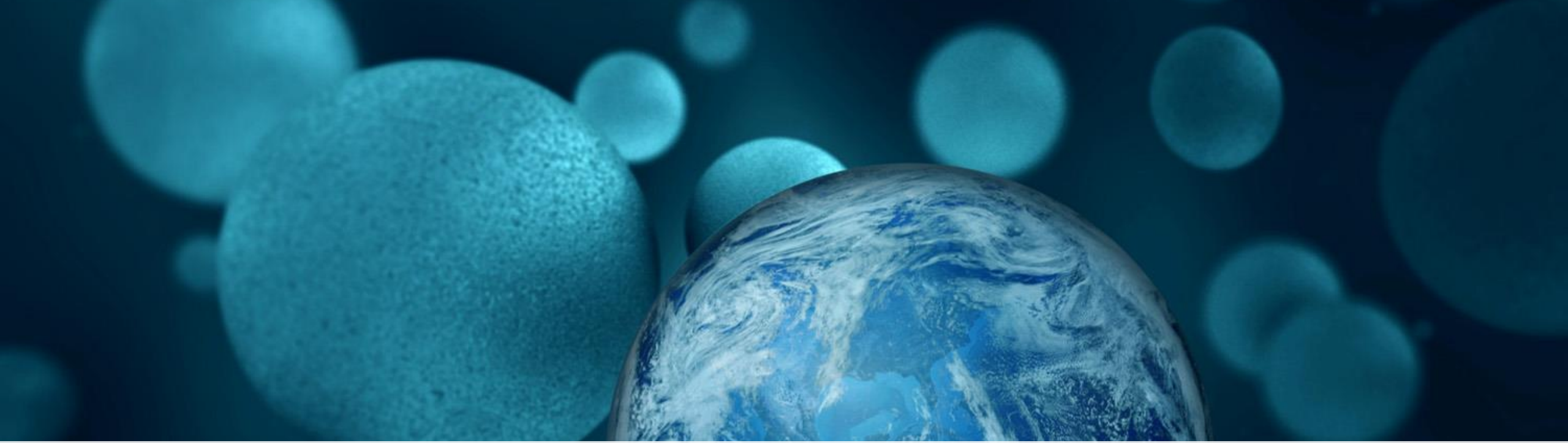

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Come Discover the Best Kept Secret in Metabolomics. Thermo Scientific Compound Discoverer Software.

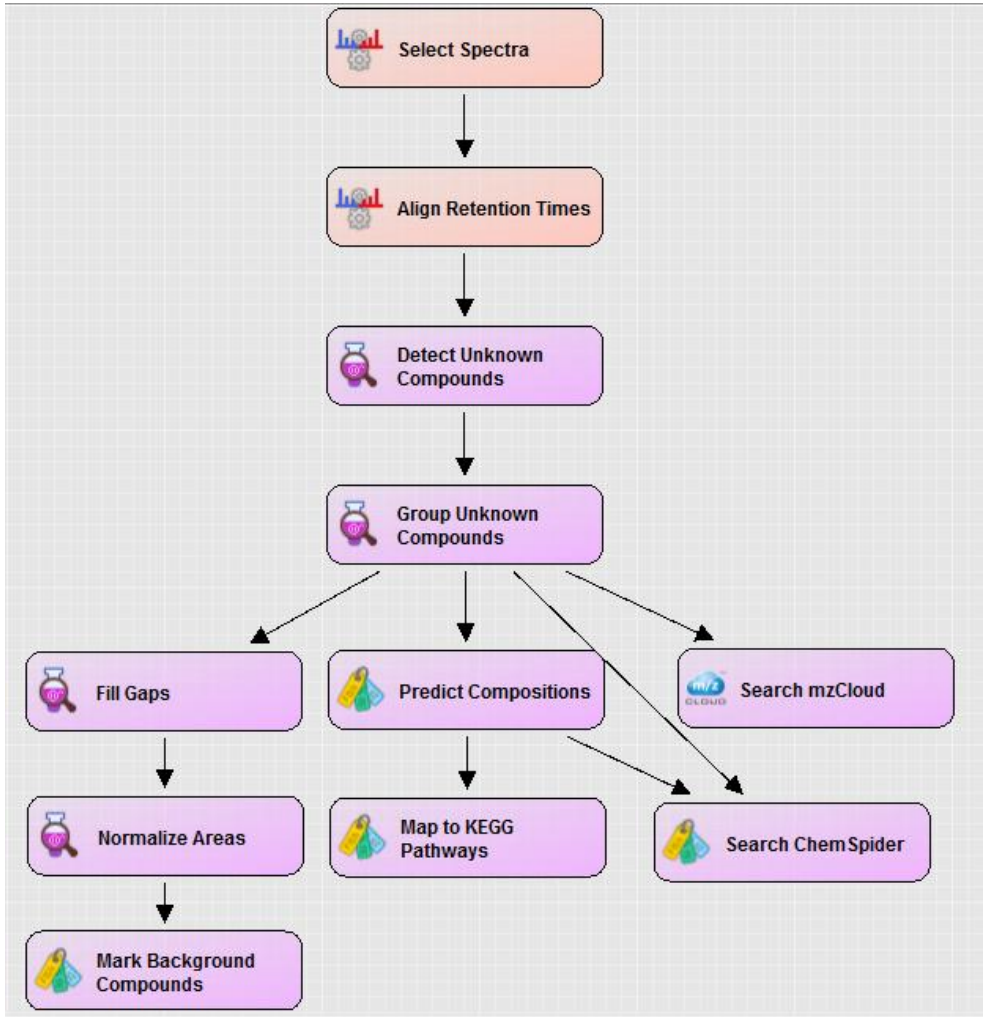
Compound Discoverer 2.1 Software

Thermo Scientific™ Compound Discoverer™ 2.1 Software

Complete small molecule structure identification in a **Next Generation** platform.

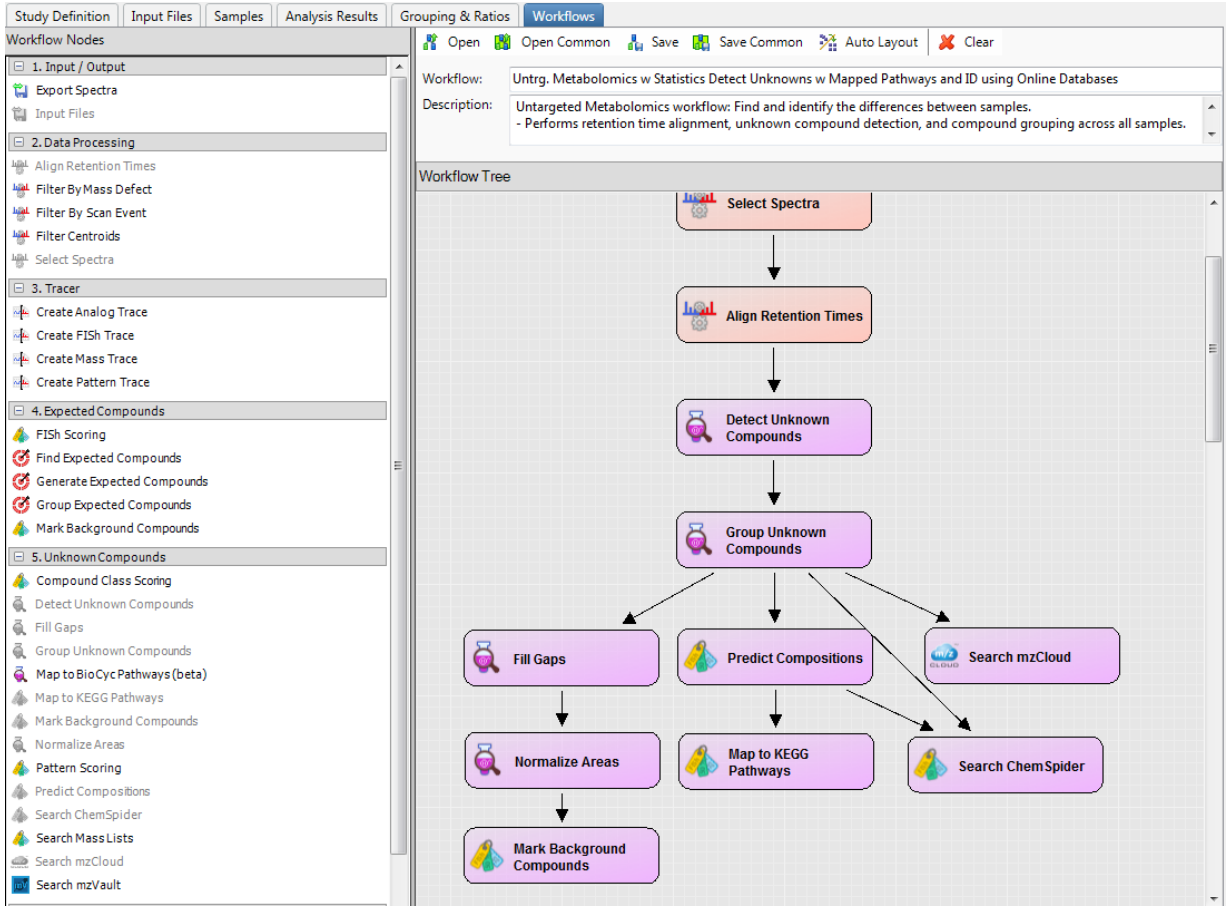
Compound Discoverer 2.1 offers flexible workflows for **Discovery Metabolomics**: Fundamental Research, Biomarker Discovery, Pharma, Environmental Research, Forensics, Foodomics, etc.

Data Processing Workflows



- Flexible data processing workflows
- Use drag & drop, “smart” connection
- Work with predefined workflows or create your own
- Option to integrate your own nodes

Data Processing Workflows



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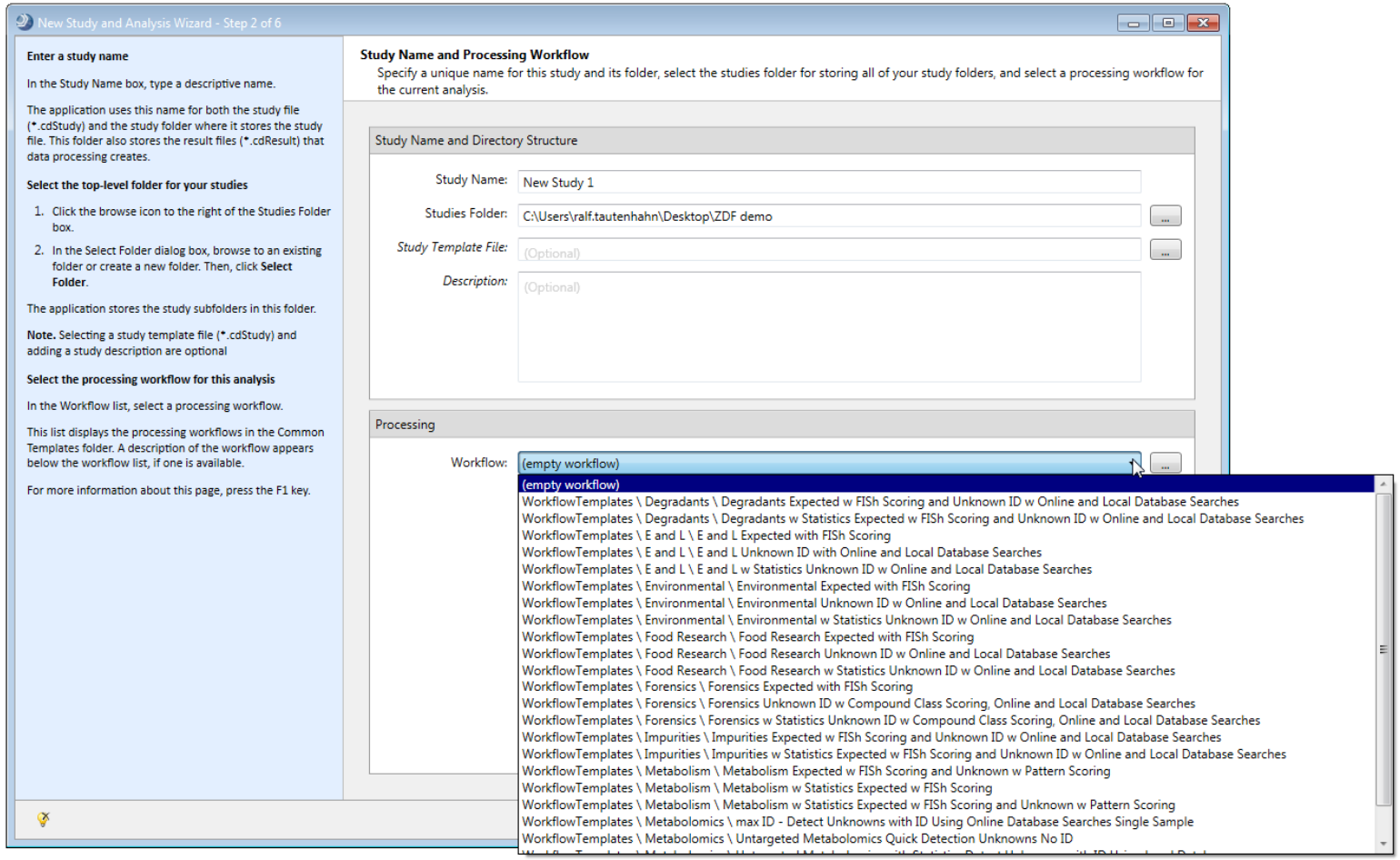
Workflow Templates



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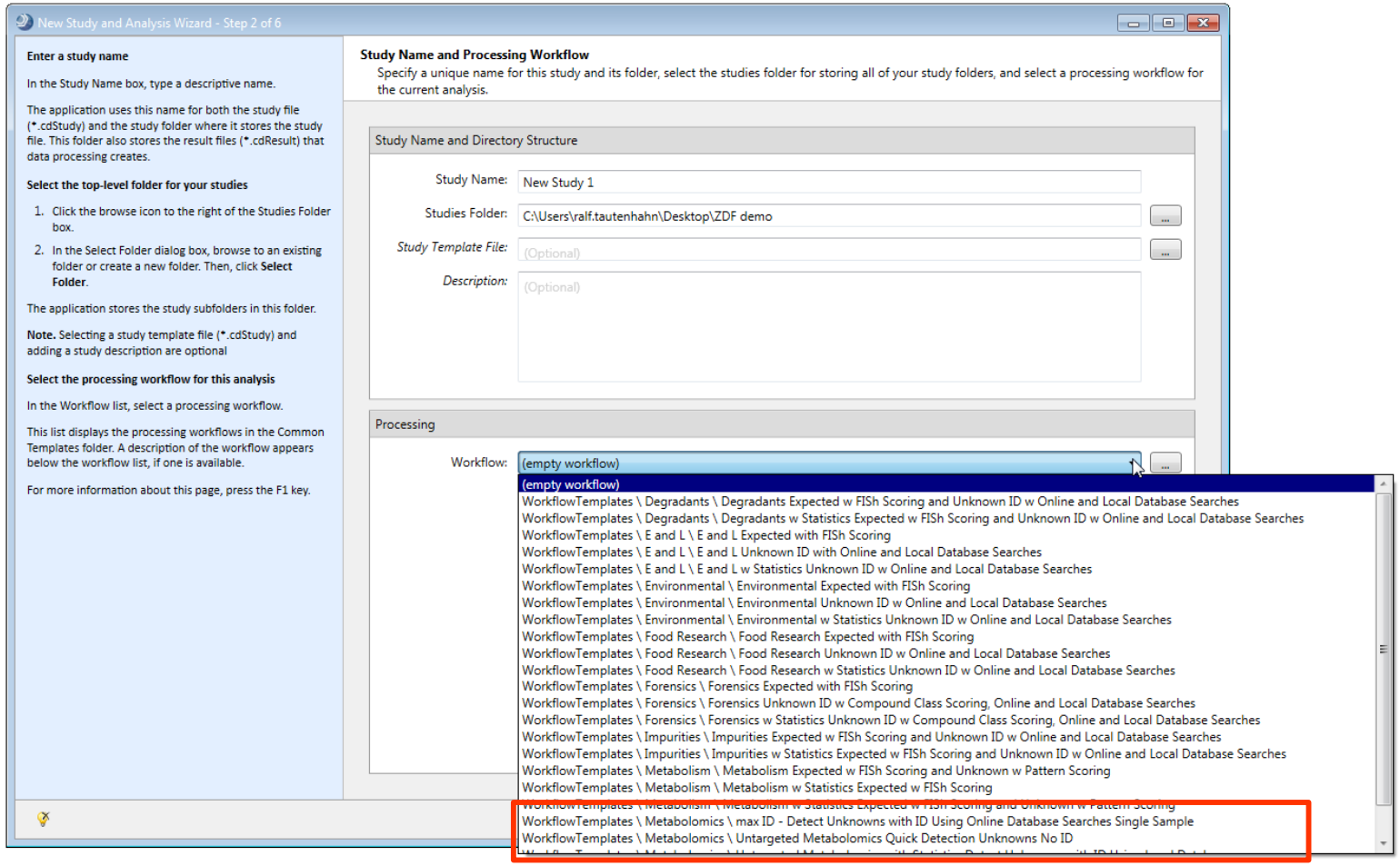
Workflow Templates for Metabolomics



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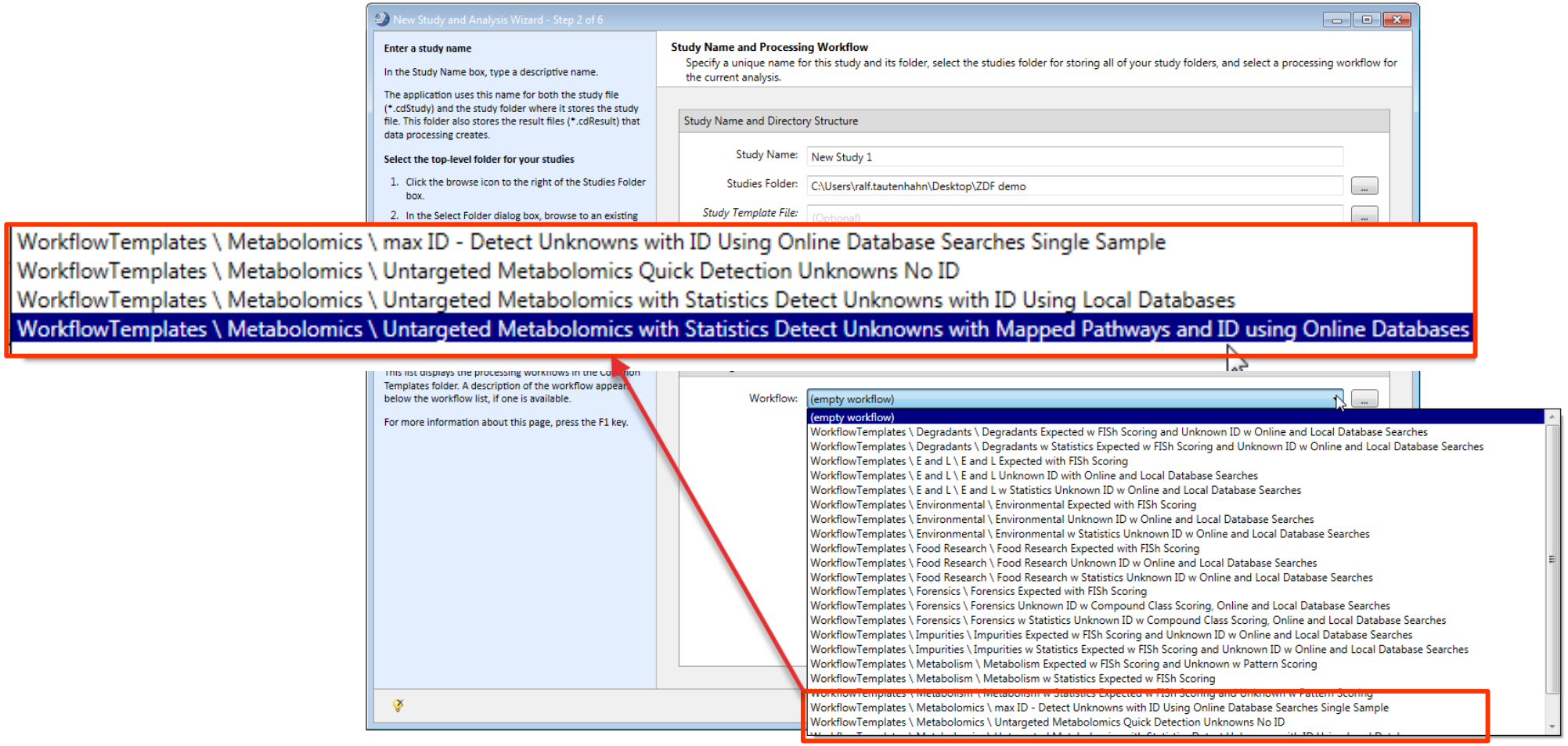
Workflow Templates for Metabolomics



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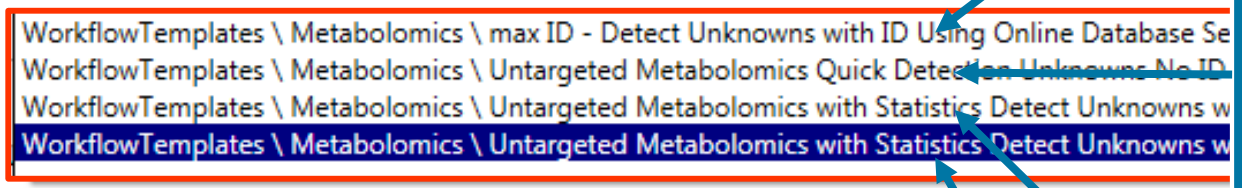
Workflow Templates for Metabolomics



Max ID workflow. Detect and identify all compounds in a single sample (with ddMS2)- even compounds with very low abundances.

