

AET

AET

40

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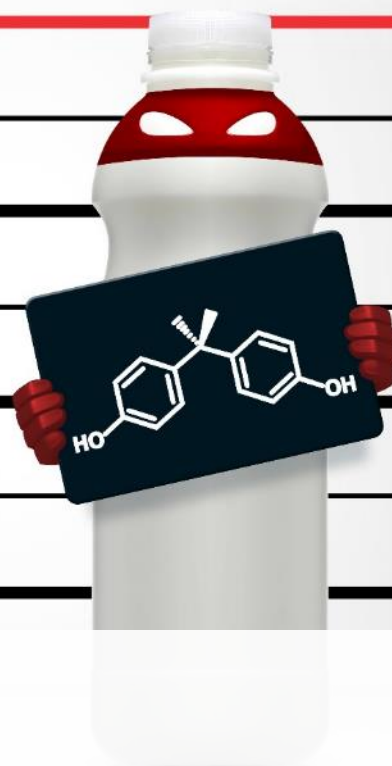
20

20

10

10

µg



ThermoFisher
SCIENTIFIC

Analytical Workflow for Extractable and Leachable Impurities

AAPS 2015 | 26 October 2015, Orlando FL
Kyle D'Silva



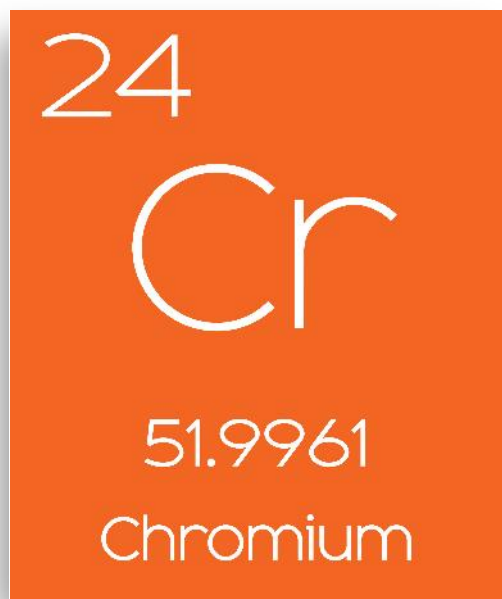
WHAT are EXTRACTABLES and LEACHABLES?



What is E&L testing for?

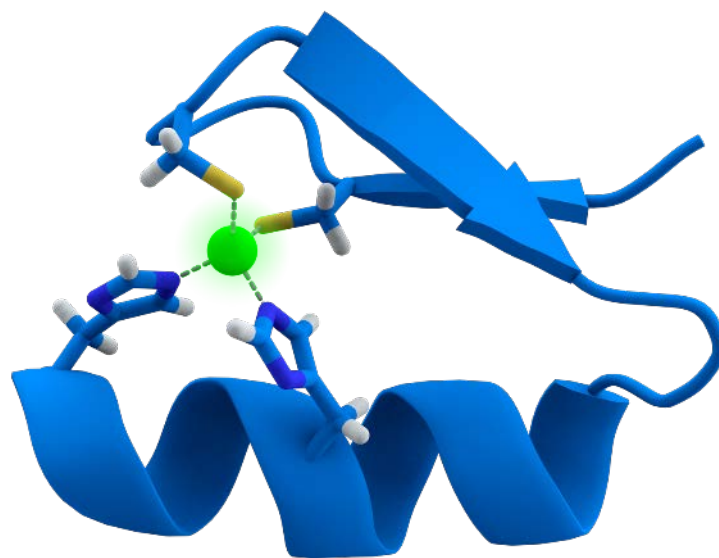


CONTAMINANT TOXICITY



Is there risk of harm
to the patient?

DRUG EFFICACY



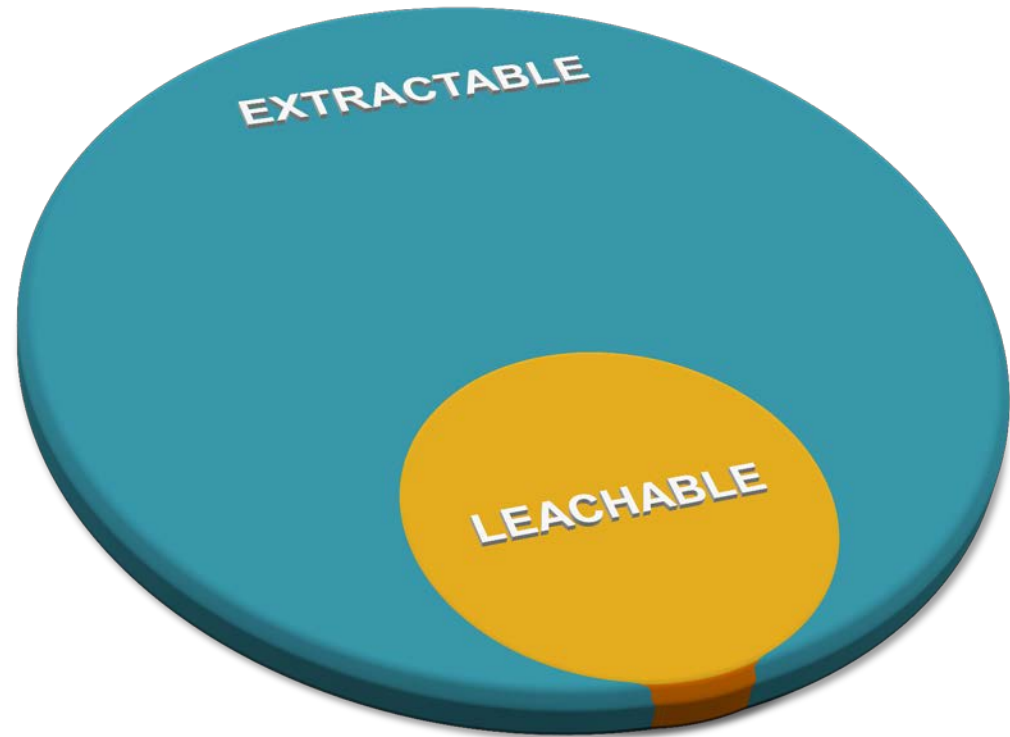
Is there impact on
drug potency?

• EXTRACTABLE

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

• LEACHABLE

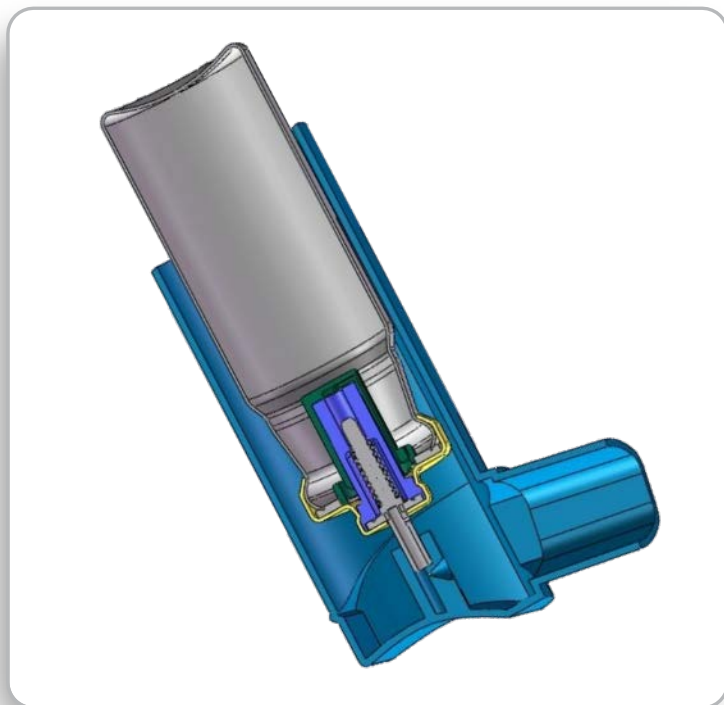
- Chemical that **migrates** from process equipment, packaging or delivery system; into drug formulation **under normal usage conditions.**



Leachables are typically a subset of extractables.

