M Phosphoric Acid



- **40 ° C for 30 days**

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O-ring Extractables Study

Semi-volatiles

Q Exactive GC GC-MS/MS

Trace 1310 GC

TraceFinder

Non-Volatiles

Q Exactive LC-MS/MS

Compound Discoverer

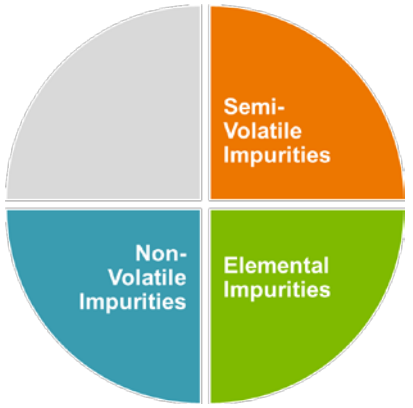
TraceFinder

MZ Cloud

Metals

iCAP Q ICP-MS

Qtegra ISDS



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GC-MS instrument conditions

Thermo Scientific™ Q Exactive™ GC
Hybrid Quadrupole-Orbitrap GC-MS/MS System

Thermo Scientific™ Trace™ 1310 GC

Thermo Scientific™ TraceFinder™

TRACE 1310 GC Parameters	
Injection Volume (mL):	1
Liner	Single gooseneck
Inlet (°C):	280
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program:	
Temperature 1 (°C):	40
Hold Time (min):	1
Temperature 2 (°C):	320
Rate (°C/min)	15
Hold Time (min):	10

Q Exactive GC MS Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source(°C):	230
Electron energy (eV):	70
Acquisition mode:	Full scan
Mass range (Da):	50-650
Mass resolution (FWHM):	60k
Lockmass (m/z):	207.03235

Integration of 3 highly successful technologies

TRACE 1310 GC

rapid heat cycling

unique modular injector and detector design

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Integration of 3 highly successful technologies

TSQ 8000 Series ExtractaBrite™ ion source technology

Routine grade robustness

Removable without breaking vacuum

Patented RF lens protects ion guide and quads

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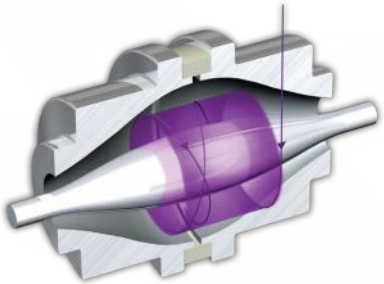
Integration of 3 highly successful technologies



Orbitrap mass analyzer technology

Incredible HR/AM performance

Highly regarded Q Exactive platform

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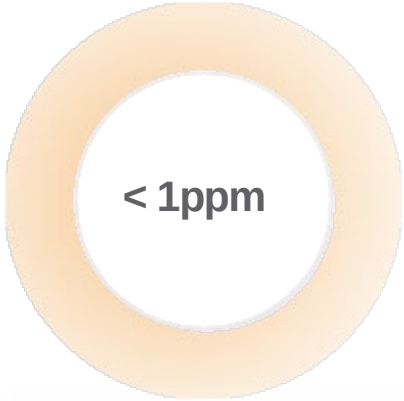
Breakthrough in GC-MS capability

The Power of Q Exactive GC

Resolution

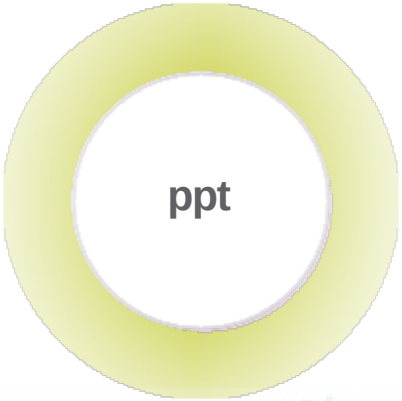
- Highest available
- Maximum selectivity
- Fast enough for GC!

Mass Accuracy



- Every scan
- All concentrations
- In complex matrix
- Across the mass range
- Everyday!

Sensitivity



- In full scan
- High selectivity
- High spectral fidelity

Dynamic Range

- Excellent coverage in sample profiling
- "Triple quad grade" quantitation in full scan

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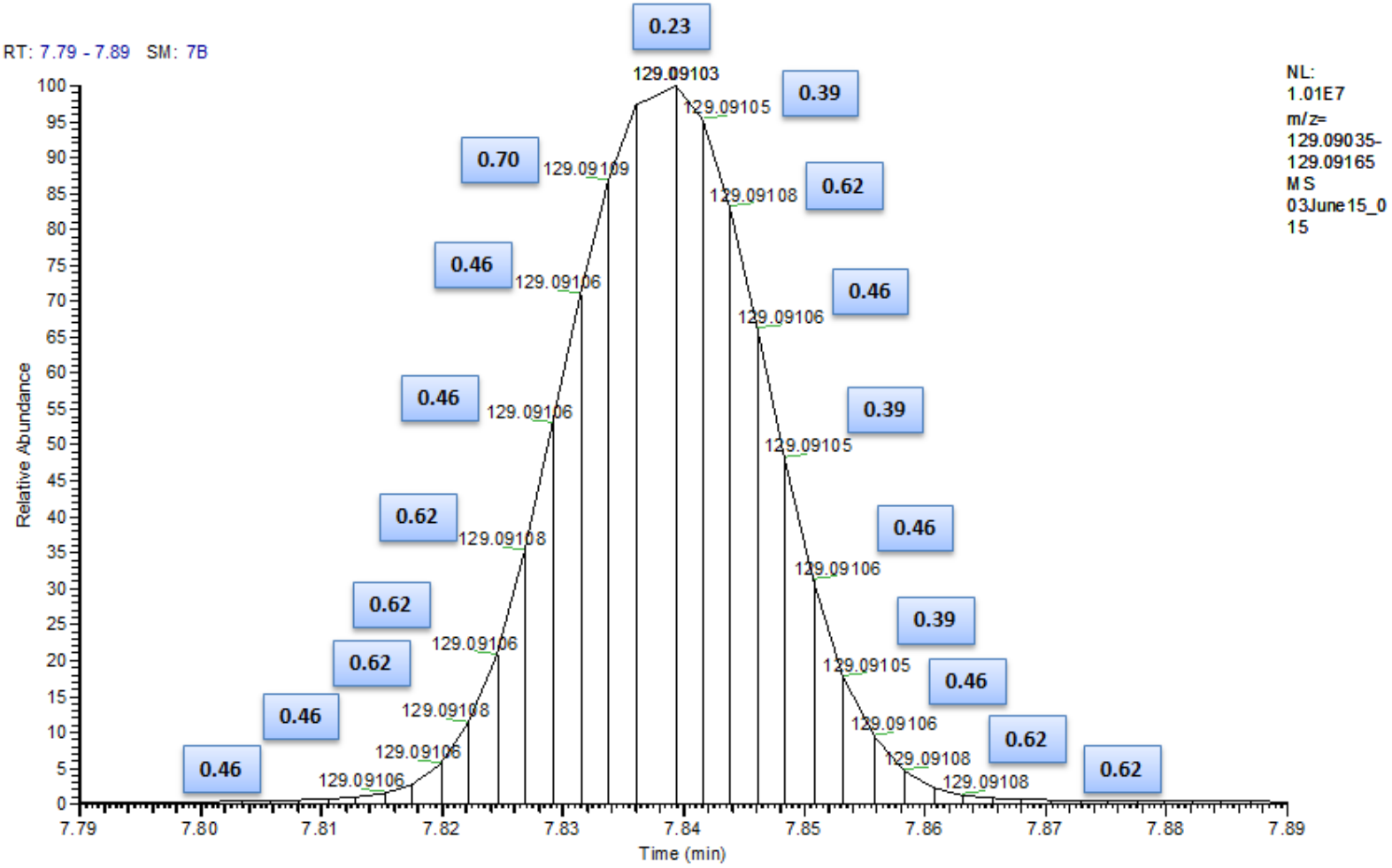
Scan speed and accurate mass error across a peak

Ethyl octanoate *m/z* 129.0910



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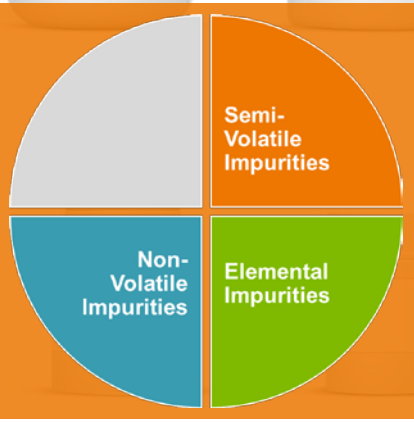
Results – Semi-volatile extractables



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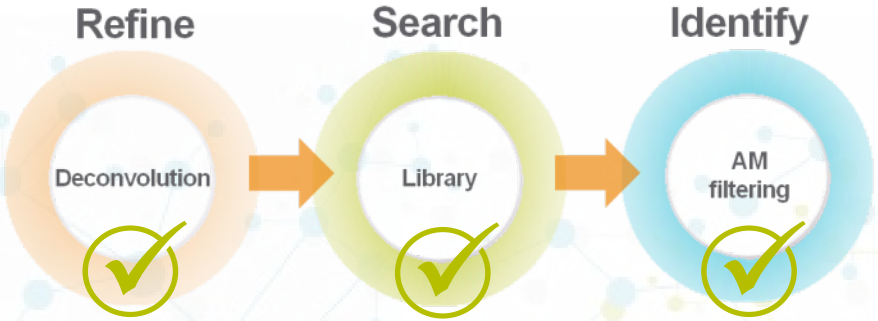
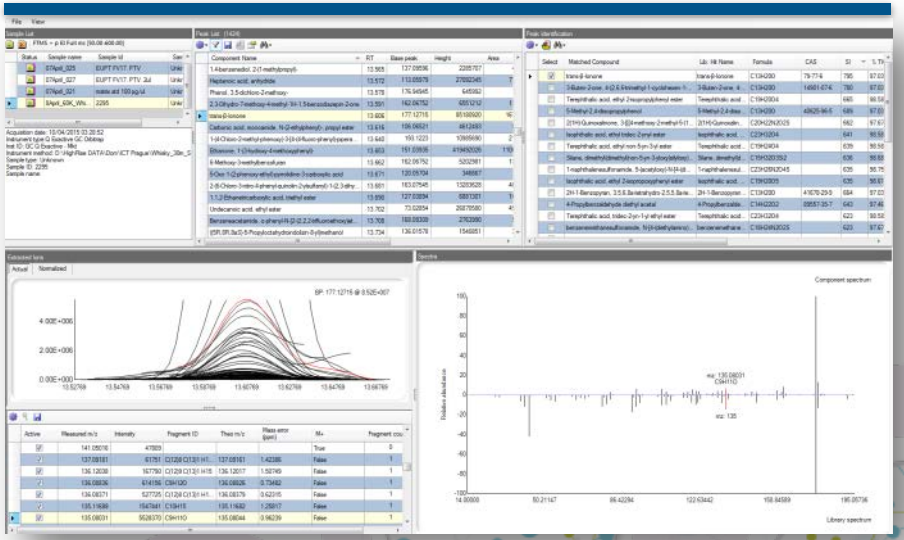
Tracefinder: automated compound detection and identification

1. Peak detection
2. Spectral deconvolution
3. Library searching (e.g. NIST, Wiley, custom)
4. % TIC explained using elemental composition and high resolution filtering with accurate mass





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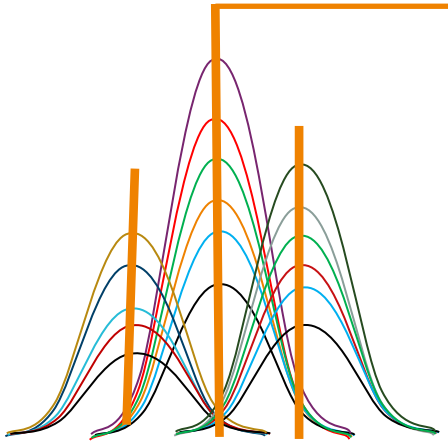
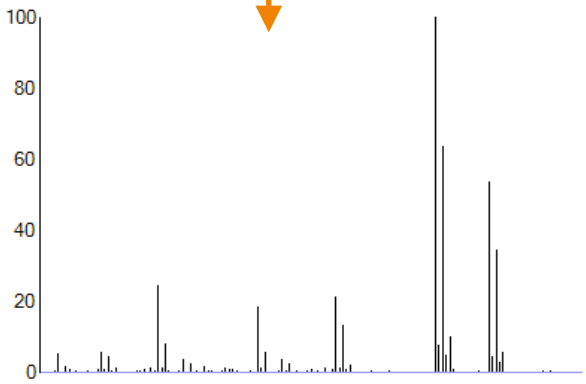
Peak Detection and Candidate Matching

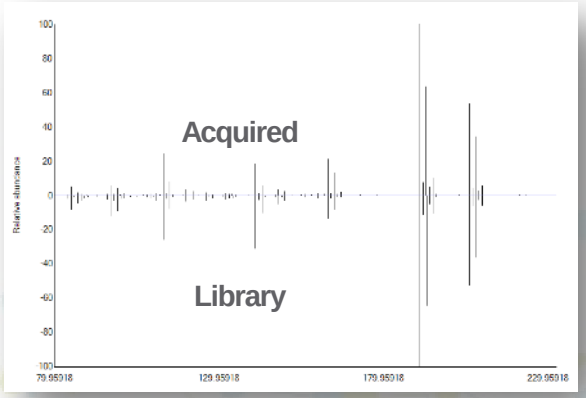


Deconvolve TIC

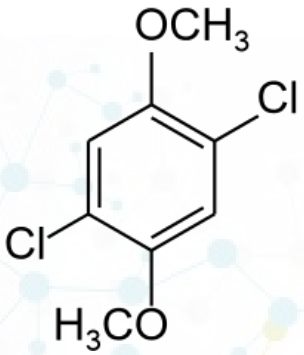


Create “clean” spectrum

Library search



Candidate Compounds



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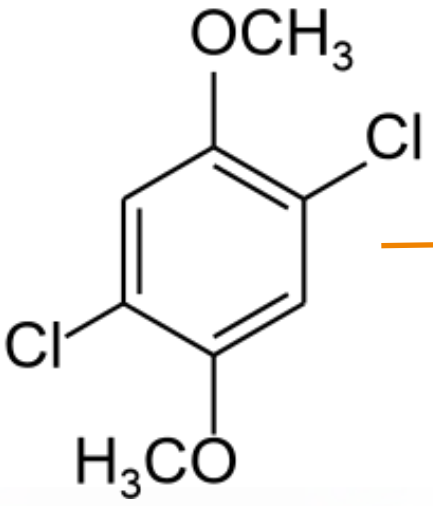
High Resolution Filtering

Candidate Compounds



Subset formulae

$C_8H_8Cl_2O_2$

Acq m/z	Fragment ID	Theo m/z	Mass Error (ppm)
147.9477	$C_5Cl_2H_2O$	147.9477	0.20277
148.9369	$C_5Cl[37]ClHO$	148.9369	0.2679
149.9448	$C_5Cl[37]ClH_2O$	149.9448	0.06602
151.9419	$C_5[37]Cl_2H_2O$	151.9418	0.72528
154.9895	$C_7ClH_4O_2$	154.9894	0.38712
155.9974	$C_7ClH_5O_2$	155.9973	0.89745
157.9943	$C_7[37]ClH_5O_2$	157.9943	0.25381
159.9479	$C_6Cl_2H_2O$	159.9477	0.87529
161.9446	$C_6Cl[37]ClH_2O$	161.9448	0.80213
162.9711	$C6Cl_2H_5O$	162.9712	0.36816
163.9745	$C_5[13]CCl_2H_5O$	163.9745	0.3342
164.9682	$C_6Cl[37]ClH_5O$	164.9682	0.24186
165.9716	$C_5CCl[37]ClH_5O$	165.9716	0.02832

$$\text{HRF Score} = \frac{\sum (m/z * \text{Intensity})_{\text{explained}}}{\sum (m/z * \text{Intensity})_{\text{observed}}} \times 100\%$$