Quick compound detection. Detect compounds in a single sample or multiple samples.

Untargeted Metabolomics workflow: Find and identify the differences between samples (offline databases).

Untargeted Metabolomics workflow: Find and identify the differences between samples (online databases).

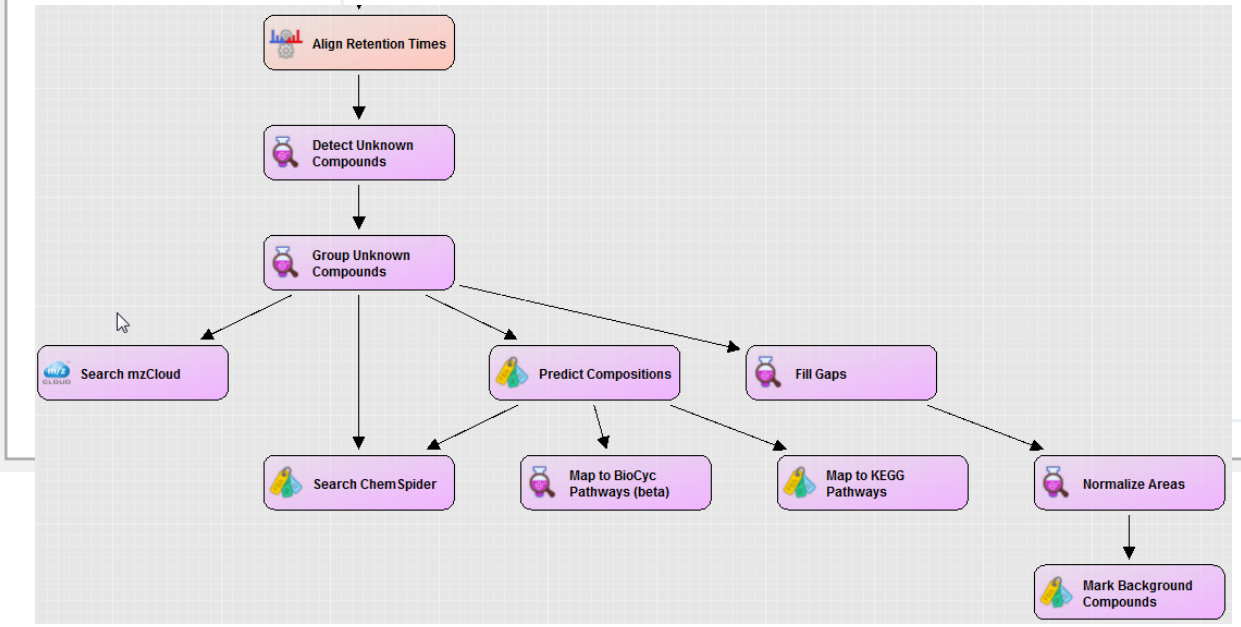
Workflow Details

Processing

Workflow: WorkflowTemplates \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with Mapped Pathways and ID using Online Databases ...

Workflow Description:

Untargeted Metabolomics workflow: Find and identify the differences between samples.
- Performs retention time alignment, unknown compound detection, and compound grouping across all samples. Predicts elemental compositions for all compounds, fills gaps across all samples, and hides chemical background (using Blank samples). Identifies compounds using mzCloud (ddMS2) and ChemSpider (exact mass or formula). Also performs similarity search for all compounds with ddMS2 data using mzCloud. Maps compounds to biological pathways using BioCyc and KEGG databases. Applies QC-based batch normalization if QC samples are available. Calculates differential analysis (t-test or ANOVA), determines p-values, adjusted p-values, ratios, fold change, CV, etc.).



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Sample Types in Compound Discoverer Software

S39	Soy_8_Replicate_2	Sample
S40	Soy_8_Replicate_3	Sample
S41	Soy_9_Replicate_1	Sample
S42	Soy_9_Replicate_2	Sample
S43	Soy_9_Replicate_3	Sample

Sample: full scan data for peak detection, alignment, statistics (required)

Sample Types in Compound Discoverer Software

S39	Soy_8_Replicate_2	Sample
S40	Soy_8_Replicate_3	Sample
S41	Soy_9_Replicate_1	Sample
S42	Soy_9_Replicate_2	Sample
S43	Soy_9_Replicate_3	Sample

Sample: full scan data for peak detection, alignment, statistics (required)

S1	Blank_1_Replicate_1	Blank
S2	Blank_1_Replicate_2	Blank
S3	Blank_1_Replicate_3	Blank

Blank: solvent or matrix blanks for background removal (optional)

Sample Types in Compound Discoverer Software

S39	Soy_8_Replicate_2	Sample
S40	Soy_8_Replicate_3	Sample
S41	Soy_9_Replicate_1	Sample
S42	Soy_9_Replicate_2	Sample
S43	Soy_9_Replicate_3	Sample

Sample: full scan data for peak detection, alignment, statistics (required)

S1	Blank_1_Replicate_1	Blank
S2	Blank_1_Replicate_2	Blank
S3	Blank_1_Replicate_3	Blank

Blank: solvent or matrix blanks for background removal (optional)

S5	QC_1	QualityControl
S6	QC_2	QualityControl
S7	QC_3	QualityControl
S8	QC_4	QualityControl
S9	QC_5	QualityControl
S10	QC_6	QualityControl

QualityControl: pooled samples for Normalization and QC (optional)

Sample Types in Compound Discoverer Software

S39	Soy_8_Replicate_2	Sample
S40	Soy_8_Replicate_3	Sample
S41	Soy_9_Replicate_1	Sample
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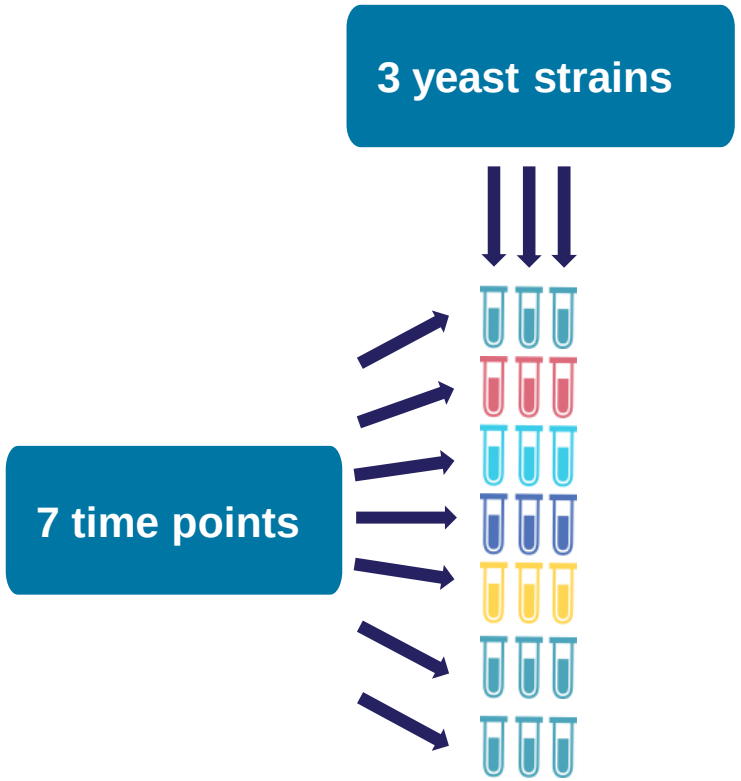
S5	QC_1	QualityControl
S6	QC_2	QualityControl
S7	QC_3	QualityControl
S8	QC_4	QualityControl
S9	QC_5	QualityControl
S10	QC_6	QualityControl

QualityControl: pooled samples for Normalization and QC (optional)

S119	Soy_ID_L1	Identification Only
S120	Soy_ID_L2	Identification Only
S121	Soy_ID_L3	Identification Only

Identification Only: one or more ddMS^2 for compound identification (optional)

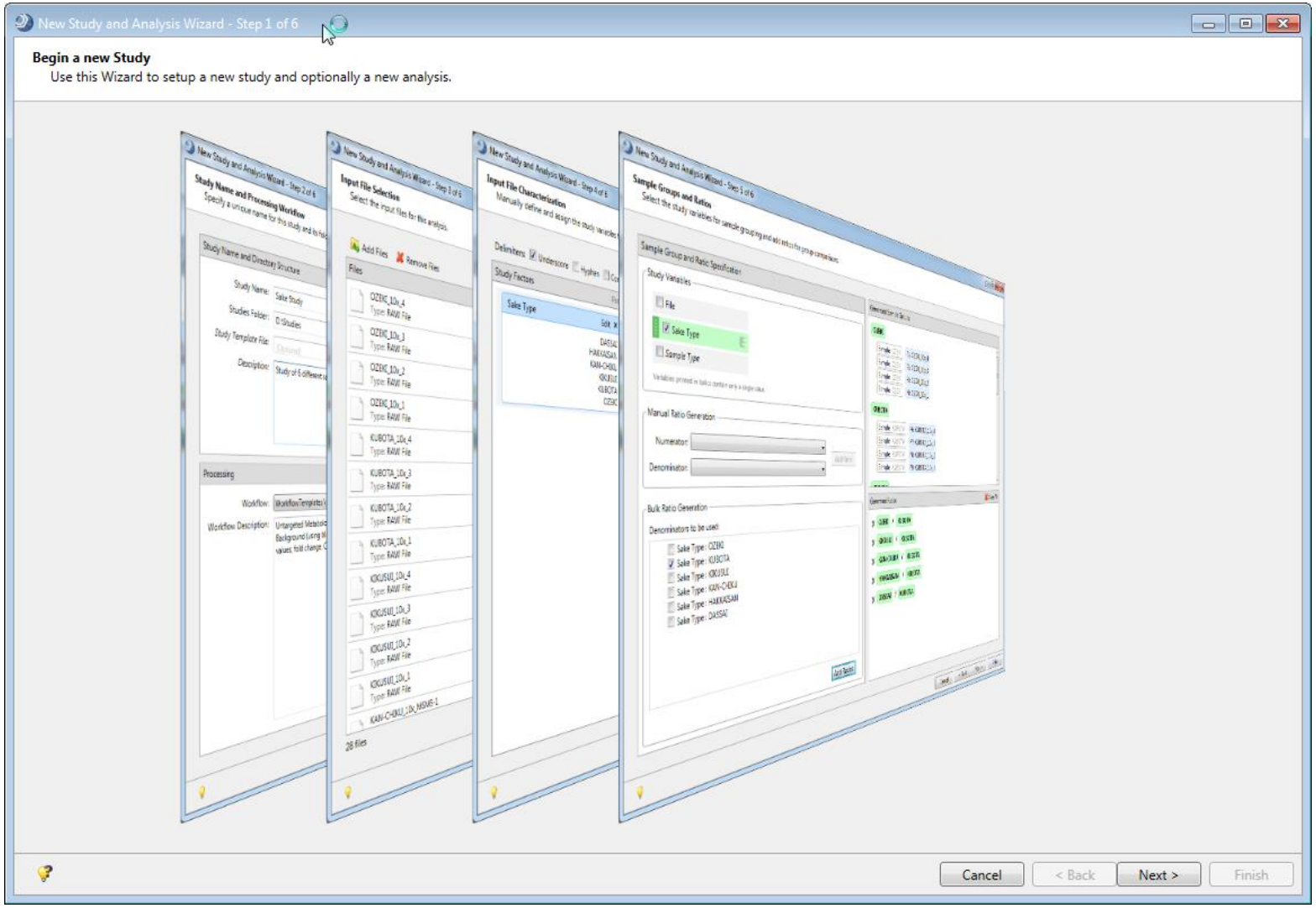
Study Factors in Compound Discoverer Software



Study factors (or study variables): Information about your samples.

Study factors are used for statistics and interactive visualizations.

Create New Study Using the Wizard in Compound Discoverer Software



Create a new study in 4 steps

- Select samples
- Select workflow
- Define sample types (optional)
- Define study factors (optional)

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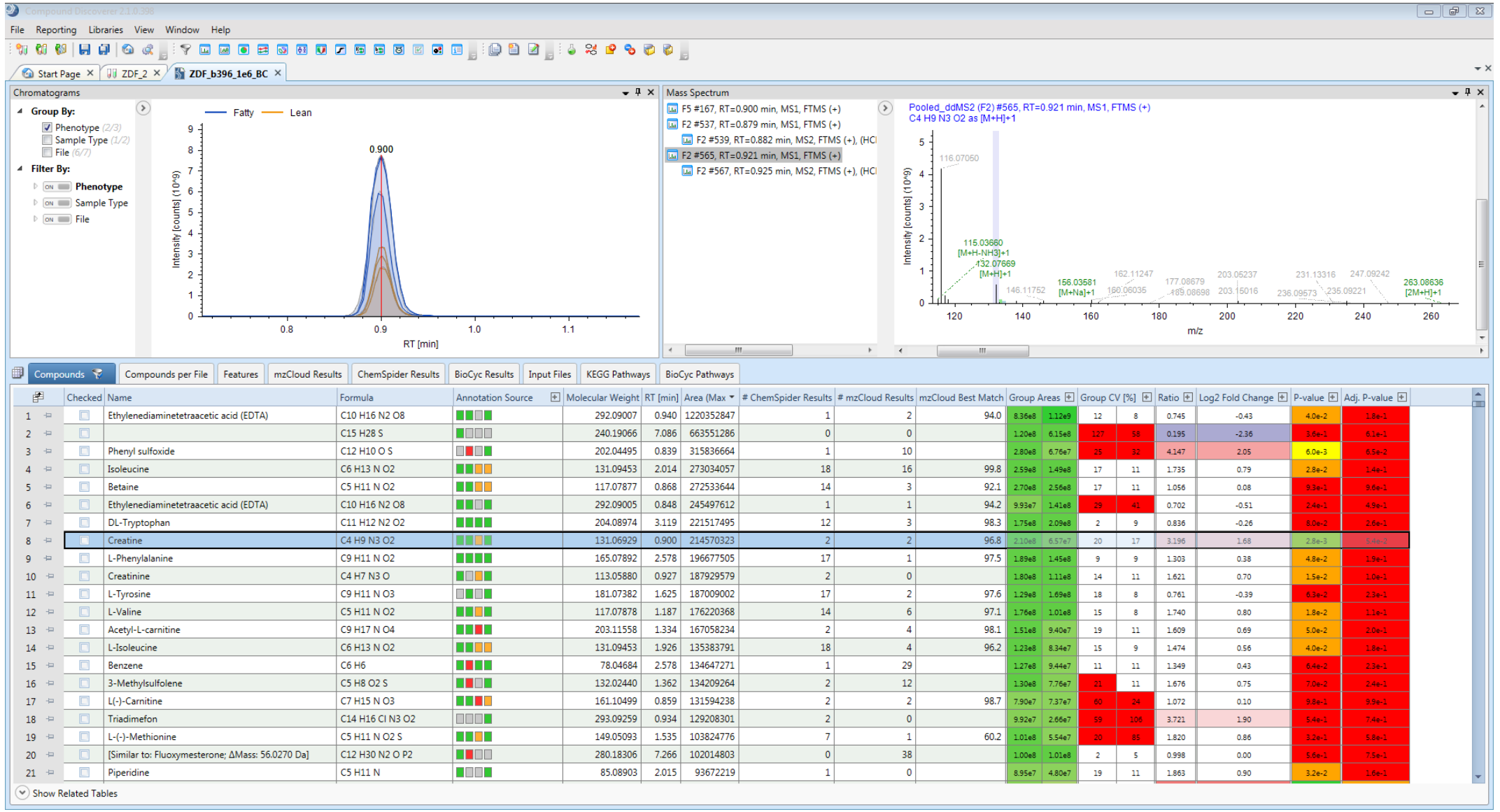
Interpreting the Results



	Checked	Name	Formula	Annotation Source	Molecular Weight	RT [min]	Area (Max	# ChemSpider Results	# mzCloud Results	mzCloud Best Match	Group Areas		Group CV [%]		Ratio	Log2 Fold Change	P-value	Adj. P-value
1	☐	Ethylenediaminetetraacetic acid (EDTA)	C10 H16 N2 O8		292.09007	0.940	1220352847	1	2	94.0	8.36e8	1.12e9	12	8	0.745	-0.43	4.0e-2	1.8e-1
2	☐		C15 H28 S		240.19066	7.086	663551286	0	0		1.20e8	6.15e8	127	58	0.195	-2.36	3.6e-1	6.1e-1
3	☐	Phenyl sulfoxide	C12 H10 O S		202.04495	0.839	315836664	1	10		2.80e8	6.76e7	25	32	4.147	2.05	6.0e-3	6.5e-2
4	☐	Isoleucine	C6 H13 N O2		131.09453	2.014	273034057	18	16	99.8	2.59e8	1.49e8	17	11	1.735	0.79	2.8e-2	1.4e-1
5	☐	Betaine	C5 H11 N O2		117.07877	0.868	272533644	14	3	92.1	2.70e8	2.56e8	17	11	1.056	0.08	9.3e-1	9.6e-1
6	☐	Ethylenediaminetetraacetic acid (EDTA)	C10 H16 N2 O8		292.09005	0.848	245497612	1	1	94.2	9.93e7	1.41e8	29	41	0.702	-0.51	2.4e-1	4.9e-1
7	☐	DL-Tryptophan	C11 H12 N2 O2		204.08974	3.119	221517495	12	3	98.3	1.75e8	2.09e8	2	9	0.836	-0.26	8.0e-2	2.6e-1
8	☐	Creatine	C4 H9 N3 O2		131.06929	0.900	214570323	2	2	96.8	2.10e8	6.57e7	20	17	3.196	1.68	2.8e-3	5.4e-2
9	☐	L-Phenylalanine	C9 H11 N O2		165.07892	2.578	196677505	17	1	97.5	1.89e8	1.45e8	9	9	1.303	0.38	4.8e-2	1.9e-1
10	☐	Creatinine	C4 H7 N3 O		113.05880	0.927	187929579	2	0		1.80e8	1.11e8	14	11	1.621	0.70	1.5e-2	1.0e-1
11	☐	L-Tyrosine	C9 H11 N O3		181.07382	1.625	187009002	17	2	97.6	1.29e8	1.69e8	18	8	0.761	-0.39	6.3e-2	2.3e-1
12	☐	L-Valine	C5 H11 N O2		117.07878	1.187	176220368	14	6	97.1	1.76e8	1.01e8	15	8	1.740	0.80	1.8e-2	1.1e-1
13	☐	Acetyl-L-carnitine	C9 H17 N O4		203.11558	1.334	167058234	2	4	98.1	1.51e8	9.40e7	19	11	1.609	0.69	5.0e-2	2.0e-1
14	☐	L-Isoleucine	C6 H13 N O2		131.09453	1.926	135383791	18	4	96.2	1.23e8	8.34e7	15	9	1.474	0.56	4.0e-2	1.8e-1
15	☐	Benzene	C6 H6		78.04684	2.578	134647271	1	29		1.27e8	9.44e7	11	11	1.349	0.43	6.4e-2	2.3e-1
16	☐	3-Methylsulfolene	C5 H8 O2 S		132.02440	1.362	134209264	2	12		1.30e8	7.76e7	21	11	1.676	0.75	7.0e-2	2.4e-1
17	☐	L(-)-Carnitine	C7 H15 N O3		161.10499	0.859	131594238	2	2	98.7	7.90e7	7.37e7	60	24	1.072	0.10	9.8e-1	9.9e-1
18	☐	Triadimefon	C14 H16 Cl N3 O2		293.09259	0.934	129208301	2	0		9.92e7	2.66e7	59	106	3.721	1.90	5.4e-1	7.4e-1
19	☐	L-(-)-Methionine	C5 H11 N O2 S		149.05093	1.535	103824776	7	1	60.2	1.01e8	5.54e7	20	85	1.820	0.86	3.2e-1	5.8e-1
20	☐	[Similar to: Fluoxymesterone; ΔMass: 56.0270 Da]	C12 H30 N2 O P2		280.18306	7.266	102014803	0	38		1.00e8	1.01e8	2	5	0.998	0.00	5.6e-1	7.5e-1
21	☐	Piperidine	C5 H11 N		85.08903	2.015	93672219	1	0		8.95e7	4.80e7	19	11	1.863	0.90	3.2e-2	1.6e-1