Extractables versus Leachables testing

EXTRACTABLE



Test the materials

LEACHABLE



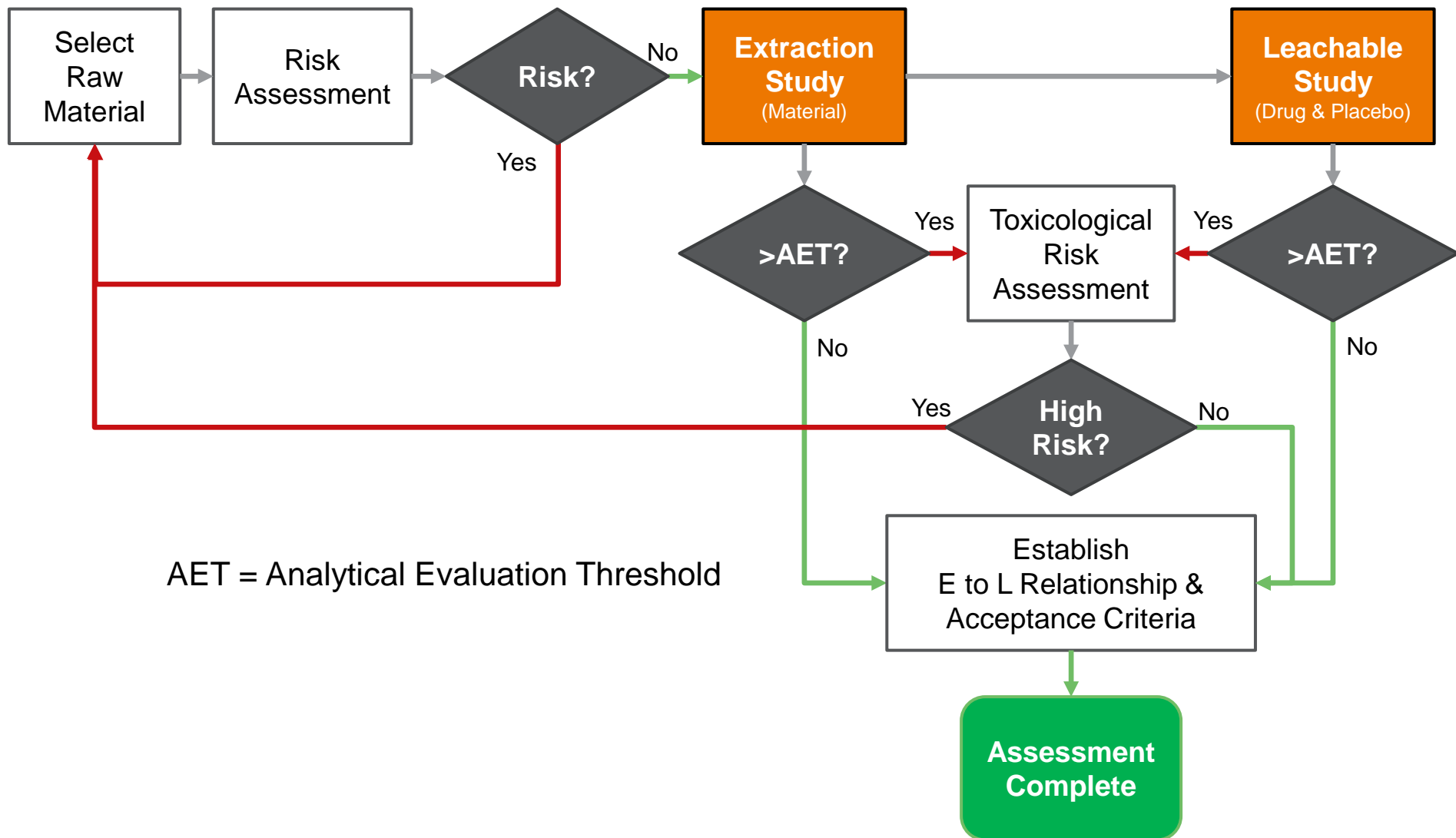
Test the product

Regulations

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

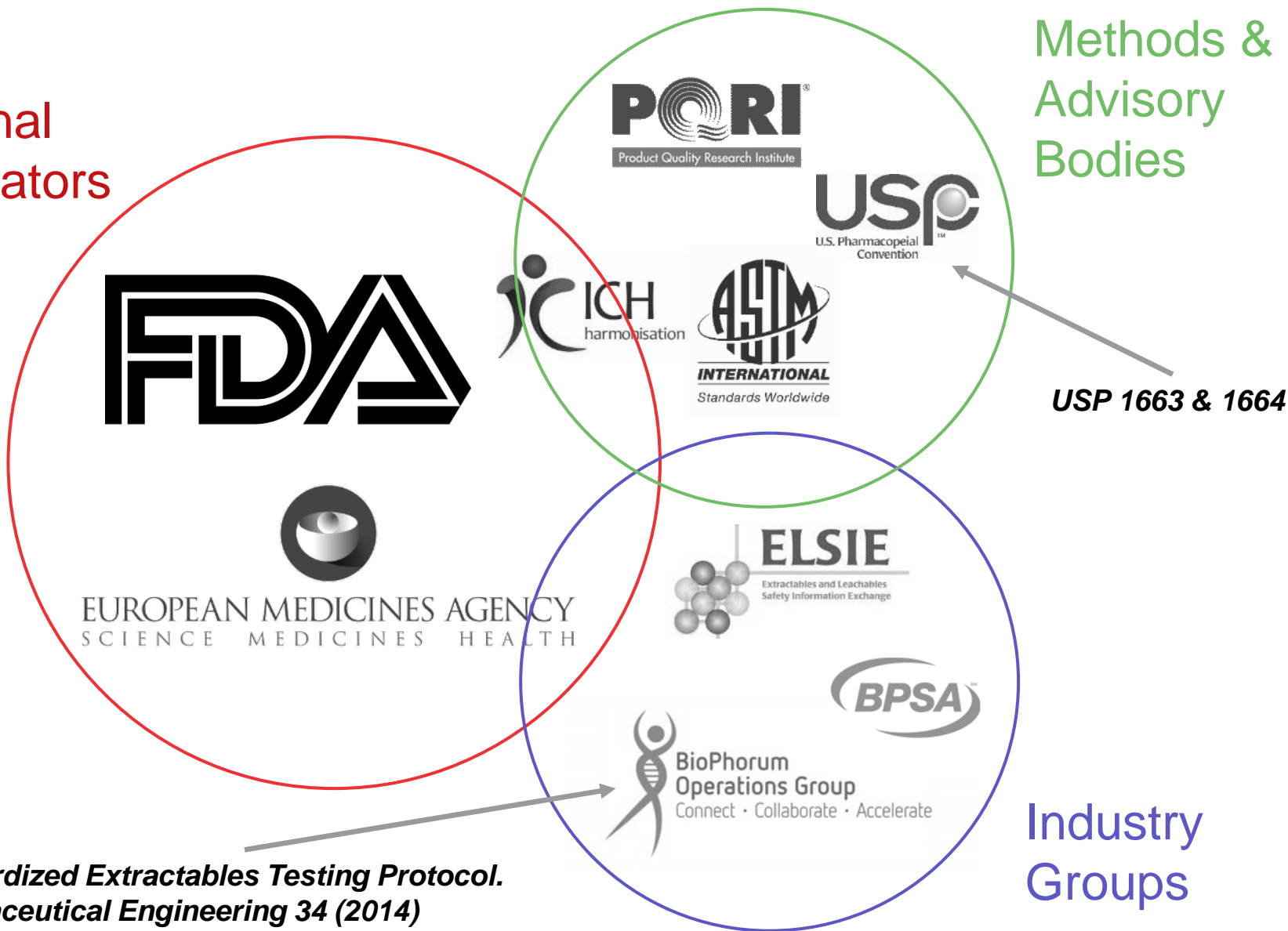


Extractables & Leachables Assessment Workflow



E&L Regulatory and method landscape

National
Regulators



Methods &
Advisory
Bodies

USP 1663 & 1664

Industry
Groups

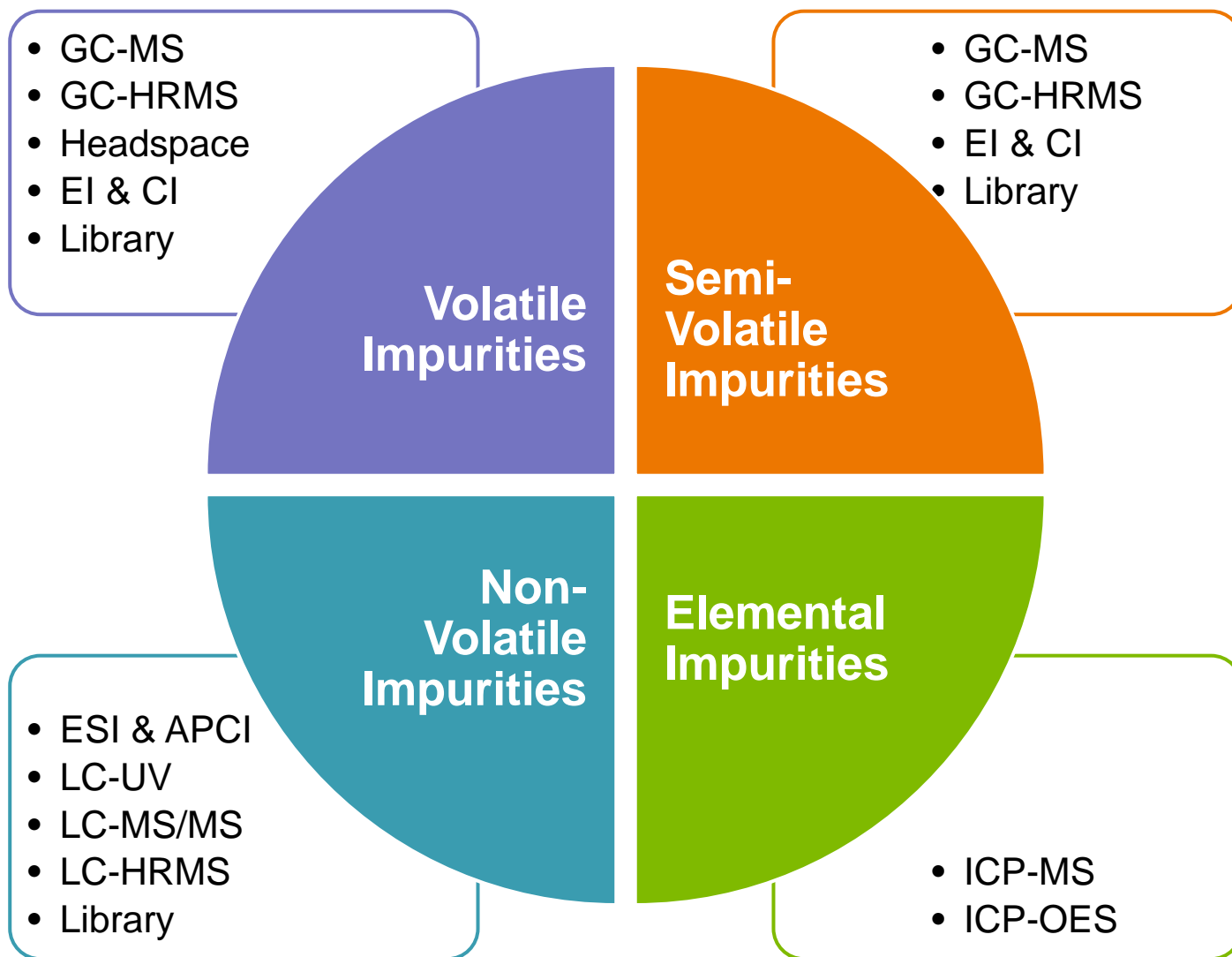
Standardized Extractables Testing Protocol.
Pharmaceutical Engineering 34 (2014)

BPOG Single-use System – Extractables Test Guidelines

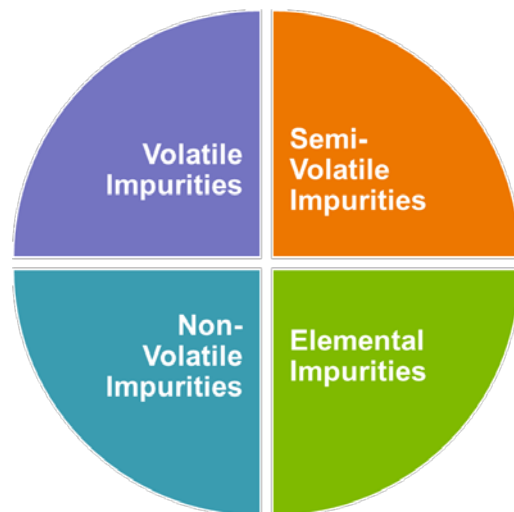
	Solvents						Time				
	50% Ethanol	1% PS-80	5M NaCl	0.5N NaOH	0.1 M Phosphoric acid	WFI ^a	Time 0 (≤ 30 min)	24 hours	7 days	21 days	70 days
							Ambient (25°C)		40°C		
Storage, Mixing, and Bioreactor Bags	X	X	X	X	X	X	X	X		X	X ^b
Tubing	X	X	X	X	X	X	X	X		X	X ^{b,c}
Tubing Connectors and Disconnectors	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)

Analysis of Extractables & Leachables



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



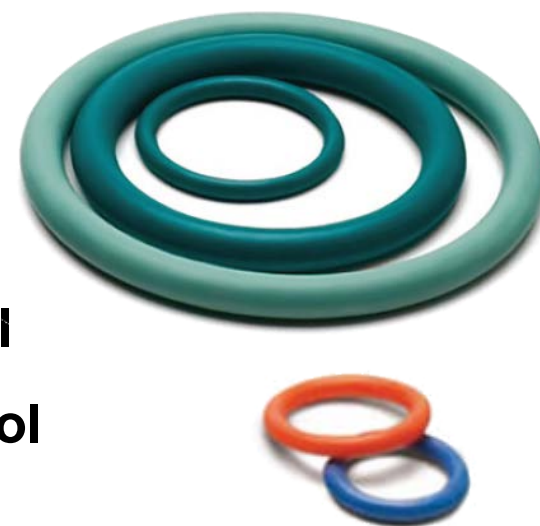
Experimental – O-Ring Extractables Study

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**



O-ring Extractables Study

Semi-volatiles



Q Exactive GC
GC-MS/MS
Trace 1310 GC
TraceFinder

Non-Volatiles

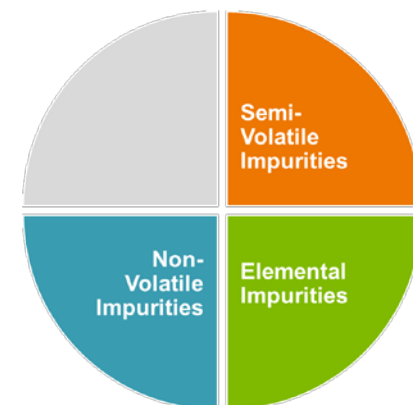


Q Exactive LC-
MS/MS
Compound
Discoverer
TraceFinder
MZ Cloud

Metals



iCAP Q ICP-MS
Qtegra ISDS



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



Integration of 3 highly successful technologies



TSQ 8000 Series ExtractaBrite™ ion source technology

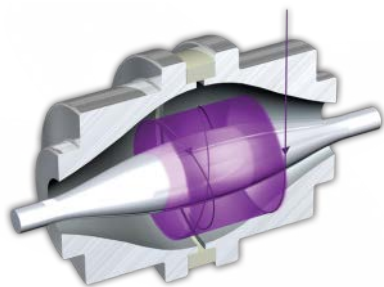
Routine grade robustness

Removable without breaking vacuum

Patented RF lens protects ion guide and quads



Integration of 3 highly successful technologies



Orbitrap mass analyzer technology

Incredible HR/AM performance

Highly regarded Q Exactive platform



Breakthrough in GC-MS capability

The Power of Q Exactive GC

Resolution

Up to
120,000 at
m/z 200

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

Mass Accuracy

< 1ppm

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

Sensitivity

ppt

- In full scan
- High selectivity
- High spectral fidelity

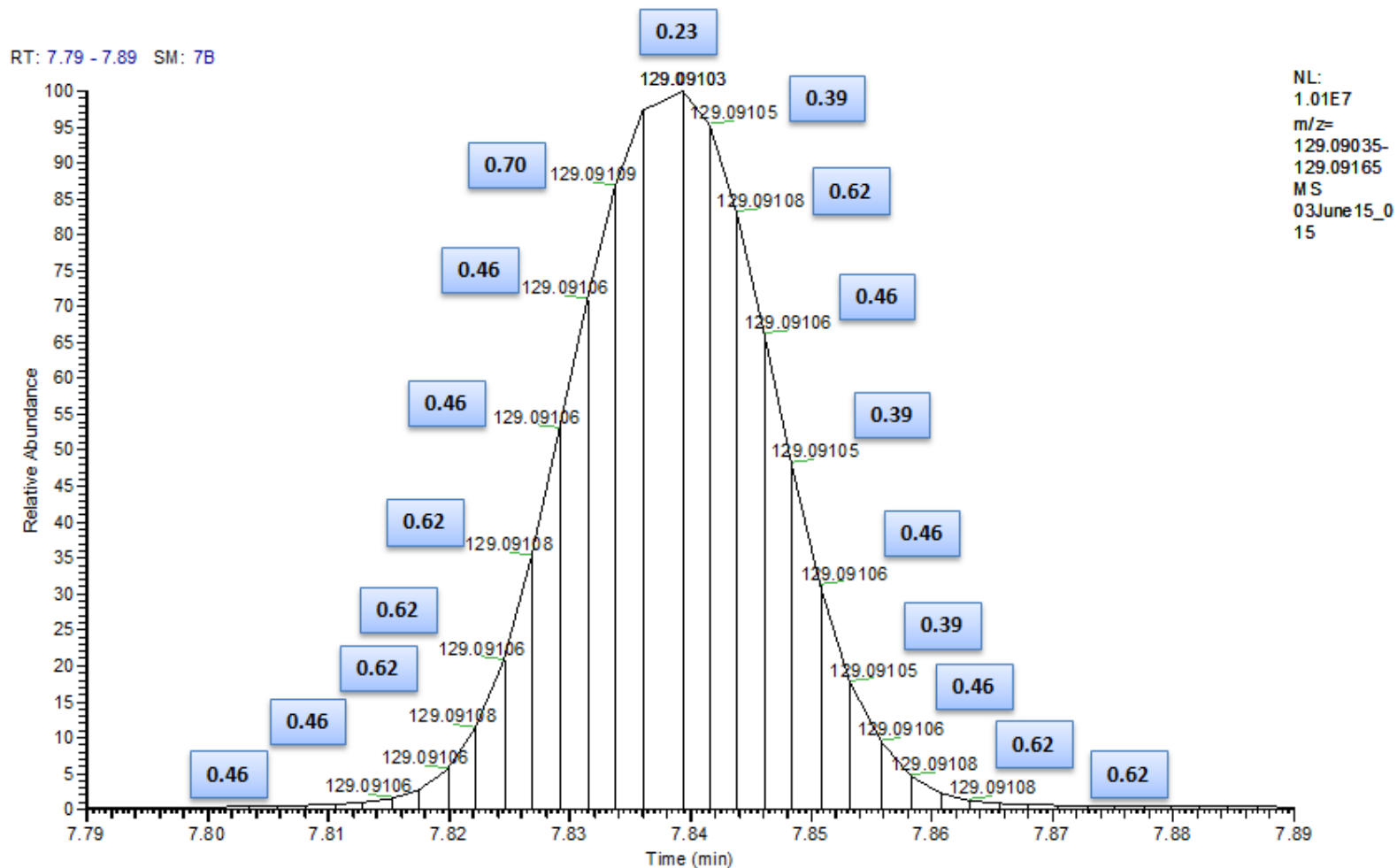
Dynamic Range

>6 orders

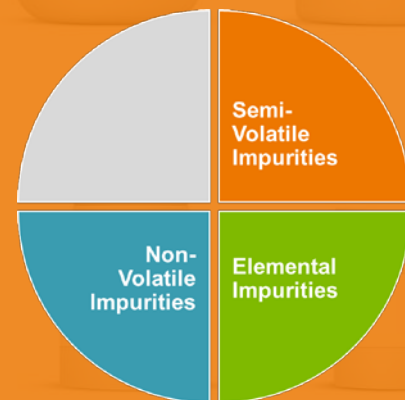
- Excellent coverage in sample profiling
- “Triple quad grade” quantitation in full scan

Scan speed and accurate mass error across a peak

Ethyl octanoate m/z 129.0910

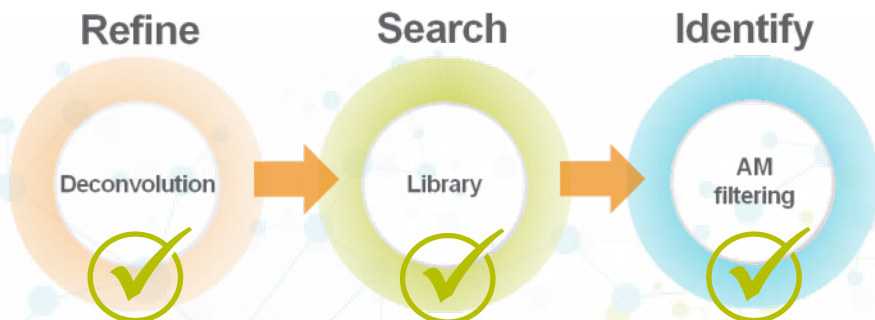
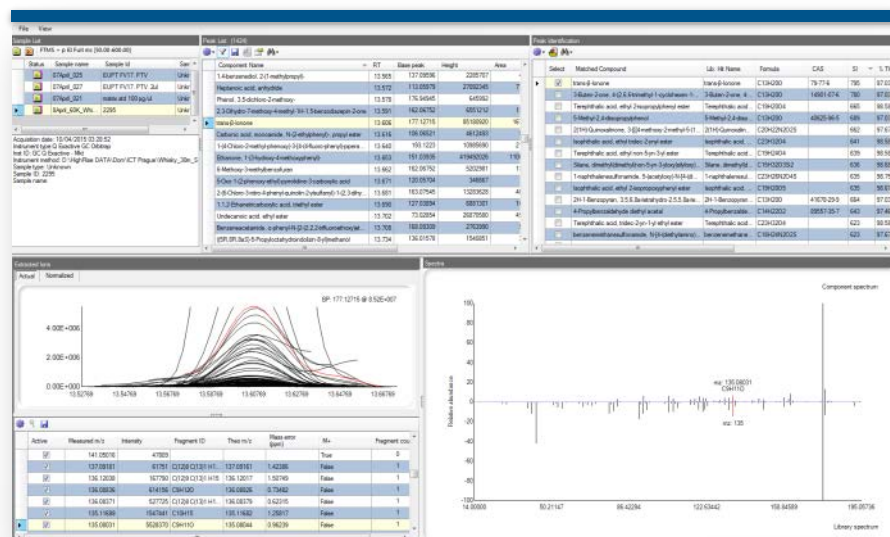


Results – Semi-volatile extractables

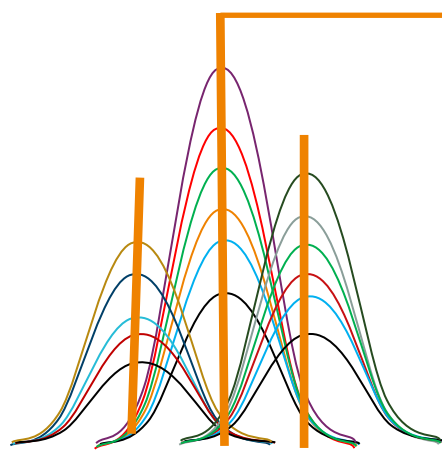


Tracefinder: automated compound detection and identification

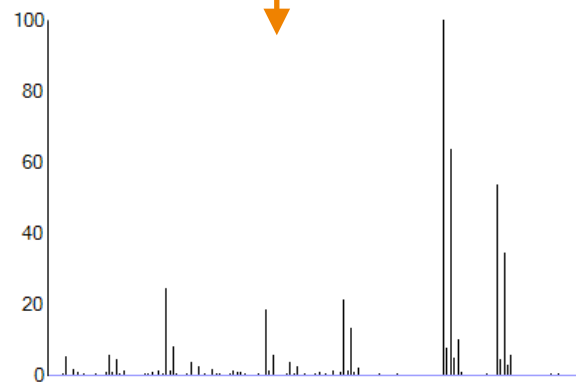
1. Peak detection
2. Spectral deconvolution
3. Library searching (e.g. NIST, Wiley, custom)
4. % TIC explained using elemental composition and high resolution filtering with accurate mass