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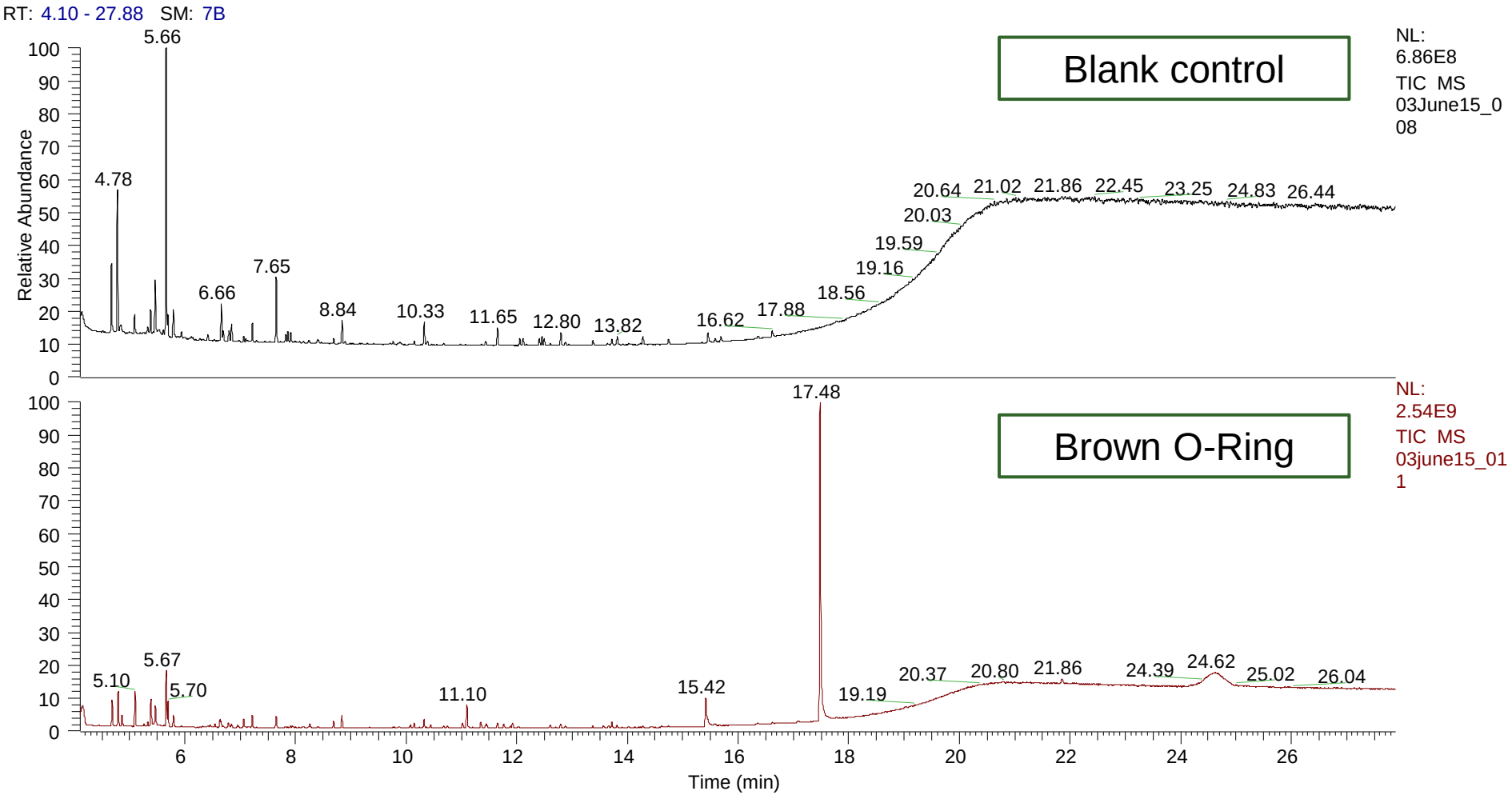
Example results



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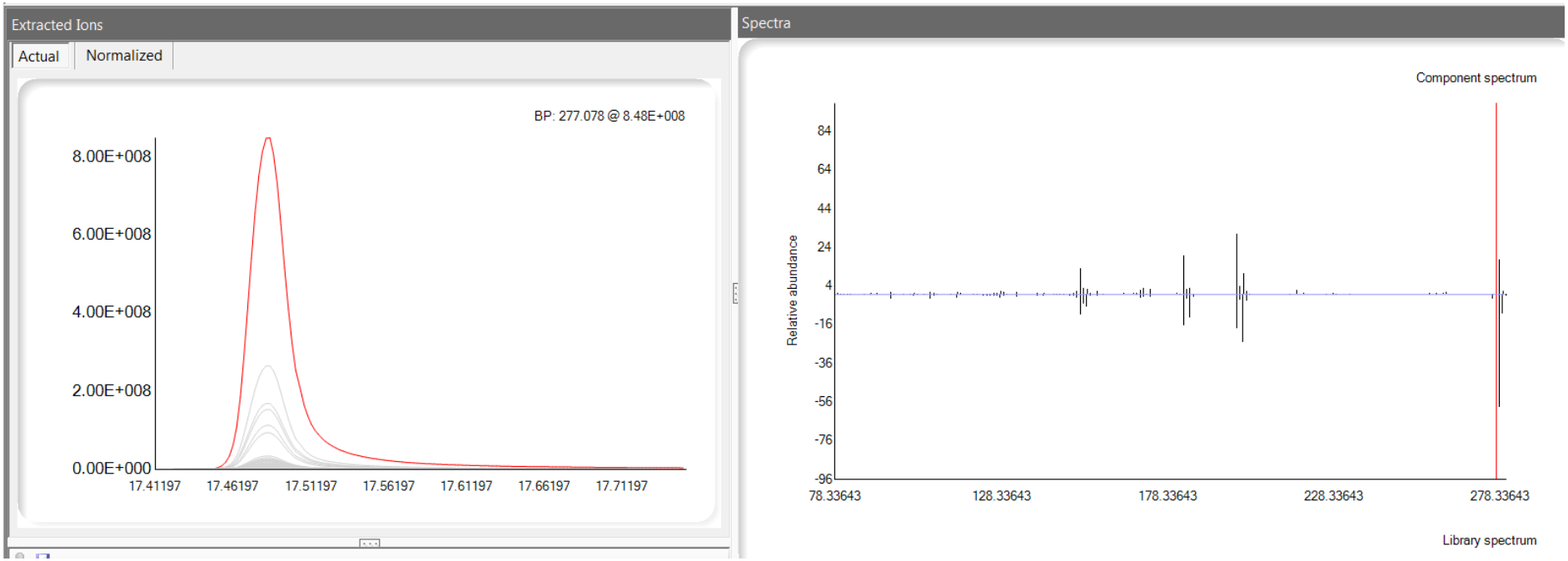
Step 1: Deconvolution of the data

- TraceFinder first performs an accurate mass deconvolution of the data.
  - Extracts all of the peaks in the chromatogram.
  - Provides a cleaned spectrum for library matching.



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Step 2: Quickly isolate the peaks of interest

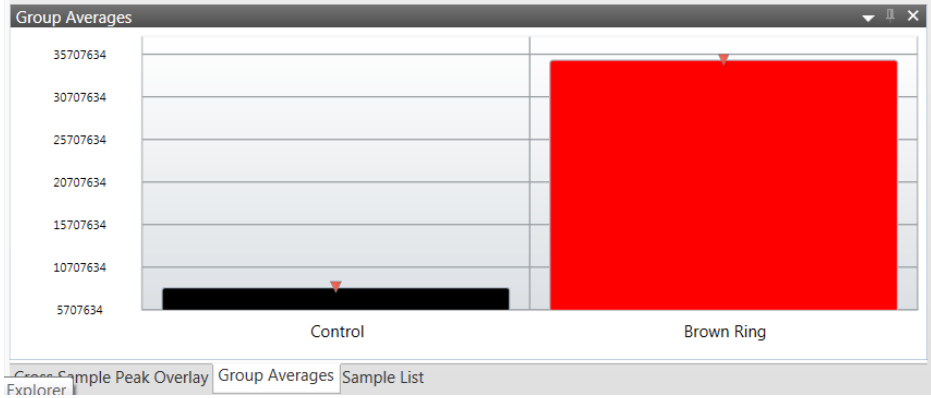
- 2051 peaks were extracted from the brown O-ring sample

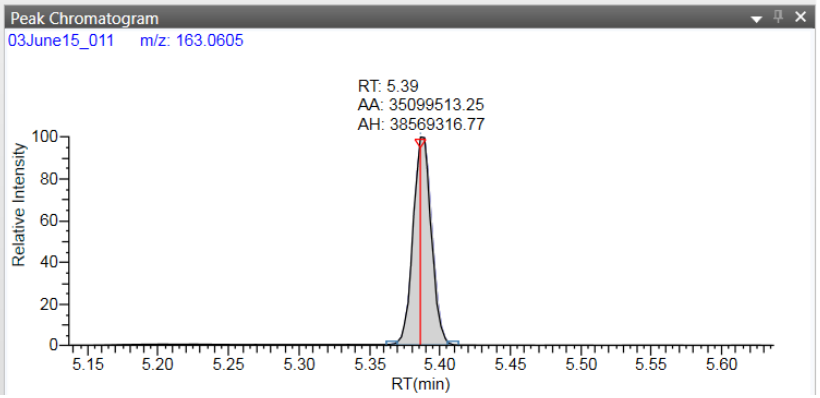
Data Review - Smithers Brown Ring 100% Ethanol [Unknown]

Heat Map

	Retention Time	M/Z	03June15_011 MS Area	03June15_008 MS Area
1	17.49	277.078	1,613,614,467	79,771
2	4.16	225.043	250,124,178	59,318,952
3	4.14	225.043	245,796,796	59,403,820
4	15.42	183.036	146,937,559	8,822
5	5.10	151.024	116,931,439	64,836,840
6	4.86	151.024	115,515,172	65,874,174
7	11.10	219.174	79,016,292	25,917
8	5.66	149.063	74,423,683	119,322,246
9	5.67	149.063	74,423,573	119,322,246
10	5.52	139.042	44,584,675	53,088,222
11	4.69	91.021	41,165,401	54,566,843
12	4.78	119.052	37,843,751	48,065,445
13	7.65	105.034	37,276,135	58,720,844
14	5.39	163.060	**35,099,513**	8,379,623
15	5.70	265.020	34,532,011	4,799,743

Heat Map indicates the peaks elevated in the sample.





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Top 10 differential peaks in Brown O-ring

Cross Sample Peak List

	Retention Time	M/Z	Control Average Area	Brown Ring Average Area	Brown Ring Fold
1	11.93	263.201	1	15,395,046	15,395,046
2	16.33	260.075	1	2,782,295	2,782,295
3	12.89	219.037	1	2,770,686	2,770,686
4	17.49	277.078	79,771	1,613,614,467	20,228
5	15.42	183.036	8,822	146,937,559	16,655
6	13.57	185.042	1,249	5,963,241	4,773
7	11.10	219.174	25,917	79,016,292	3,049
8	11.02	221.154	2,586	4,183,057	1,617
9	11.46	185.042	6,077	6,840,822	1,126
10	18.01	183.036	4,685	4,161,114	888

Peaks list can also be sorted by fold difference compared with control to isolate the differential peaks that could be low or high intensity.

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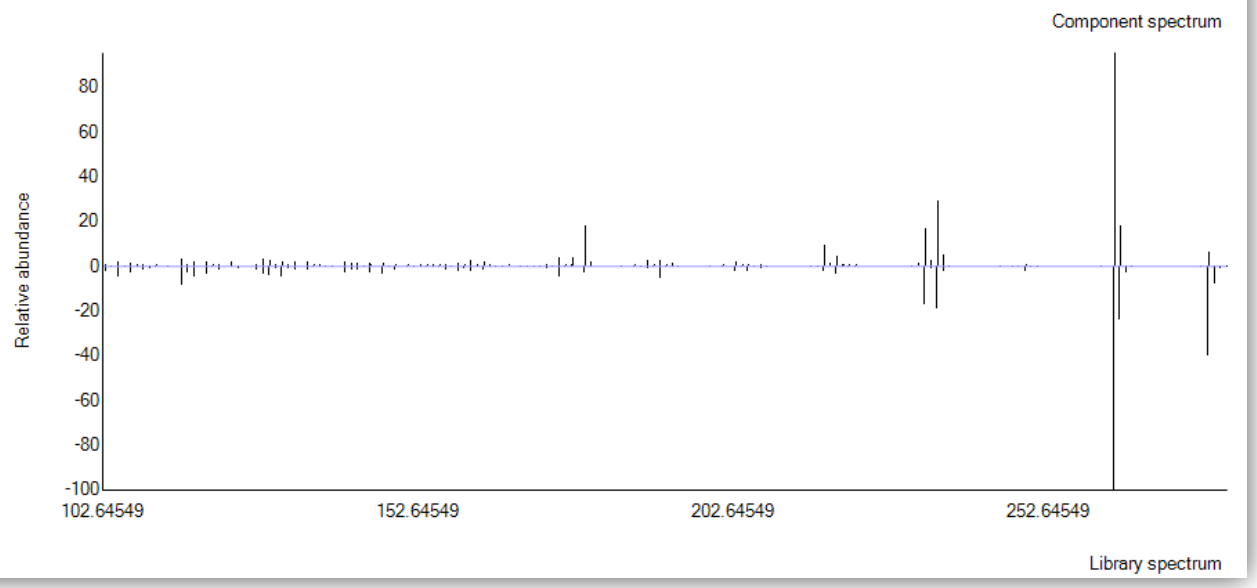
Step 3: Identify the compound – searching NIST 14

Peak Identification

Score	Matched Compound	Formula	CAS	SI	HRF Score	M+ m/z	M+	M+ Lib	% Elements
94.4	**1,4-Dihydrophenacetic acid,...**	**C18H30O2**		**728**	**99.4959**	**278.22403**	**Yes**	**Yes**	**100**
70.4	1,5-Dioxaspiro[5.6]dodeca-7,...	C18H32O2Si2		524	99.8259	336.19353	No	Yes	100
57.7	Benzoic acid, 3,5-bis(1,1-dim...	C17H26O3	1620-64-0	706	58.9058	278.18764	No	Yes	100
56.8	3,5-di-tert-Butyl-4-hydroxyph...	C17H26O3	20170-32-5	659	58.9058	278.18764	No	Yes	100
55.4	Benzenemethanol, 3,5-bis(1,1...	C17H26O3	14387-17-8	591	58.9058	278.18764	No	Yes	100
51.3	Monoallyl phthalate, TBDMS ...	C17H24O4Si		517	52.4488	320.14383	No	No	100
44.5	2,6-Bis(tert-butyl)phenol, TMS...	C17H30OSi	10416-73-6	514	35.6312	278.20604	No	Yes	100
42.4	12-Cyclohex-3-enyl-3-methyl...	C23H24N2O		533	29.2285	344.18831	No	Yes	100
41.6	6-Oxo-5-phenyl-2,3,5,6-tetrah...	C16H13N3O	87365-22-8	525	27.7606	263.10531	No	Yes	100

Spectra



- 26 Hits from NIST are sorted based on:
  - Spectral matching
  - High Resolution
  - Filtering (HRF) score.

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Step 3: Identify the compound – searching NIST 14

Combined SI and HRF values give an overall score (%) to quickly and confidently identify the compound. Eliminates other hits that would be valid if only SI used.