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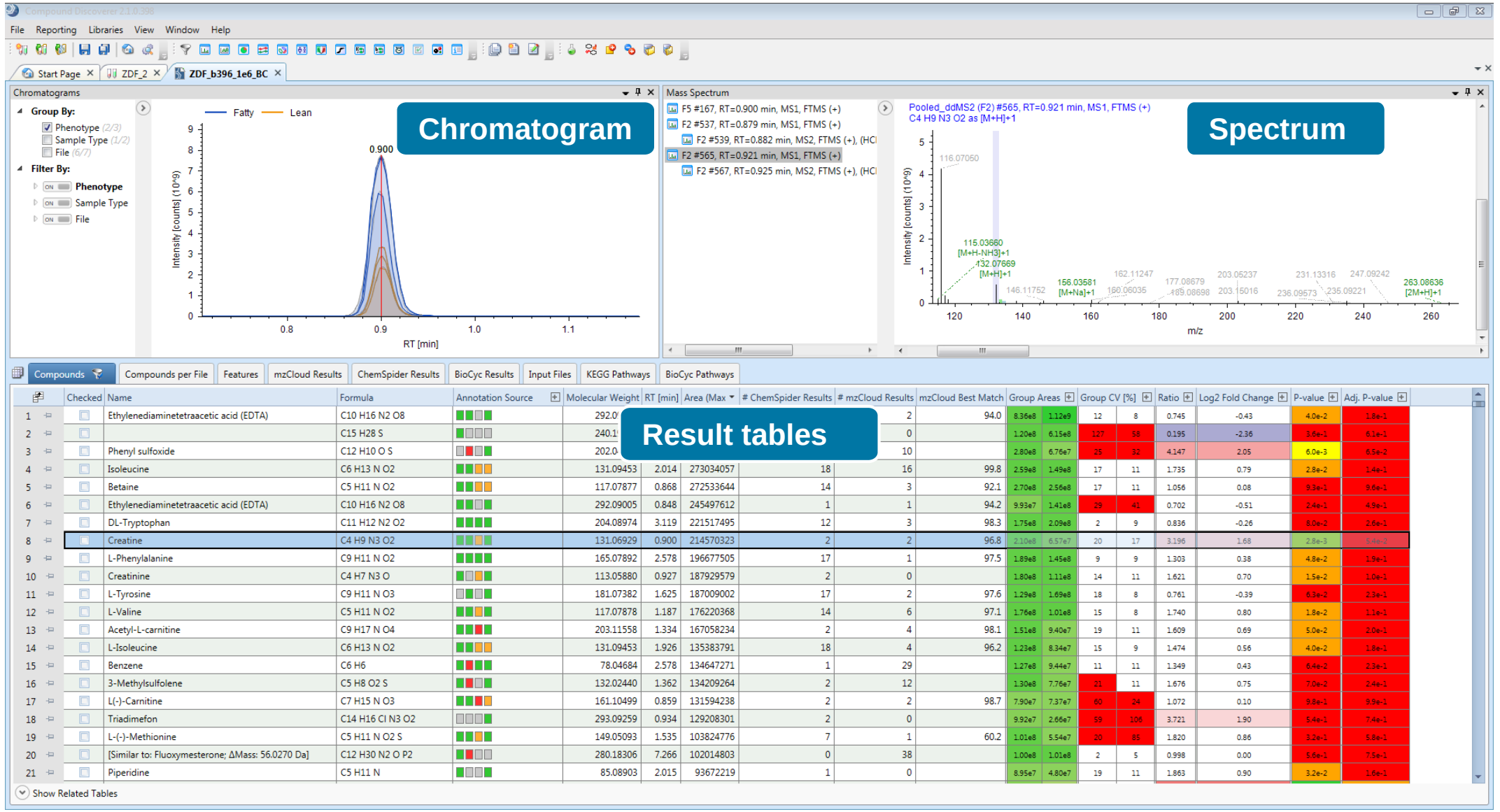
Interpreting the Results



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2			C15 H28 S		240.1				0		1.20e8	6.15e8	127	58	0.195	-2.36	3.6e-1	6.1e-1
3		Phenyl sulfoxide	C12 H10 O S		202.0				10		2.80e8	6.76e7	25	32	4.147	2.05	6.0e-3	6.5e-2
4		Isoleucine	C6 H13 N O2		131.09453	2.014	273034057	18	16	99.8	2.59e8	1.49e8	17	11	1.735	0.79	2.8e-2	1.4e-1
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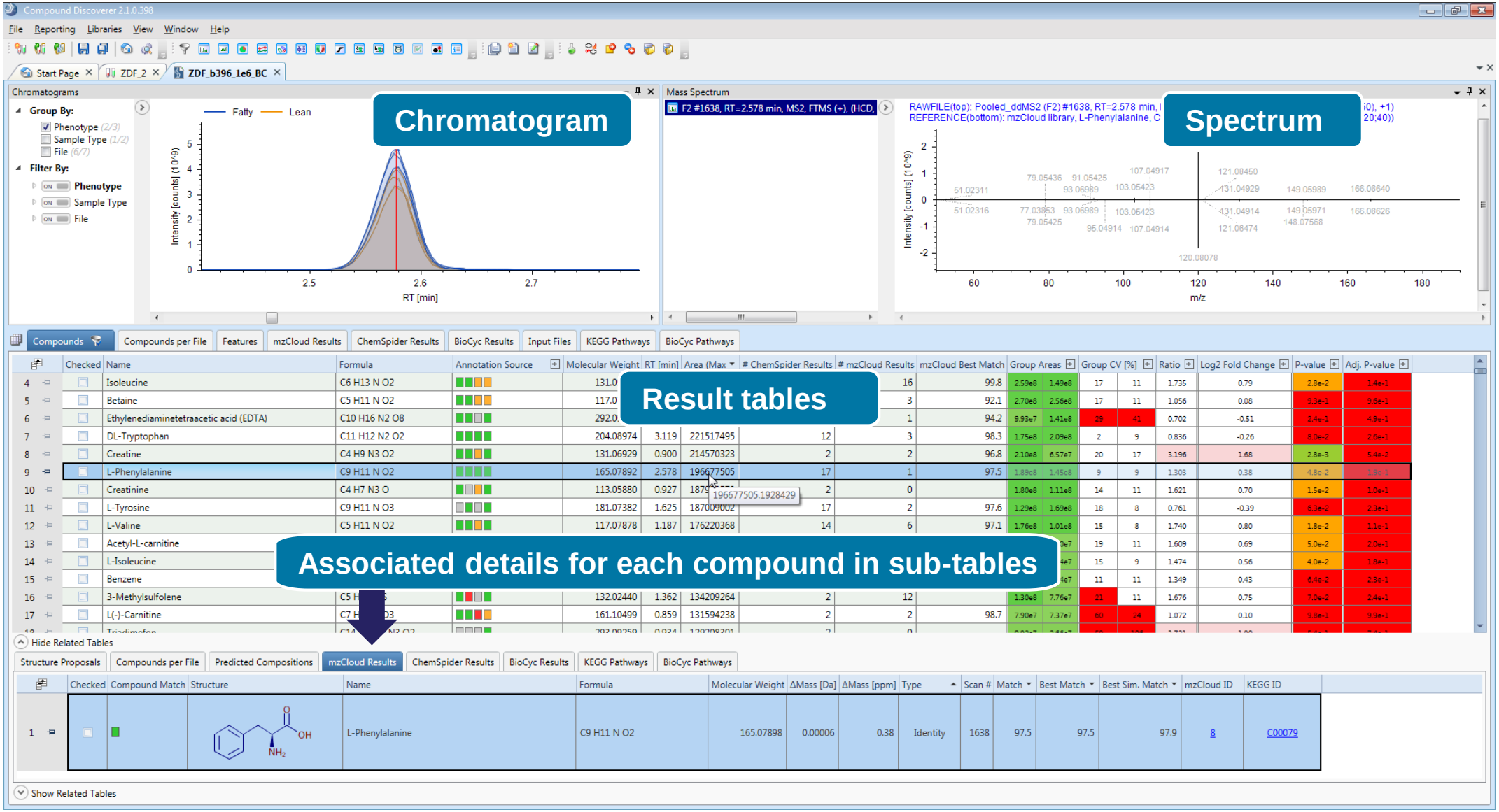
Interpreting the Results



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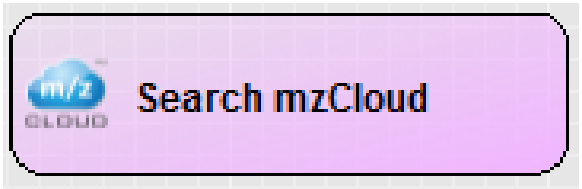
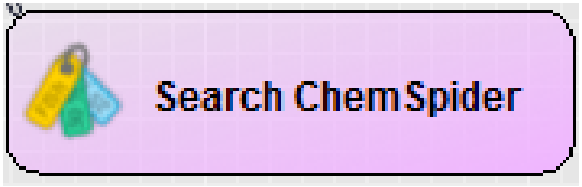
Identifying Unknowns – Spectral Libraries and Compound Databases



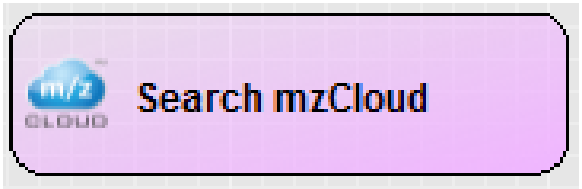
Online spectral library

> 2 million spectra



Chemical structure database with >480 data sources, 59 million structures

Identifying Unknowns – Spectral Libraries and Compound Databases



Online spectral library
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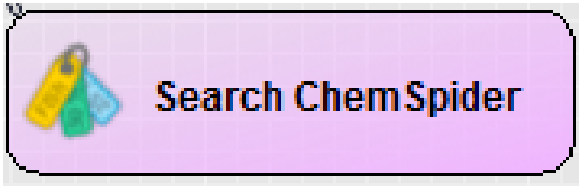
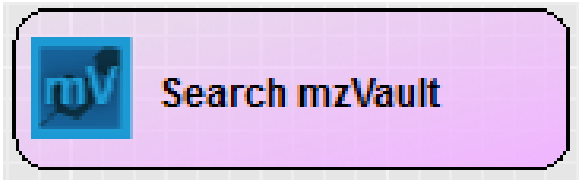
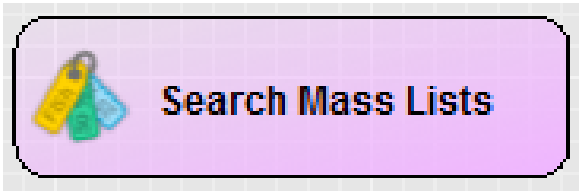


Chemical structure database with >480 data sources, 59 million structures

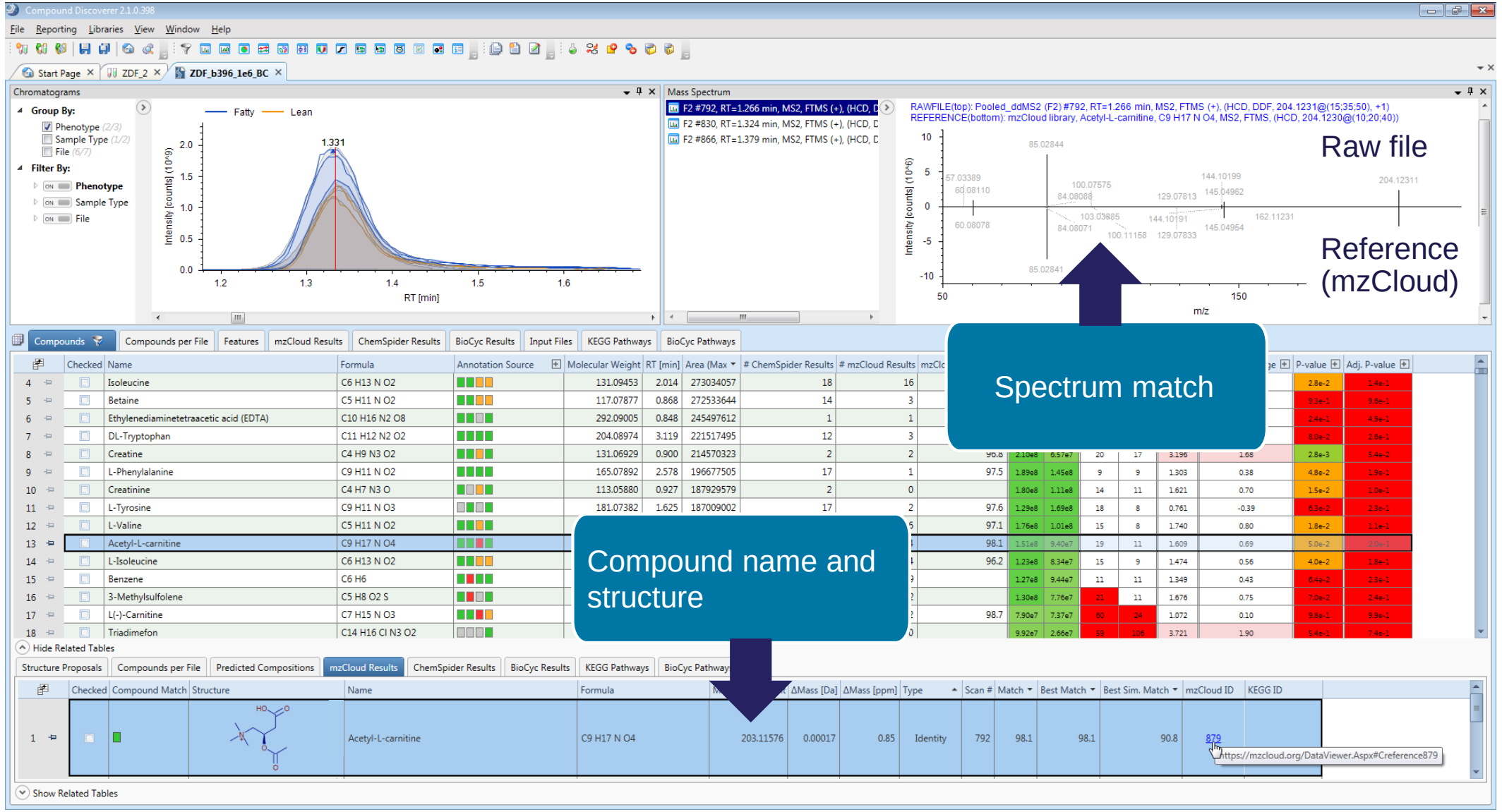


Local spectral libraries



Local databases

Identifying Unknowns: mzCloud



mzCloud is a trademark of HighChem LLC.

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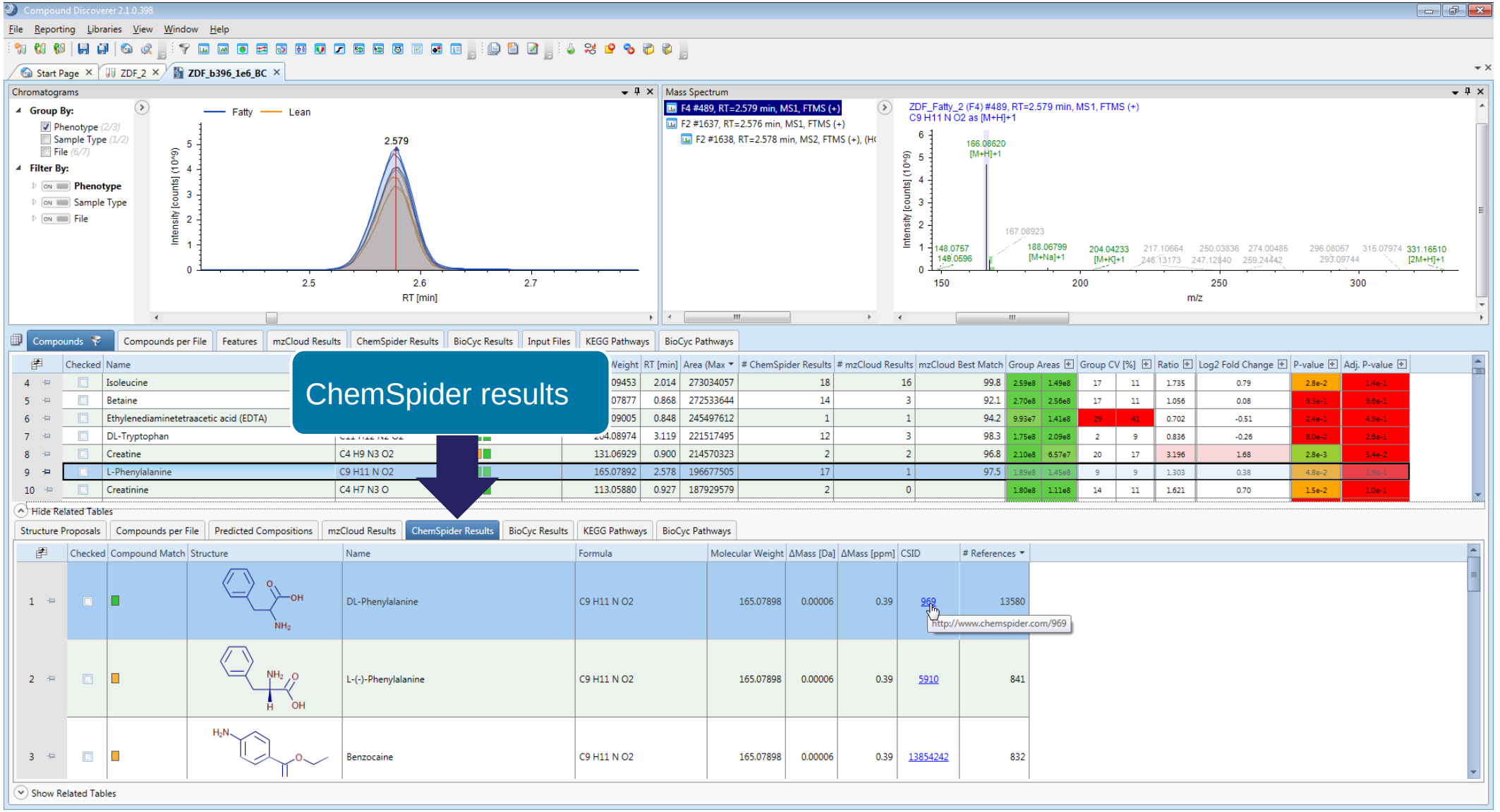
Identifying Unknowns: ChemSpider



ChemSpider is a trademark of Royal Society of Chemistry.

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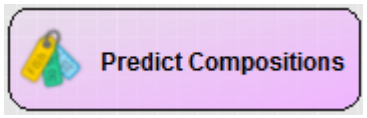
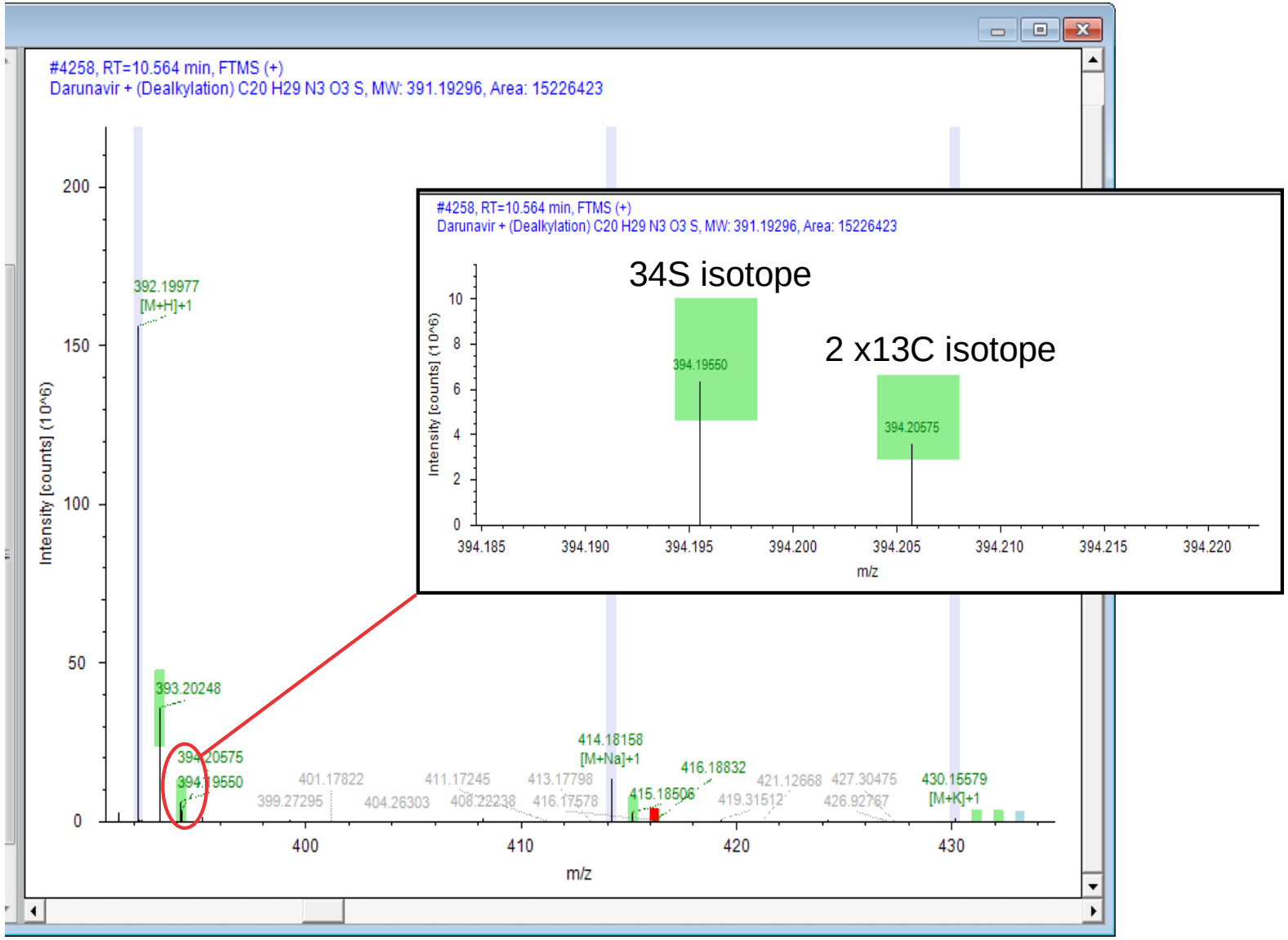
Identifying Unknowns: Predict Elemental Composition Using Very High Resolution Data