Peak Detection and Candidate Matching

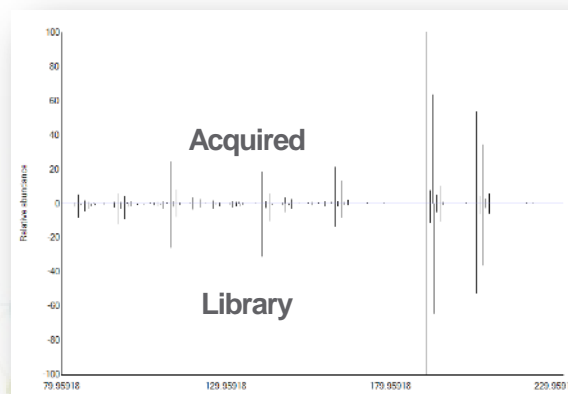


Deconvolve TIC

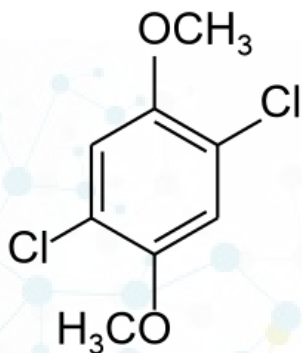


Create "clean" spectrum

Library
search

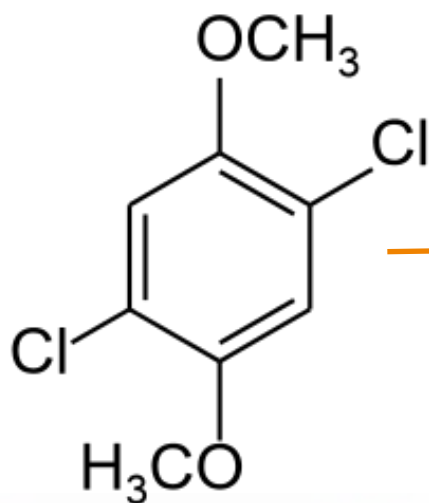


Candidate
Compounds



High Resolution Filtering

Candidate
Compounds



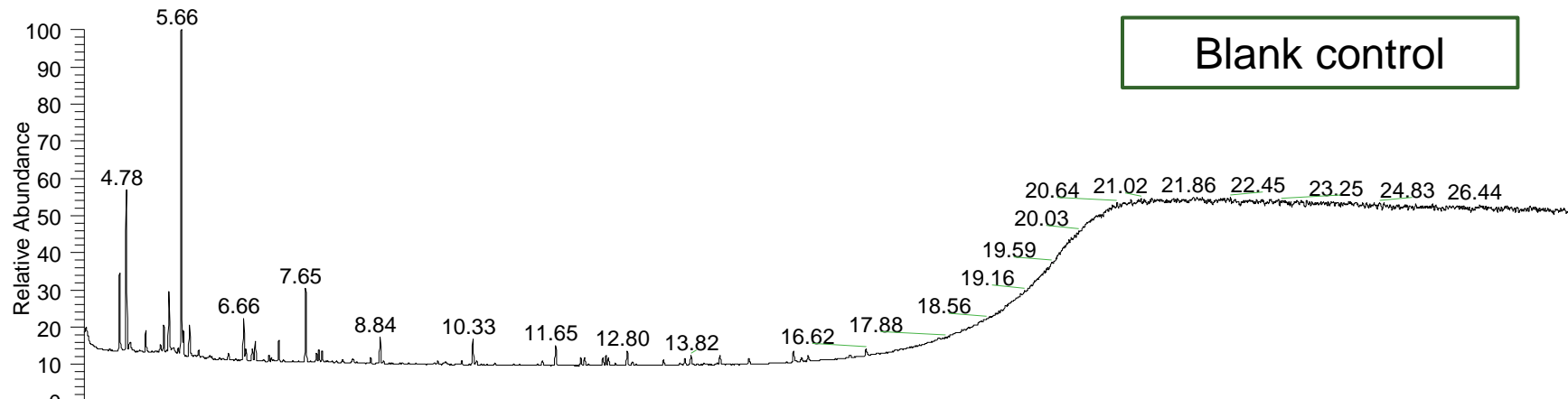
Subset
formulae

Acq m/z	Fragment ID	Theo m/z	Mass Error (ppm)
147.9477	C ₅ Cl ₂ H ₂ O	147.9477	0.20277
148.9369	C ₅ Cl[37]ClHO	148.9369	0.2679
149.9448	C ₅ Cl[37]ClH ₂ O	149.9448	0.06602
151.9419	C ₅ [37]Cl ₂ H ₂ O	151.9418	0.72528
154.9895	C ₇ ClH ₄ O ₂	154.9894	0.38712
155.9974	C ₇ ClH ₅ O ₂	155.9973	0.89745
157.9943	C ₇ [37]ClH ₅ O ₂	157.9943	0.25381
159.9479	C ₆ Cl ₂ H ₂ O	159.9477	0.87529
161.9446	C ₆ Cl[37]ClH ₂ O	161.9448	0.80213
162.9711	C ₆ Cl ₂ H ₅ O	162.9712	0.36816
163.9745	C ₅ [13]CCl ₂ H ₅ O	163.9745	0.3342
164.9682	C ₆ Cl[37]ClH ₅ O	164.9682	0.24186
165.9716	C ₅ CCl[37]ClH ₅ O	165.9716	0.02832

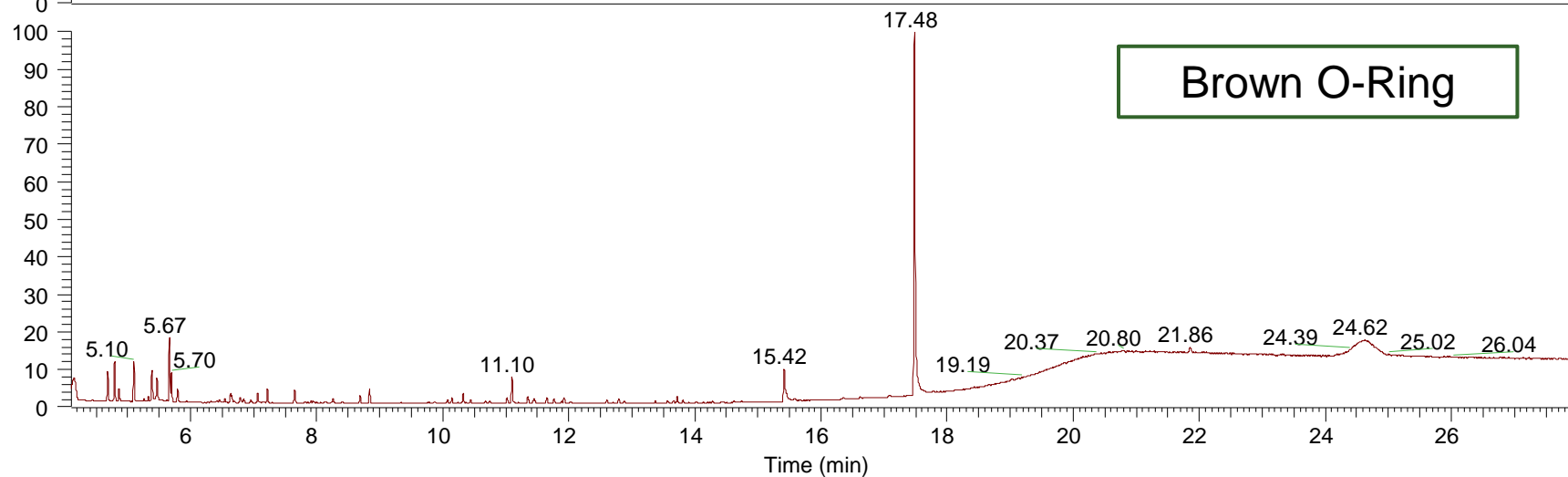
$$\text{HRF Score} = \frac{\sum (m/z * \text{Intensity})_{\text{explained}}}{\sum (m/z * \text{Intensity})_{\text{observed}}} \times 100\%$$

Example results

RT: 4.10 - 27.88 SM: 7B



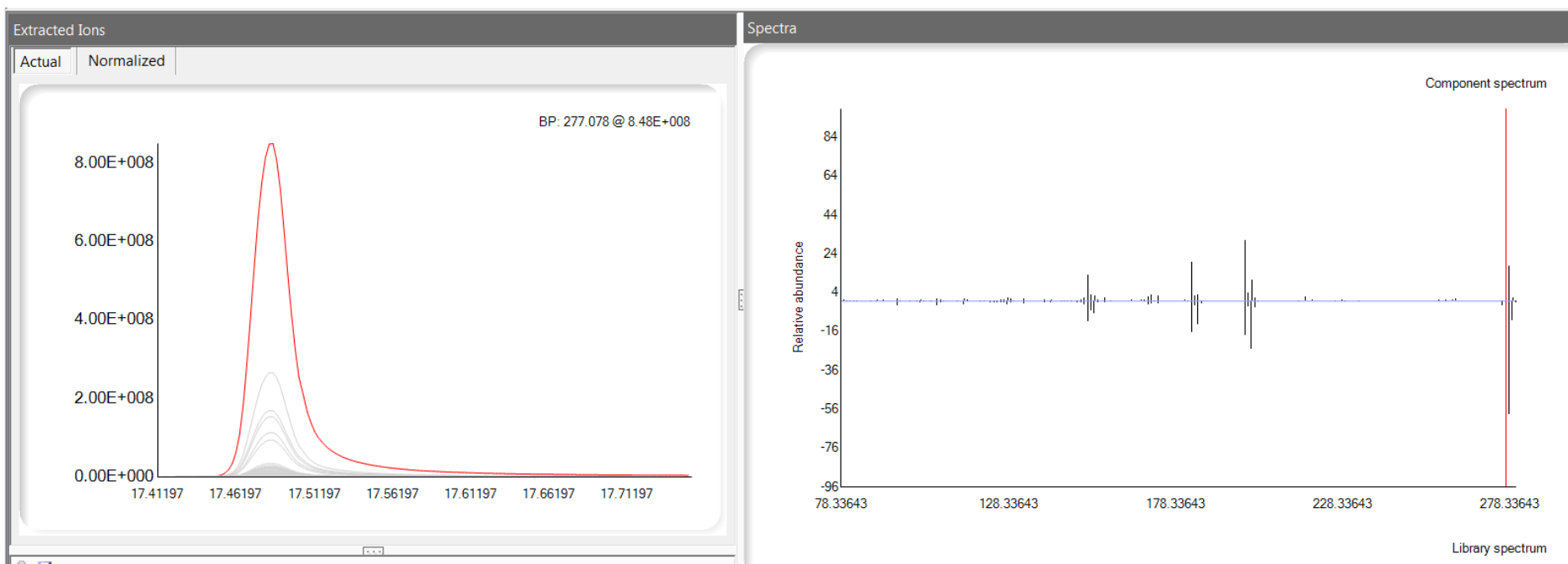
NL:
6.86E8
TIC MS
03June15_0
08



NL:
2.54E9
TIC MS
03june15_01
1

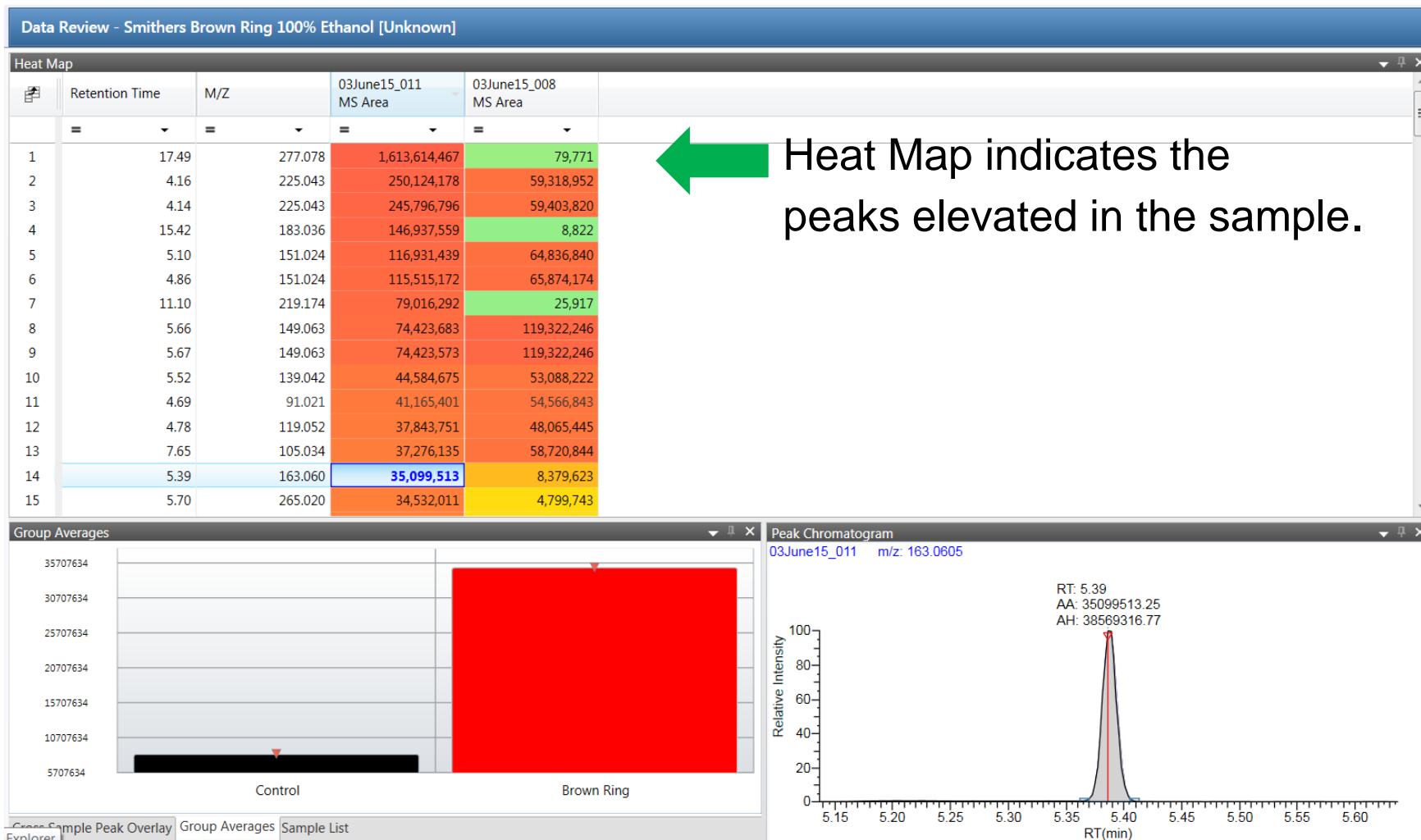
Step 1: Deconvolution of the data

- 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.



Step 2: Quickly isolate the peaks of interest

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

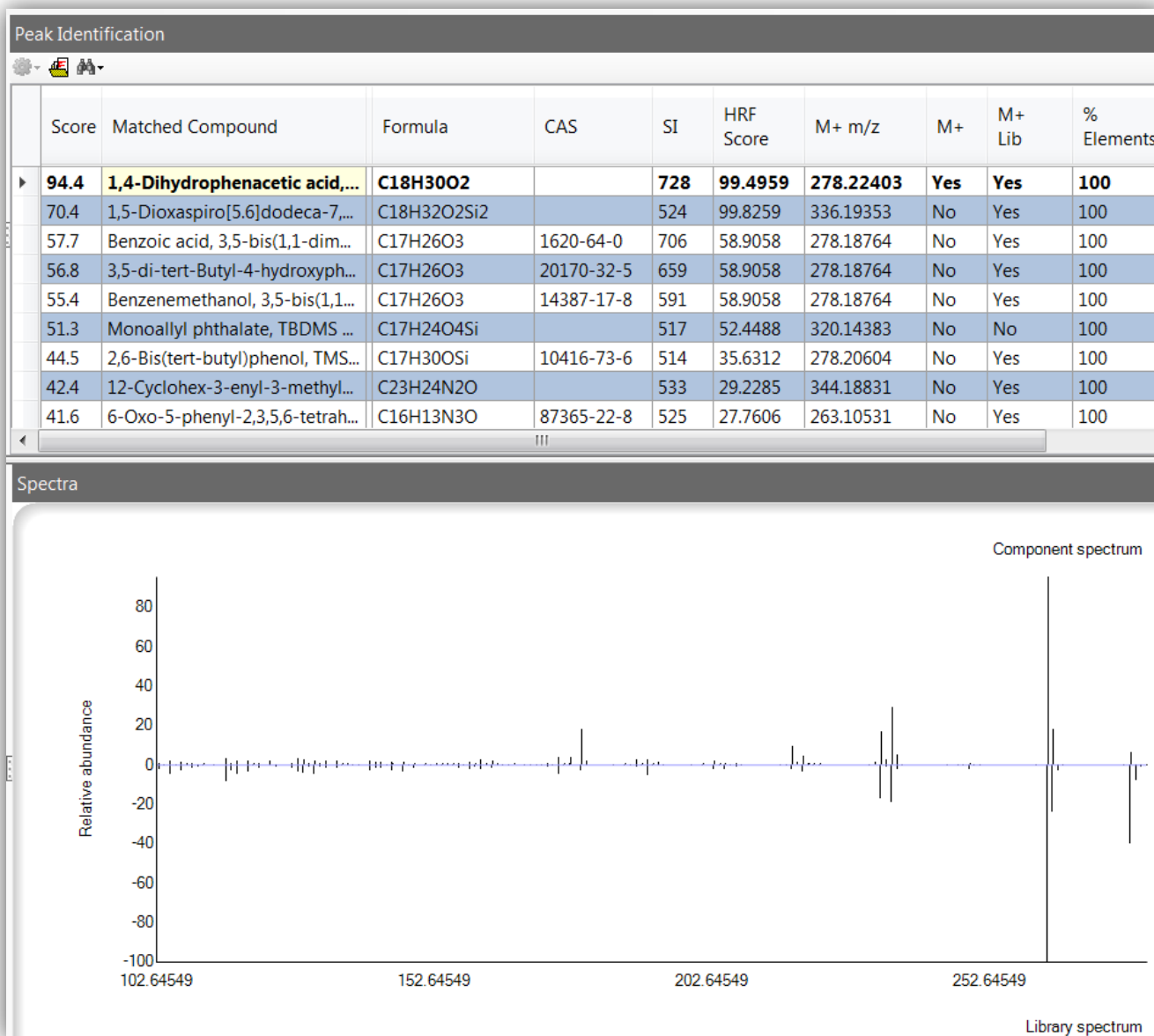


Top 10 differential peaks in Brown O-ring

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.