Pea entification

Score	Matched Compound	Formula	CAS	SI	HRF Score	M+ m/z	M+	M+ Lib	% Elements
94.4	**1,4-Dihydrophenacetic acid,...**	**C18H30O2**		**728**	**99.4959**	**278.22403**	**Yes**	**Yes**	**100**
70.4	1,5-Dioxaspiro[5.6]dodeca-7,...	C18H32O2Si2		524	99.8259	336.19353	No	Yes	100
57.7	Benzoic acid, 3,5-bis(1,1-dim...	C17H26O3	1620-64-0	706	58.9058	278.18764	No	Yes	100
56.8	3,5-di-tert-Butyl-4-hydroxyph...	C17H26O3	20170-32-5	659	58.9058	278.18764	No	Yes	100
55.4	Benzenemethanol, 3,5-bis(1,1...	C17H26O3	14387-17-8	591	58.9058	278.18764	No	Yes	100
51.3	Monoallyl phthalate, TBDMS ...	C17H24O4Si		517	52.4488	320.14383	No	No	100
44.5	2,6-Bis(tert-butyl)phenol, TMS...	C17H30OSi	10416-73-6	514	35.6312	278.20604	No	Yes	100
42.4	12-Cyclohex-3-enyl-3-methyl...	C23H24N2O		533	29.2285	344.18831	No	Yes	100
41.6	6-Oxo-5-phenyl-2,3,5,6-tetrah...	C16H13N3O	87365-22-8	525	27.7606	263.10531	No	Yes	100

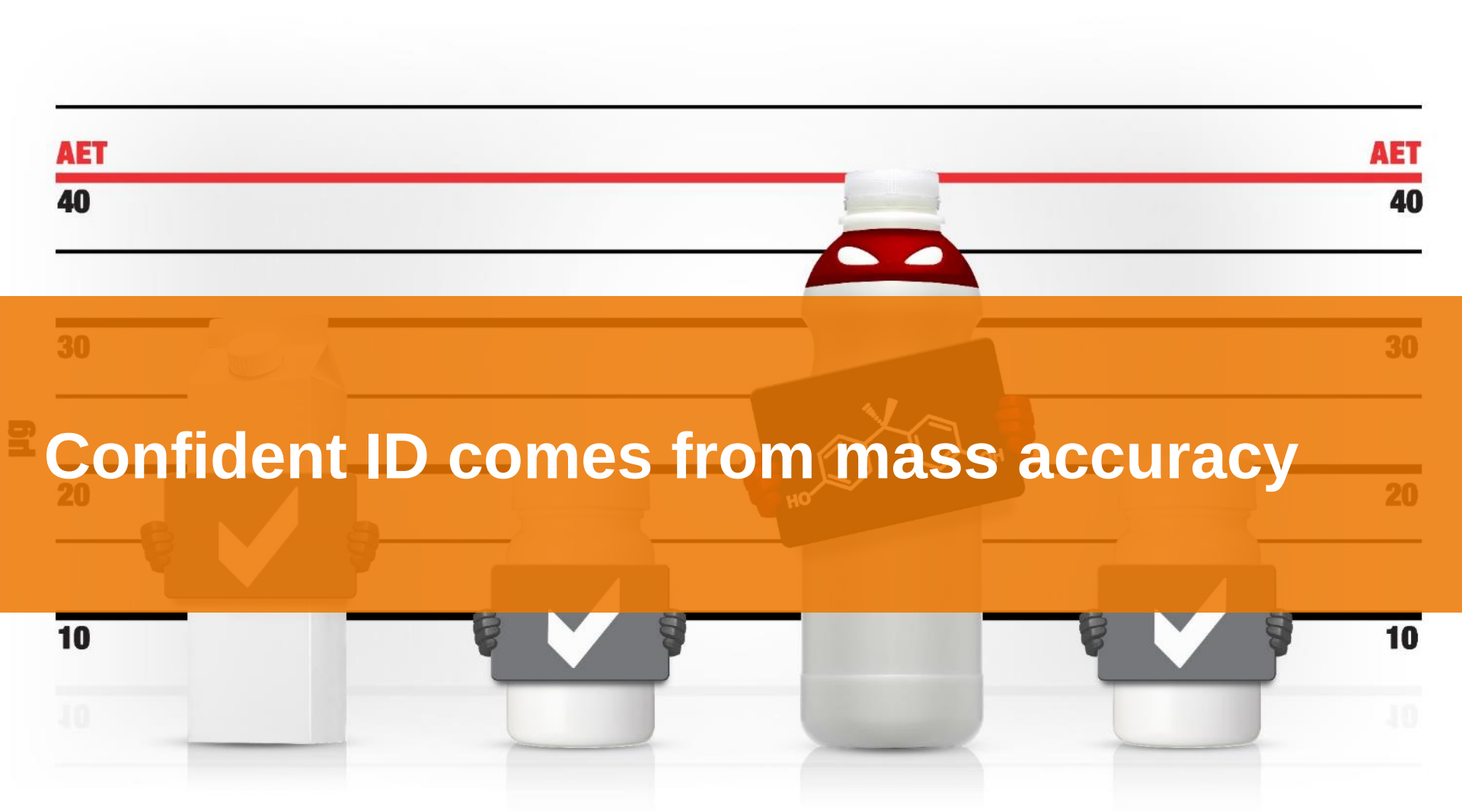
ThermoFisher SCIENTIFIC

Step 4: Fragments can be explained with < 1ppm mass accuracy

Measured m/z	Area	Fragment ID	Theo m/z	Mass error (ppm)
278.22412	956521	C(12)18 H30O2	278.22403	0.32348
264.20401	2825159	C(12)16 C(13)1 H27O2	264.2039	0.39818
263.20071	15464145	C(12)17 H27O2	263.20055	0.6079
249.18506	17789	C(12)16 H25O2	249.1849	0.64209
236.17262	717539	C(12)14 C(13)1 H23O2	236.1726	0.06436
235.16931	4502672	C(12)15 H23O2	235.16925	0.25514
234.19318	420047	C(12)15 C(13)1 H25O	234.19334	0.70369
233.19005	2618908	C(12)16 H25O	233.18999	0.2573
233.15364	179702	C(12)15 H21O2	233.1536	0.17156
232.18231	164562	C(12)16 H24O	232.18216	0.64604
222.15691	15338	C(12)13 C(13)1 H21O2	222.15695	0.20166
221.15359	145557	C(12)14 H21O2	221.1536	0.04522
220.17767	117435	C(12)14 C(13)1 H23O	220.17769	0.11264
220.14131	70817	C(12)13 C(13)1 H19O2	220.1413	0.02362
219.17430	717976	C(12)15 H23O	219.17434	0.1825
219.13797	551886	C(12)14 H19O2	219.13795	0.09127
218.16208	227603	C(12)14 C(13)1 H21O	218.16204	0.16135
217.15871	1435532	C(12)15 H21O	217.15869	0.0921
207.13797	43932	C(12)13 H19O2	207.13795	0.09655
205.15869	28947	C(12)14 H21O	205.15869	0
205.12231	94843	C(12)13 H17O2	205.1223	0.04875

ThermoFisher SCIENTIFIC

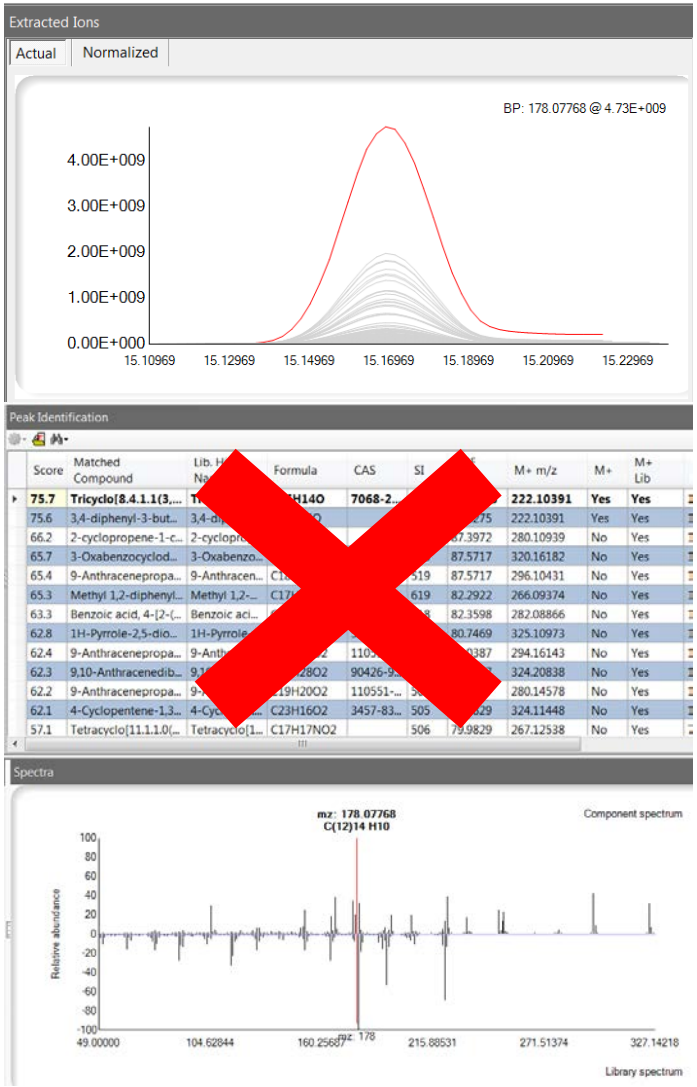
Identifying without a library hit?



Confident ID comes from mass accuracy

ThermoFisher SCIENTIFIC

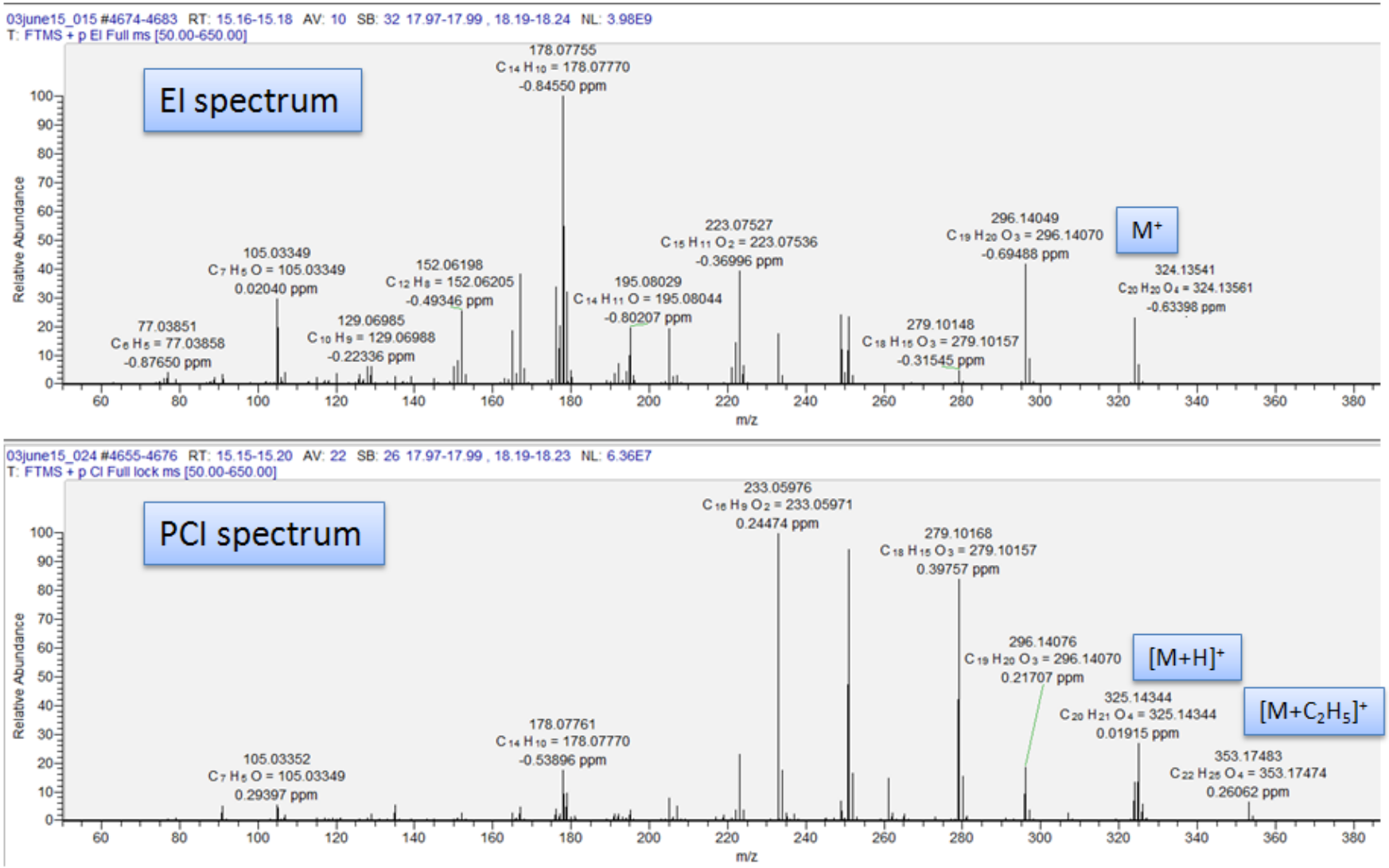
Black O ring peak at 15.17 minutes – no clear NIST hit



No clear match with low score at 75.7%

ThermoFisher SCIENTIFIC

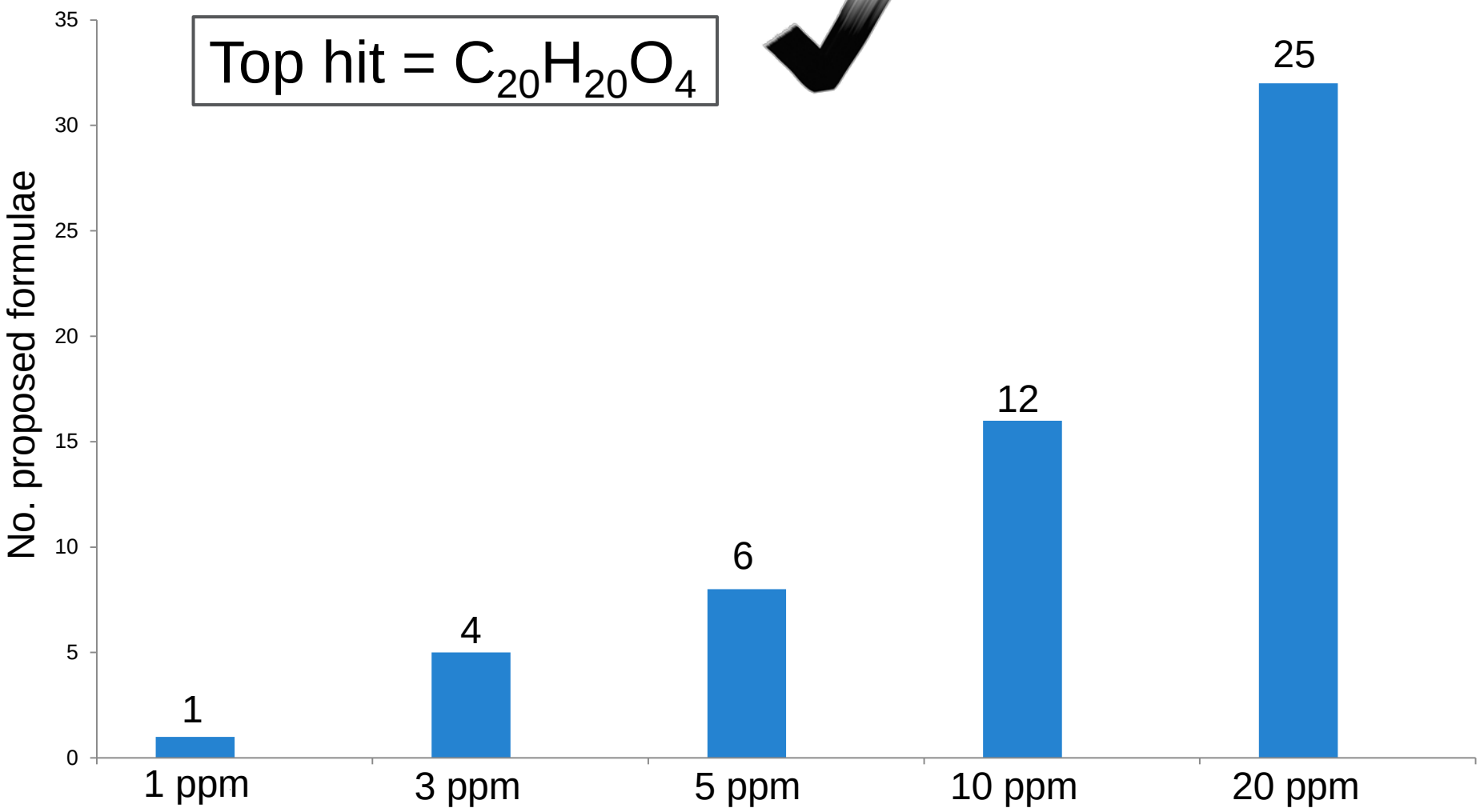
EI & PCI spectra for peak at 15.17 mins.