TrueComposition™ algorithm uses

- Exact mass
- Isotopic pattern
- *Fine* isotopic pattern
- MS^2 data



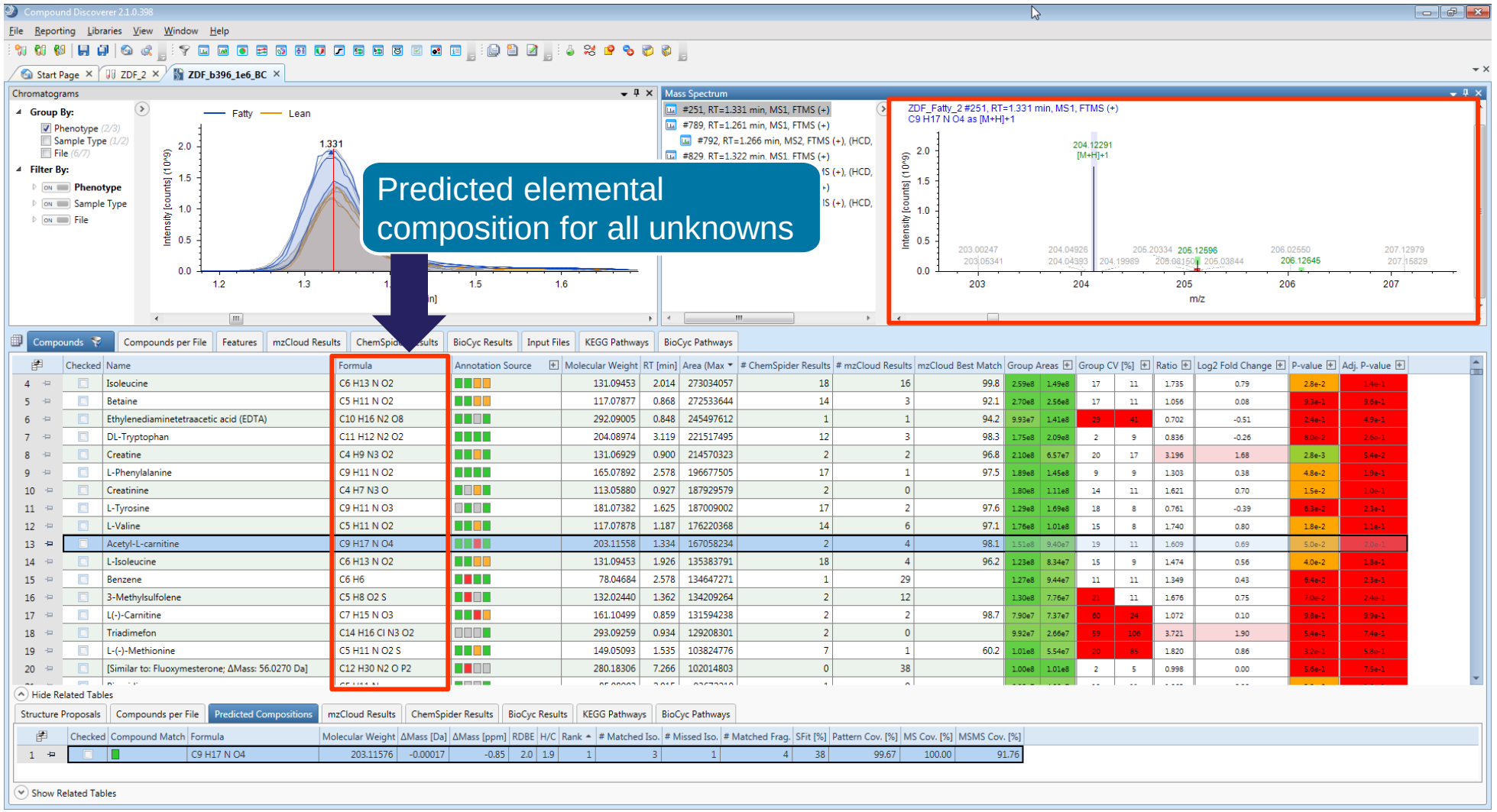
ThermoFisher SCIENTIFIC

Identifying Unknowns: Predict Elemental Composition Using Very High Resolution Data

Predict Compositions

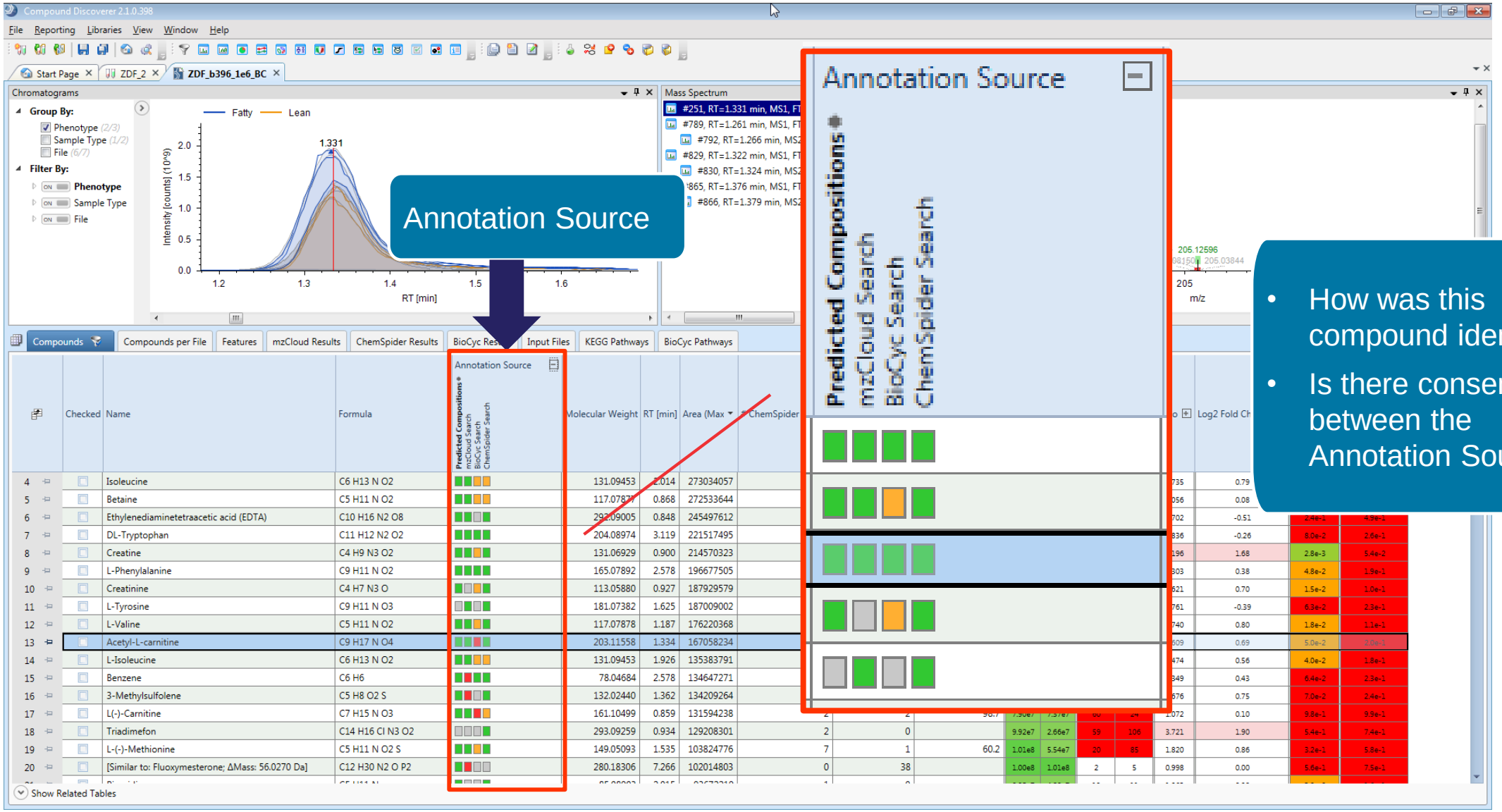
TrueComposition™ algorithm uses

- Exact mass
- Isotopic pattern
- *Fine* isotopic pattern
- MS^2 data



ThermoFisher SCIENTIFIC

Identifying Unknowns: How to Interpret the Results from Multiple Sources?



- How was this compound identified ?
- Is there consensus between the Annotation Sources ?

ThermoFisher SCIENTIFIC

Identifying Unknowns: How to Interpret the Results from Multiple Sources?

Assign compound name and formula based on multiple data sources

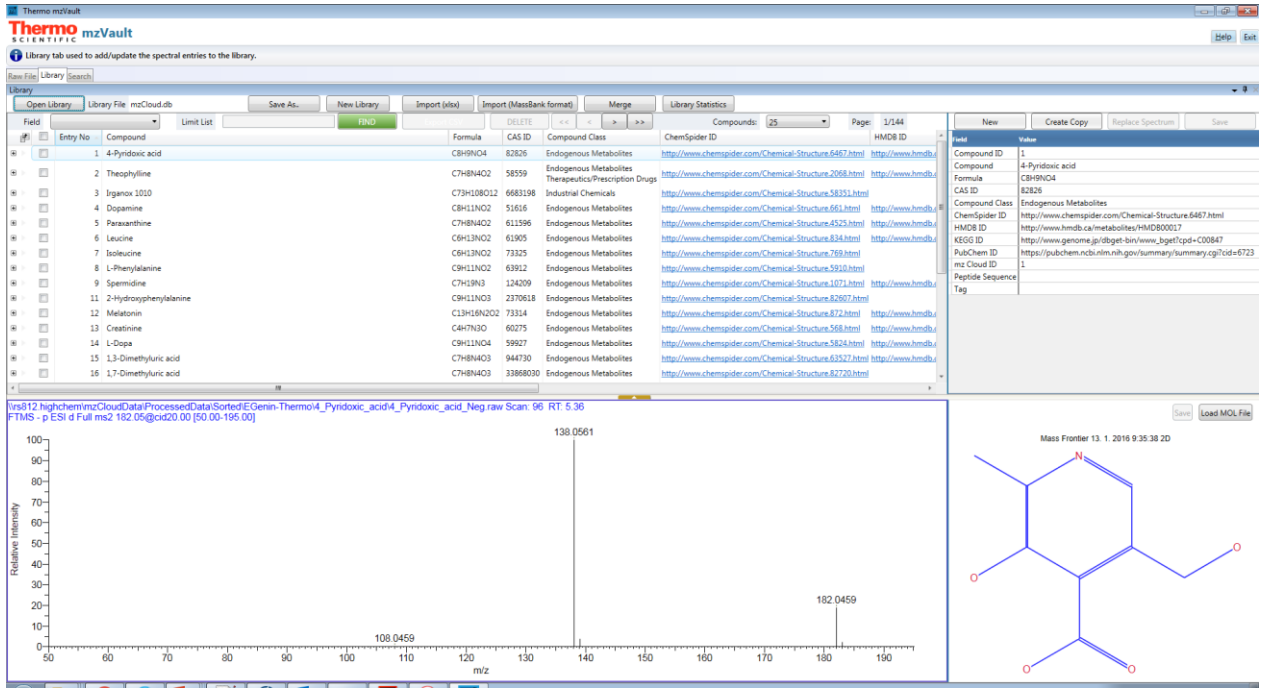
1. General Settings	
Mass Tolerance	5 ppm
2. Data Sources	
Data Source #1	mzCloud Search
Data Source #2	Predicted Compositions
Data Source #3	MassList Match
Data Source #4	ChemSpider Search

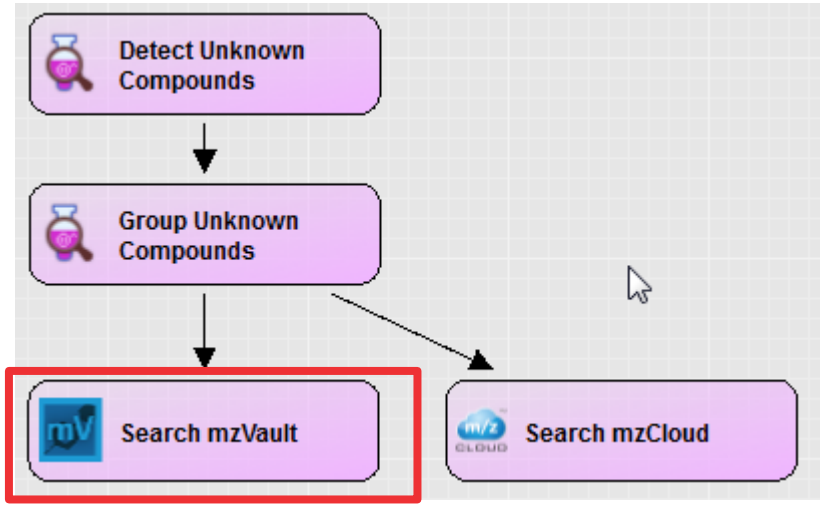
Users can define priorities

	Checked	Name	Formula	Annotation Source (Predicted Compositions, mzCloud Search, BioCyc Search, ChemSpider Search)
2		L(-)-Carnitine	C7 H15 N O3	
3		Acetyl-L-carnitine	C9 H17 N O4	
4		DL-Tryptophan	C11 H12 N2 O2	
5		L-Tyrosine	C9 H11 N O3	
6		DL-Glutamine	C5 H10 N2 O3	
7		L-Phenylalanine	C9 H11 N O2	
8		L-Valine	C5 H11 N O2	
9		Creatine	C4 H9 N3 O2	
10		L-Histidine	C6 H9 N3 O2	
			C10 H19 N O4	
			C6 H13 N O2	
		nate	C10 H14 N5 O7 P	
14		DL-Arginine	C6 H14 N4 O2	
15		PEG n6	C12 H26 O7	
16		Hexanoylcarnitine	C13 H25 N O4	
17		Ethylenediaminetetraacetic acid (EDTA)	C10 H16 N2 O8	

Identifying Unknowns: Local Spectral Libraries (mzVault)

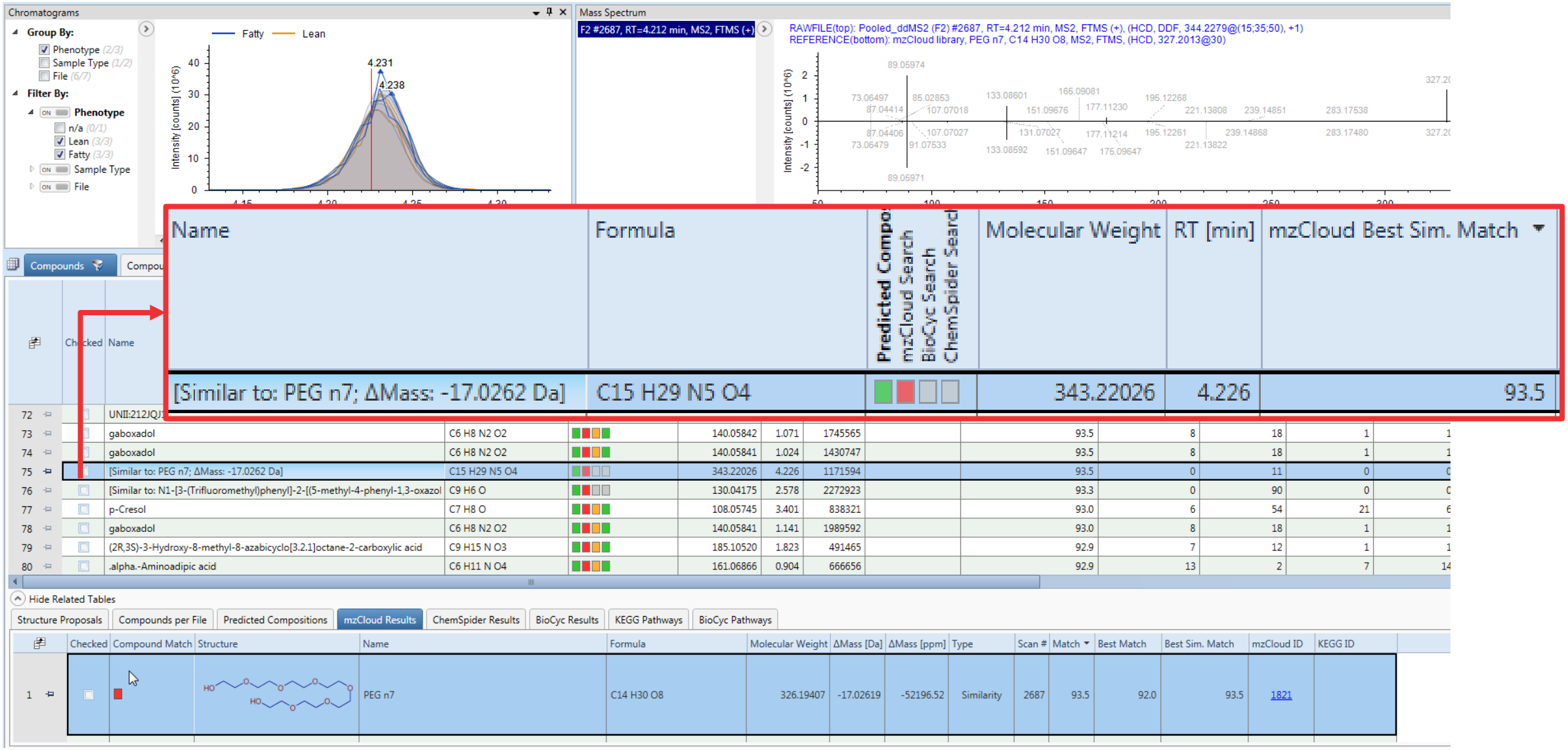
- **mzVault** – support for local spectral libraries
  - Compound Discoverer 2.1 comes with a local version of the mzCloud™ database
  - Custom spectral libraries can created and edited using mzVault application