Step 3: Identify the compound – searching NIST 14



- 26 Hits from NIST are sorted based on:

- Spectral matching
- High Resolution
- Filtering (HRF) score.

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.

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-tetra...	C16H13N3O	87365-22-8	525	27.7606	263.10531	No	Yes	100

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

Identifying without a library hit?

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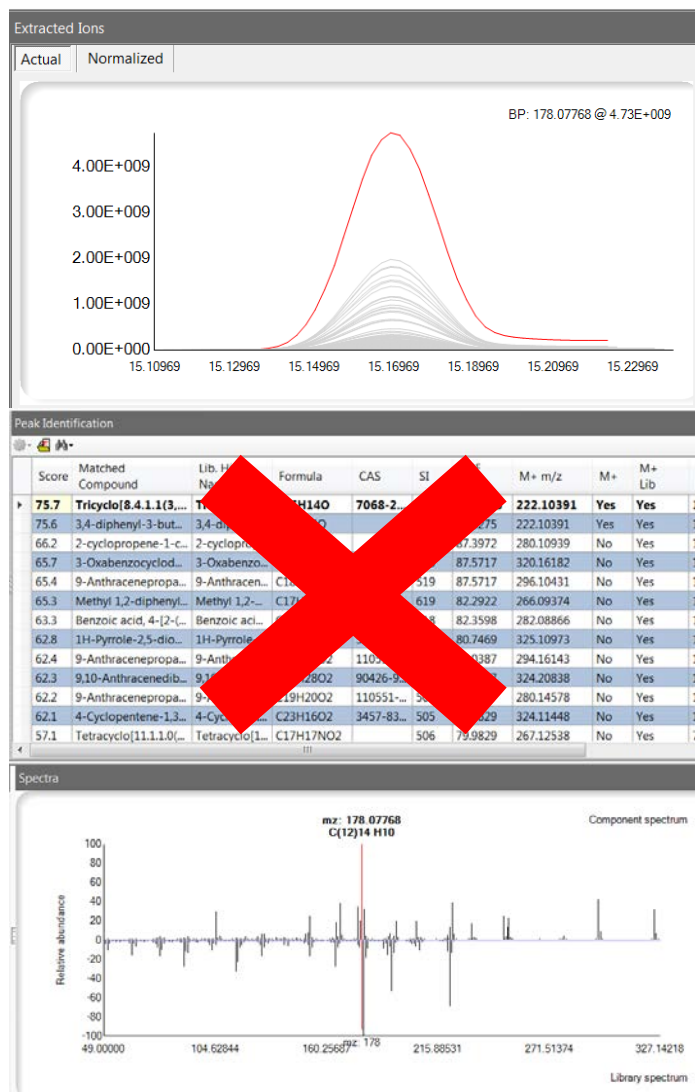
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10

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Confident ID comes from mass accuracy

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

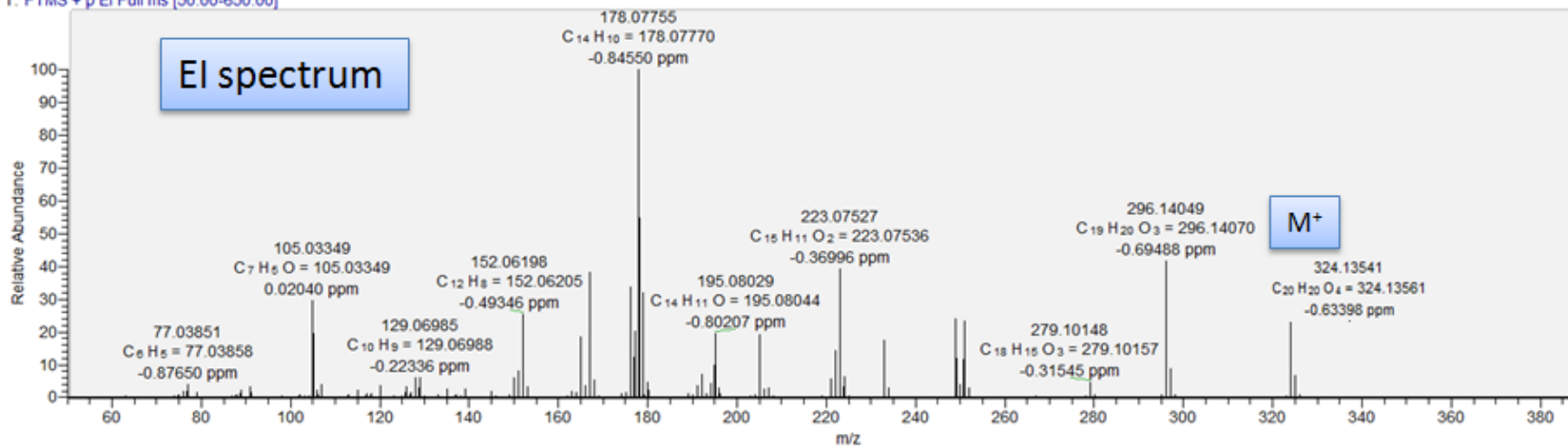


No clear match
with low score
at 75.7%

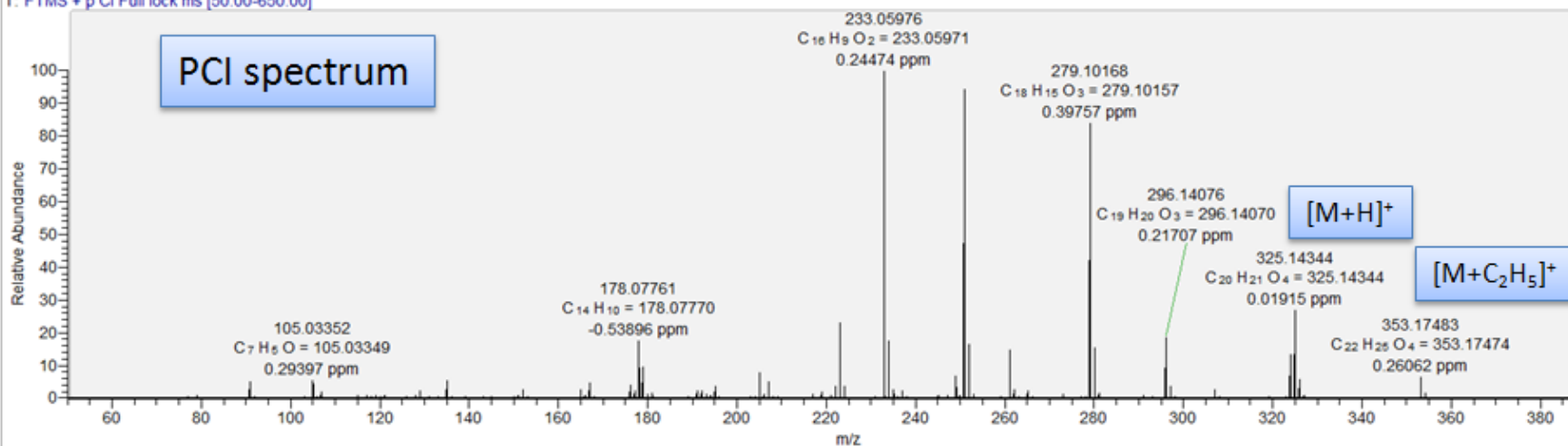


EI & PCI spectra for peak at 15.17 mins.

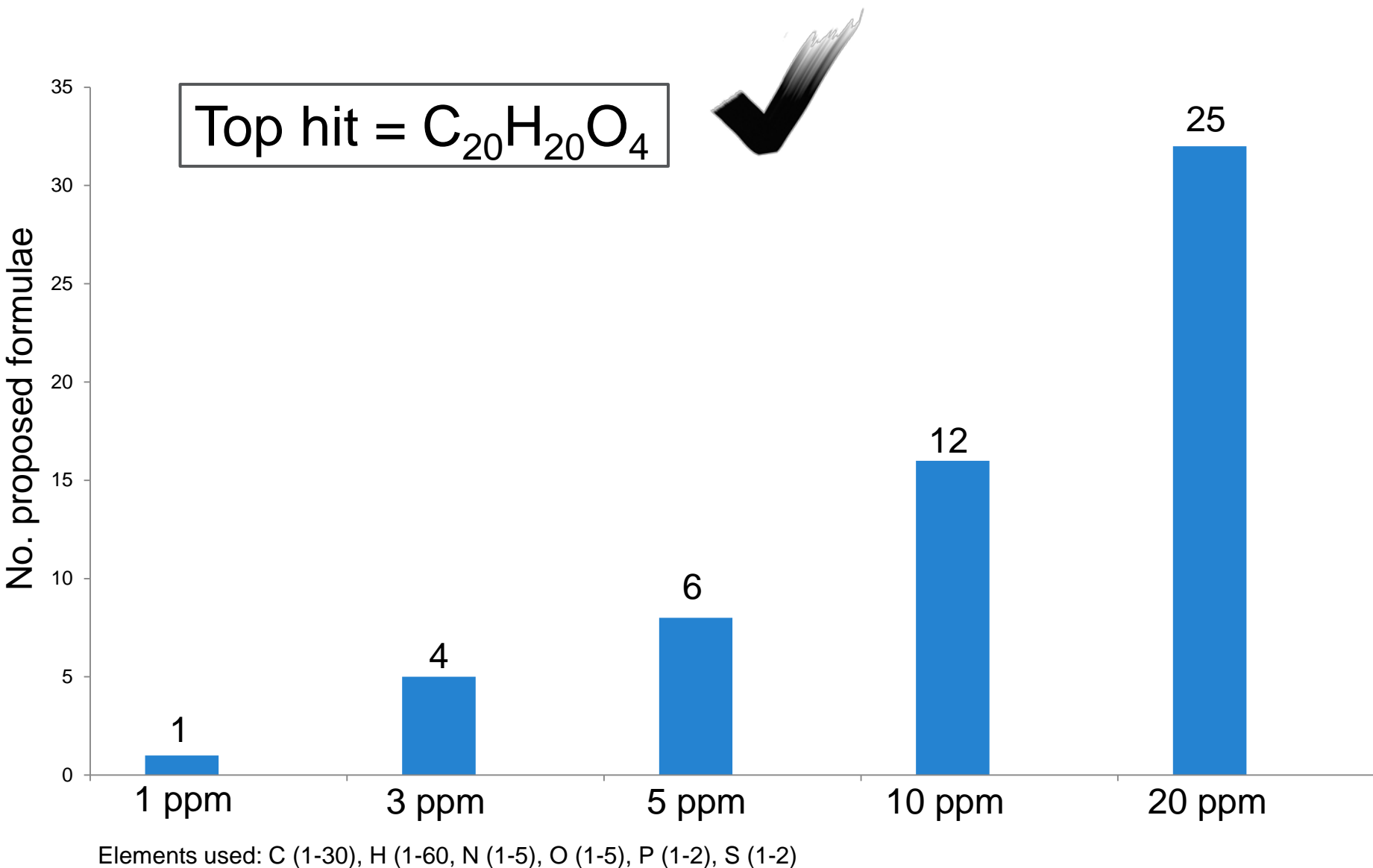
03june15_015 #4674-4683 RT: 15.16-15.18 AV: 10 SB: 32 17.97-17.99, 18.19-18.24 NL: 3.98E9
T: FTMS + p EI Full ms [50.00-650.00]



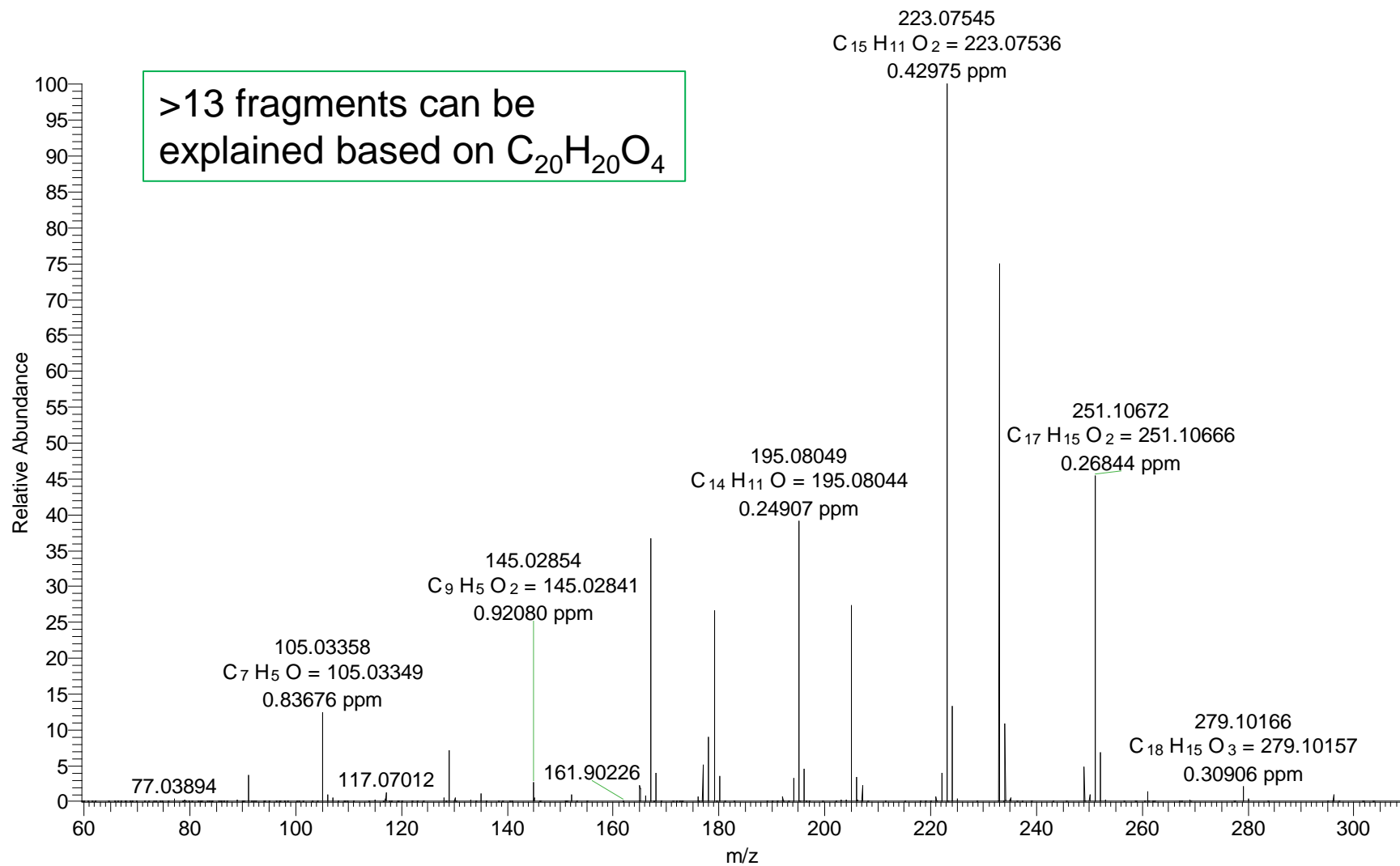
03june15_024 #4655-4676 RT: 15.15-15.20 AV: 22 SB: 26 17.97-17.99, 18.19-18.23 NL: 6.36E7
T: FTMS + p CI Full lock ms [50.00-650.00]



No. of proposed formulae for m/z 324.13541



MS/MS m/z 325.14 to support proposed formula



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

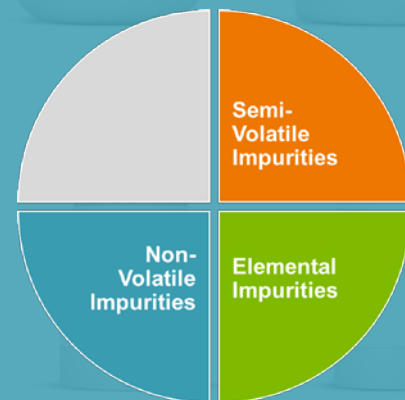
Peaks listed are significantly different compared to control.