ThermoFisher SCIENTIFIC

No. of proposed formulae for *m/z* 324.13541

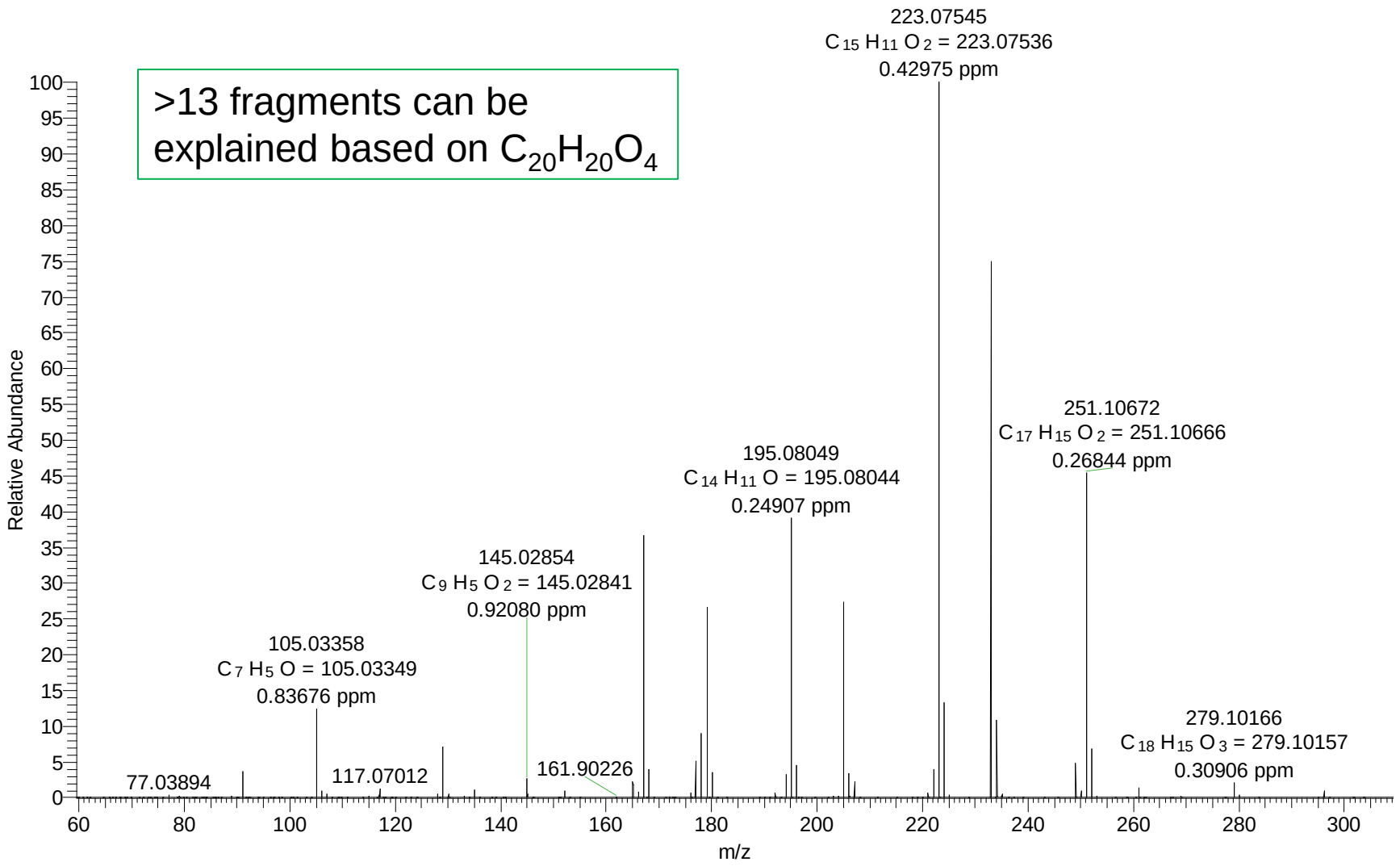
Top hit = $C_{20}H_{20}O_4$



Elements used: C (1-30), H (1-60, N (1-5), O (1-5), P (1-2), S (1-2)

ThermoFisher SCIENTIFIC

MS/MS *m/z* 325.14 to support proposed formula



ThermoFisher SCIENTIFIC

Summary of identifications in Black and Brown O-rings

Sample	RT (min)	Base Peak (m/z)	Search Index	HRF	Compound Name	Formula	Base Peak Mass Accuracy (ppm)	Molecular ion Mass Accuracy (ppm)
Black O Ring	15.17	178.07754	No match	No match	Unknown	$C_{20}H_{20}O_4$	0.88	0.66
	15.29	178.07754	No match	No match	Unknown	$C_{20}H_{20}O_4$	0.11	0.22
	18.08	171.13806	673	99.965	Tetraethylene glycol bis (2-ethylhexanoate)	$C_{24}H_{46}O_7$	0.64	-
	23.47	219.17435	777	99.805	Irganox 1076	$C_{35}H_{62}O_3$	0.03	1.02
	14.94	280.10939	536	99.8458	ethyl 1-hydroxy-2,3-diphenylcycloprop-2-ene-1-carboxylate	$C_{18}H_{16}O_3$	0.39	0.39
	16.50	126.09145	652	99.7409	9-Octadecenamide	$C_{18}H_{35}NO$	0.87	0.63
Brown O Ring	17.48	277.07790	806	98.7	Triphenylphosphine oxide	$C_{18}H_{15}OP$	0.85	0.06
	15.42	183.03595	831	99.7455	Triphenylphosphine	$C_{18}H_{15}P$	0.68	0.91
	11.10	219.1743	796	99.7775	4-tert-butyl-2,6-diisopropylphenol	$C_{16}H_{26}O$	0.18	0.21
	11.35	149.02341	831	98.4127	Diethyl phthalate	$C_{12}H_{14}O_4$	0.60	0.9
	13.57	185.04198	813	93.8	Diphenyl sulfide	$C_{12}H_{10}S$	0.21	0.05
	16.00	155.07025	690	100	di(butoxyethyl)adipate	$C_{18}H_{34}O_6$	0.13	1.02

Peaks listed are significantly different compared to control.

ThermoFisher SCIENTIFIC

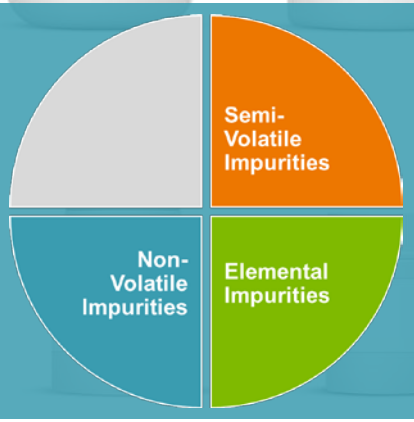
Summary of identifications in White and Red O-rings

Sample	RT (min)	Base Peak (m/z)	Search Index	HRF	Compound Name	Formula	Base Peak Mass Accuracy (ppm)	Molecular ion Mass Accuracy (ppm)
White O Ring	11.93	263.20074	711	99.8611	1,4 Dihydrophenacetic acid, 3,5-di-t-butyl, ethyl ester	$C_{18}H_{30}O_2$	0.72	0.43
	7.65	101.02344	781	100	Butanedioic acid, diethyl ester	$C_8H_{14}O_4$	0.54	-
Red O Ring	10.44	163.07549	775	99.6123	Ethanone, 1-[4-(1-hydroxy-1-methylethyl)phenyl	$C_{11}H_{14}O_2$	0.85	0.38
	15.09	87.044	740	99.8332	Methyl stearate	$C_{19}H_{38}O_2$	1.26	0.06
	16.00	155.07025	690	100	di(butoxyethyl)adipate	$C_{18}H_{34}O_6$	0.13	1.02

Peaks listed are significantly different compared to control.

ThermoFisher SCIENTIFIC

Results – Non-Volatile extractables



ThermoFisher SCIENTIFIC

LC-MS/MS instrument conditions

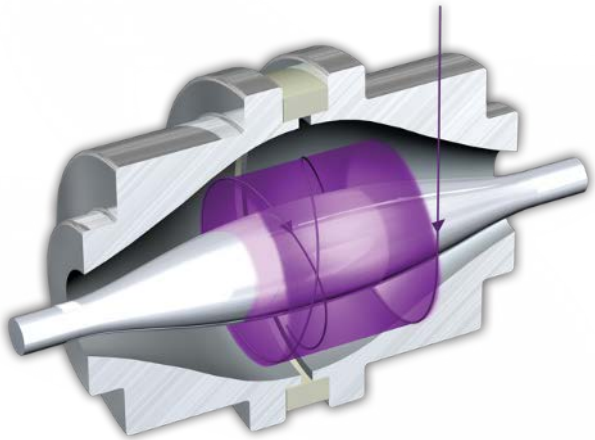
- Equipped with a HESI II ion source.
- Full scan MS and top3 data-dependent HCD MS/MS data were collected.
- Resolutions of 17,500 and 70,000
- Polarity switching.

Thermo Scientific™ Q Exactive Plus™

Hybrid Quadrupole-Orbitrap LC-MS/MS System.

Thermo Scientific™ UltiMate™ 3000

UHPLC System



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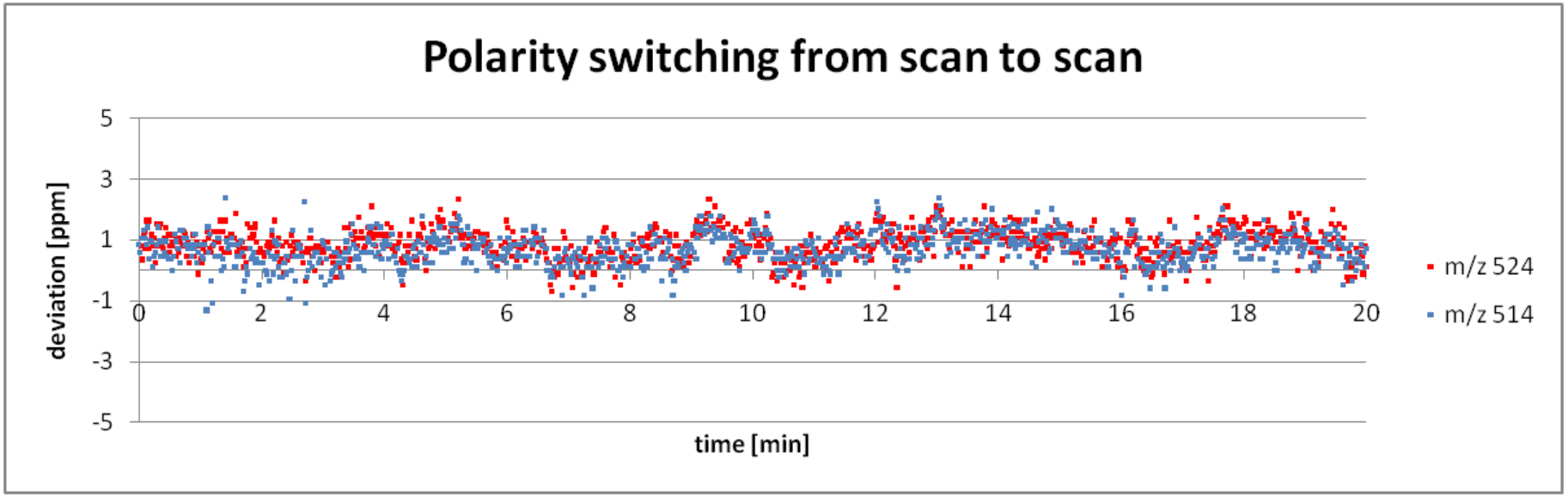
Mass Accuracy with Polarity Switching (External Calibration)



1 positive + 1 negative scan in <1 second

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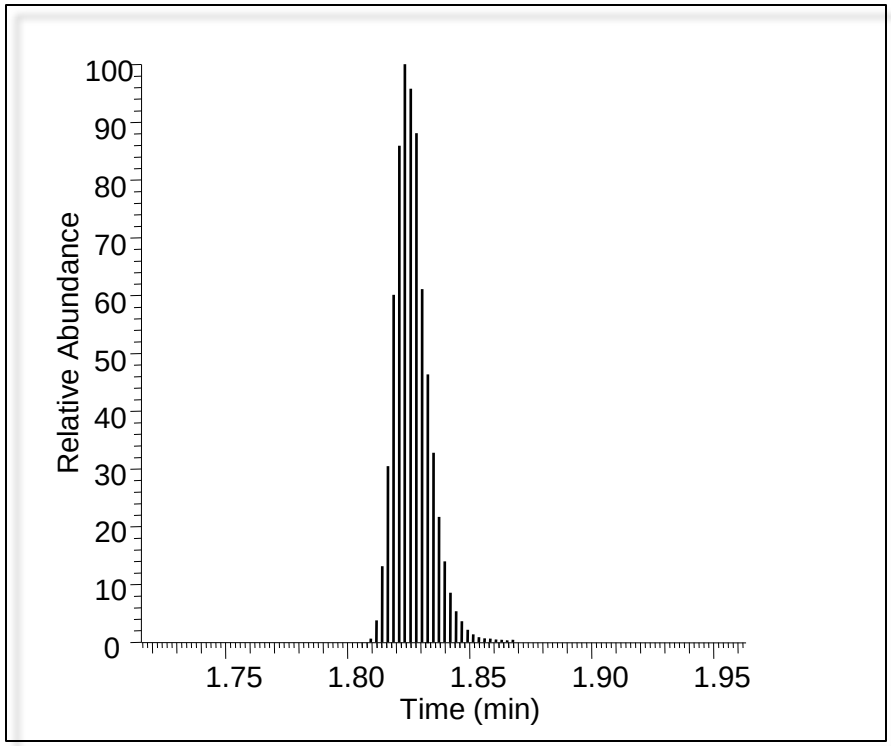
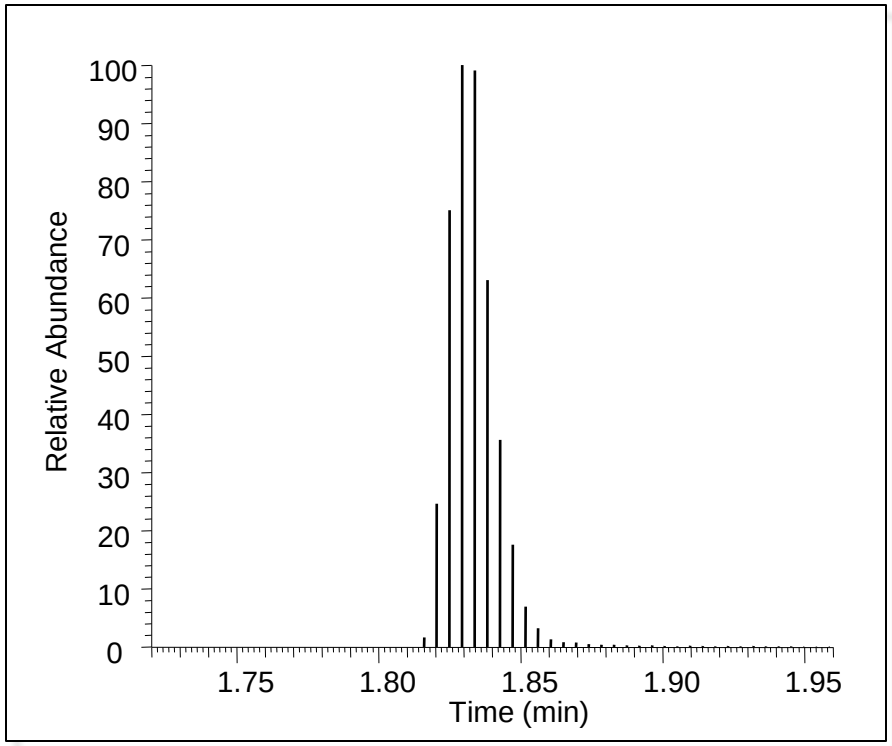
MS Scanning Speeds to meet UHPLC Separation