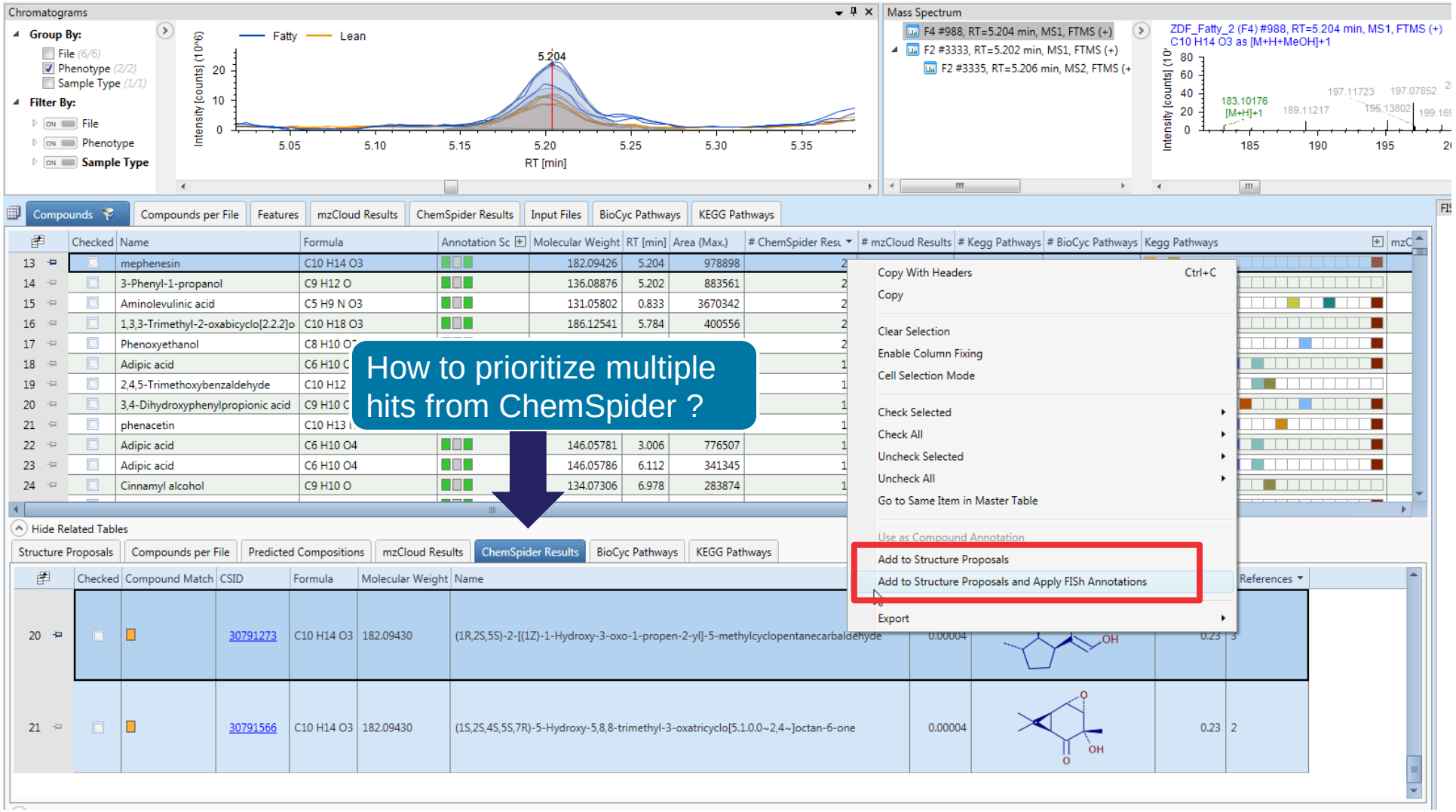
ThermoFisher SCIENTIFIC

Identifying Unknowns: Similarity Search Using mzCloud



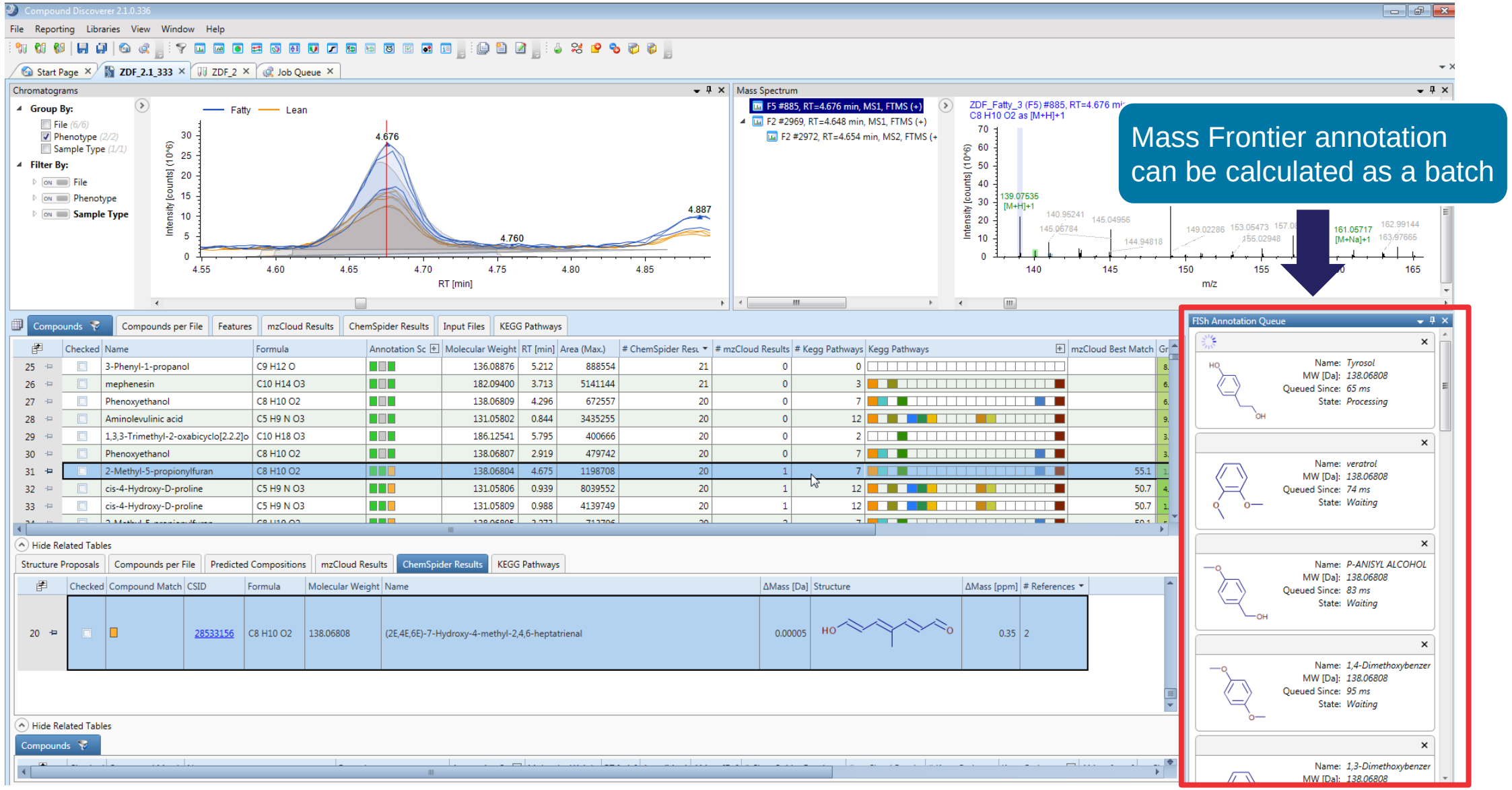
ThermoFisher SCIENTIFIC

Mass Frontier Annotation (FISh) for Structure Proposals



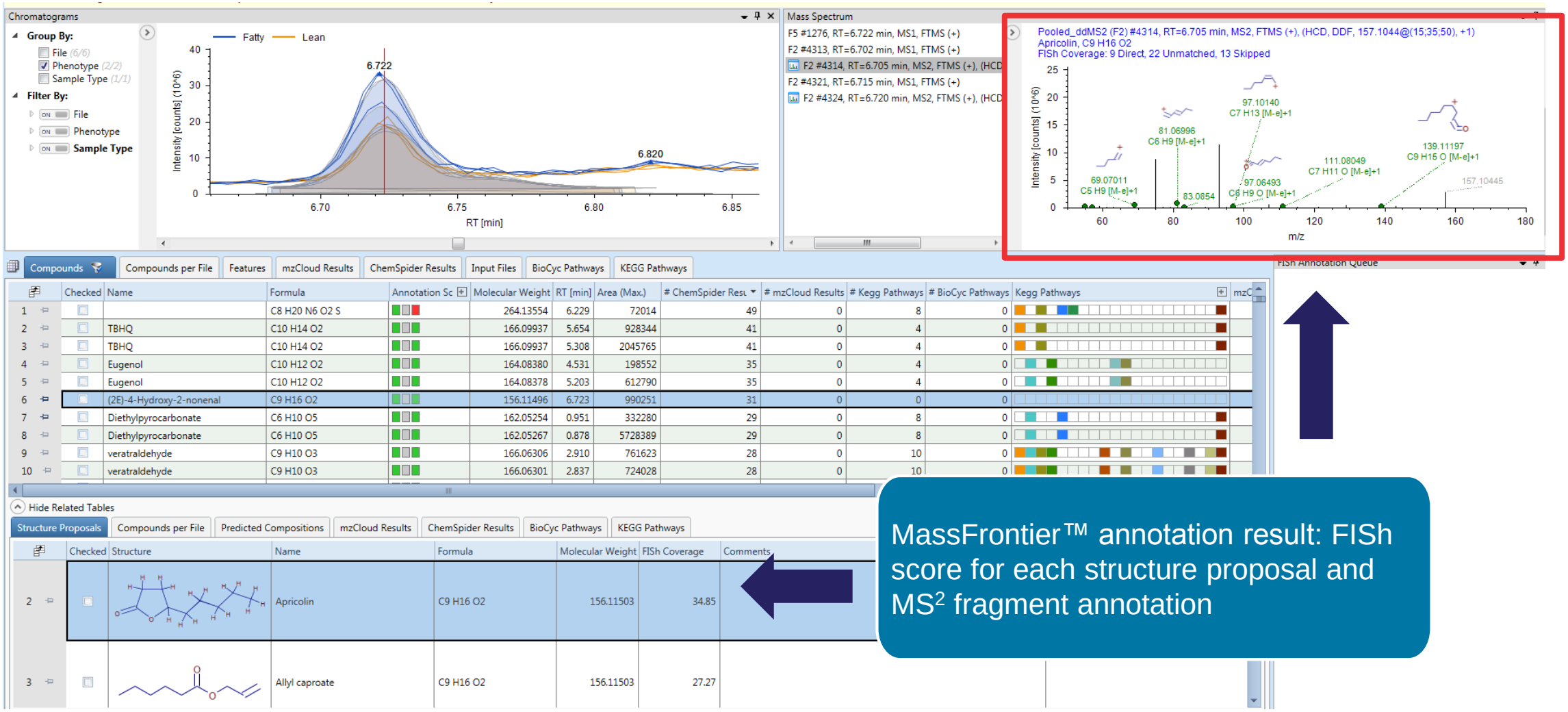
ThermoFisher SCIENTIFIC

Mass Frontier Annotation (FISh) for Structure Proposals



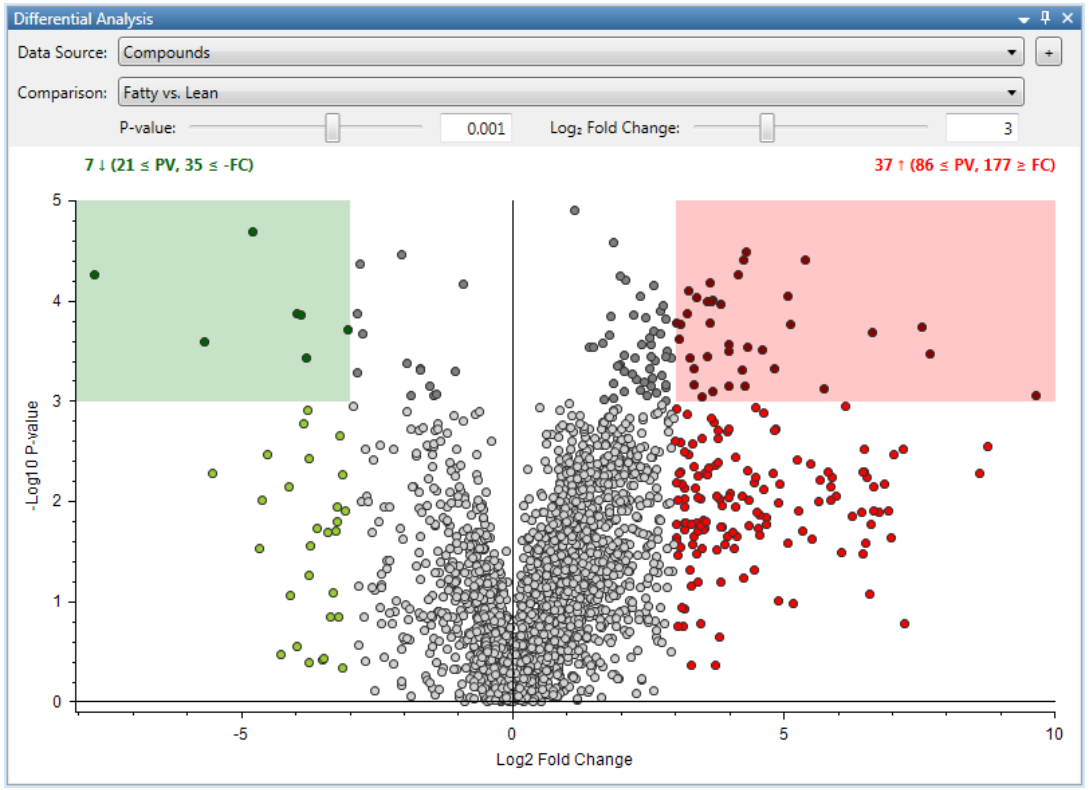
ThermoFisher SCIENTIFIC

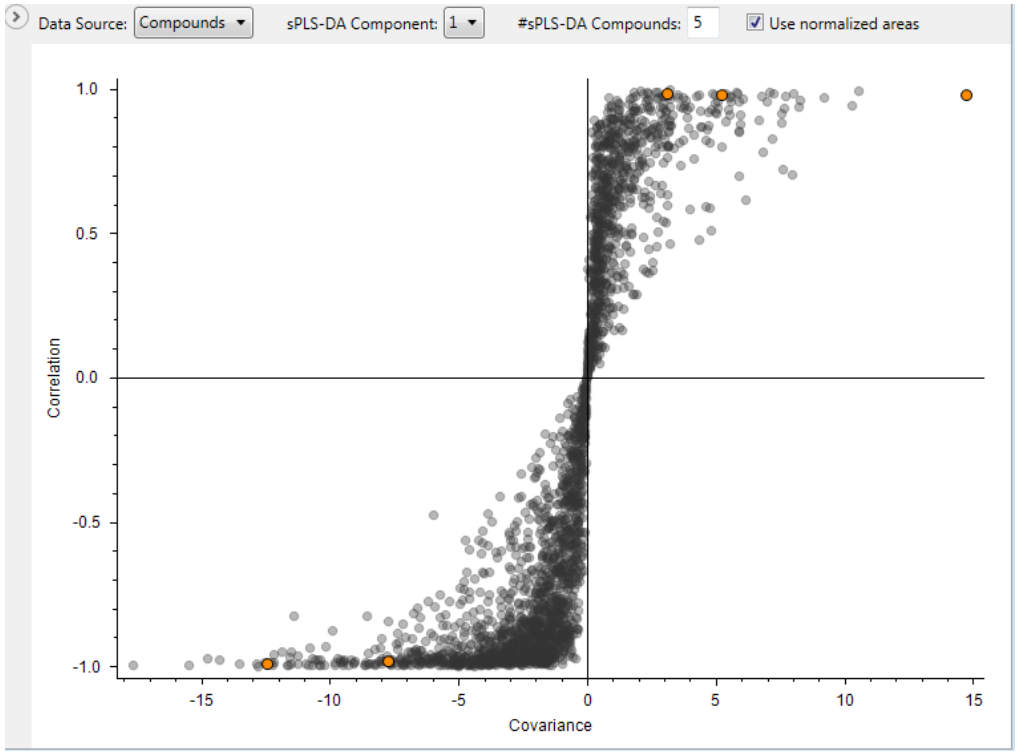
Mass Frontier Annotation (FISh) for Structure Proposals



ThermoFisher SCIENTIFIC

Compound Discoverer Software: Statistics

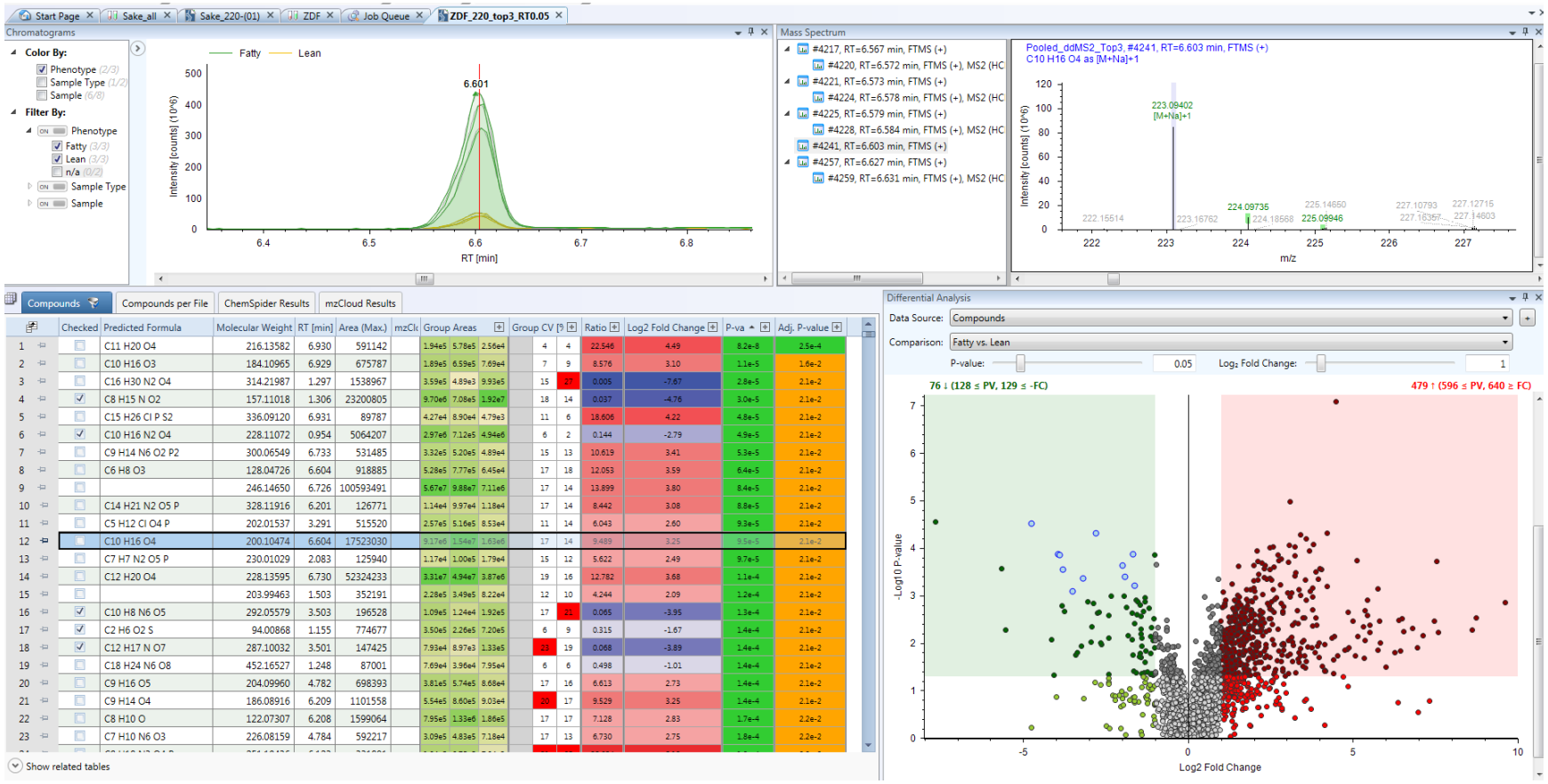




Differential Analysis, volcano plots, ANOVA, PCA, PLS-DA …

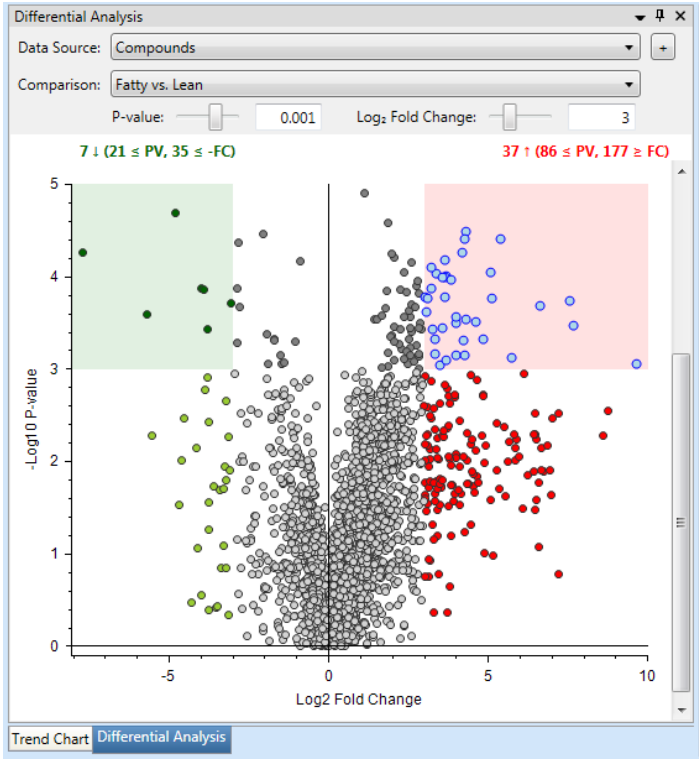
ThermoFisher SCIENTIFIC

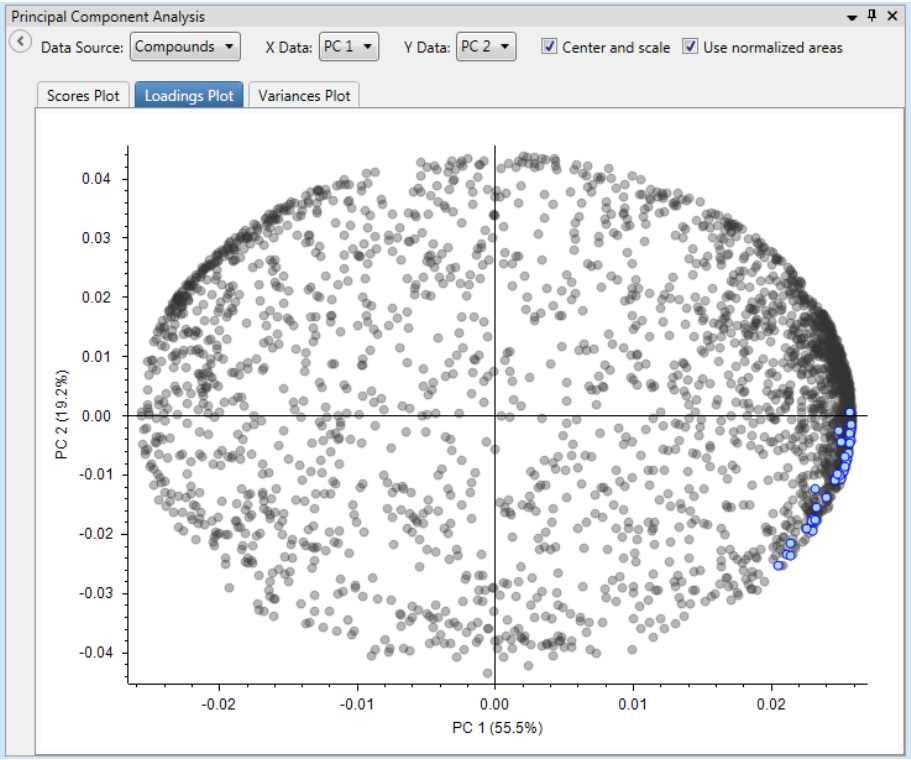
Compound Discoverer Software: Statistics

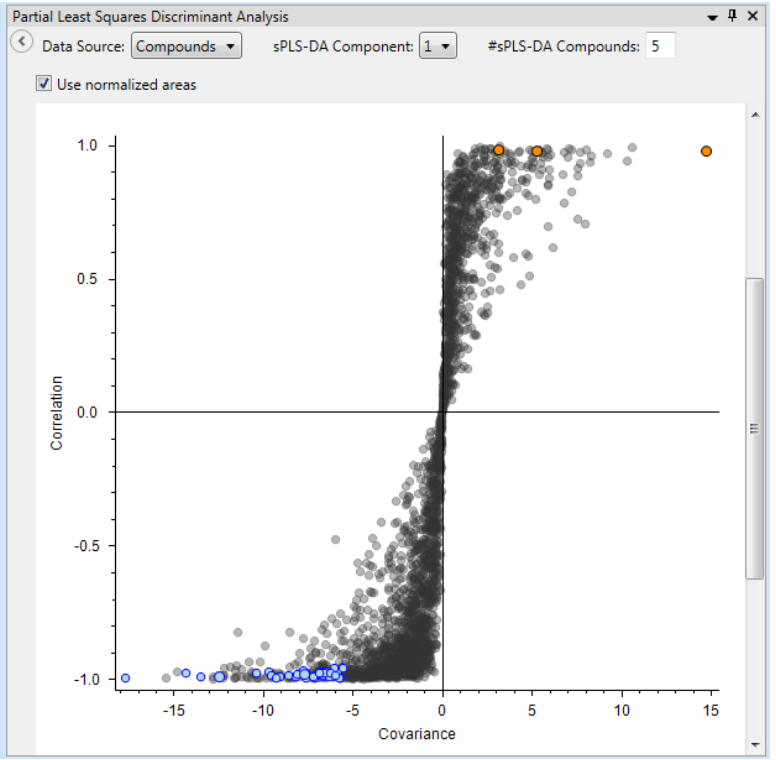


- Volcano plots, PCA, PLS-DA ,… are interactive
- Results are always directly linked to raw data.