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 ₈ H 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.

Results – Non-Volatile extractables



LC-MS/MS instrument conditions



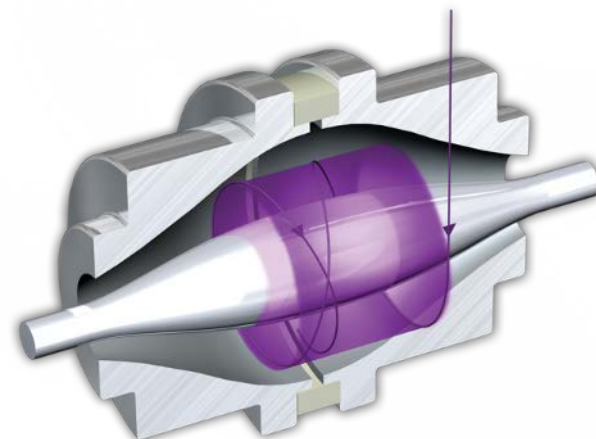
- Equipped with a HESI II ion source.
- Full scan MS and top3 data-dependent HCD MS/MS data were collected.
- Resolutions of 17,500 and 70,000
- Polarity switching.

Thermo Scientific™ Q Exactive Plus™

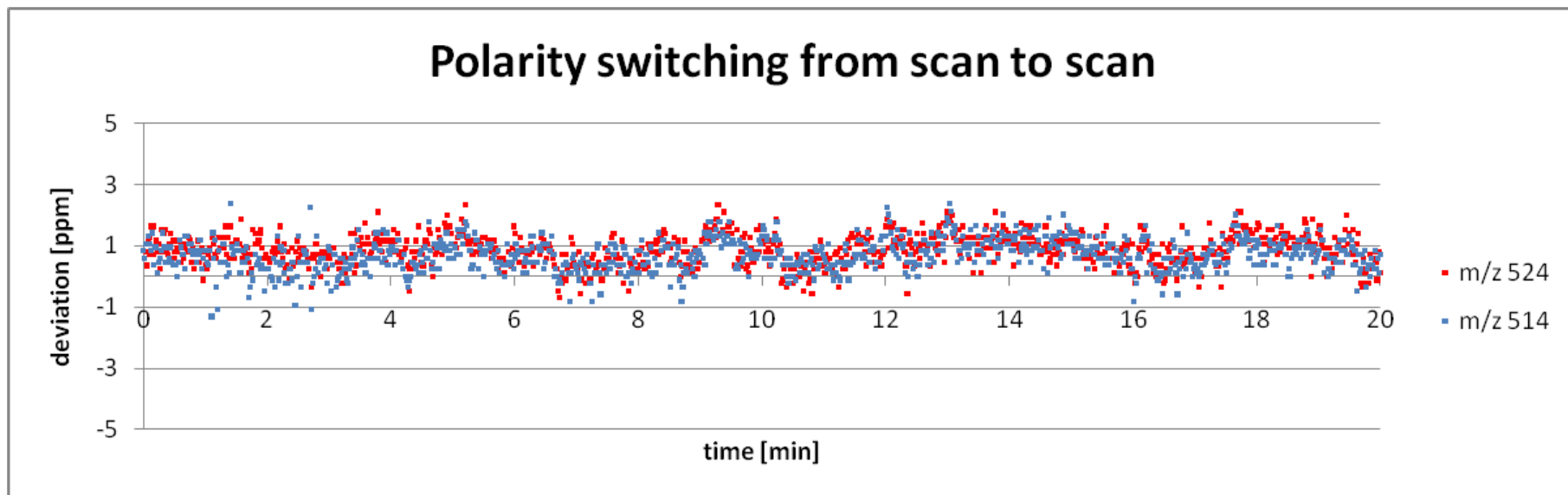
Hybrid Quadrupole-Orbitrap LC-MS/MS System.

Thermo Scientific™ UltiMate™ 3000

UHPLC System



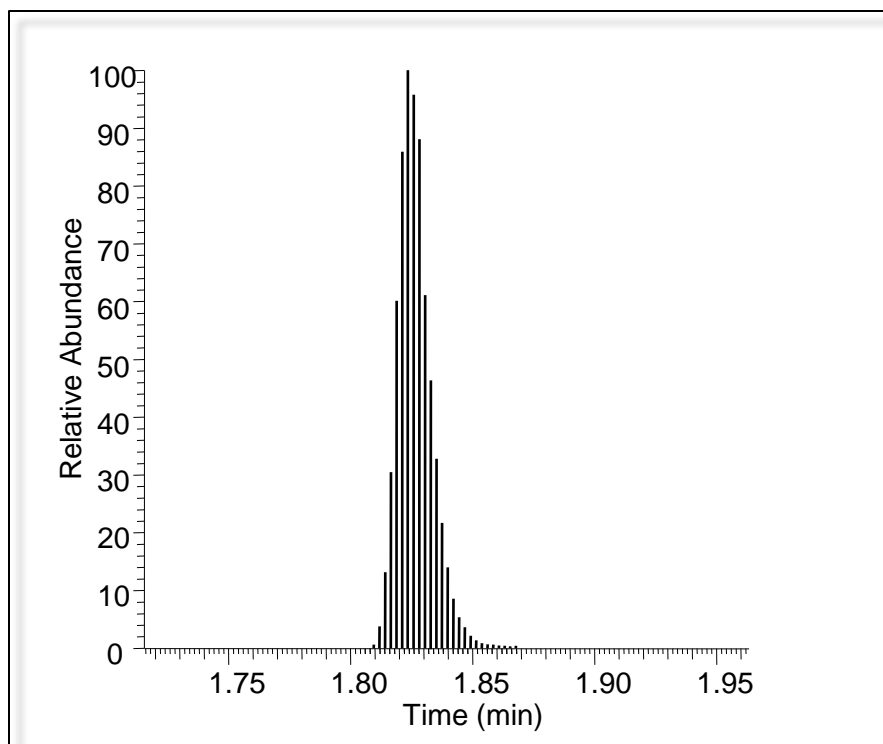
Mass Accuracy with Polarity Switching (External Calibration)



1 positive + 1 negative scan in <1 second

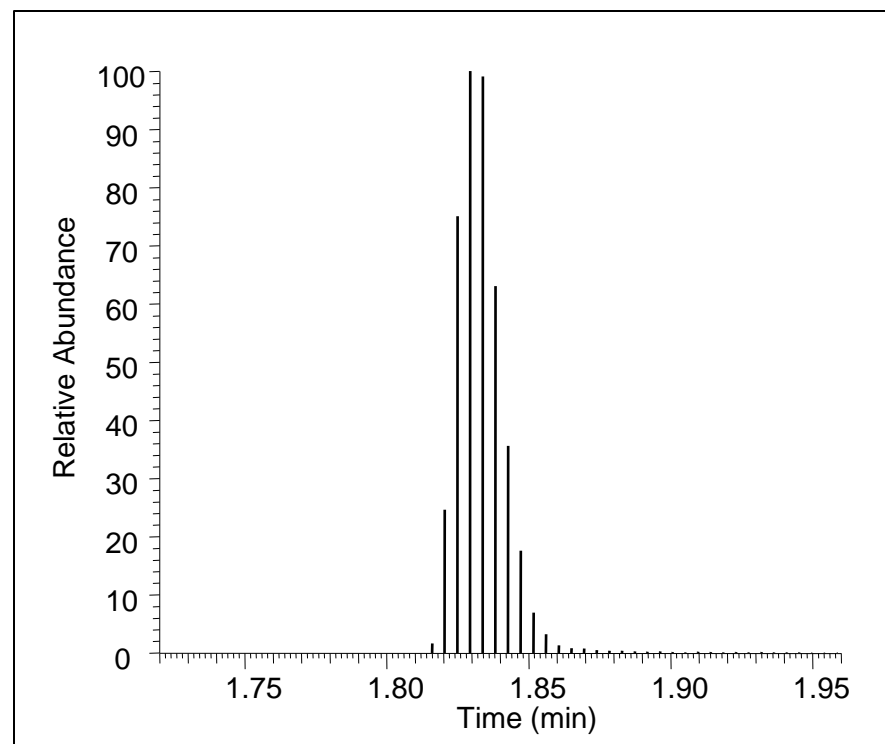
MS Scanning Speeds to meet UHPLC Separation

Resolution Setting: 35,000



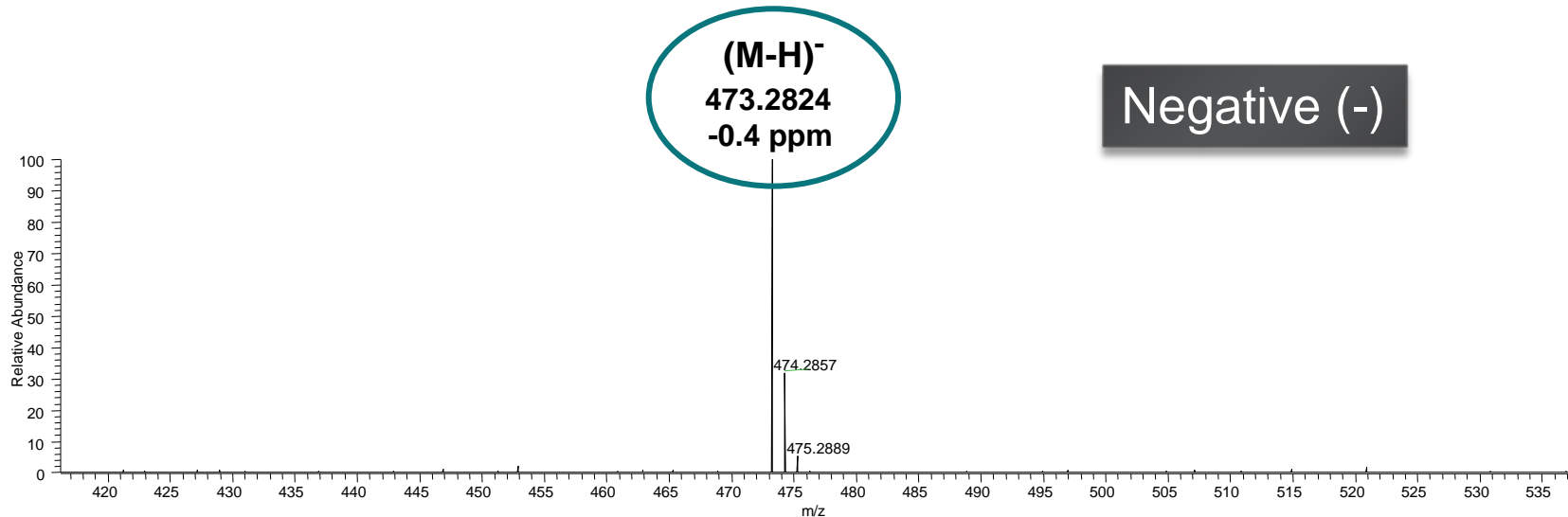
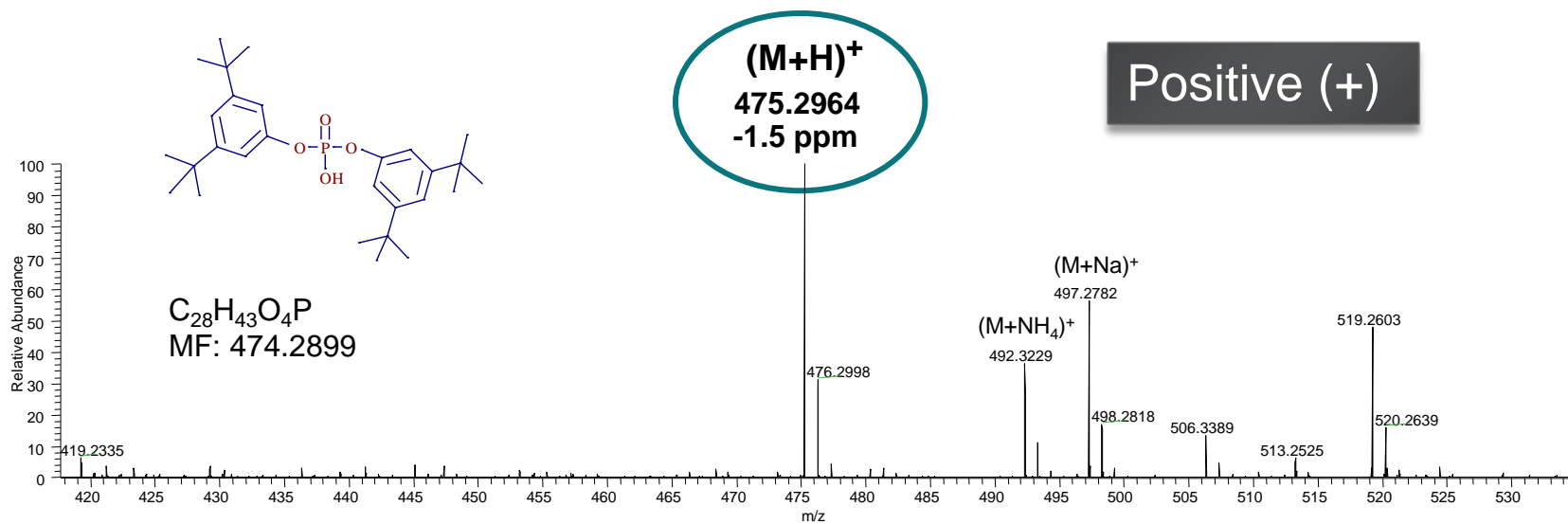
Peakwidth (FWHM) ~ 1 sec
Scans/peak = 21