Resolution Setting: 35,000



Peakwidth (FWHM) ~ 1 sec
Scans/peak = 21

Resolution Setting: 70,000



Peakwidth (FWHM) ~ 1 sec
Scans/peak = 11

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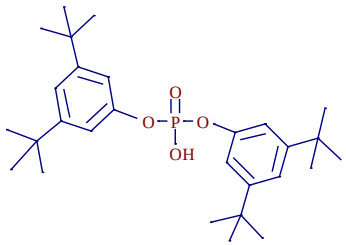
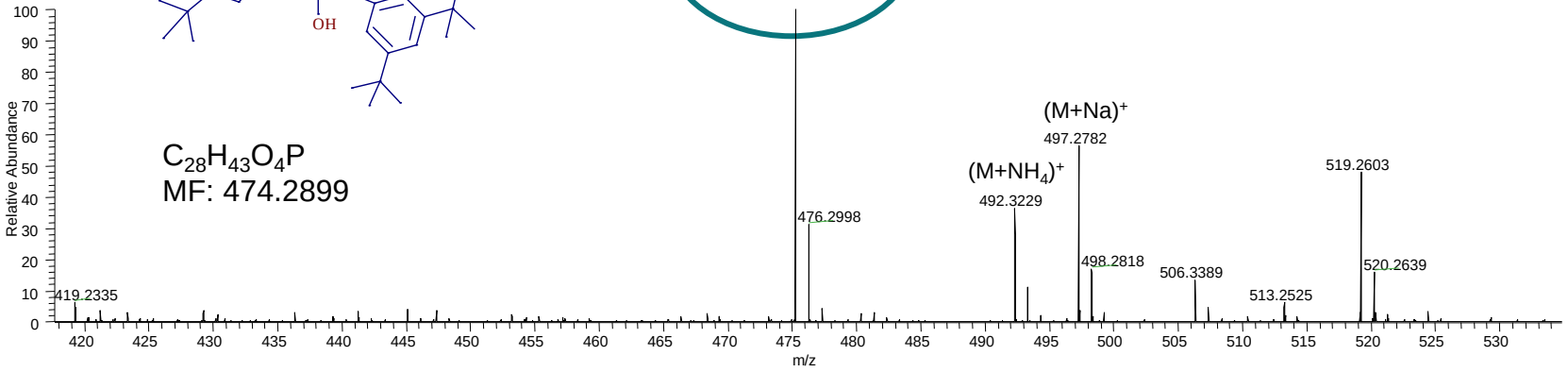
Polarity Switching maintains High Mass Accuracy



$C_{28}H_{43}O_4P$
MF: 474.2899

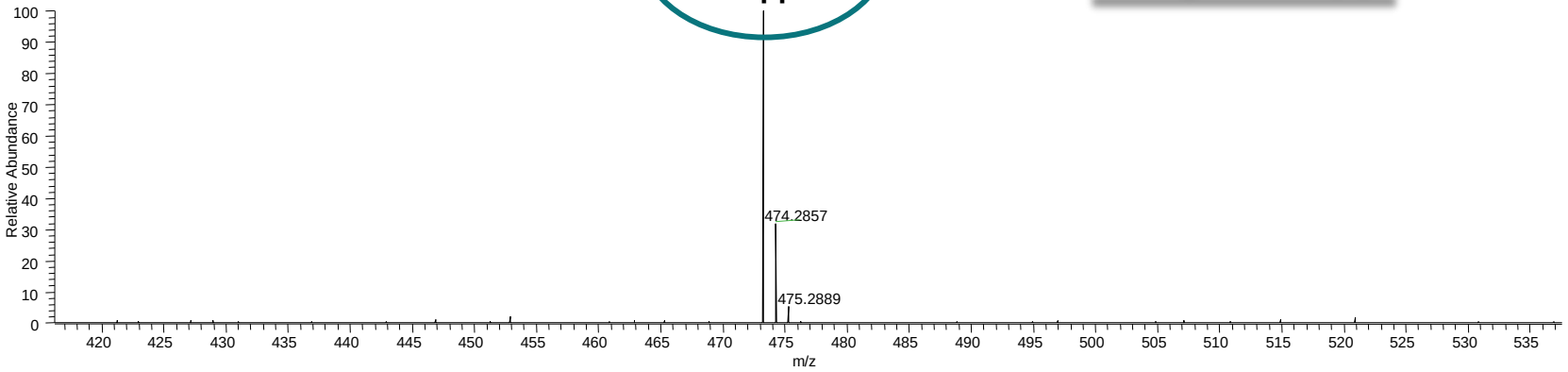
$(M+H)^+$
475.2964
-1.5 ppm

Positive (+)



$(M-H)^-$
473.2824
-0.4 ppm

Negative (-)



ThermoFisher SCIENTIFIC

Data Analysis For Unknown Quanfirmation

Thermo Scientific™
TraceFinder™ Software

Quantitation |
Deconvolution | Searching

Thermo Scientific™
Compound Discoverer™

Advanced Unknown
Identification | Metabolism

Thermo Scientific™
SIEVE™ Software

Differential Analysis | PCA

Small Molecule Unknown
ID & Quantification

Spectral Interpretation

Thermo Scientific™
Mass Frontier™ Software

Libraries & Mass Tables

High-Resolution Accurate-Mass
MS/MS Spectral Libraries

Validated Cloud Library

MZ Cloud™

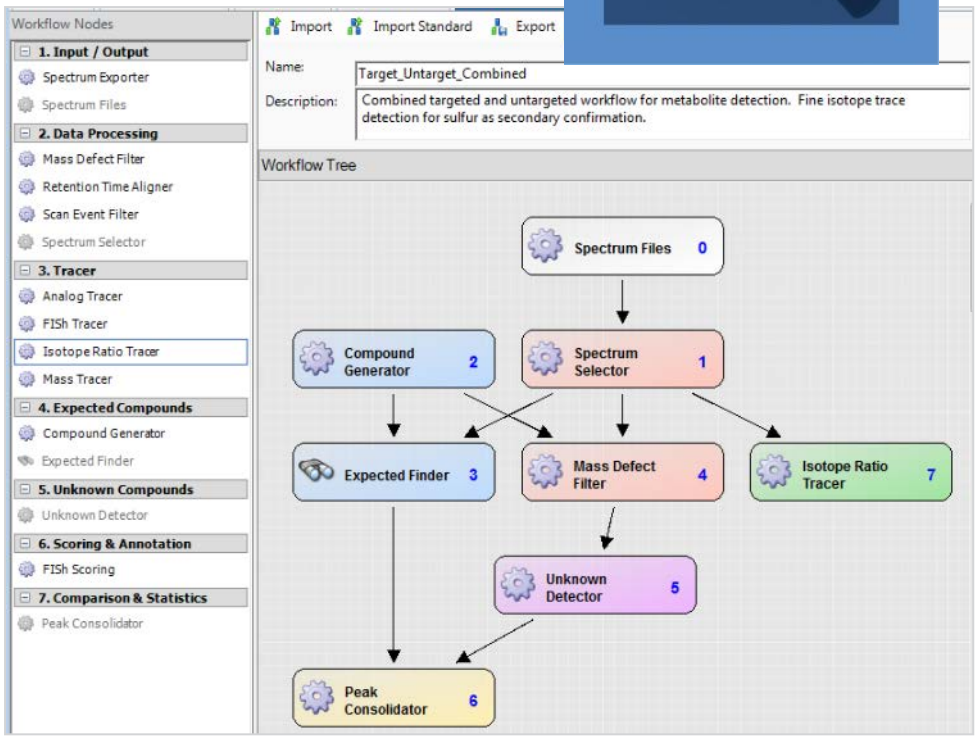
ThermoFisher SCIENTIFIC

Unknown analysis of small molecules

Thermo Scientific™ Compound Discoverer™

Customizable node based small molecule structure analysis software

- CD v.1 is for “known parent” workflows structure analysis
- CD v.2 will include “unknown” workflows structure analysis (E&L). **Coming end 2015.**



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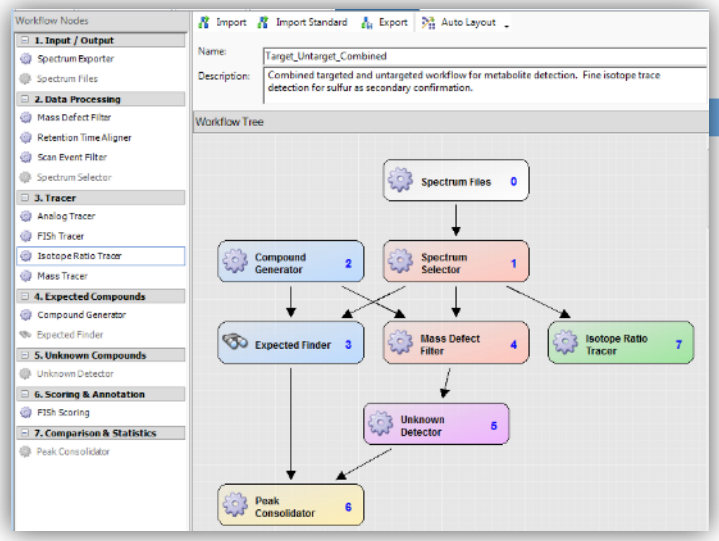
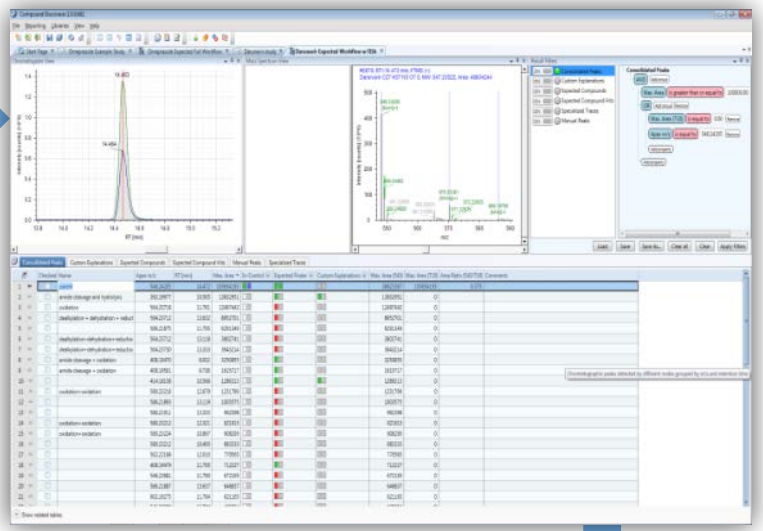
Compound Discoverer Workflow

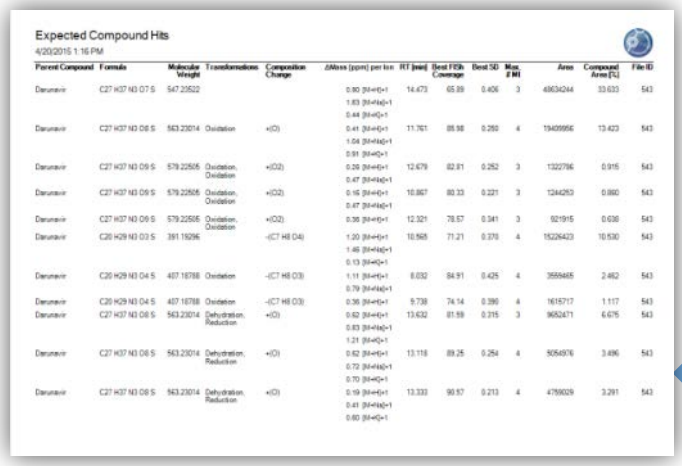
Process



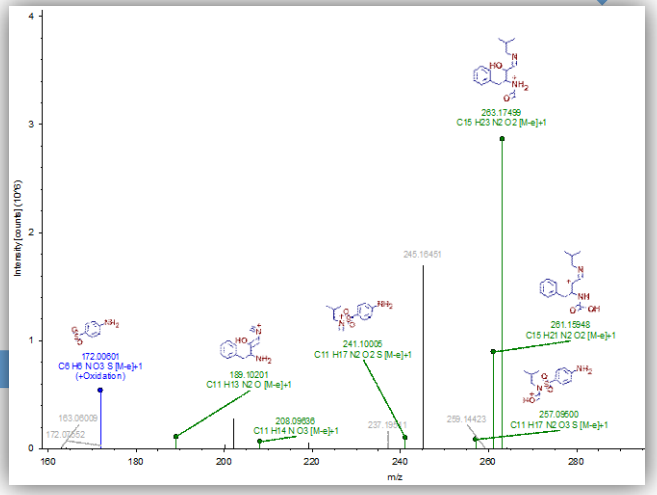
Review results



Report



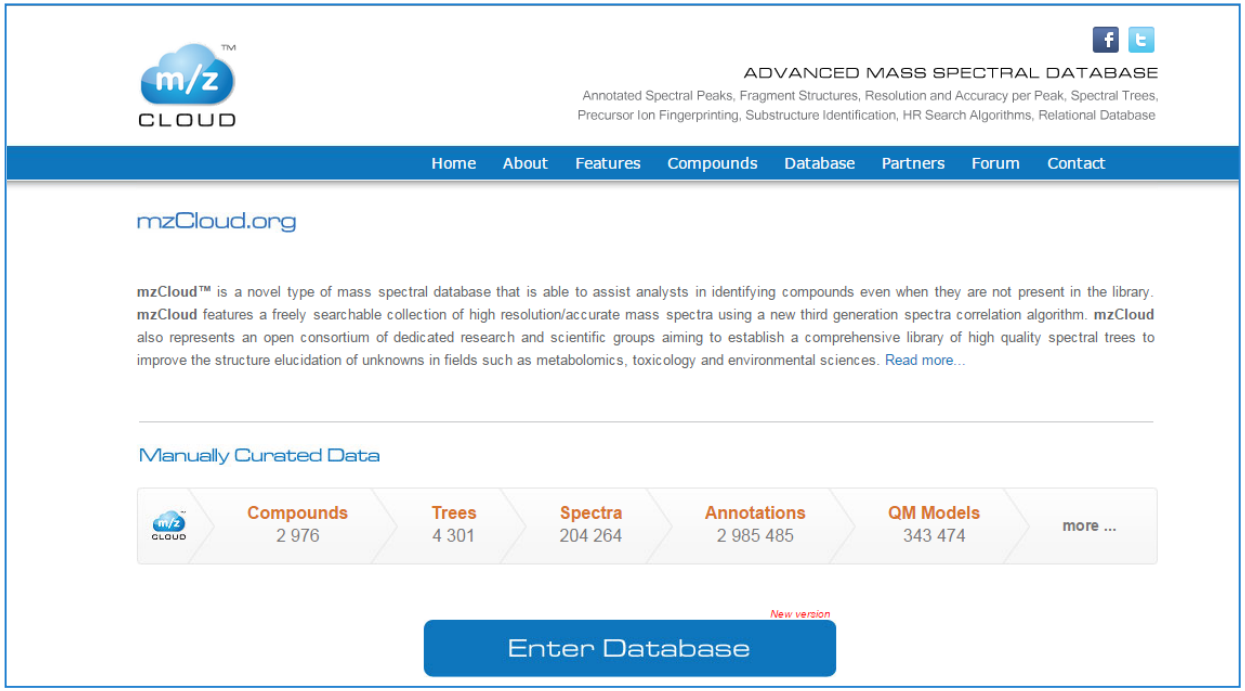
Elucidate structure

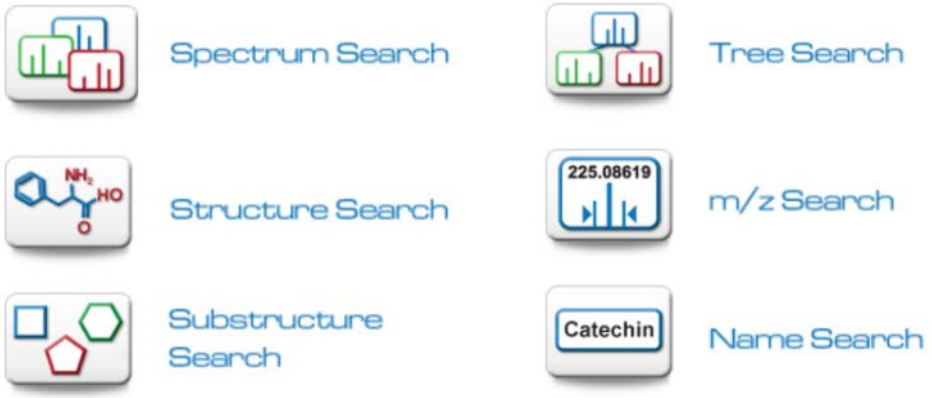


ThermoFisher SCIENTIFIC

www.mzcloud.org

- mzCloud™
  - Free
  - Advanced high resolution mass spectral database
  - Search spectrum, name, structure, substructure, and m/z
  - Identify compounds even when they are not present in the library through substructure search

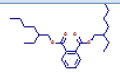
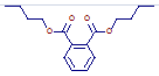
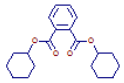
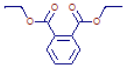




ThermoFisher SCIENTIFIC

High Resolution E&L Database

- Contains >1200 common E&L related compounds.
- Works with **TraceFinder** and **Compound Discoverer** for targeted screening.
- New E&L related compounds are added frequently.