Compound Discoverer Software: Statistics





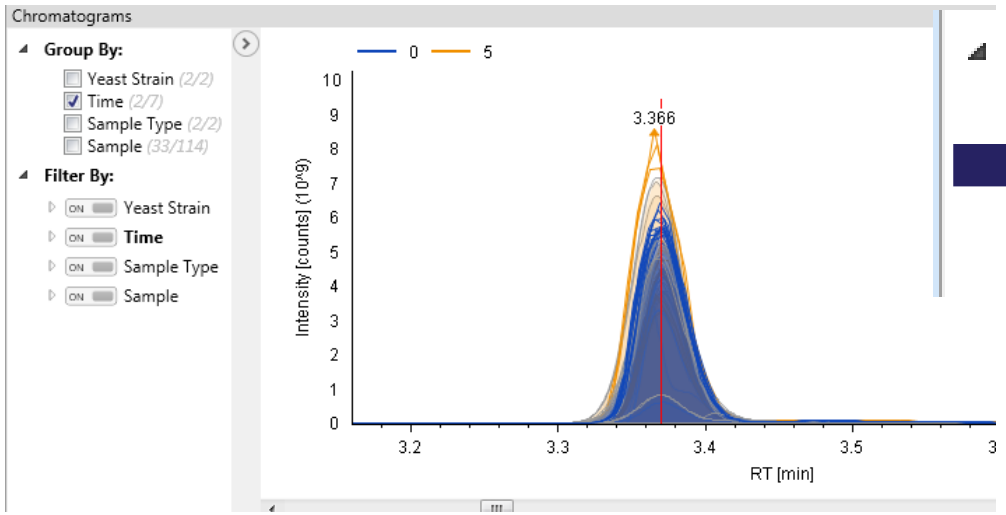


Checked	Name	Formula	Annotati	Molecular Weight	RT [min]
☐	DL-Tryptophan	C11 H12 N2 O2		204.08974	3.119
☑	Creatine	C4 H9 N3 O2		131.06929	0.900
☑	L-Phenylalanine	C9 H11 N O2		165.07892	2.578
☑	Creatinine	C4 H7 N3 O		113.05880	0.927
☐	L-Tyrosine	C9 H11 N O3		181.07382	1.625

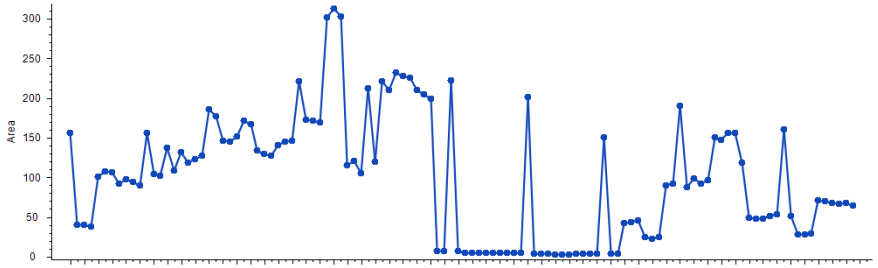
- Use tracking feature to navigate through complex datasets
- Interpret results from univariate and multivariate analysis

ThermoFisher SCIENTIFIC

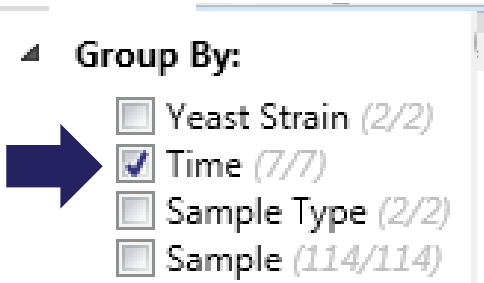
Utilize Study Factors for Statistics and Interactive Visualizations



Tryptophan



Relative abundance in 114 samples
(7 time points, 2 yeast strains x biol. replicates)

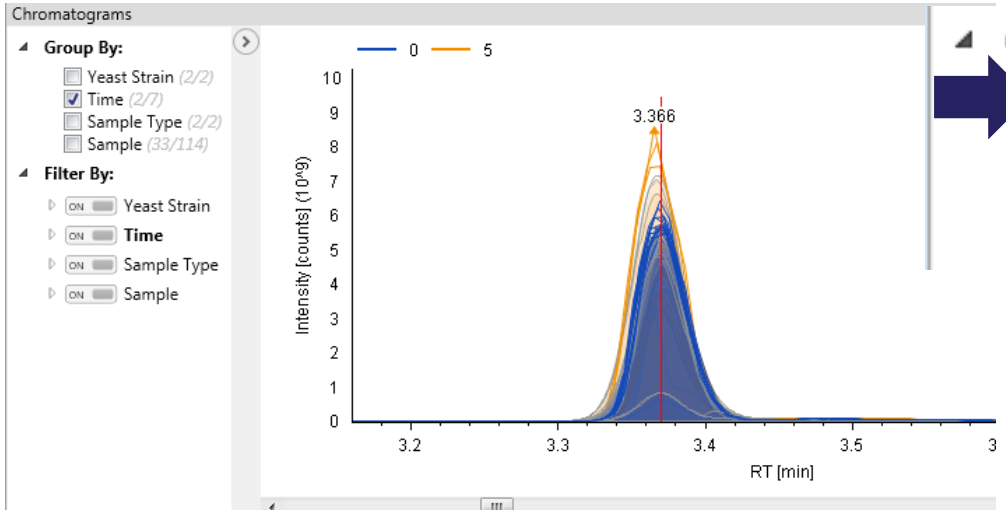


2 yeast strains

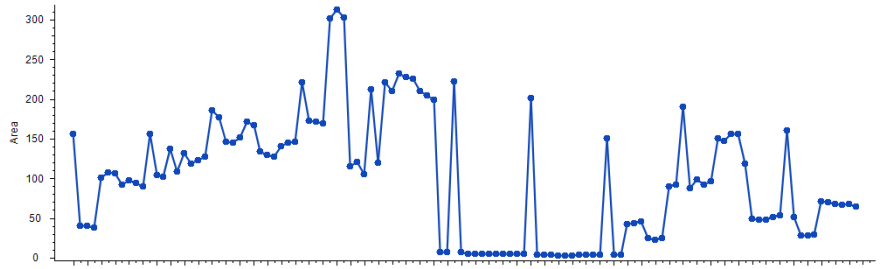
7 time points

Plot as box-whisker chart grouped by 7 time points …

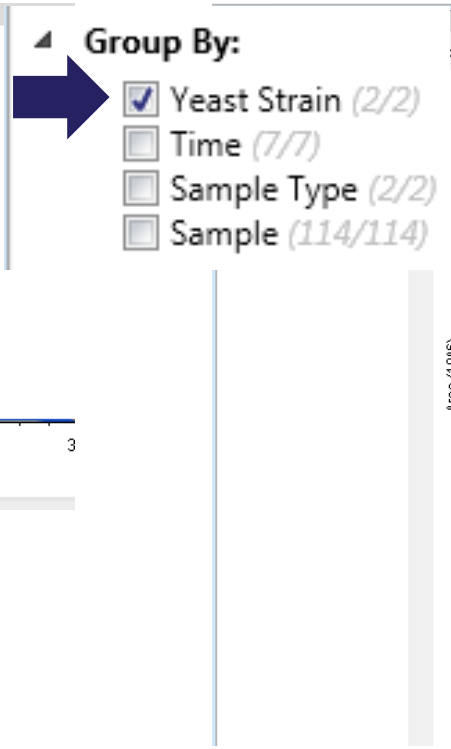
Utilize Study Factors for Statistics and Interactive Visualizations

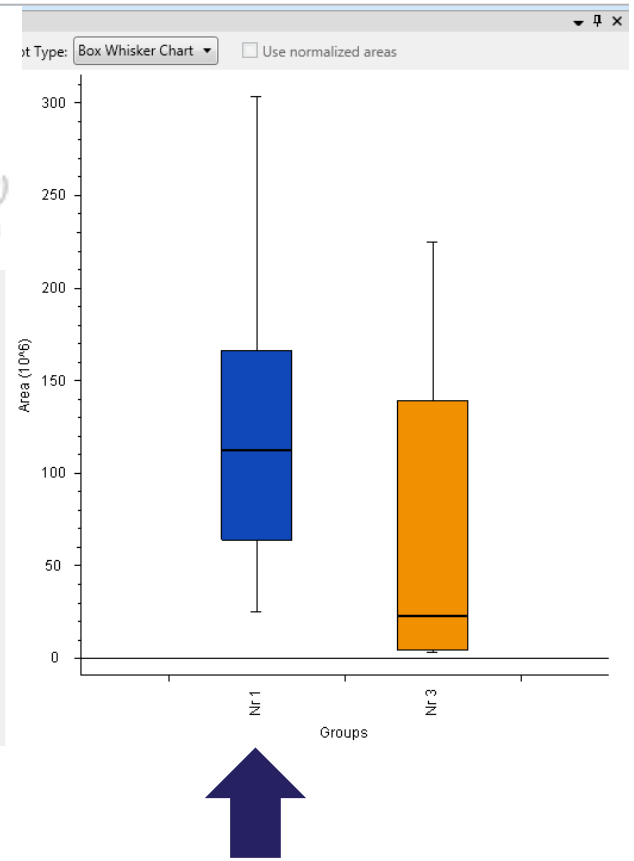


Tryptophan



Relative abundance in 114 samples
(7 time points, 2 yeast strains x biol. replicates)



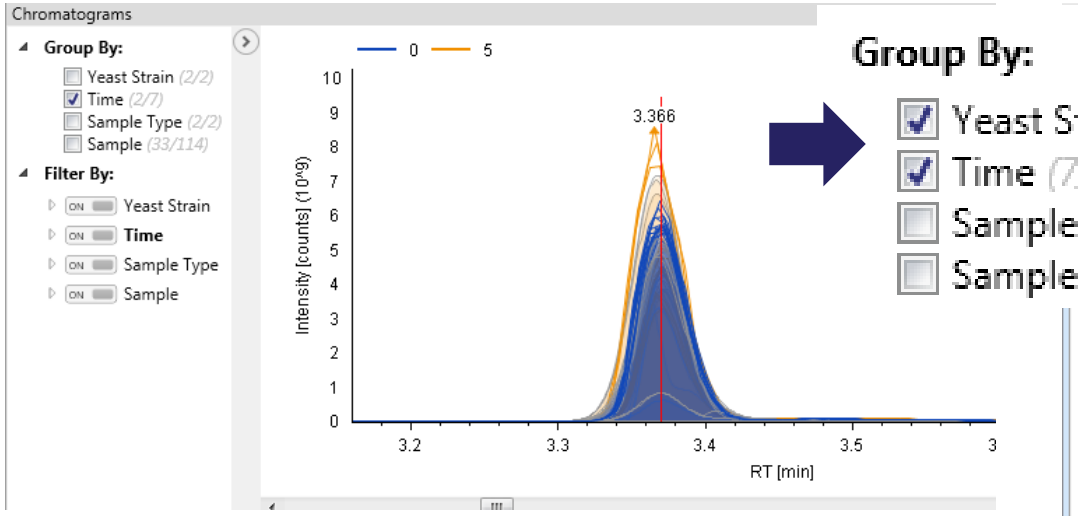


… or grouped by the two yeast strains

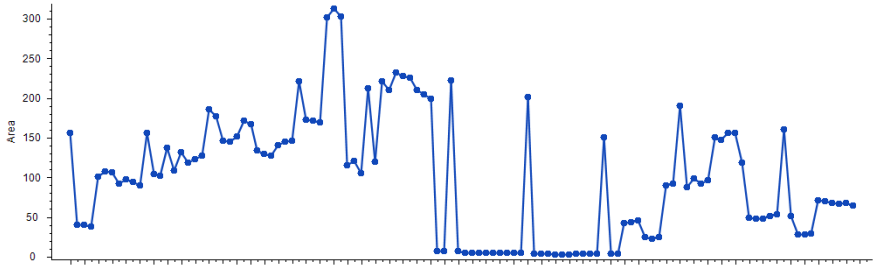
2 yeast strains

7 time points

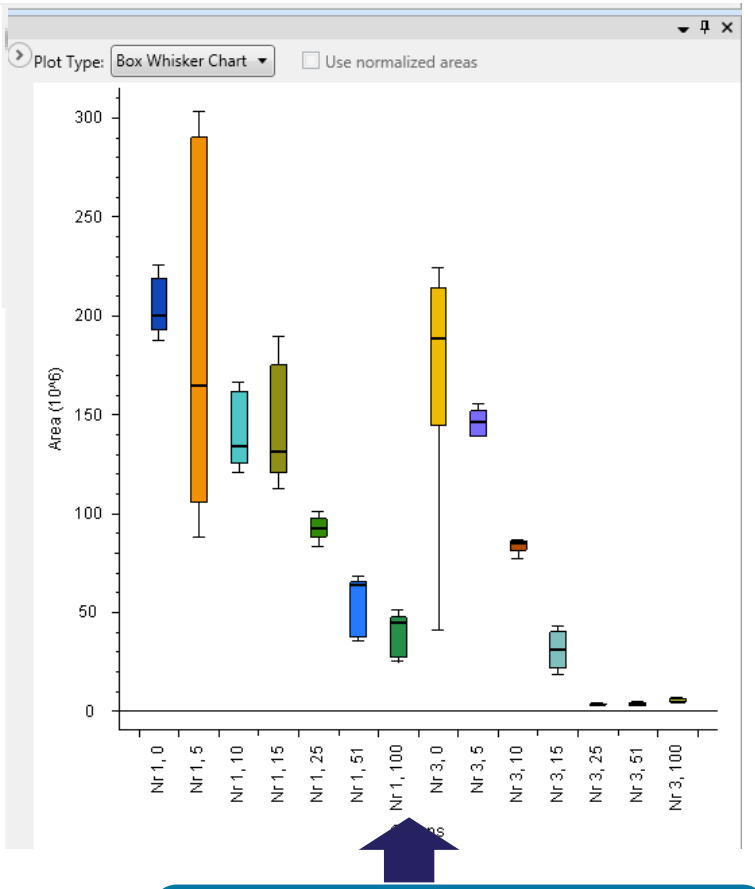
Utilize Study Factors for Statistics and Interactive Visualizations



Tryptophan



Relative abundance in 114 samples
(7 time points, 2 yeast strains x biol. replicates)



… or both at the same time

2 yeast strains

7 time points

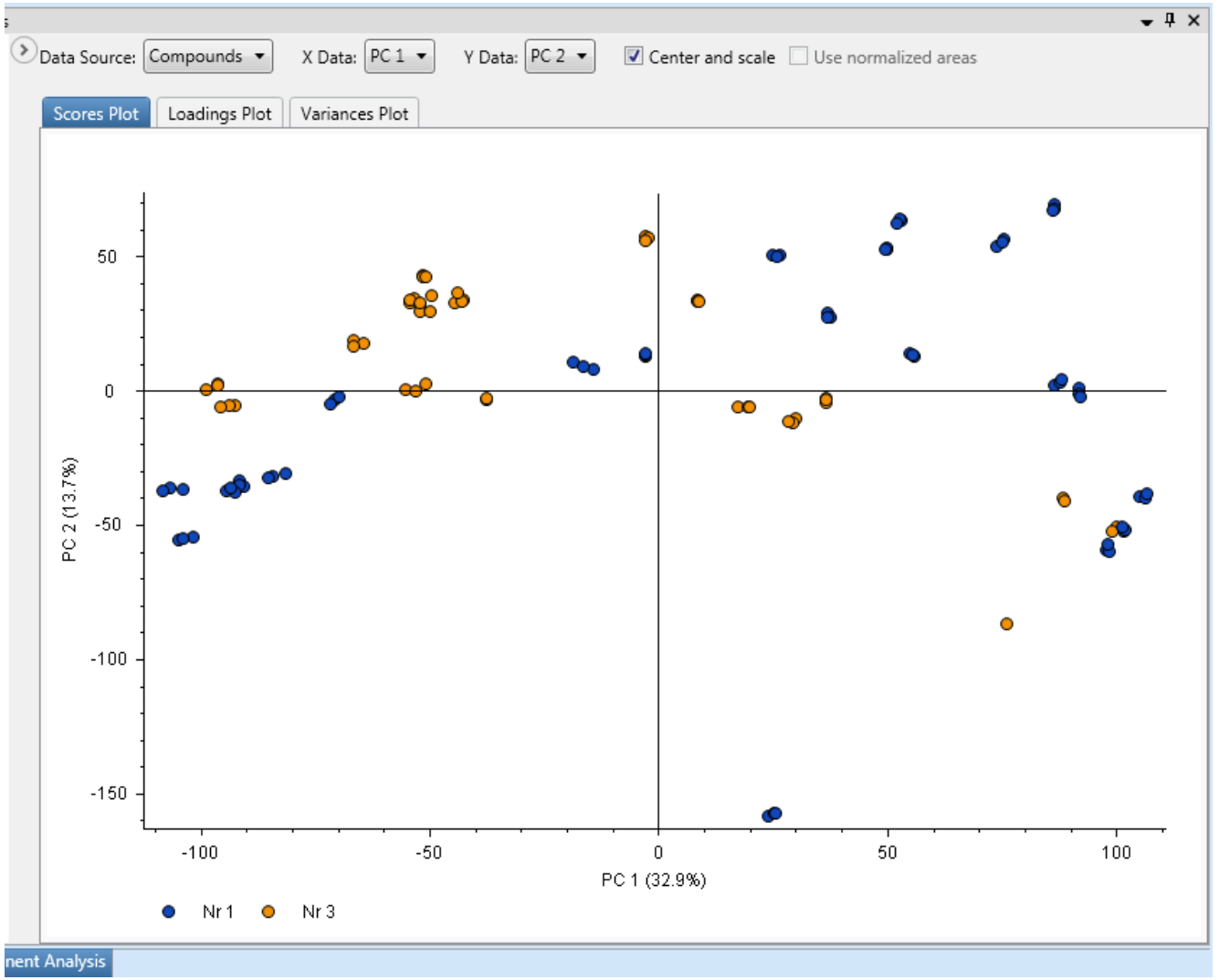
ThermoFisher SCIENTIFIC

Utilize Study Factors for Statistics and Interactive Visualizations - PCA

Color By:

- [x] Yeast Strain *(2/2)*
- [ ] Time *(7/7)*
- [ ] Sample Type *(2/2)*
- [ ] Sample *(114/114)*

PCA scores plot – samples colored using the two yeast strains …



2 yeast strains

7 time points

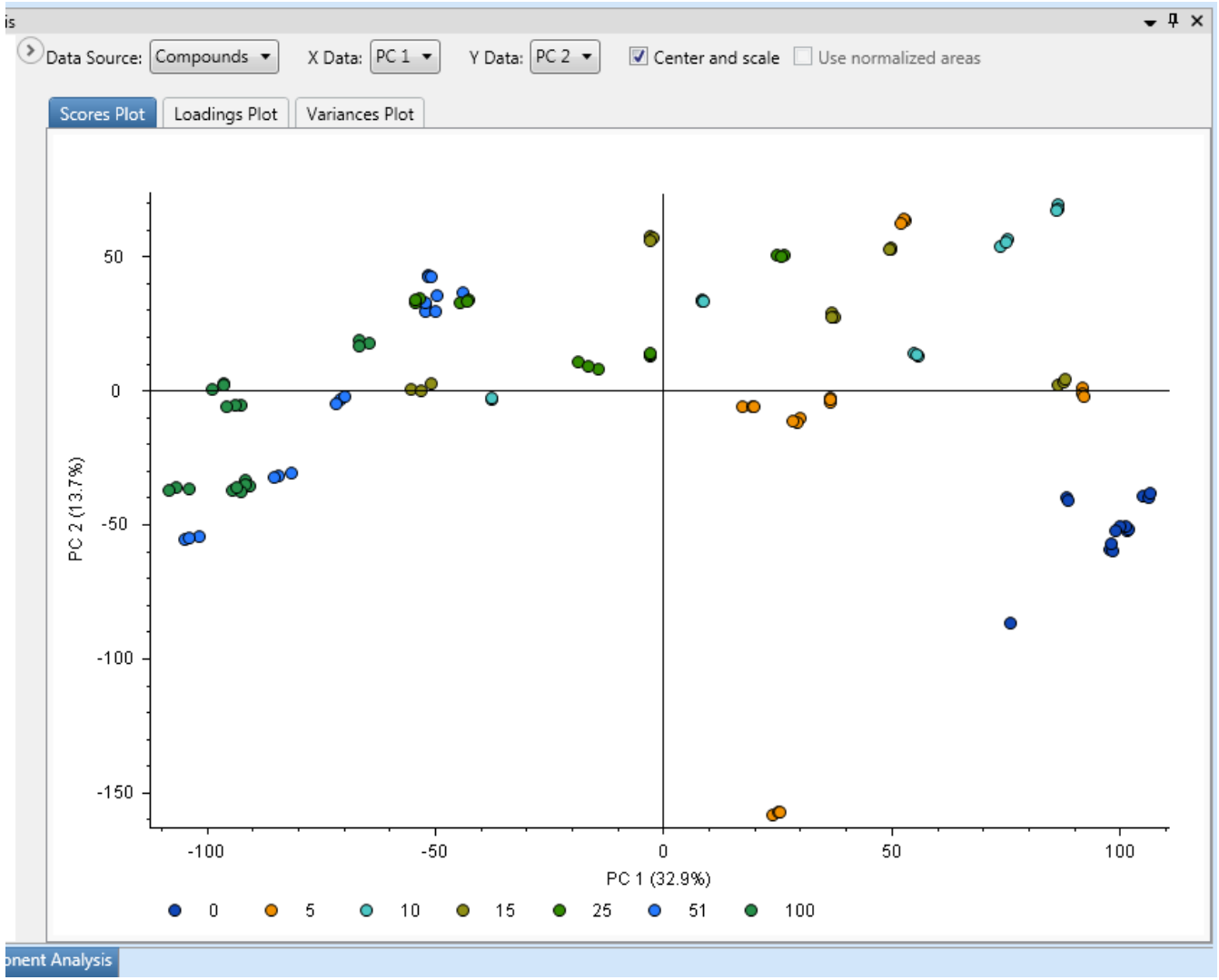
ThermoFisher SCIENTIFIC

Utilize Study Factors for Statistics and Interactive Visualizations - PCA

Color By:

- [ ] Yeast Strain *(2/2)*
- [x] Time *(7/7)*
- [ ] Sample Type *(2/2)*
- [ ] Sample *(114/114)*

PCA scores plot – samples colored using the 7 time points …



2 yeast strains

7 time points

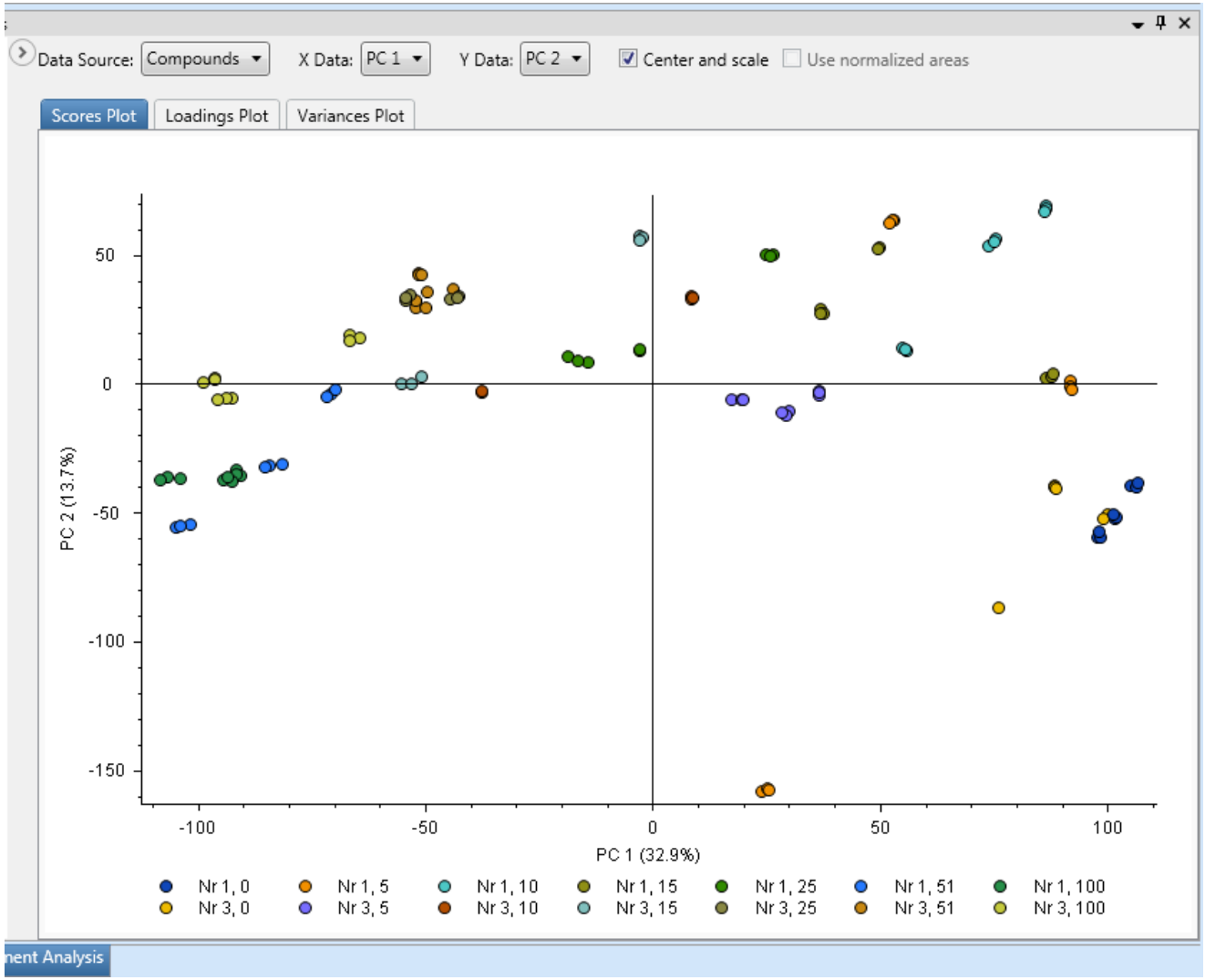
ThermoFisher SCIENTIFIC

Utilize Study Factors for Statistics and Interactive Visualizations - PCA

Color By:

- [x] Yeast Strain *(2/2)*
- [x] Time *(7/7)*
- [ ] Sample Type *(2/2)*
- [ ] Sample *(114/114)*

PCA scores plot – samples colored using both yeast strain and time point information



2 yeast strains

7 time points

ThermoFisher SCIENTIFIC

Compound Discoverer Software – QC-based Normalization

PROTOCOL

Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry

Warwick B Dunn[1–3], David Broadhurst[2,4], Paul Begley[2], Eva Zelena[2], Sue Francis-McIntyre[2], Nadine Anderson[2], Marie Brown[2], Joshau D Knowles[5], Antony Halsall[2], John N Haselden[6], Andrew W Nicholls[6], Ian D Wilson[7], Douglas B Kell[2], Royston Goodacre[1,2] & The Human Serum Metabolome (HUSERMET) Consortium

- Large experiments with hundreds of samples often require data acquisition in multiple batches
- QC metrics and filtering using pooled samples as QCs
- Correct “batch-effects” – independently for each compound

Compound Discoverer 2.1 Software – QC-based Normalization

- QC-based normalization
  - State of the art method for normalization (correction of batch-effects)
  - Based on pooled samples used as QCs
  - Correction applied independently for each (unknown) compound
  - Also filters compounds based on QC criteria

PROTOCOL

Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry

Warwick B Dunn[1–3], David Broadhurst[2,4], Paul Begley[2], Eva Zelena[2], Sue Francis-McIntyre[2], Nadine Anderson[2], Marie Brown[2], Joshau D Knowles[5], Antony Halsall[2], John N Haselden[6], Andrew W Nicholls[6], Ian D Wilson[7], Douglas B Kell[2], Royston Goodacre[1,2] & The Human Serum Metabolome (HUSERMET) Consortium

[1]Manchester Centre for Integrative Systems Biology, Manchester Interdisciplinary Biocentre, University of Manchester, Manchester, UK. [2]School of Chemistry, Manchester Interdisciplinary Biocentre, University of Manchester, Manchester, UK. [3]Centre for Advanced Discoveries and Experimental Therapeutics, Manchester Biomedical Research Centre and School of Biomedicine, Manchester, UK. [4]Department of Medicine, Katz Group Centre for Pharmacy & Health, University of Alberta, Edmonton, Alberta, Canada. [5]School of Computer Science, The University of Manchester, Manchester, UK. [6]Department of Investigative Preclinical Toxicology, GlaxoSmithKline, Hertfordshire, UK. [7]Department of Clinical Pharmacology, Drug Metabolism and Pharmacokinetics, AstraZeneca, Cheshire, UK. Correspondence should be addressed to W.B.D. (warwick.dunn@manchester.ac.uk).

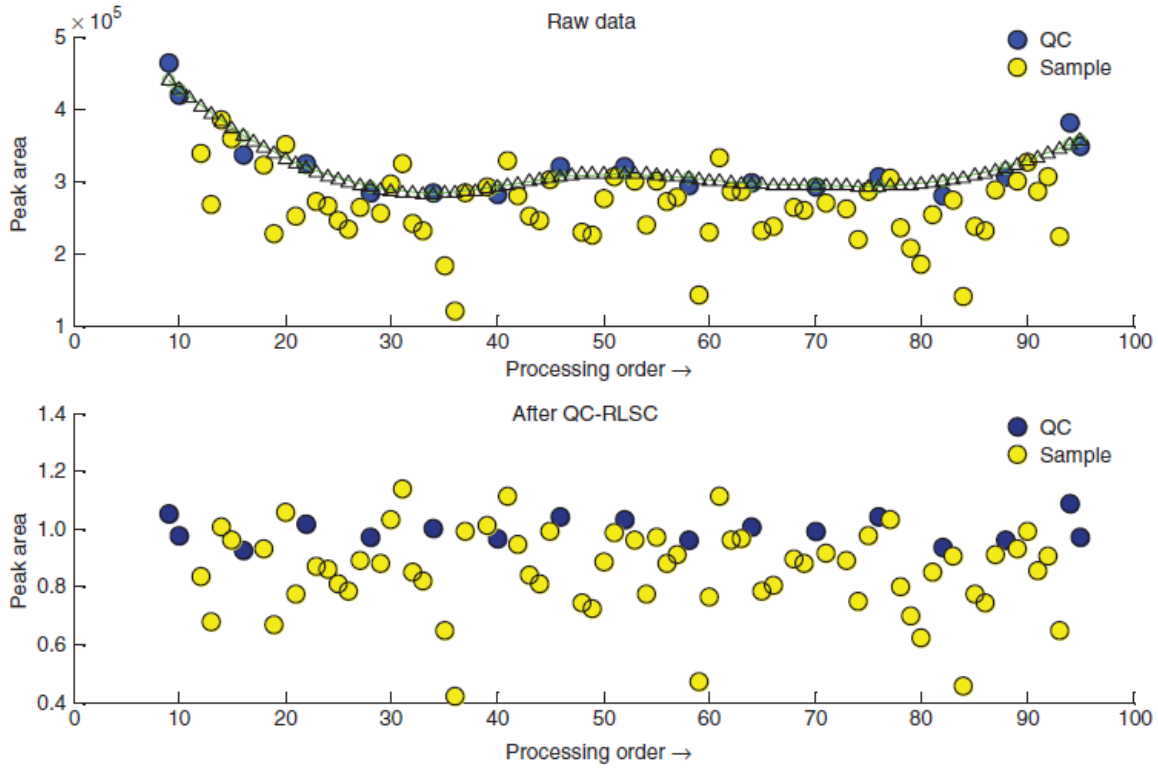


Figure 2 | The QC-RLSC protocol for a metabolic feature detected in UPLC-MS (ES+) with signal attenuation across a given analytical batch. A cross-validated LOESS curve (upper plot) is fitted to the QC samples, the correction curve interpolated (triangles), to which the total data set for that peak is corrected (lower plot).

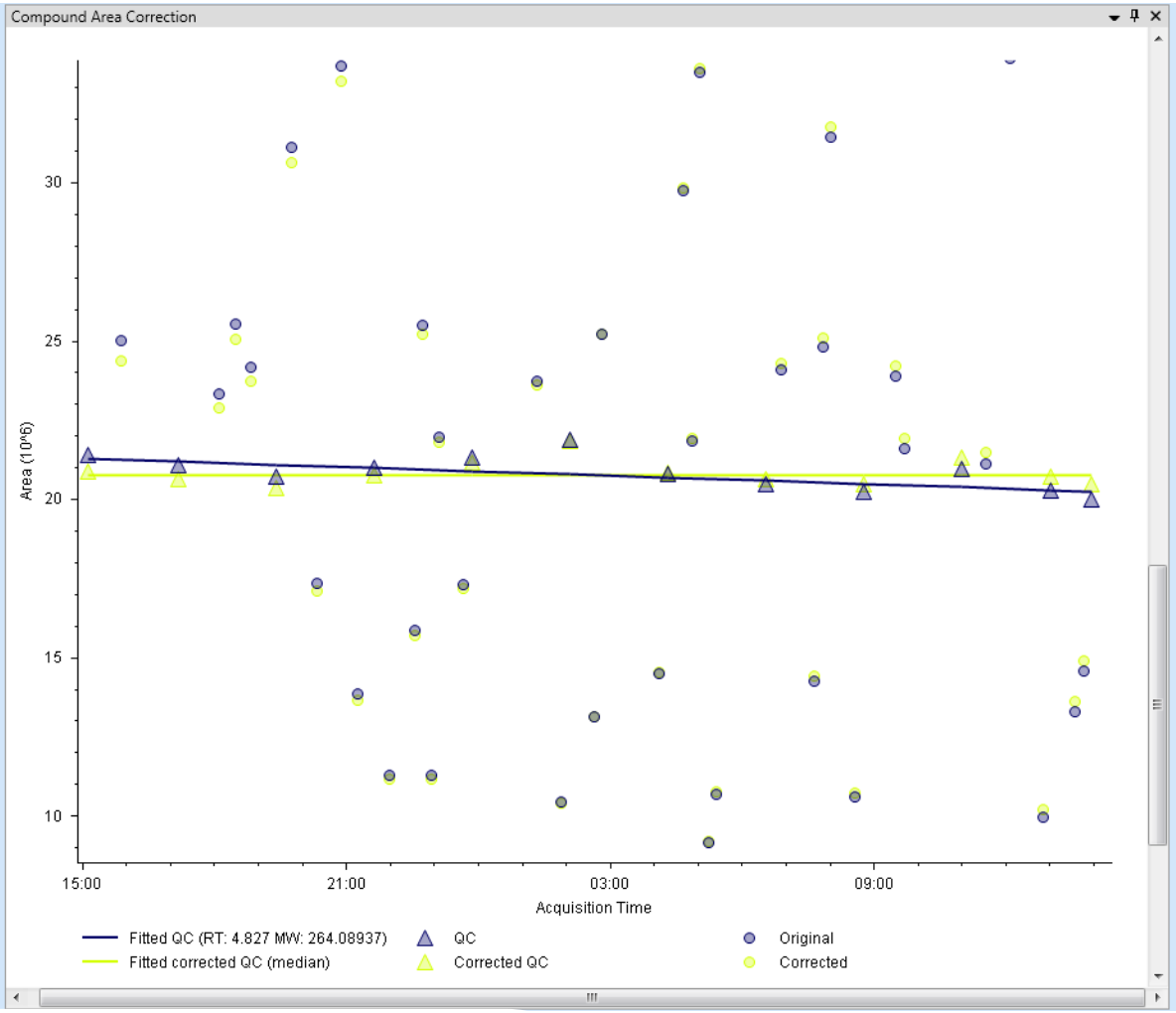
ThermoFisher SCIENTIFIC

Compound Discoverer 2.1 Software – QC-based Normalization

Compounds | Compounds per File | Features | mzCloud Results | ChemSpider Results | Input Files | BioCyc Pathways | KEGG Pathways

	Checked	Name	Formula	Annotation Sc	Molecular Weight	RT [min]	Area (Max.)	# ChemSpider Results	# mzC
45					143.07341	1.761	67188588	0	
46		Ethopabate	C12 H15 N O4		237.09979	4.490	67080371	4	
47		Leu-pro	C11 H20 N2 O3		228.14706	1.109	67079827	2	
48			C17 H30 N2 O8		390.19965	2.307	65531510	0	
49		Vorinostat	C14 H20 N2 O3		264.14691	3.080	63658499	6	
50		N-Acetyl-L-leucine	C8 H15 N O3		173.10512	3.400	62193714	7	
51			C14 H22 N2 O4		282.15746	3.083	61301753	1	
52			C14 H21 N O5		283.14135	1.278	60537885	0	
53		Vorinostat	C14 H20 N2 O3		264.14695	3.120	59530224	6	
54		gamma-Glutamylleucine	C11 H20 N2 O5		260.13675	1.283	58596541	2	
55		N-Acetyl-L-leucine	C8 H15 N O3		173.10512	3.640	56052561	7	
56			C17 H30 N2 O8		390.19972	2.413	54782014	0	
57		Pilocarpidine (d)	C10 H14 N2 O2		194.10539	1.625	51843100	3	
58		Phthalazino[2,3-b]phthalazine-5,12(	C16 H12 N2 O2		264.08937	4.827	51782643	4	
59		gamma-Glutamylleucine	C11 H20 N2 O5		260.13674	1.691	51488459	2	
60			C7 H18 N5 O3 P		251.11531	1.044	50252524	0	
61		1-Methyl-2,3,4,9-tetrahydro-1H-bet	C13 H14 N2 O2		230.10520	3.719	49733004	3	
62		FENAMIC ACID	C13 H11 N O2		213.07862	3.781	49614478	3	
63		FENAMIC ACID	C13 H11 N O2		213.07865	3.675	49614478	3	
64			C11 H11 N		157.08901	3.428	48066675	0	
65		leu-phe	C15 H22 N2 O3		278.16251	4.179	47851674	3	
66		(5S)-5-Acetyl-1-(phenylacetyl)-2-pyr	C14 H15 N O3		245.10488	3.106	47497618	1	
67			C14 H17 N O4		263.11545	3.089	47413311	1	
68		Methyl alpha-aspartylphenylalaninat	C14 H18 N2 O5		294.12102	2.538	46994047	9	
69		Isoprenaline	C11 H17 N O3		211.12067	1.847	46235067	6	
70		leu-phe	C15 H22 N2 O3		278.16260	4.274	46055569	3	
71		1-Trimethylammonio-3-(3-indolyl)pr	C14 H18 N2 O2		246.13646	4.185	46036209	1	
72		gamma-Glutamylleucine	C11 H20 N2 O5		260.13674	2.032	45578677	2	
73		Guaiacol	C7 H8 O2		124.05236	1.067	45282171	13	
74		Tetraacetylethylenediamine	C10 H16 N2 O4		228.11077	2.045	45151607	5	
75			C11 H11 N		157.08902	3.518	44668359	0	

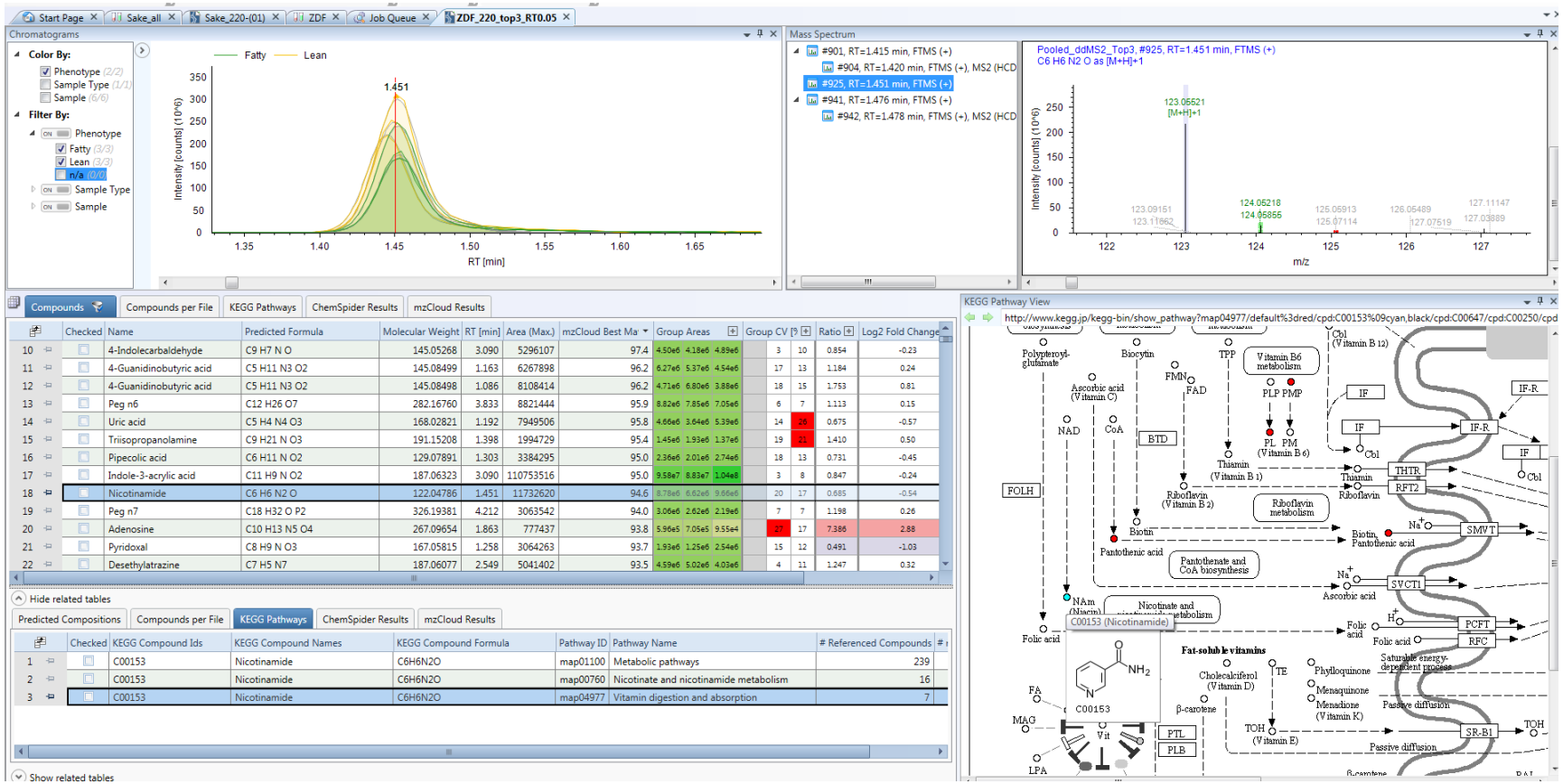
Show Related Tables



Compound area correction plot for each compound

ThermoFisher SCIENTIFIC

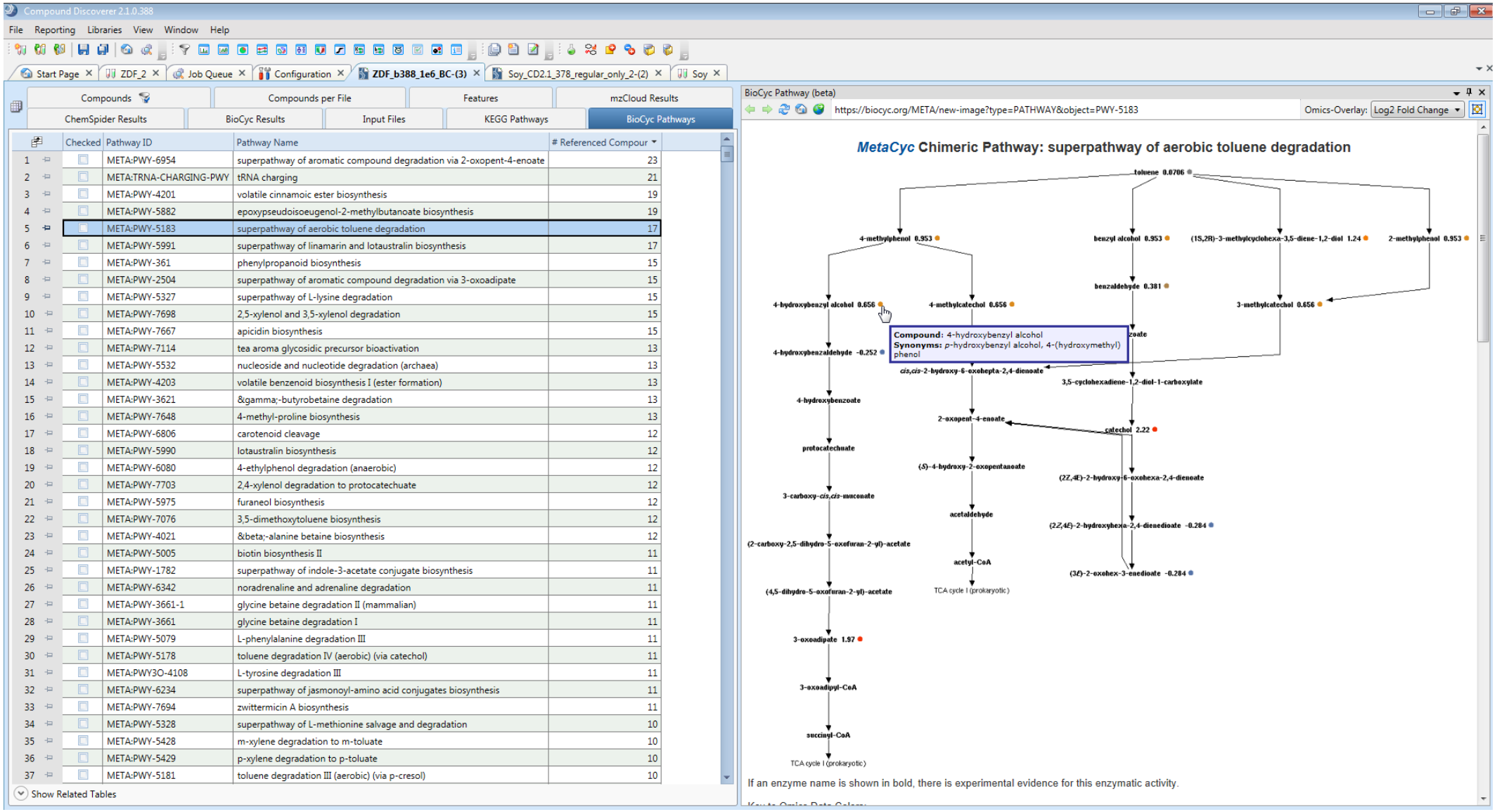
Compound Discoverer 2.1 Software: Pathway Mapping using KEGG and BioCyc