Resolution Setting: 70,000

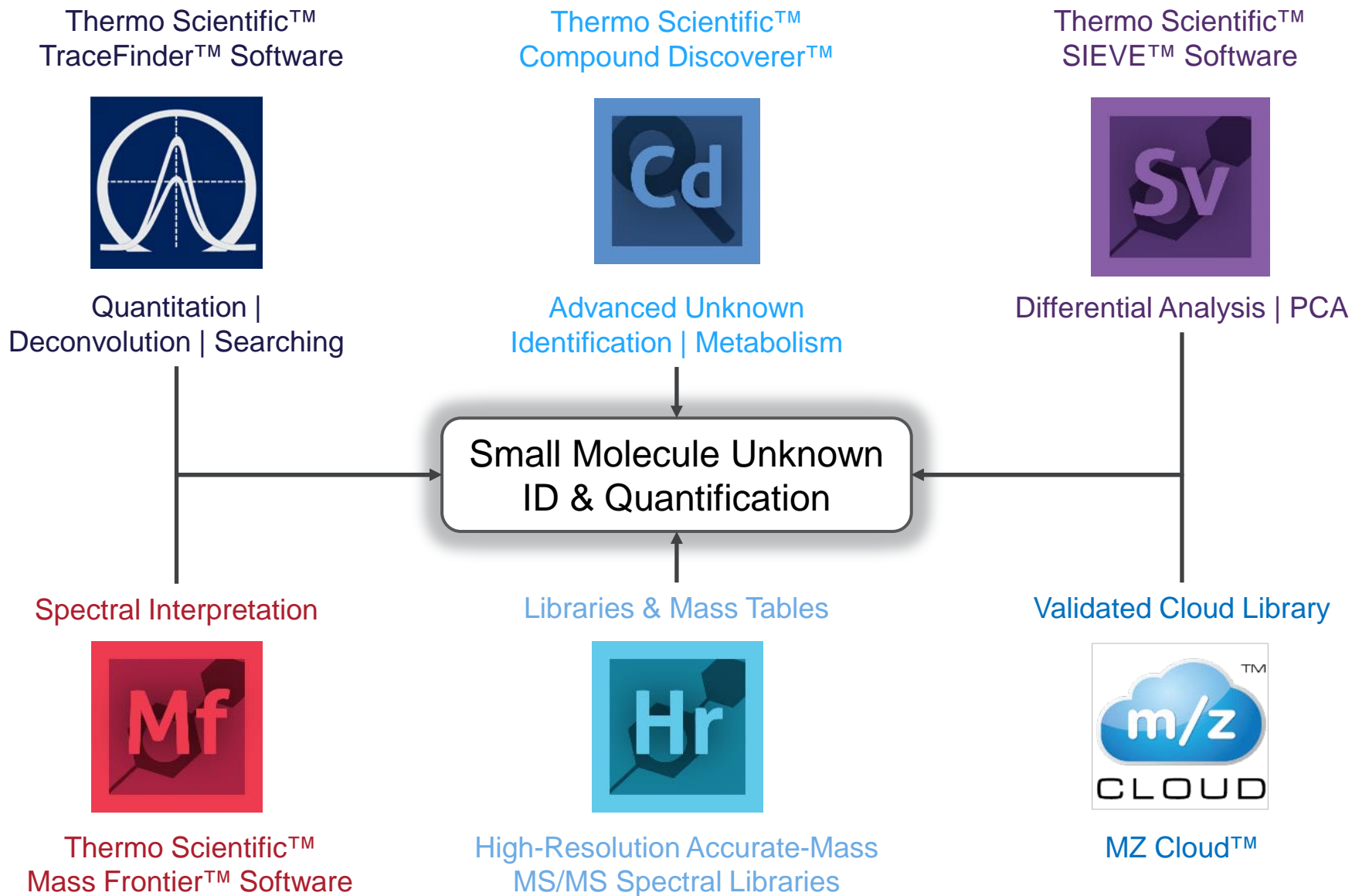


Peakwidth (FWHM) ~ 1 sec
Scans/peak = 11

Polarity Switching maintains High Mass Accuracy



Data Analysis For Unknown Quantification

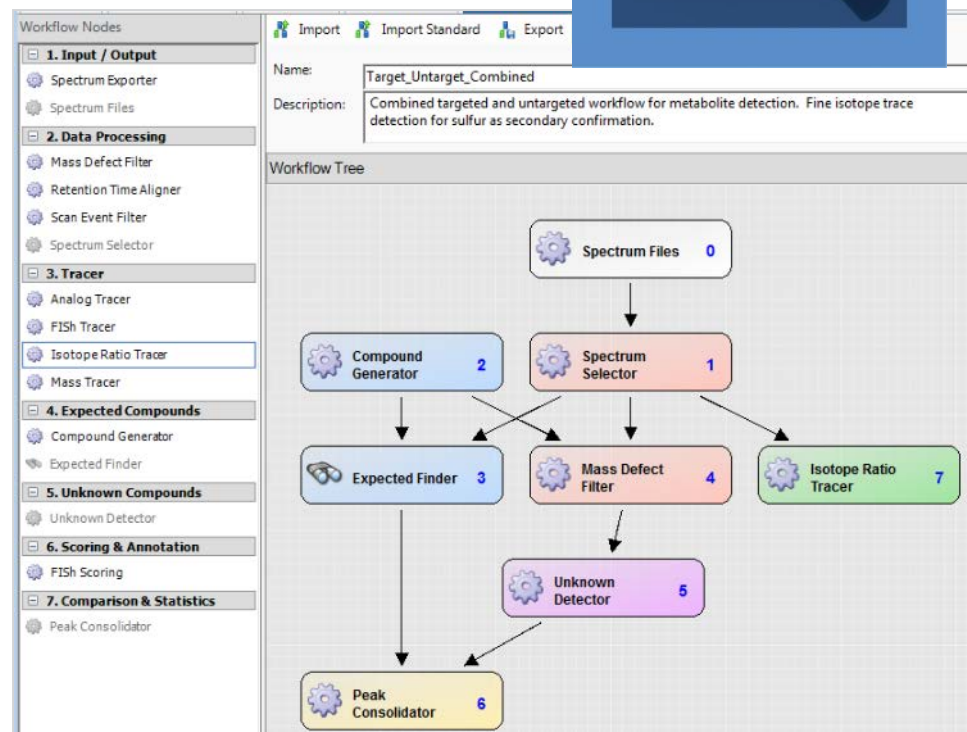


Unknown analysis of small molecules

Thermo Scientific™ Compound Discoverer™

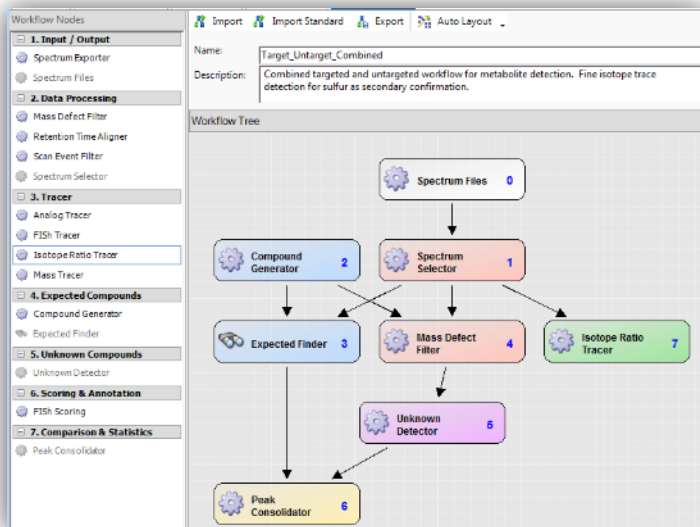
Customizable node based small molecule structure analysis software

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

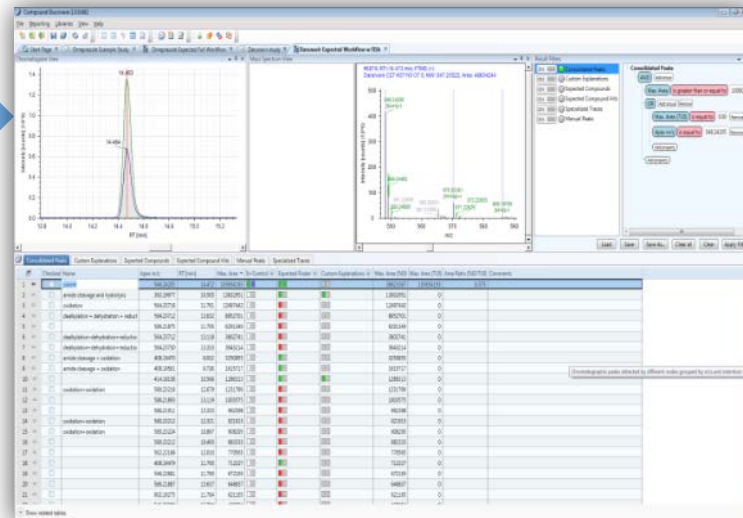


Compound Discoverer Workflow

Process



Review results



Report

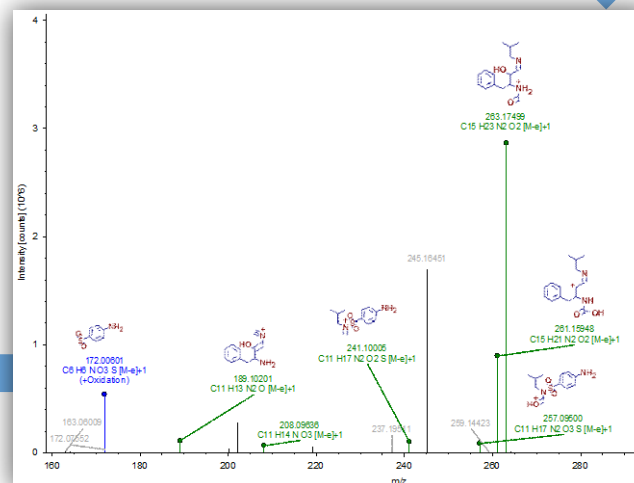
Expected Compound Hits

4/20/2015 1:16 PM


Parent Compound	Formula	Molecular Weight	Transformation	Composition Change	ΔMass (ppm per unit)	RT [min]	Best FISH Coverage	Best MS [M]	Area	Compound Area [%]	File ID	
Darunavir	C27 H37 N3 O7 S	547.2552			0.90 [M+1]	14.473	65.89	4.06	3	4953244	33.633	543
Darunavir	C27 H37 N3 O8 S	563.22014	Oxidation	+O	0.41 [M+1]	11.761	85.98	0.260	4	19409966	13.423	543
Darunavir	C27 H37 N3 O9 S	579.22506	Oxidation	+O2	0.20 [M+1]	12.679	82.81	0.262	3	1322786	0.915	543
Darunavir	C27 H37 N3 O9 S	579.22506	Oxidation	+O2	0.16 [M+1]	10.867	83.33	0.221	3	1344283	0.880	543
Darunavir	C27 H37 N3 O9 S	579.22506	Oxidation	+O2	0.20 [M+1]	12.321	79.57	0.341	3	921916	0.636	543
Darunavir	C29 H39 N3 O3 S	391.18296	Oxidation	+C2 H6 O4	1.20 [M+1]	10.968	71.21	0.376	4	15224423	10.630	543
Darunavir	C29 H39 N3 O4 S	407.18788	Oxidation	+C2 H8 O3	1.11 [M+1]	8.632	84.91	0.426	4	3558468	2.482	543
Darunavir	C29 H39 N3 O4 S	407.18788	Oxidation	+C2 H8 O3	0.36 [M+1]	9.738	74.14	0.396	4	1615717	1.117	543
Darunavir	C27 H37 N3 O8 S	563.22014	Dehydration, Reduction	+O	0.62 [M+1]	13.632	81.59	0.315	3	9620471	6.675	543
Darunavir	C27 H37 N3 O8 S	563.22014	Dehydration, Reduction	+O	0.20 [M+1]	13.118	89.26	0.264	4	3054976	3.496	543
Darunavir	C27 H37 N3 O8 S	563.22014	Dehydration, Reduction	+O	0.70 [M+1]	13.333	90.57	0.213	4	4798029	3.291	543
Darunavir	C27 H37 N3 O8 S	563.22014	Dehydration, Reduction	+O	0.43 [M+1]							





Elucidate structure



- 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




ADVANCED MASS SPECTRAL DATABASE
 Annotated Spectral Peaks, Fragment Structures, Resolution and Accuracy per Peak, Spectral Trees, Precursor Ion Fingerprinting, Substructure Identification, HR Search Algorithms, Relational Database

[Home](#) [About](#) [Features](#) [Compounds](#) [Database](#) [Partners](#) [Forum](#) [Contact](#)



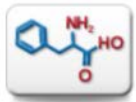



mzCloud.org

mzCloud™ is a novel type of mass spectral database that is able to assist analysts in identifying compounds even when they are not present in the library. mzCloud features a freely searchable collection of high resolution/accurate mass spectra using a new third generation spectra correlation algorithm. mzCloud also represents an open consortium of dedicated research and scientific groups aiming to establish a comprehensive library of high quality spectral trees to improve the structure elucidation of unknowns in fields such as metabolomics, toxicology and environmental sciences. [Read more...](#)

Manually Curated Data

	Compounds 2 976	Trees 4 301	Spectra 204 264	Annotations 2 985 485	QM Models 343 474	more ...
---	---------------------------	-----------------------	---------------------------	---------------------------------	-----------------------------	----------

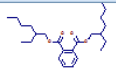
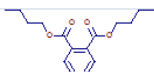
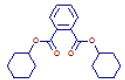
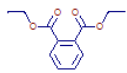



New version

	Spectrum Search		Tree Search
	Structure Search		m/z Search
	Substructure Search		Name Search

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.

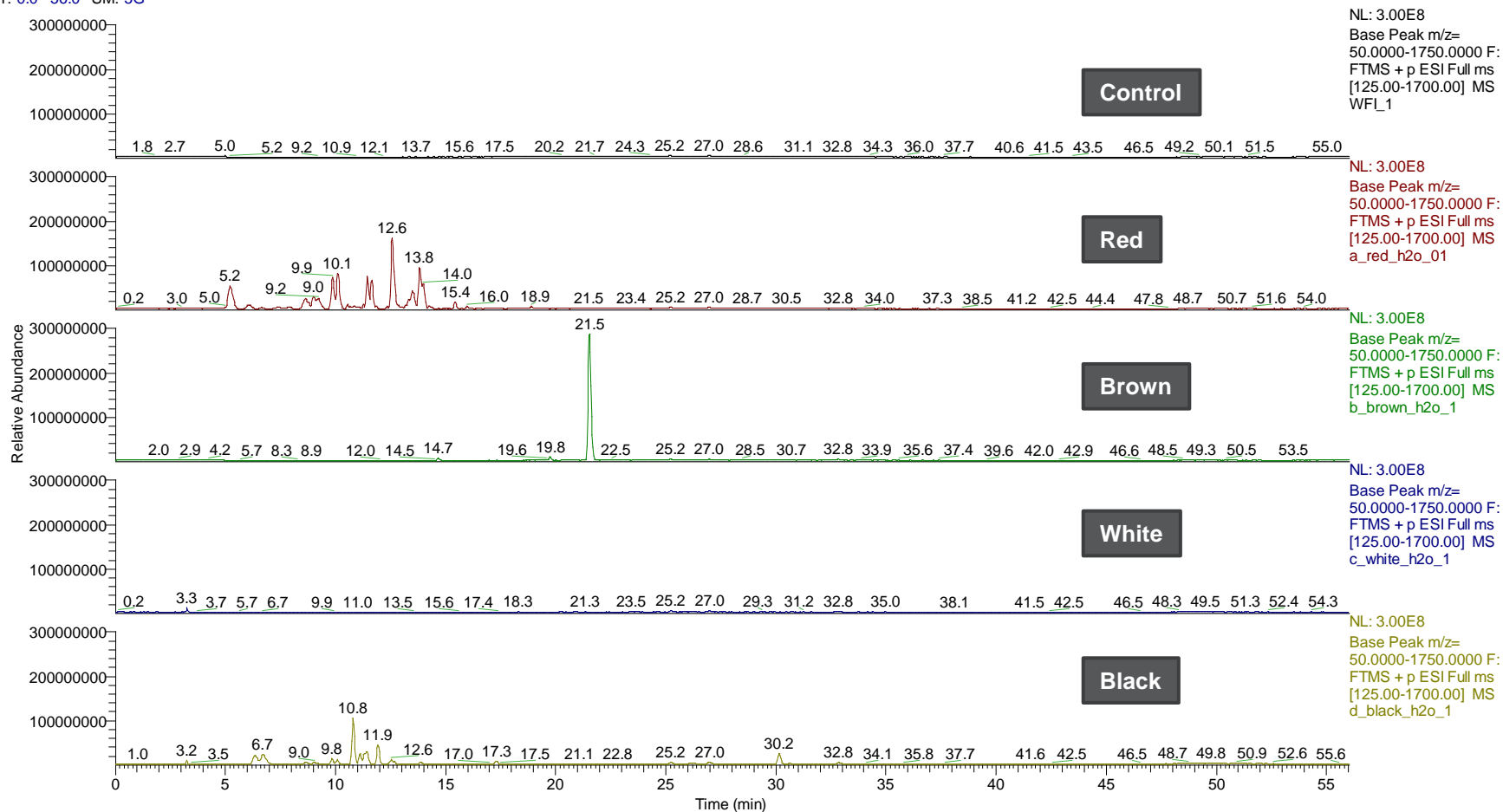


Commercial Name	Chemical Name	Class	CAS No.	Chemical Formula	Formula weight	[M+H] ⁺	[M-H] ⁻	M+NH ₄ ⁺	M+Na ⁺	M+K ⁺	Structure	mzCloud link
dioctyl phthalate	ethylhexylphthalate	PL	117-81-7	C ₂₄ H ₃₈ O ₄	390.27701	391.28429	389.26973	408.31083	413.26623	429.24017		https://mzcloud.org/DataViewer.aspx#CReference2
Dibutyl phthalate	Dibutyl phthalate	PL	84-74-2	C ₁₆ H ₂₂ O ₄	278.1518	279.15908	277.14452	296.18562	301.14102	317.11496		https://mzcloud.org/DataViewer.aspx#CReference2
	Dicyclohexyl phthalate		84-61-7	C ₂₀ H ₂₆ O ₄	330.18311	331.19039	329.17583	348.21693	353.17233	369.14627		https://mzcloud.org/DataViewer.aspx#CReference2
	Diethyl phthalate		84-66-2	C ₁₂ H ₁₄ O ₄	222.08921	223.09649	221.08193	240.12303	245.07843	261.05237		https://mzcloud.org/DataViewer.aspx#CReference2
	Dimethyl phthalate		131-11-3	C ₁₀ H ₁₀ O ₄	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	C ₅ H ₁₀ O ₃	118.06239	119.07027	117.05571	136.09681	141.05221	157.02615		https://mzcloud.org/DataViewer.aspx#CReference3
	6-Aminocaproic acid		60-32-2	C ₆ H ₁₃ N ₂ O ₂	131.09463	132.10191	130.08735	149.12845	154.08385	170.05779		https://mzcloud.org/DataViewer.aspx#CReference3

O-Ring Extractables - Water

E:\Parker-O-Ring Leachable\WFL1 06/24/15 00:17:23
Accucore C18 150X2.1 2.6 um A: H2O/0.1% FA B: ACN/0.1% FA WFI Control