Commercial Name	Chemical Name	Class	CAS No.	Chemical Formula	Formula weight	$(M•H)^-$	$(M-H)^-$	$M•NH_4^-$	$M•Na^-$	$M•K^-$	Structure	mzCLoud link
dioctyl phthalate	ethylhexylphthalate	PL	117-81-7	C24H38O4	390.27701	391.28429	389.26973	408.31083	413.26623	429.24017		https://mzcloud.org/DataViewer.aspx#CReference2
Dibutyl phthalate	Dibutyl phthalate	PL	84-74-2	C16H22O4	278.1518	279.15908	277.14452	296.18562	301.14102	317.11496		https://mzcloud.org/DataViewer.aspx#CReference2
	Dicyclohexyl phthalate		84-61-7	C20H26O4	330.18311	331.19039	329.17583	348.21693	353.17233	369.14627		https://mzcloud.org/DataViewer.aspx#CReference2
	Diethyl phthalate		84-66-2	C12H14O4	222.08921	223.09649	221.08193	240.12303	245.07843	261.05237		https://mzcloud.org/DataViewer.aspx#CReference2
	Dimethyl phthalate		131-11-3	C10H10O4	194.05791	195.06519	193.05063	212.09173	217.04713	233.02107		https://mzcloud.org/DataViewer.aspx#CReference2
	2-HYDROXY-3-METHYLBUTYRIC ACID		4026-18-0	C5H10O3	118.06299	119.07027	117.05571	136.09681	141.05221	157.02615		https://mzcloud.org/DataViewer.aspx#CReference3
	6-Aminocaproic acid		60-32-2	C6H13NO2	131.09463	132.10191	130.08735	149.12845	154.08385	170.05779		https://mzcloud.org/DataViewer.aspx#CReference3

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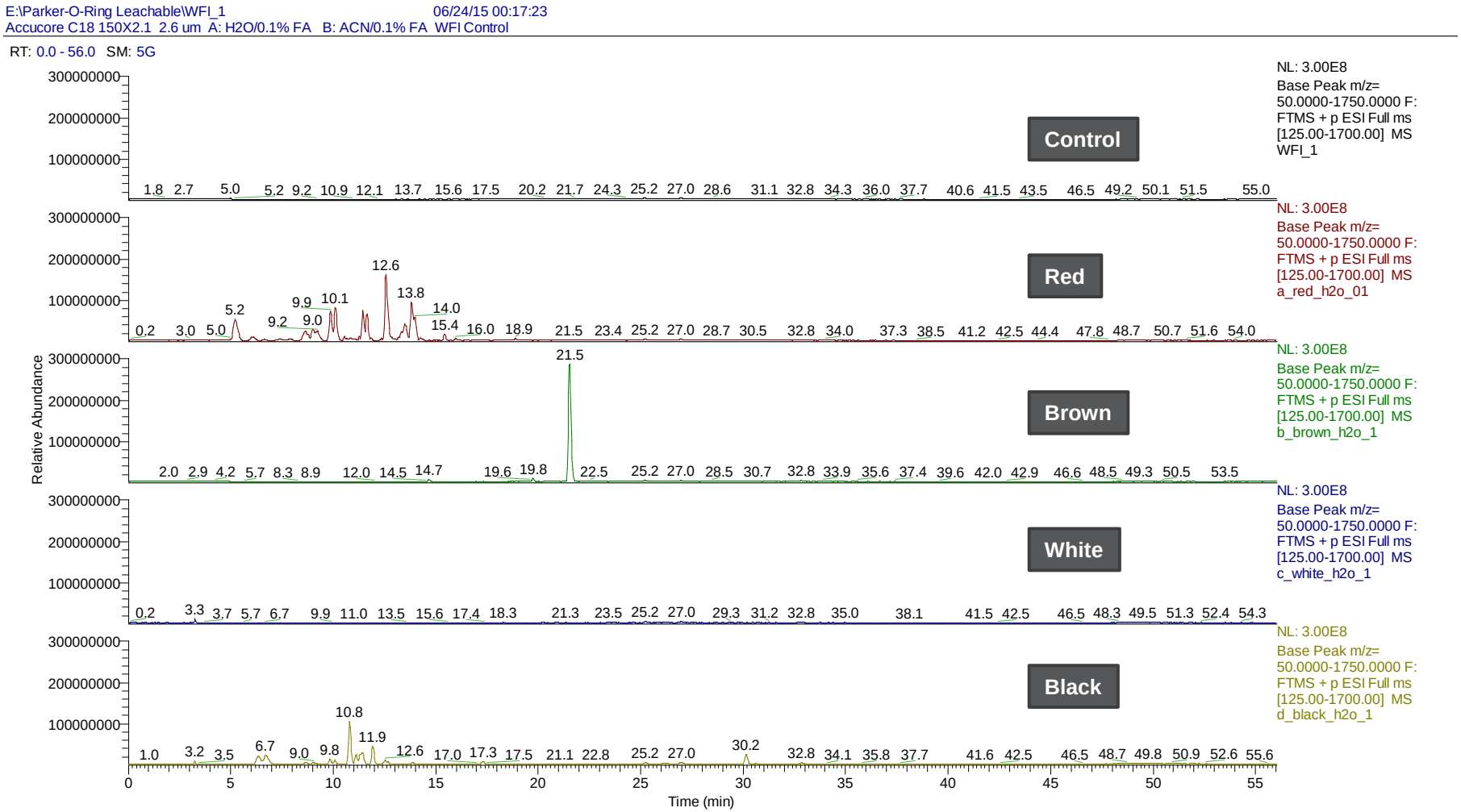
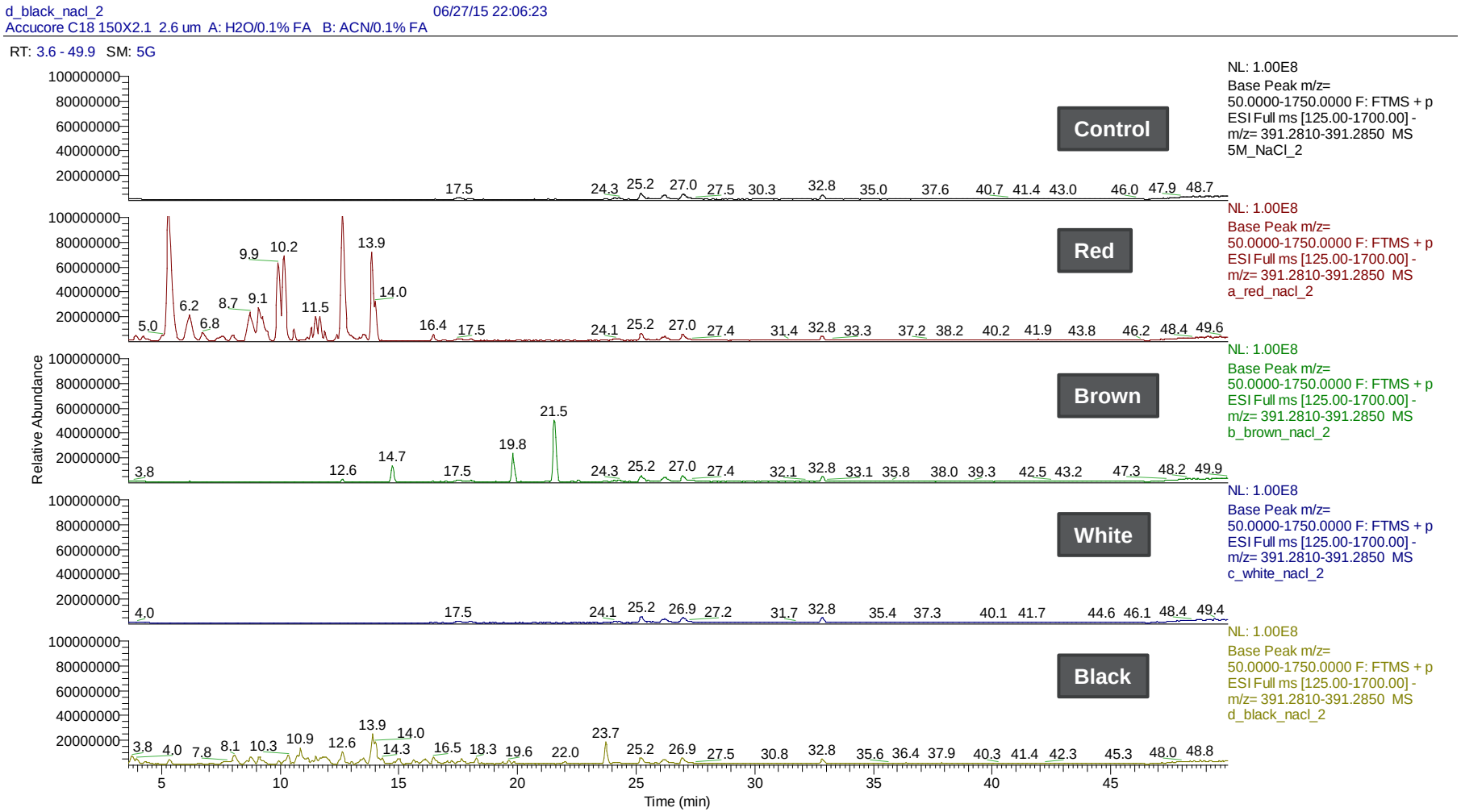
O-Ring Extractables - Water