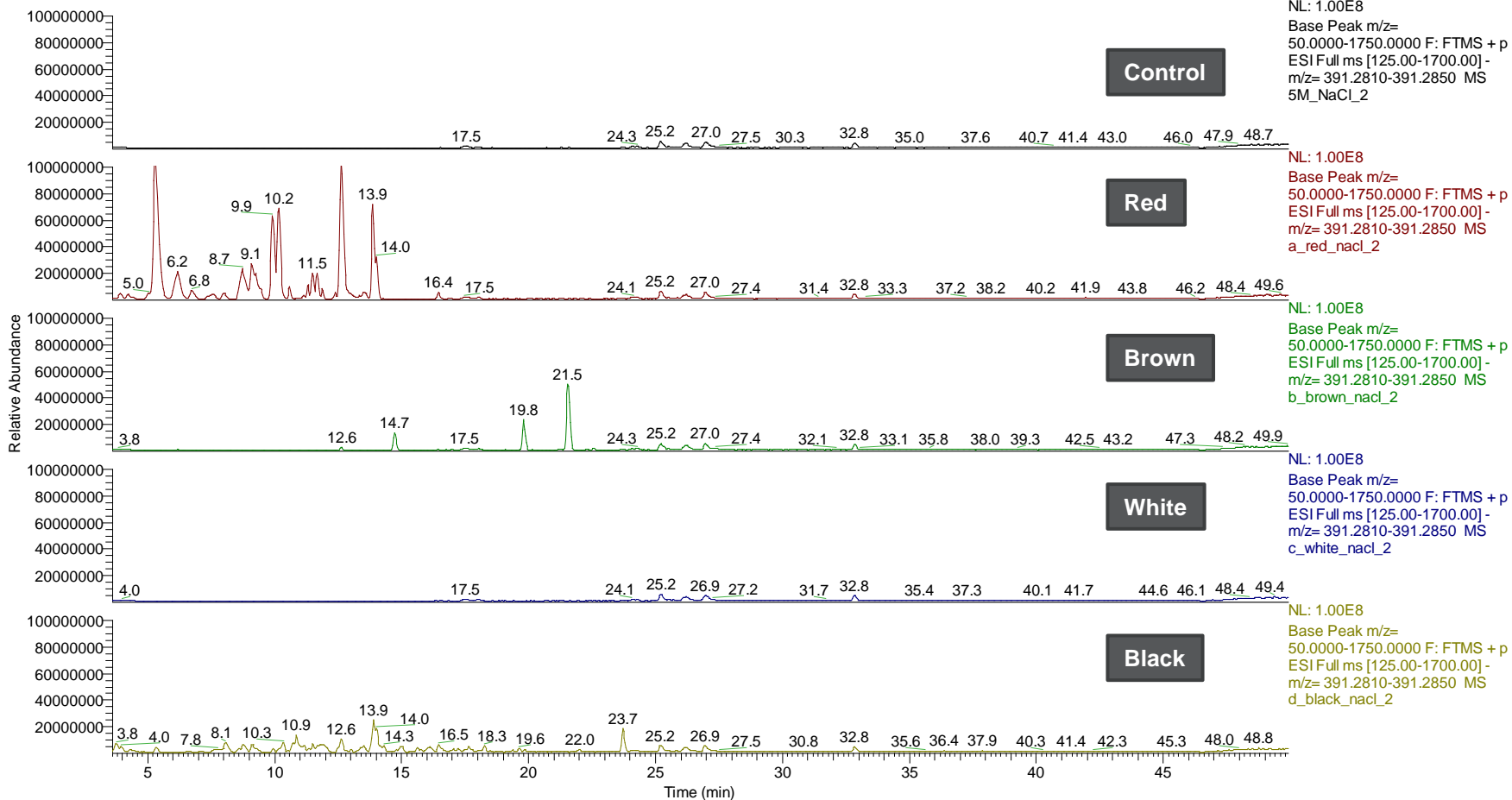
RT: 0.0 - 56.0 SM: 5G



O-Ring Extractables – 5M NaCl

d_black_nacl_2 06/27/15 22:06:23
Accucore C18 150X2.1 2.6 um A: H2O/0.1% FA B: ACN/0.1% FA

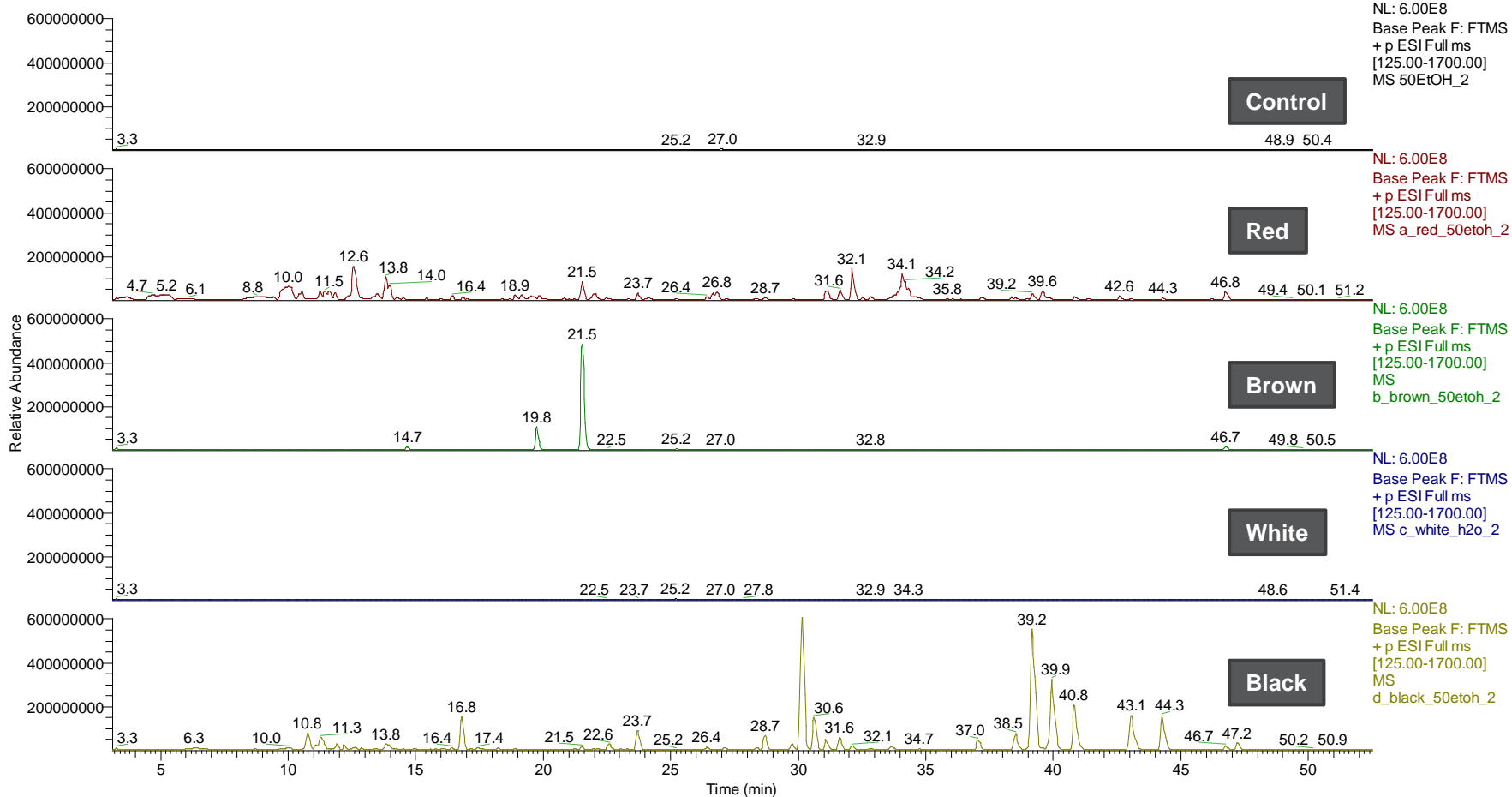
RT: 3.6 - 49.9 SM: 5G



O-Ring Extractables – 50% Ethanol

E:\Parker-O-Ring Leachable\50EtOH_2 06/25/15 04:50:58
Accucore C18 150X2.1 2.6 um A: H2O/0.1% FA B: ACN/0.1% FA WFI Control

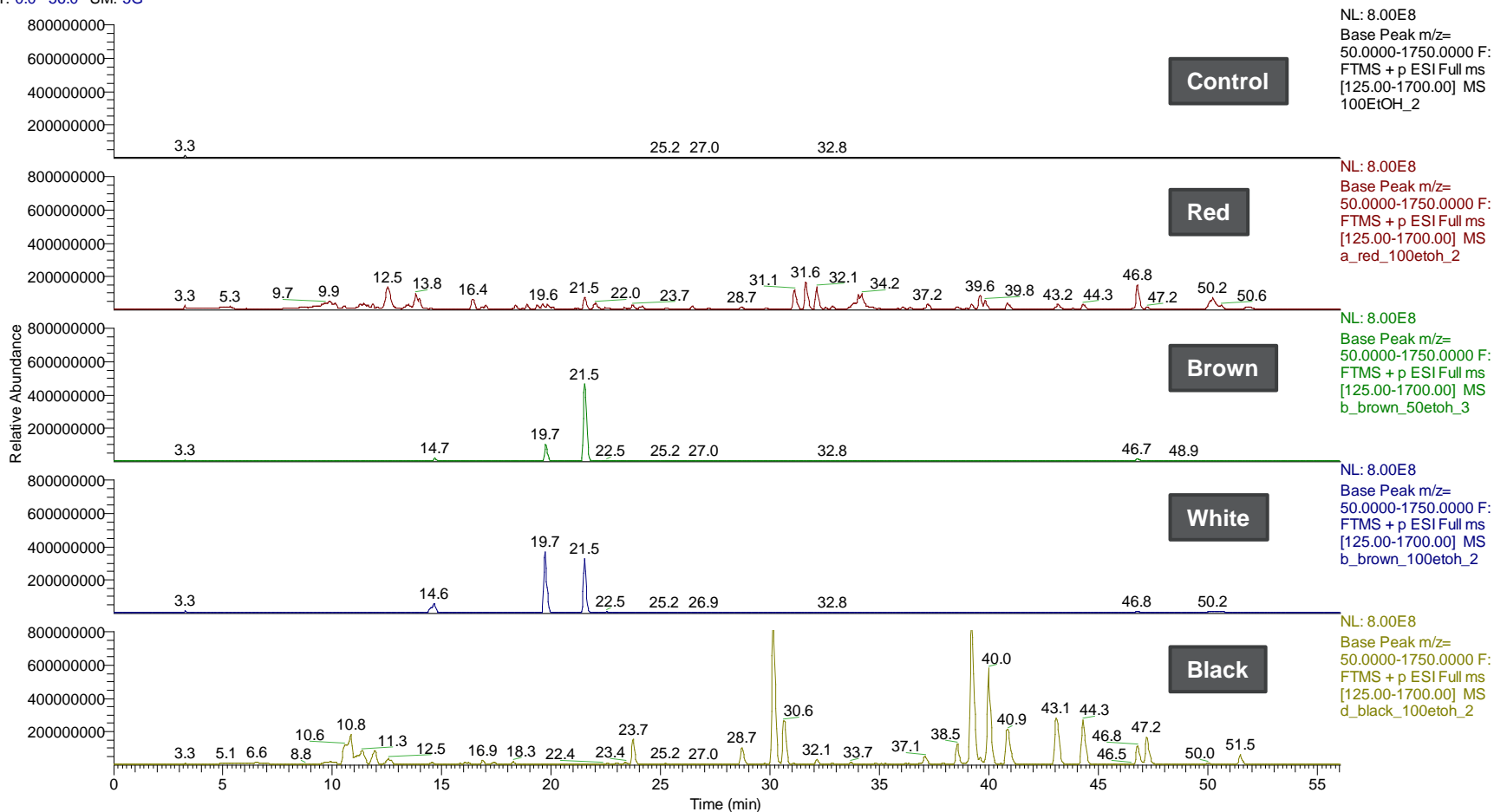
RT: 3.1 - 52.5 SM: 5G



O-Ring Extractables – 100% Ethanol

E:\Parker-O-Ring Leachable\100EtOH_2 06/26/15 04:17:58
Accucore C18 150X2.1 2.6 um A: H2O/0.1% FA B: ACN/0.1% FA WFI Control

RT: 0.0 - 56.0 SM: 5G



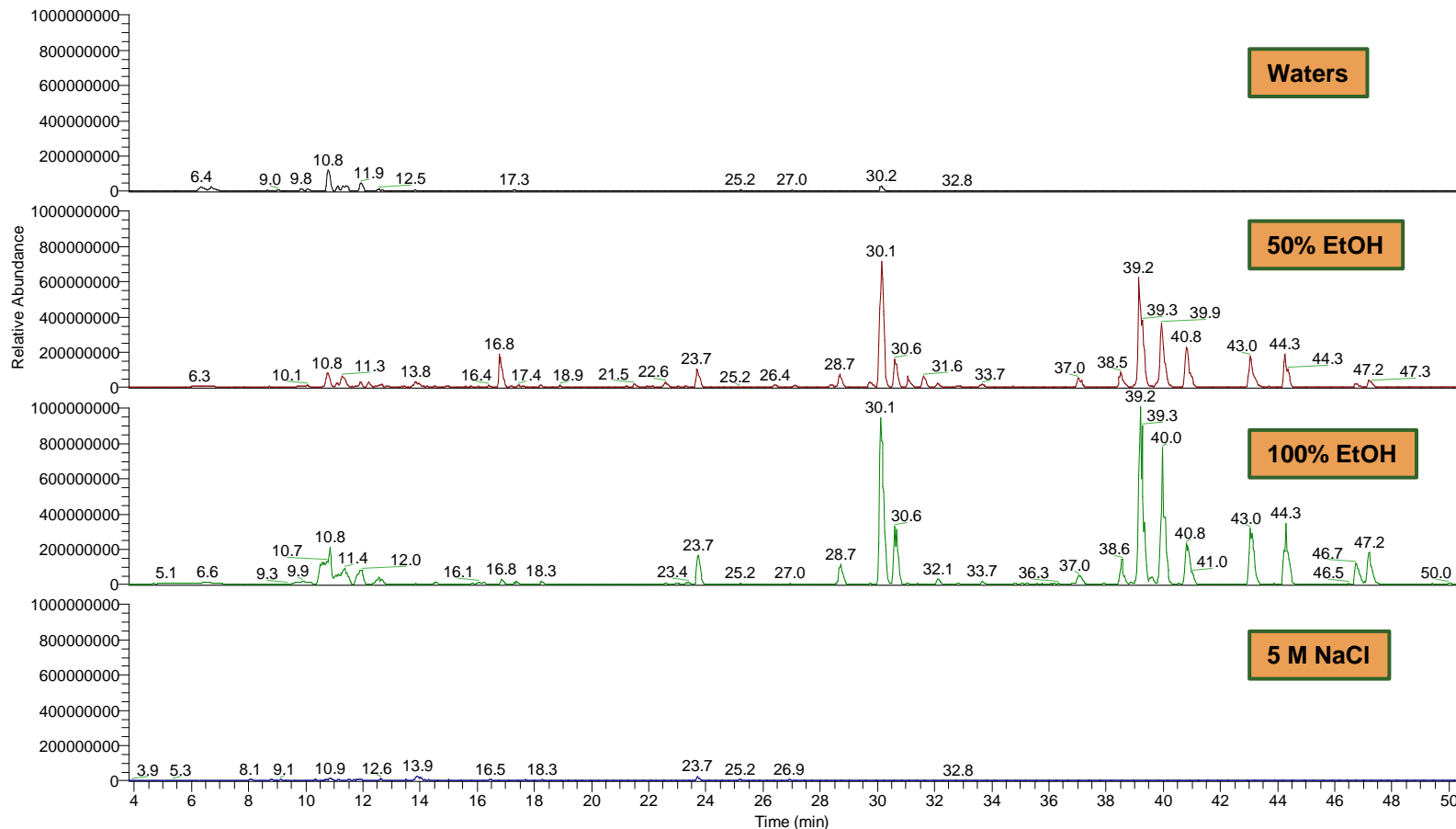
BPI+ of Black O-ring in Four Extraction Solutions

d_black_50etoh_2

06/25/15 23:12:07

Accucore C18 150X2.1 2.6 um A: H2O/0.1% FA B: ACN/0.1% FA WFI Control

RT: 3.8 - 50.3



NL: 1.00E9
Base Peak F: FTMS
+ p ESI Full ms
[125.00-1700.00]
MS D_black_H2O_2

NL: 1.00E9
Base Peak F: FTMS
+ p ESI Full ms
[125.00-1700.00]
MS
d_black_50etoh_2

NL: 1.00E9
Base Peak F: FTMS
+ p ESI Full ms
[125.00-1700.00]
MS
d_black_100etoh_2

NL: 1.00E9
Base Peak F: FTMS
+ p ESI Full ms
[125.00-1700.00]
MS d_black_nacl_2

Compound Discoverer Workflow for O-Ring leachables

Workflow Nodes

Workflow Tree

Compound Discoverer 2.0.0.220

File Reporting Libraries View Help

Start Page Fusion Omeprazole Impurity ID Omeprazole Wine Bag E&L Unknown_with_ElemComp_ChemSpider_mzCloud_HideBackground_Pos_Mode_b192 O-ring leachables ID

Add Files Remove Files Open Containing Folder New Analysis Open Analysis Template

Study Definition Input Files Samples Analysis Results Grouping & Ratios Workflows

Workflow Nodes

- 1. Input / Output
 - Export Spectra
 - Input Files
- 2. Data Processing
 - Align Retention Times
 - Filter By Mass Defect
 - Filter By Scan Event
 - Filter Centroids
 - Select Spectra
- 3. Tracer
 - Create Analog Trace
 - Create FISH Trace
 - Create Mass Trace
 - Create Pattern Trace
- 4. Expected Compounds
 - FISH Scoring
 - Find Expected Compounds
 - Generate Expected Compounds
 - Group Expected Compounds
 - Mark Background Compounds
- 5. Unknown Compounds
 - Detect Unknown Compounds
 - Fill Gaps
 - Group Unknown Compounds
 - Map to KEGG Pathways
 - Mark Background Compounds
 - Normalize Areas
 - Pattern Scoring
 - Predict Compositions
 - Search ChemSpider
 - Search Mass Lists
 - Search mzCloud
- 6. Comparison & Statistics
 - Merge Features
- 7. Post-Processing
 - Compounds Descriptive Statistics
 - Differential Analysis

Workflow: O-ring Leachable LCMS

Description:

Workflow Tree

```
graph TD; N0[Input Files 0] --> N1[Select Spectra 1]; N0 --> N2[Create Analog Trace 2]; N0 --> N3[Create Analog Trace 3]; N1 --> N4[Create Mass Trace 4]; N1 --> N5[Align Retention Times 5]; N2 --> N6[Create Mass Trace 6]; N3 --> N6; N5 --> N7[Detect Unknown Compounds 7]; N4 --> N7; N7 --> N8[Group Unknown Compounds 8]; N8 --> N9[Search mzCloud 9]; N8 --> N10[Search ChemSpider 10]; N8 --> N11[Search Mass Lists 11]; N8 --> N12[Fill Gaps 12]; N8 --> N13[Predict Compositions 13]; N9 --> N14[Differential Analysis 14]; N11 --> N15[Compounds Descriptive Statistics 15];
```

Post-Processing Nodes

- Differential Analysis 14
- Compounds Descriptive Statistics 15

Analysis

Processing Step

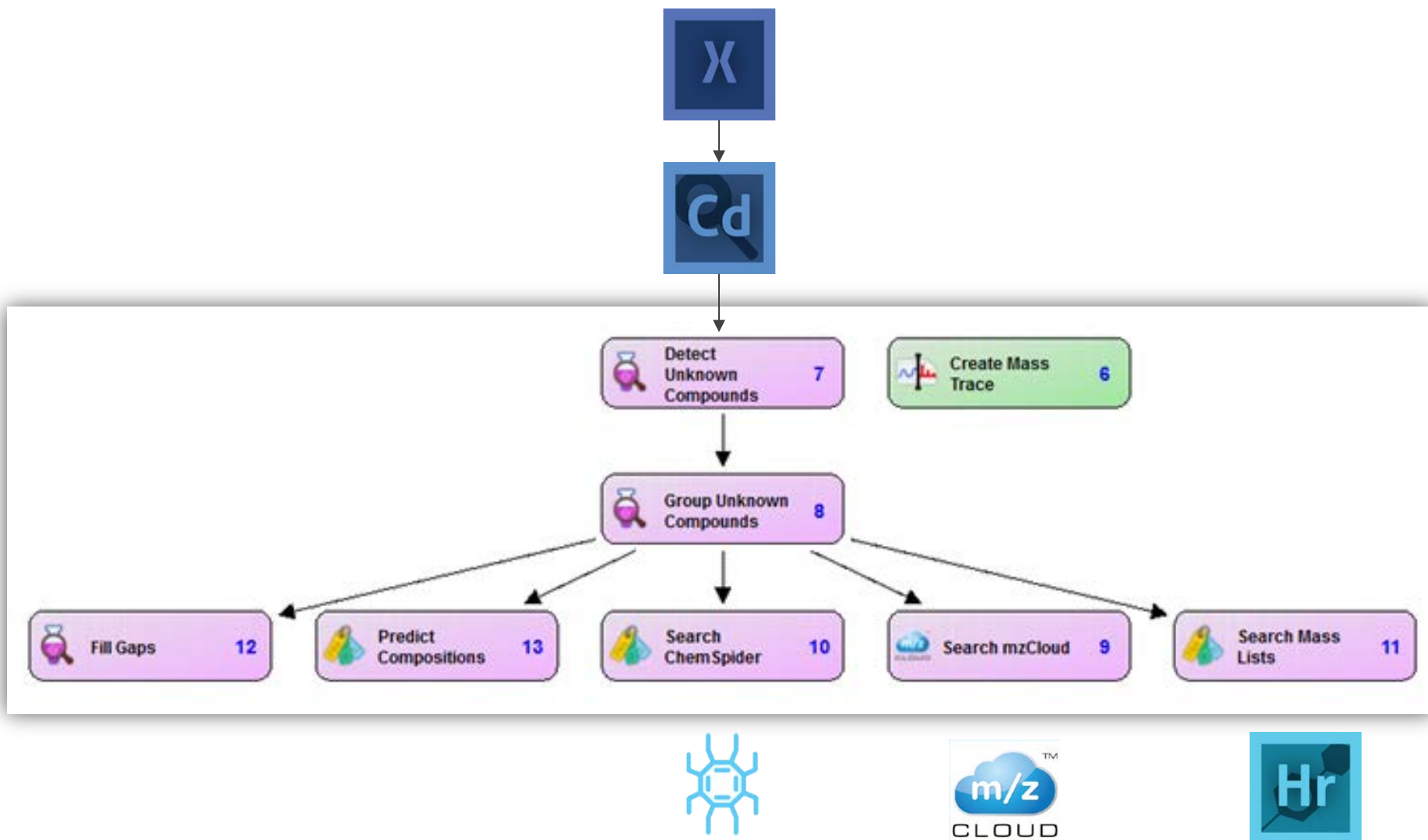
Workflow: O-ring Leachable LCMS

Result File: WFI_1cdResult

Files for Analysis: (62)

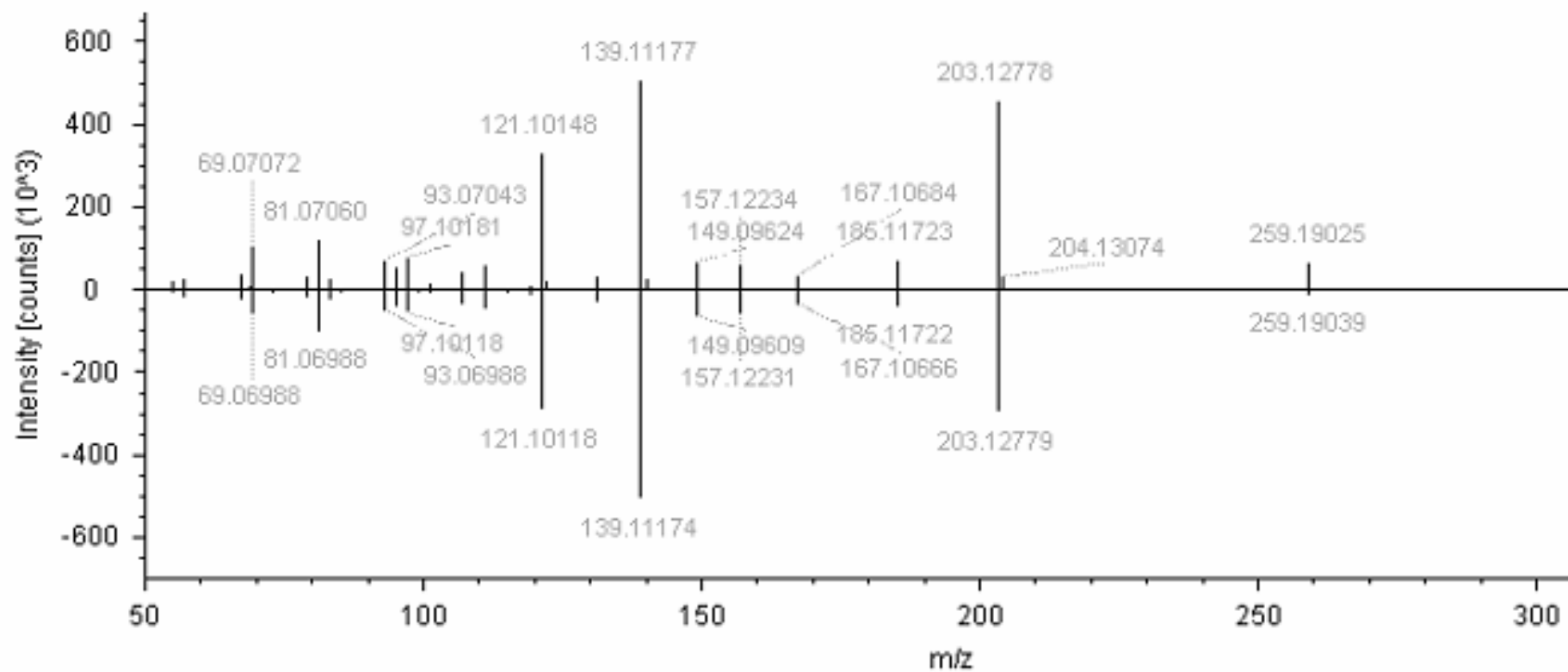
File	Sample Type
F1 WFI_1	[Control]
F2 WFI_2	[Control]
F3 WFI_3	[Control]
F4 A_Red_H2O_02	[Sample]
F5 A_Red_H2O_03	[Sample]
F6 A_Red_H2O_04	[Sample]
F7 B_Brown_H2O_1	[Sample]
F8 B_Brown_H2O_2	[Sample]
F9 B_Brown_H2O_3	[Sample]
F10 C_White_H2O_1	[Sample]
F11 C_White_H2O_2	[Sample]
F12 C_White_H2O_3	[Sample]
F13 D_Black_H2O_1	[Sample]
F14 D_Black_H2O_2	[Sample]
F15 D_Black_H2O_3	[Sample]
F16 A_Red_H2O_1	[Sample]
F17 A_Red_H2O_2	[Sample]
F18 A_Red_H2O_3	[Sample]
F19 A_Red_50EtOH_1	[Sample]
F20 A_Red_50EtOH_3	[Sample]
F21 A_Red_50EtOH_4	[Sample]
F22 B_Brown_50EtOH_1	[Sample]
F23 B_Brown_50EtOH_2	[Sample]
F24 B_Brown_50EtOH_3	[Sample]
F25 C_White_50EtOH_1	[Sample]
F26 C_White_50EtOH_2	[Sample]
F27 C_White_50EtOH_3	[Sample]
F28 D_Black_50EtOH_1	[Sample]
F29 D_Black_50EtOH_2	[Sample]
F30 D_Black_50EtOH_3	[Sample]
F31 100EtOH_1	[Control]
F32 100EtOH_2	[Control]

Automated Identification – Compound Discoverer



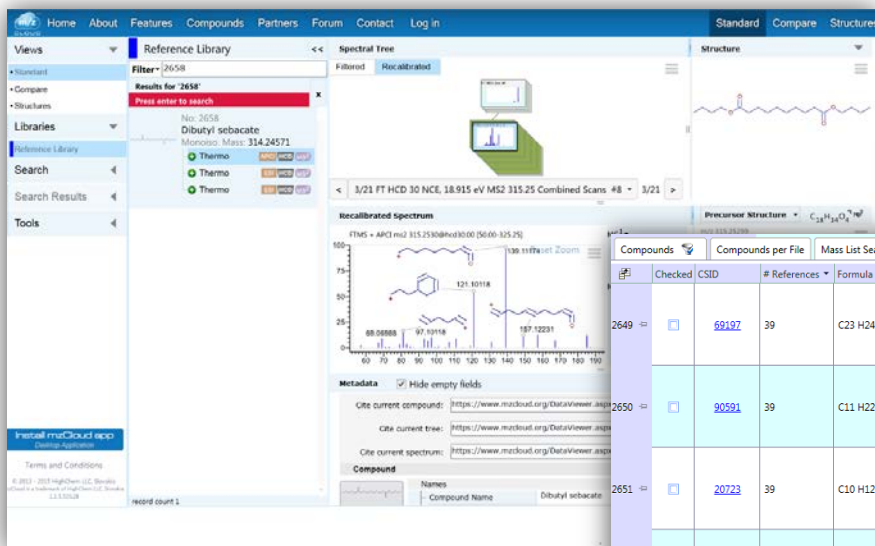
Data are directly mirror-plotted versus mzCloud

RAWFILE(top): Solvent_Blank_2, #8204, RT=29.612 min, FTMS (+), MS2 (HCD, DDF, 315.25@35.00, z=+1)
REFERENCE(bottom): mzCloud library C18 H34 O4 Dibutyl sebacate FTMS (+) MS2 (HCD 315.25@30.00)



Parallel identification through multiple reference sources

mzCloud Library Spectrum



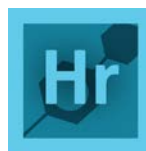
ChemSpider Search Results

Compounds	Checked	CSID	# References	Formula	Molecular Weight	Structure	Name
2649	<input type="checkbox"/>	69197	39	C23 H24 O4	364.16745		2,2-Propanediyl-di-4-1-phenylene bis(2-methylacrylate)
2650	<input type="checkbox"/>	90591	39	C11 H22 N2 O	198.17322		N-(2,6,6-Tetramethyl-4-piperidinyl)acetamide
2651	<input type="checkbox"/>	20723	39	C10 H12 N2	160.10005		1-Ethyl-2-methyl-1H-benzimidazole
2652	<input type="checkbox"/>	17679	39	C11 H12 O2	176.08372		
2653	<input type="checkbox"/>	4445035	39	C15 H14 O2	226.09938		

Show related tables

E&L Library Results

Compounds	Checked	Formula	Molecular Weight	RT [min]	Structure	Name	Annotation	Reference List Name
1	<input type="checkbox"/>	C18 H15 O P	278.08605	0.000		Triphenylphosphine oxide		Converted EandL_List_v1
2	<input type="checkbox"/>	C10 H18 O4	202.12051	0.000		Sebacic acid		Converted EandL_List_v1
3	<input type="checkbox"/>	C28 H27 N	377.21435	0.000		4-(1-phenylethyl)-N-[4-(1-phenylethyl)phenyl]acetamide		Converted EandL_List_v1
4	<input type="checkbox"/>	C20 H32 O2	304.24023	0.000		(all-Z)-581114-Eicosatetraenoic acid		Converted EandL_List_v1
5	<input type="checkbox"/>	C21 H40 O4	356.29266	0.000		Dihexyl azelate		Converted EandL_List_v1

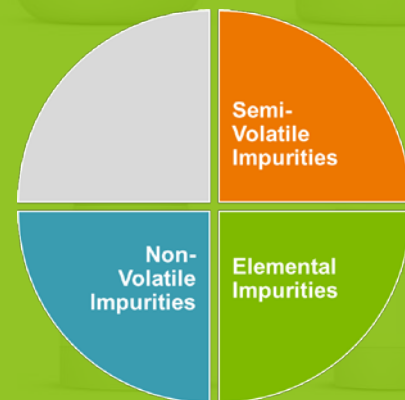


Components identified confidently

- 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

Results – Elemental extractables



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



iCAP Q ICP-MS Instrument Configuration



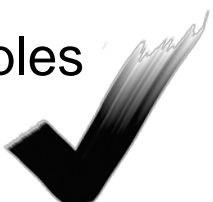
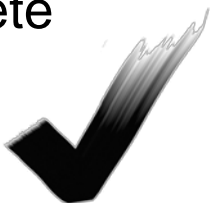
- **Measurement Mode:**

- 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)

0.1%, 10 dissolved in ultra pure water.

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
 - **$\pm 30\%$ of prepared concentration**
- Accuracy Check: Spike recovery of a 0.2 mg/L solution after complete sample acidification routine
 - **$\pm 20\%$ of expected concentration**
- Drift Check: Standard 2 (500x dilution) was analyzed every 10 samples
 - **$\pm 30\%$ of prepared concentration**



Quality data, automatically tested

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	-

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	-		-	-	-	-	-	-	-	-	-

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



Summary – Extractable workflow

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.



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

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

The screenshot shows the Thermo Scientific website's community page for Extractables and Leachables. The page is titled "Extractables and Leachables" and features a navigation menu with options like "Products", "Communities", "Services", "Support", and "About Us". The main content area includes a video player with the text "No more unknowns" and "Identify all extractables and leachables contaminants with HPLC workflows and advanced compound identification". Below the video, there is a section titled "Join the Community" with a "Subscribe Now" button. The page also features a "Free cloud-based mass spectral database" section with a "Visit Site" button and a "A history of GC-MS" section with a "Watch this 3 minute video" button. The page is designed with a clean, professional layout and includes a search bar and a "Give Feedback" button on the right side.

www.thermoscientific.com/Leachables

Thank you