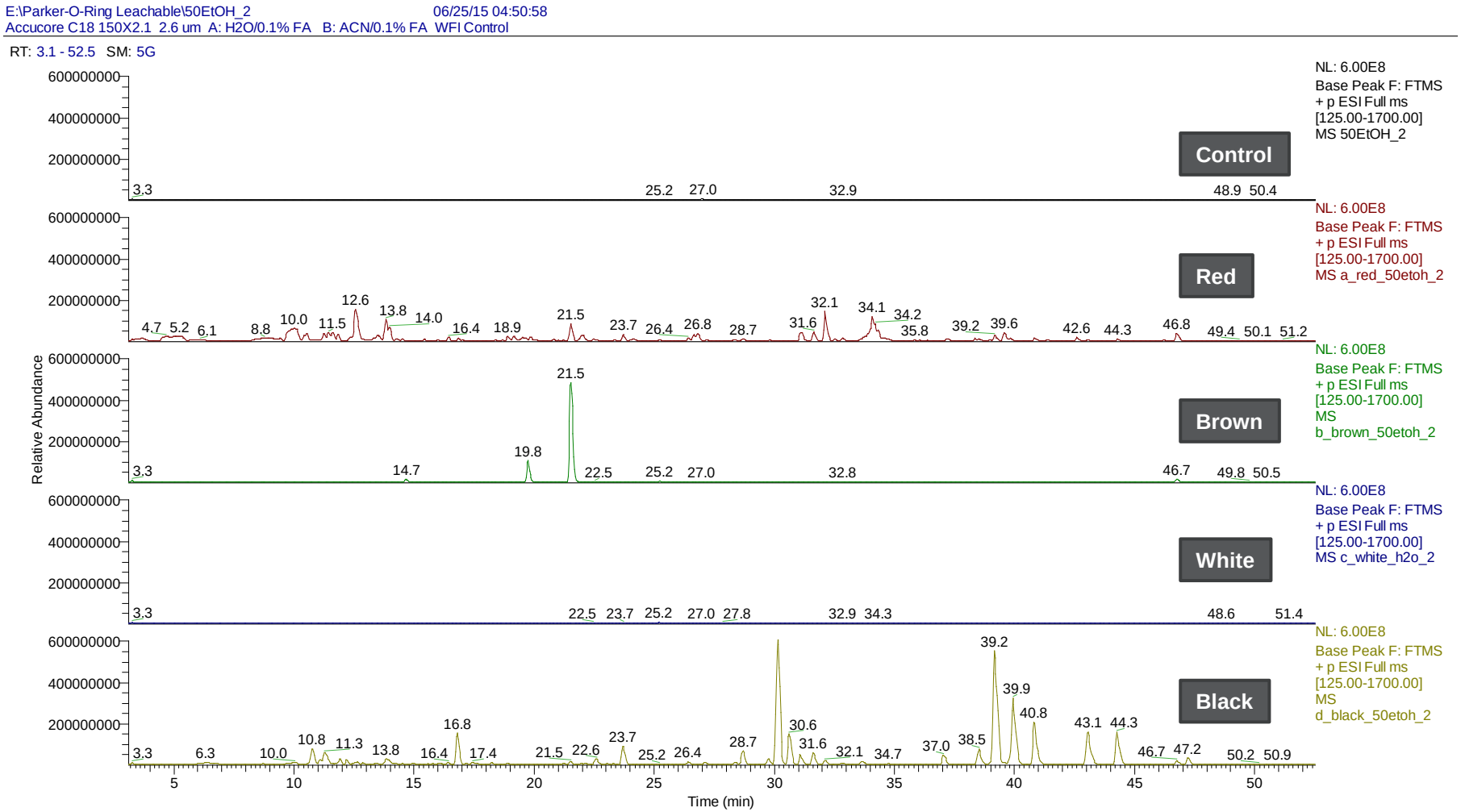


O-Ring Extractables – 5M NaCl

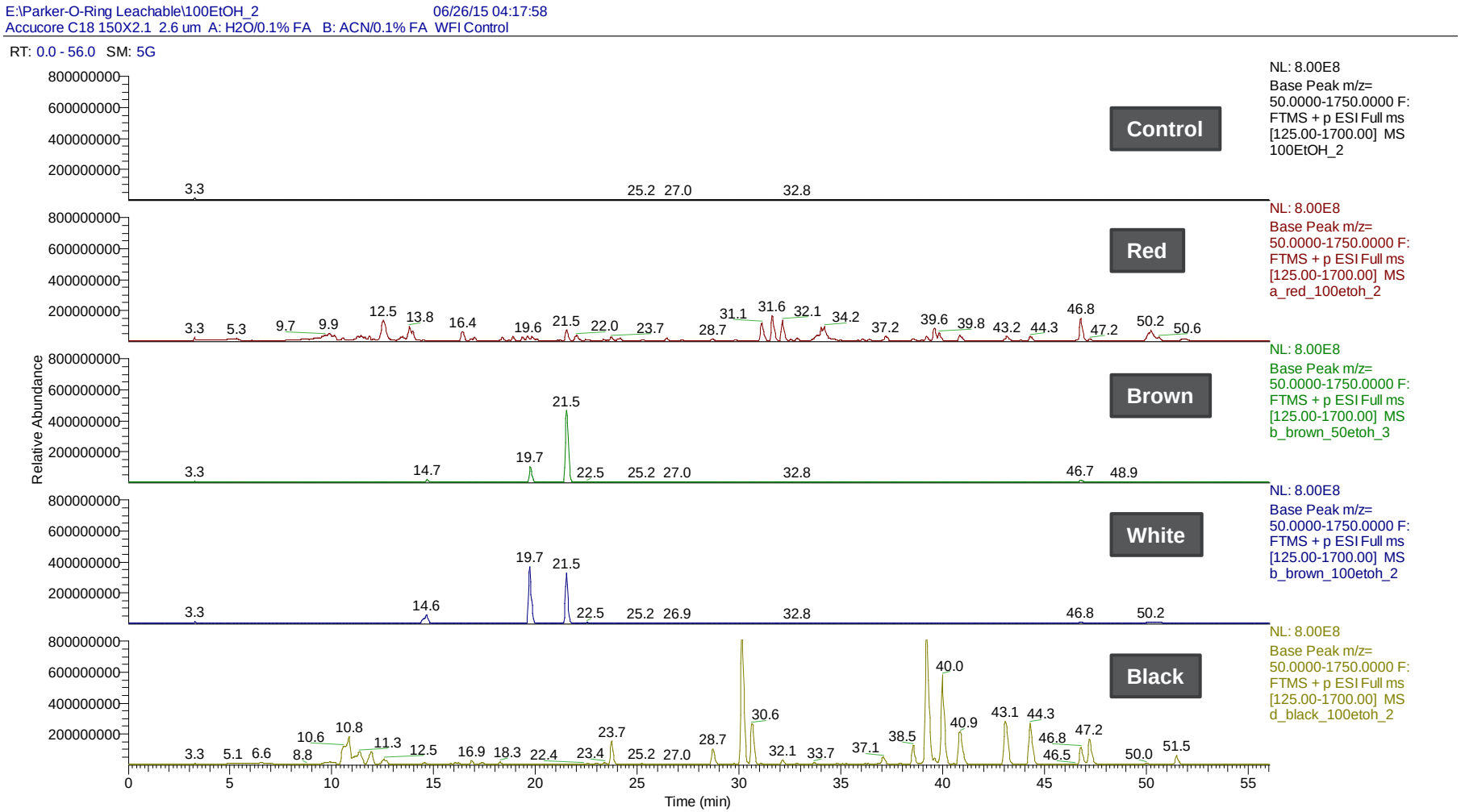


ThermoFisher SCIENTIFIC

O-Ring Extractables – 50% Ethanol

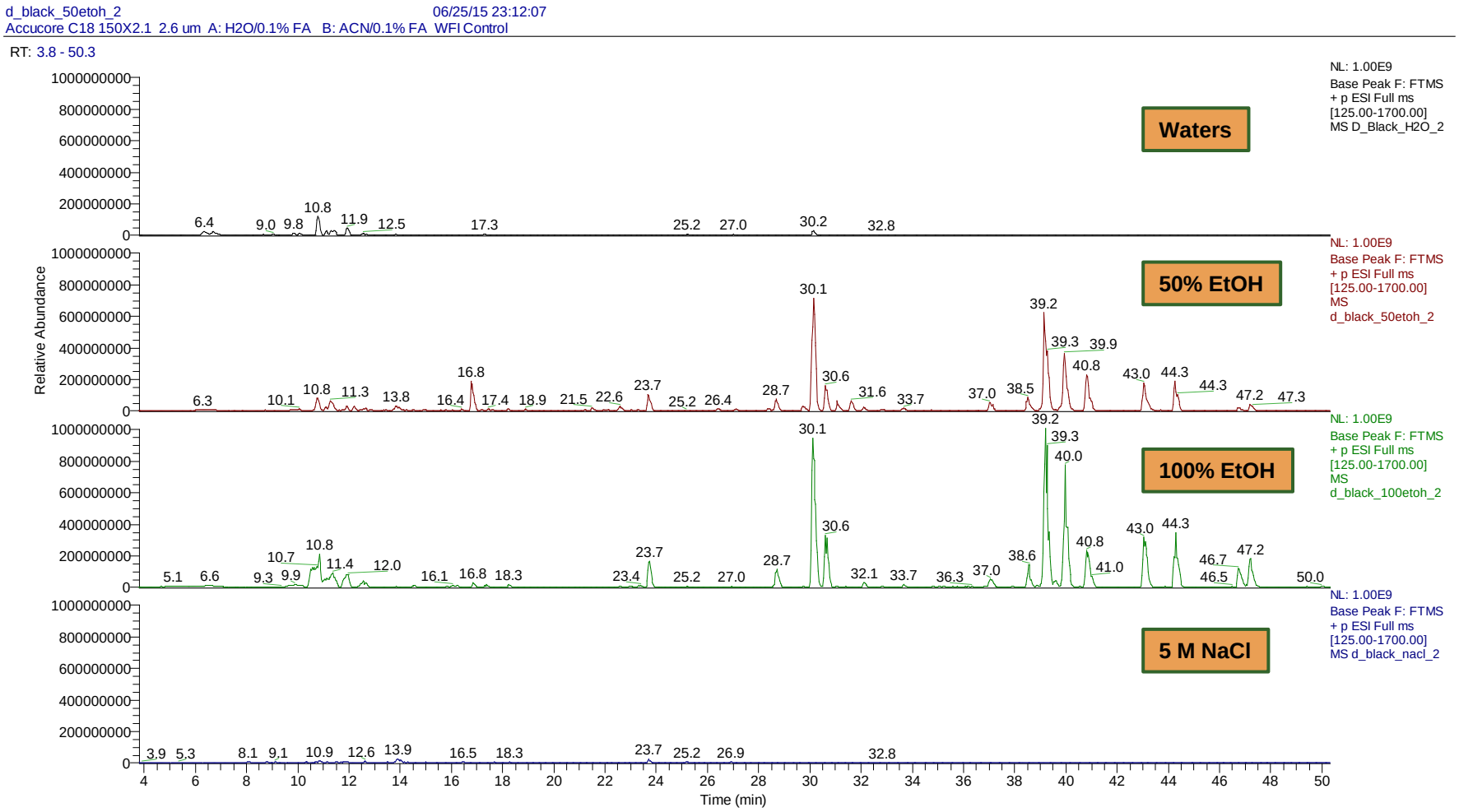


O-Ring Extractables – 100% Ethanol



ThermoFisher SCIENTIFIC

BPI+ of Black O-ring in Four Extraction Solutions

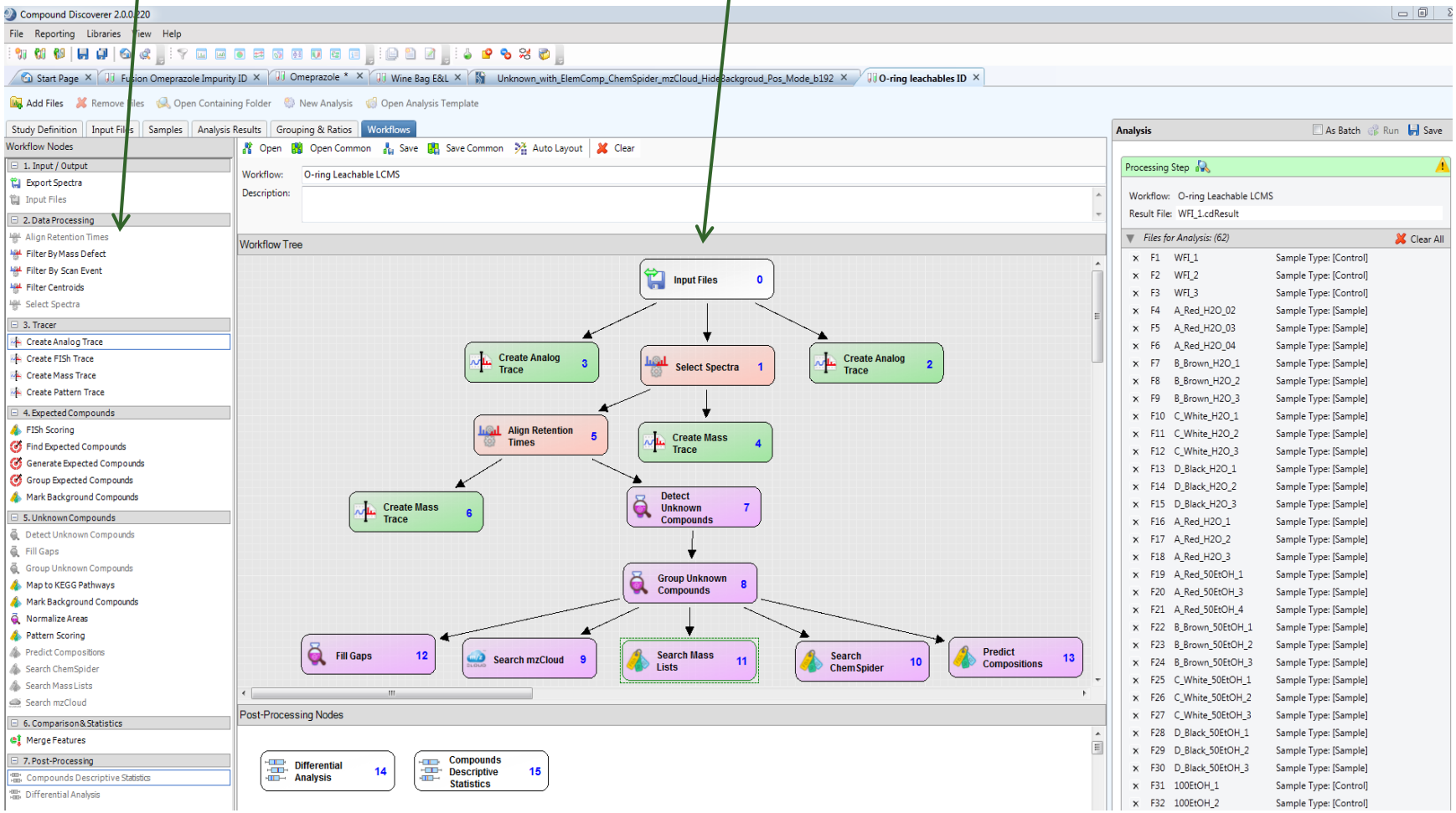


ThermoFisher SCIENTIFIC

Compound Discoverer Workflow for O-Ring leachables

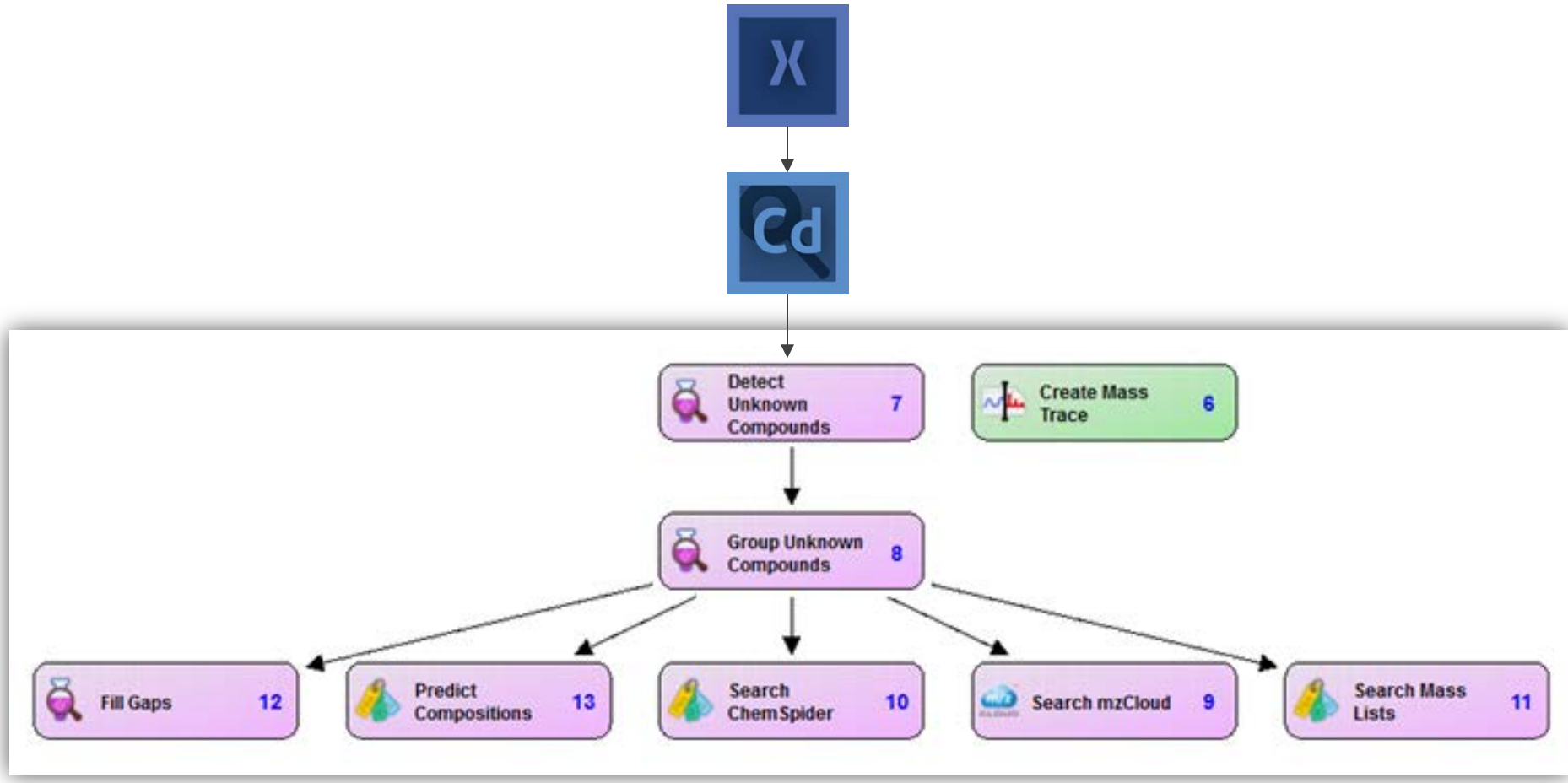
Workflow Nodes

Workflow Tree



ThermoFisher SCIENTIFIC

Automated Identification – Compound Discoverer



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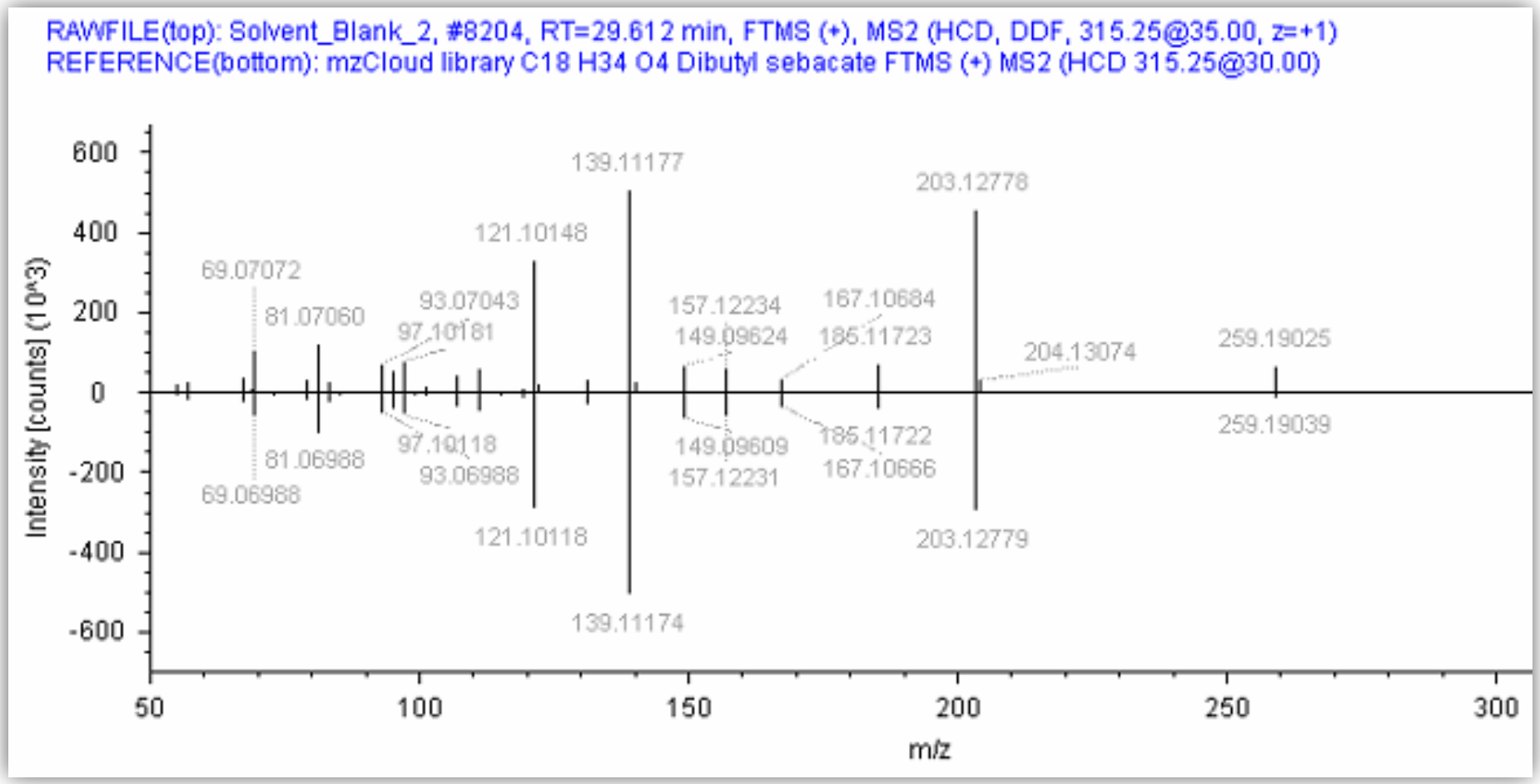
Data are directly mirror-plotted versus mzCloud



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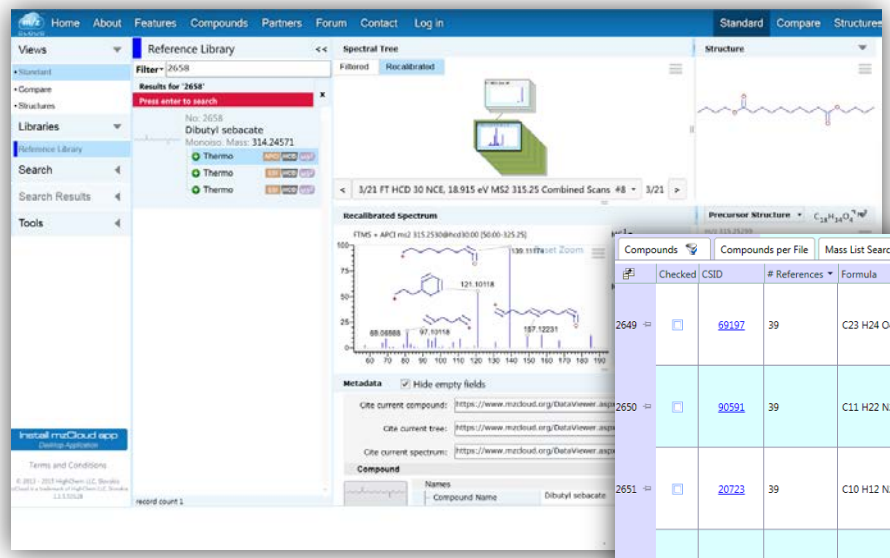
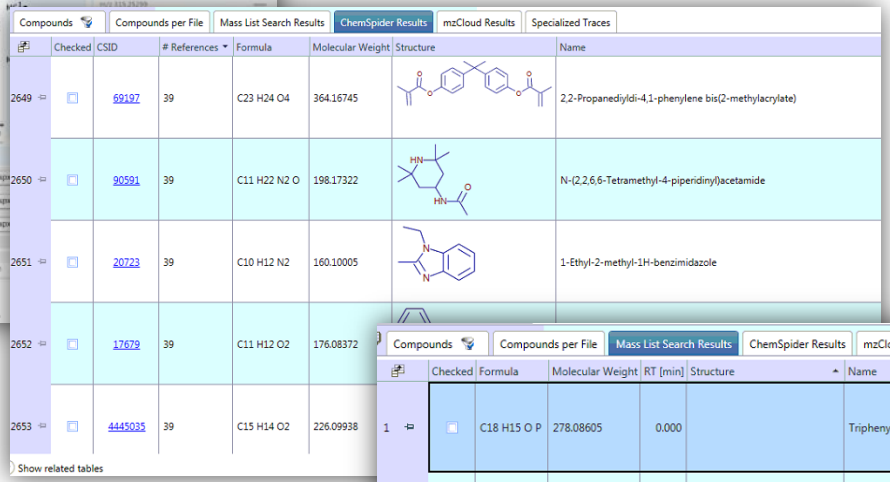
Parallel identification through multiple reference sources

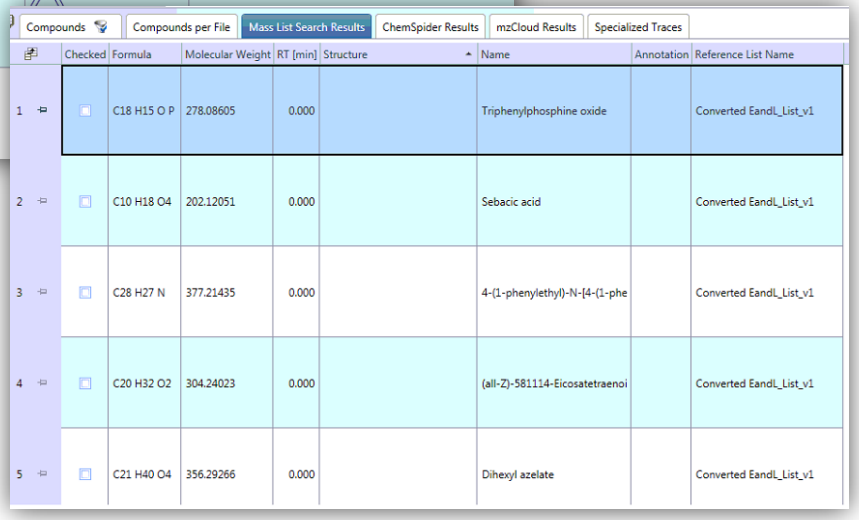
mzCloud Library Spectrum



ChemSpider Search Results



E&L Library Results



Components identified confidently

- Compound Discoverer allows rapid identification with confident searching of multiple databases in parallel
- Targeted search compounds can be easily confirmed.
- High confidence Orbitrap data (<1ppm) combine with mzCloud libraries

O-Ring Type	Study Solution	Peak ID	RT (min)	Measured $(M+H)^+$	Calculated (M+H)+	Elemental Composition	Error (PPM)	Name
Brown	WFI	1	1.3	173.0792		C6H14O4Na		PEG
Brown	WFI	2	1.5	217.1052		C8H18O5Na		PEG
Brown	WFI	3	1.8	261.1312		C10H22O6Na		PEG
Brown	WFI	4	3.5	171.0995		C7H16O3Na		Glycol ether
Brown	WFI	5	6.7	185.1152		C8H18O3Na		Glycol ether
Brown	WFI	6	9	277.1145		C19H18P		Diphenyl(phenylmethyl)phosphine (isomer?)
Brown	WFI	7	14.1	353.1458		C25H22P	1.5	2-(Diphenylphosphino)-2'-methylbiphenyl
Brown	WFI	8	14.3	279.0936		C18H16OP	0.97	Triphenylphosphine oxide

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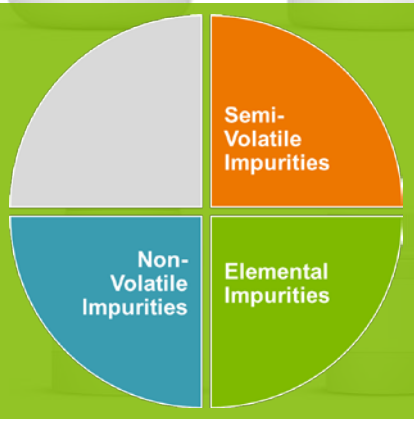
Results – Elemental extractables



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Elemental analysis

- Thermo Scientific™ iCAP Q™ Series ICP-MS
  - Qcell™ technology for interference reduction
  - Sub ppt detection limits
  - >9 orders dynamic range
  - Robust design to compete with almost any sample matrix
  - Fully compliant Thermo Scientific™ Qtegra™ ISDS software
  - Automatic- dilution
  - Full USP 233 & ICH Q3D method capabilities

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iCAP Q ICP-MS Instrument Configuration

- Measurement Mode:
  - KED; pure He @ 4.5 mL min-1
  - Internal standard added online
  - 30ppb Sc, 10 ppb Ga, Y and Lu in 2% HNO3 (4% EtOH added for 50% EtOH samples)

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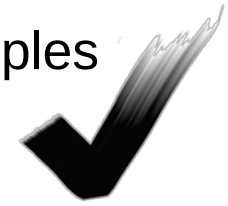
Analysis and QC checks following USP 233

- Calibration Range: between 1000-fold and 10-fold dilution of lower limit
  - **R^2 better than 0.99**

- Sensitivity Verification: Analysis @ 0.5x lower limit
  - **±30% of prepared concentration**

- Accuracy Check: Spike recovery of a 0.2 mg/L solution after complete sample acidification routine
  - **±20% of expected concentration**

- Drift Check: Standard 2 (500x dilution) was analyzed every 10 samples
  - **±30% of prepared concentration**



Quality data, automatically tested

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Results Water and 5M NaCl

- Reported is concentration in undiluted sample solution
- Most elements were not detectable

	Water					5M NaCl					
	^{63}Cu	^{66}Zn	^{111}Cd	^{123} Sb	^{208}Pb	^{56}Fe	^{63}Cu	^{66}Zn	^{75}As	^{123}Sb	^{208}Pb
	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}
Black	-	0.64	0.022	-	0.03	0.05	0.011	0.71	-	0.002	0.001
White	0.007	-	-	0.002	0.09	0.007	0.008	0.08	-	0.009	0.005
Brown	-	-	-	-	-	0.015	-	0.005	-	0.007	0.0002
Red	0.004	-	0.006	-	0.02	0.01	0.018	0.026	0.1	0.04	-

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Results 100% EtOH

- Reported is concentration in undiluted sample solution
- Most elements were not detectable

	100% EtOH						50% EtOH				
	^{63}Cu		^{66}Zn	^{111}Cd	^{123}Sb	^{208}Pb	^{63}Cu	^{66}Zn	^{111}Cd	^{123}Sb	^{208}Pb
	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}	ng·mL^{-1}
E36092	-		0.03	-	-	-	-	0.3	-	-	-
FF3502	-		-	-	0.41	-	-	-	-	-	-
HF355-2	-		-	-	-	-	-	-	-	-	-
S 11382	-		-	-	-	-	-	-	-	-	-

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Drift Check

- 6.5 hour run, 100 samples, 11 drift checks

Analyte	Av. Recovery [%]	RSD [%]
7Li	100.9	4.2
9Be	100.9	9.2
51V	99.0	1.2
52Cr	99.2	1.8
55Mn	92.9	0.8
59Co	95.0	0.7
60Ni	98.5	1.3
63Cu	99.4	1.3
66Zn	97.6	0.8
75As	100.1	4.8
78Se	97.7	2.7
95Mo	97.6	1.4
101Ru	97.6	1.6

Analyte	Av. Recovery [%]	RSD [%]
103Rh	99.2	1.6
105Pd	95.7	3.1
111Cd	97.0	4.0
115In	93.7	1.0
121Sb	102.0	2.5
182W	100.0	2.6
189Os	95.0	2.6
193Ir	100.3	1.5
195Pt	99.4	1.4
202Hg	96.4	1.7
205Tl	97.6	12.6
208Pb	98.1	1.3

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Summary – Extractable workflow

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Summary

- GC & LC Orbitrap have superior HRAM, enabling confident identification of extractable unknowns.
- Empowering software workflows simplify and automate the process of complex identification.
- Free cloud libraries allow quick qualification of unknowns.
- Analysis of elemental impurities at the lowest levels is possible with iCAP Q.

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Webinars – SAVE THE DATES

1. **Testing for leachables in pharmaceutical contact closure materials; a complete ICP-MS, GC-MS & LC-MS workflow.**

   - Wednesday 4th November 2015 | 10:30 EST 16:30 CET
   - Dr. Andrew Feilden, Chemistry Operations Director, Smithers Rapra

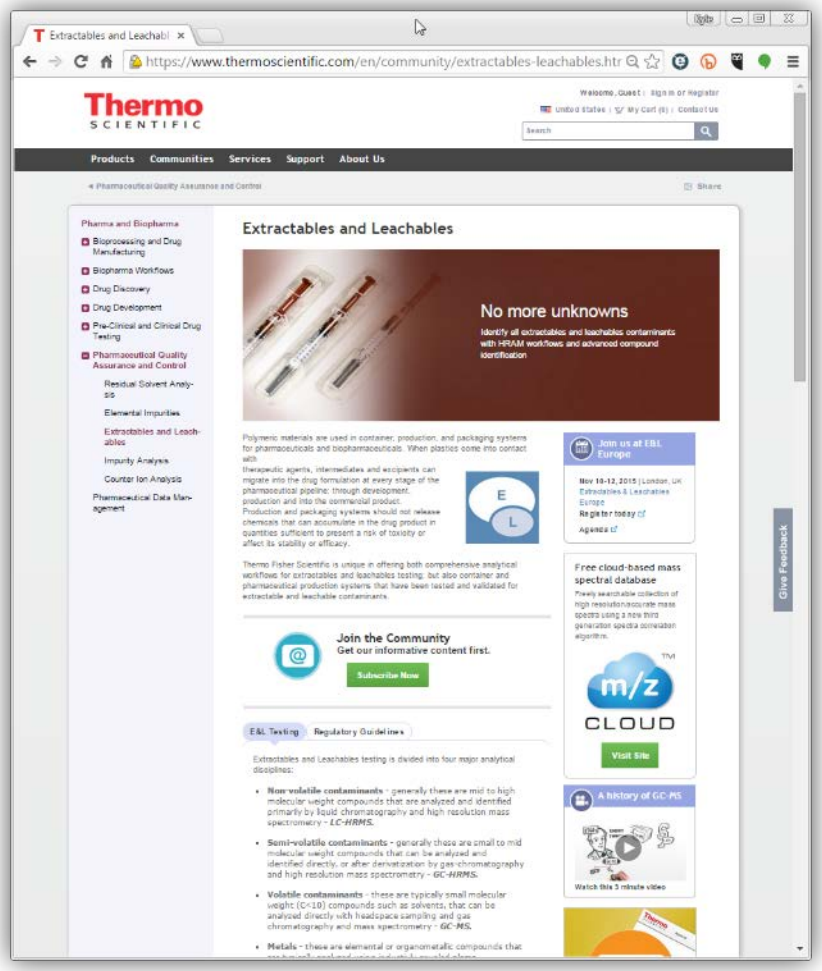
2. **Using GC & LC Orbitrap mass spectrometry to confidently identify leached packaging and process impurities.**

   - Wednesday 2nd December 2015 | 10:30 EST 16:30 CET
   - Dr. Kate Comstock & Mr. Dominic Roberts, Thermo Fisher Scientific

bit.ly/IdentifyLeachables

E&L Resources

- Webinars
- Applications
- Blogs
- Regulatory updates
- White papers
- And more…



www.thermoscientific.com/Leachables

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Thank you

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