KEGG and BioCyc pathways: global and context-specific

ThermoFisher SCIENTIFIC

Pathway Mapping to BioCyc with Omics Data Overlay



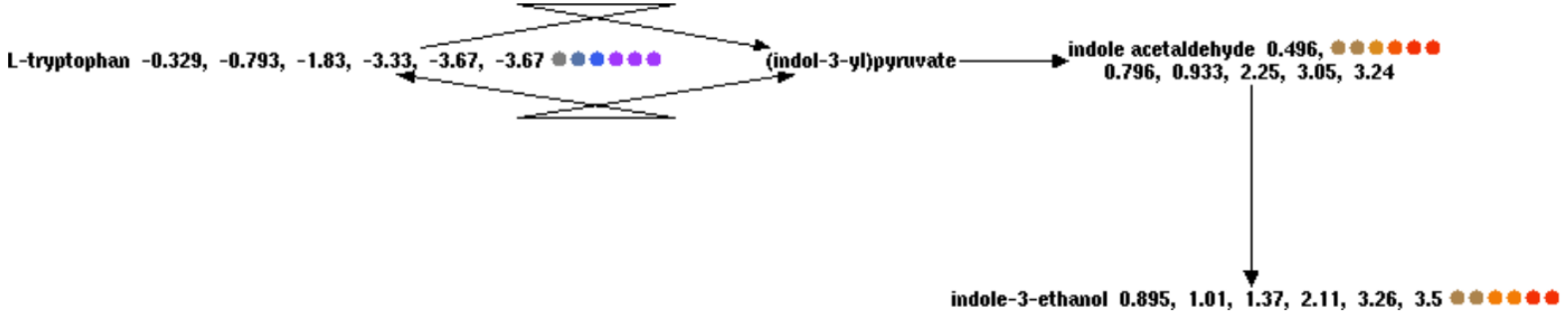
ThermoFisher SCIENTIFIC

Pathway Mapping to BioCyc with Omics Data Overlay

Add to SmartTable

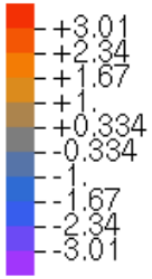
Saccharomyces cerevisiae S288c Pathway: tryptophan degradation VIII (to tryptophol)

More Detail | Less Detail



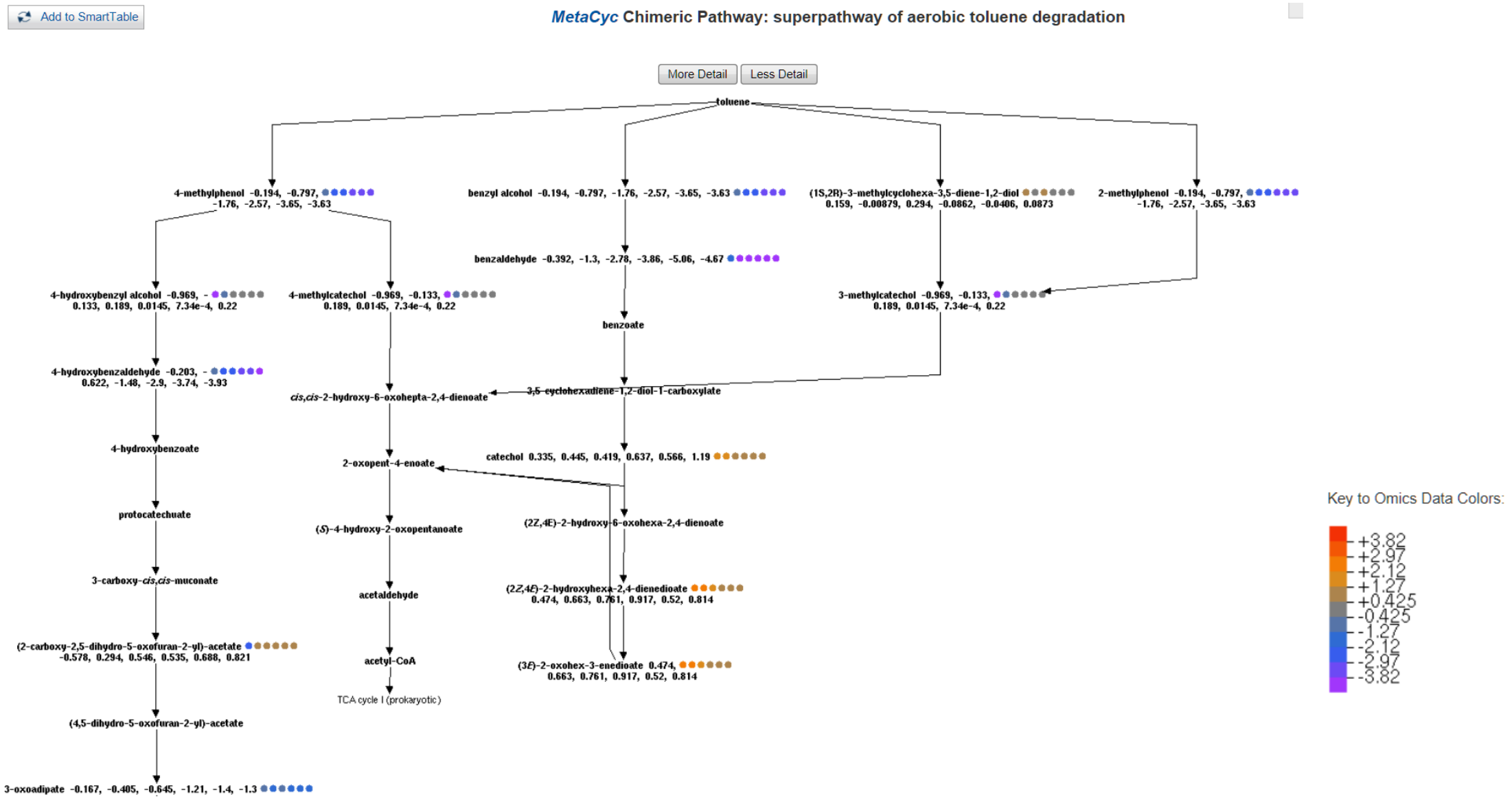
If an enzyme name is shown in bold, there is experimental evidence for this enzymatic activity.

Key to Omics Data Colors:



Synonyms: Ehrlich pathway

Pathway Mapping to BioCyc with Omics Data Overlay



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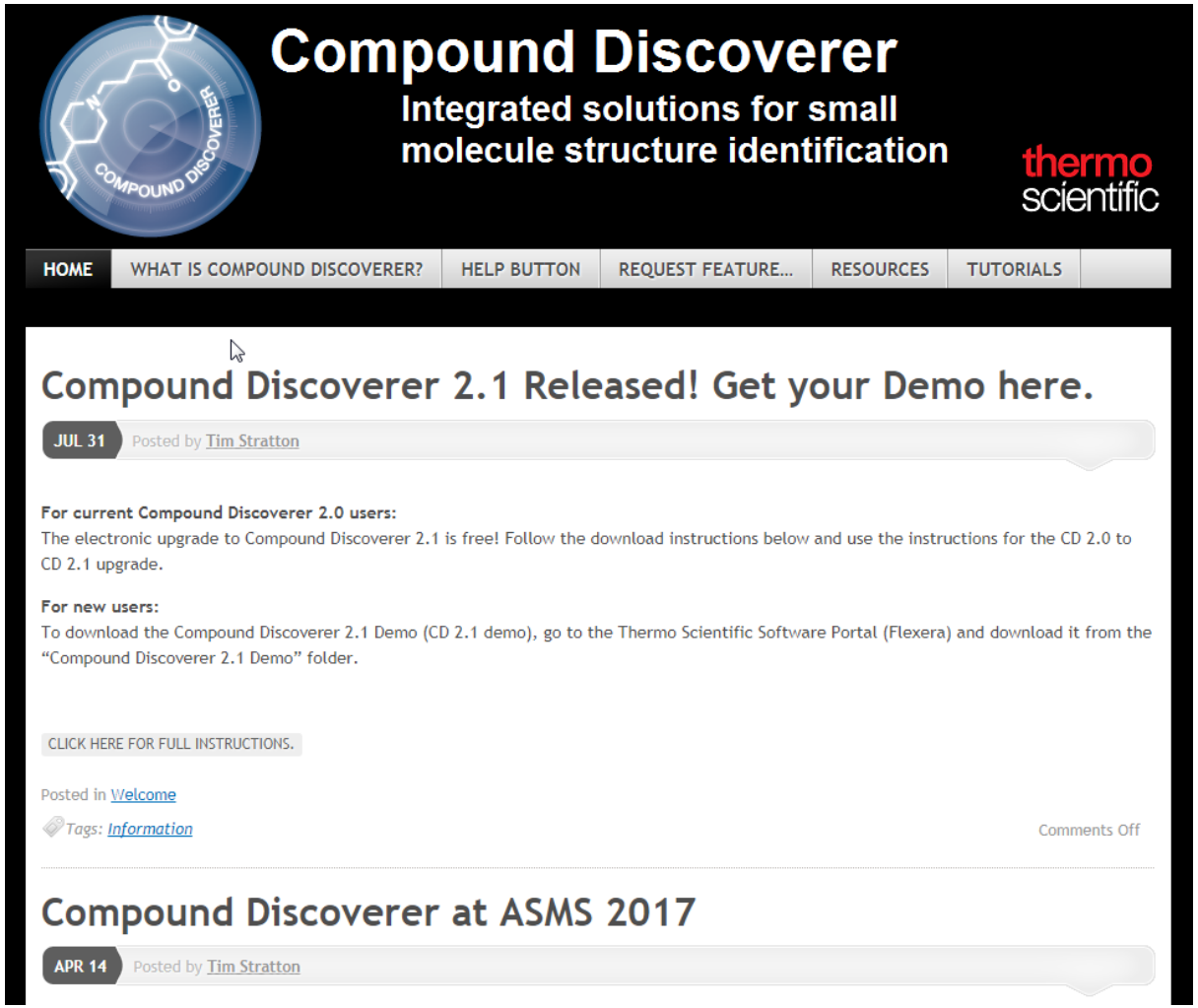
Compound Discoverer website



mycompounddiscoverer.com

- News on Compound Discoverer
- Quick Videos
- Request a feature
- "Help button" - contact the team

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Appendix

Compound Discoverer 2.1 Software – QC-based Normalization

Study File ID	File Name	Creation Date	Instrument Name	Ref. File ID	Sample Type
F1	Blank_1_Replicate_1.raw	10/4/2016 2:34 PM	Q Exactive HF Orbitrap	F5	Blank
F2	Blank_1_Replicate_2.raw	10/4/2016 2:45 PM	Q Exactive HF Orbitrap	F5	Blank
F3	Blank_1_Replicate_3.raw	10/4/2016 2:57 PM	Q Exactive HF Orbitrap	F5	Blank
F5	QC_1.raw	10/4/2016 3:08 PM	Q Exactive HF Orbitrap	F35	Quality Control
F98	Soy_28_Replicate_1.raw	10/4/2016 3:19 PM	Q Exactive HF Orbitrap	F84	Sample
F41	Soy_9_Replicate_1.raw	10/4/2016 3:30 PM	Q Exactive HF Orbitrap	F84	Sample
F91	Soy_25_Replicate_3.raw	10/4/2016 3:41 PM	Q Exactive HF Orbitrap	F84	Sample
F28	Soy_4_Replicate_3.raw	10/4/2016 3:52 PM	Q Exactive HF Orbitrap	F84	Sample
F32	Soy_6_Replicate_1.raw	10/4/2016 4:03 PM	Q Exactive HF Orbitrap	F35	Sample
F38	Soy_8_Replicate_1.raw	10/4/2016 4:15 PM	Q Exactive HF Orbitrap	F35	Sample
F69	Soy_18_Replicate_2.raw	10/4/2016 4:26 PM	Q Exactive HF Orbitrap	F84	Sample
F30	Soy_5_Replicate_2.raw	10/4/2016 4:37 PM	Q Exactive HF Orbitrap	F84	Sample
F42	Soy_9_Replicate_2.raw	10/4/2016 4:48 PM	Q Exactive HF Orbitrap	F84	Sample
F60	Soy_15_Replicate_2.raw	10/4/2016 4:59 PM	Q Exactive HF Orbitrap	F84	Sample
F6	QC_2.raw	10/4/2016 5:10 PM	Q Exactive HF Orbitrap	F5	Quality Control
F51	Soy_12_Replicate_2.raw	10/4/2016 5:33 PM	Q Exactive HF Orbitrap	F84	Sample
F52	Soy_12_Replicate_3.raw	10/4/2016 5:44 PM	Q Exactive HF Orbitrap	F84	Sample
F92	Soy_26_Replicate_1.raw	10/4/2016 5:55 PM	Q Exactive HF Orbitrap	F84	Sample
F109	Soy_31_Replicate_3.raw	10/4/2016 6:06 PM	Q Exactive HF Orbitrap	F84	Sample
F21	Soy_2_Replicate_2.raw	10/4/2016 6:17 PM	Q Exactive HF Orbitrap	F84	Sample
F117	Soy_34_Replicate_2.raw	10/4/2016 6:28 PM	Q Exactive HF Orbitrap	F84	Sample
F112	Soy_32_Replicate_3.raw	10/4/2016 6:39 PM	Q Exactive HF Orbitrap	F84	Sample
F48	Soy_11_Replicate_2.raw	10/4/2016 6:50 PM	Q Exactive HF Orbitrap	F84	Sample
F26	Soy_4_Replicate_1.raw	10/4/2016 7:02 PM	Q Exactive HF Orbitrap	F84	Sample
F97	Soy_27_Replicate_3.raw	10/4/2016 7:13 PM	Q Exactive HF Orbitrap	F84	Sample
F7	QC_3.raw	10/4/2016 7:24 PM	Q Exactive HF Orbitrap	F5	Quality Control

- Use sample type “Quality Control” for QC samples
- Correct order is detected automatically
- Needs one QC sample at the beginning of the sequence and one at the end and at constant intervals (every 5-10 injections)

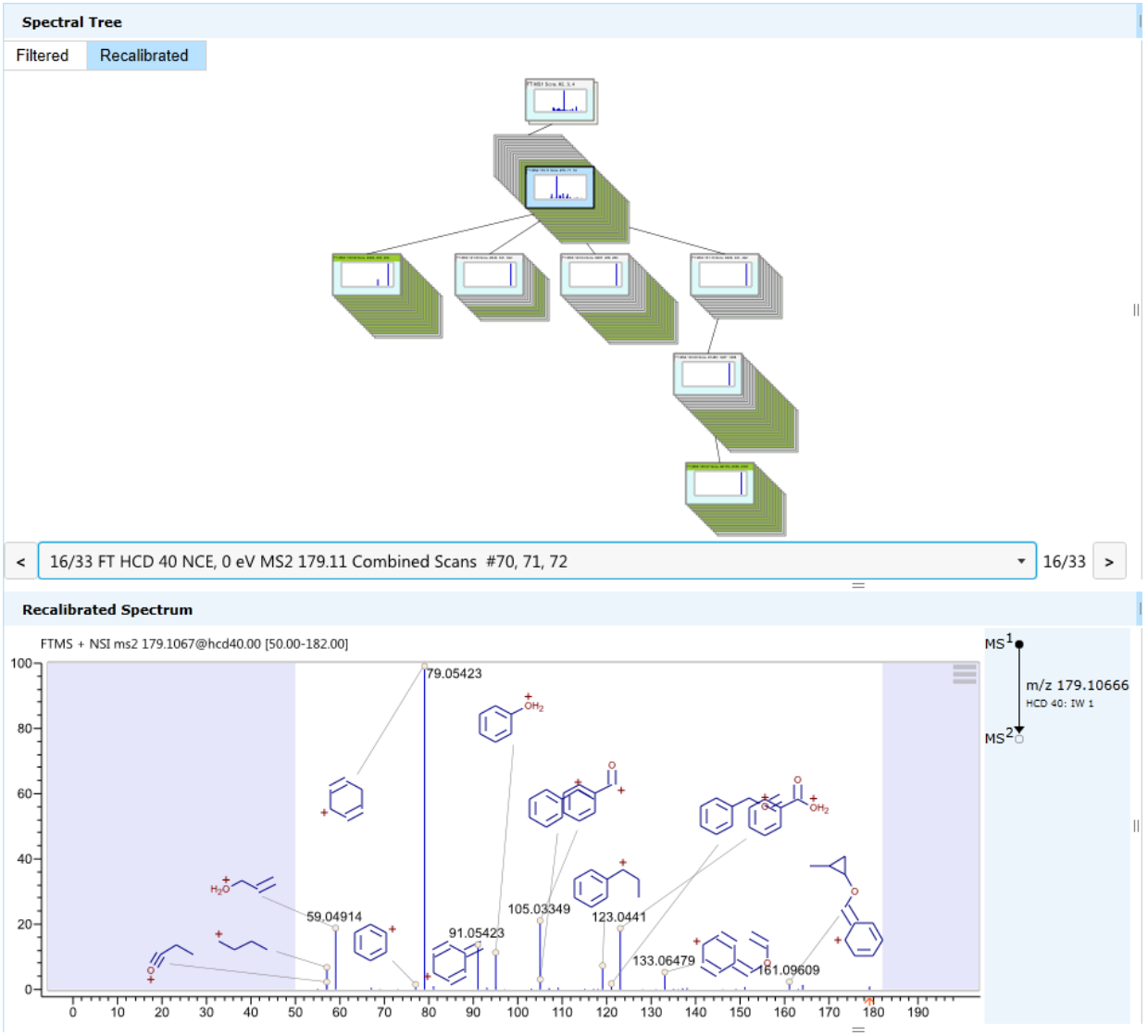
Compound Discoverer 2.1 Software – mzCloud Search Using Stepped Collision Energy

m/z CLOUD Advanced Mass Spectral Database

6,819 (+44)	10,672 (+64)	2,382,231 (+32,439)	706,837 (+1,210)
compounds	trees	spectra	QM models

(+ added in the last 14 days)

> 10,000 spectral trees
> 2.4 million spectra



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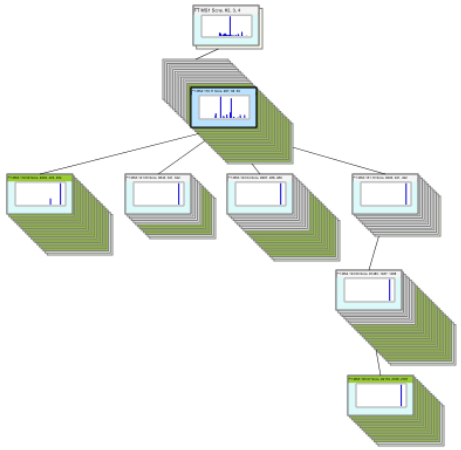
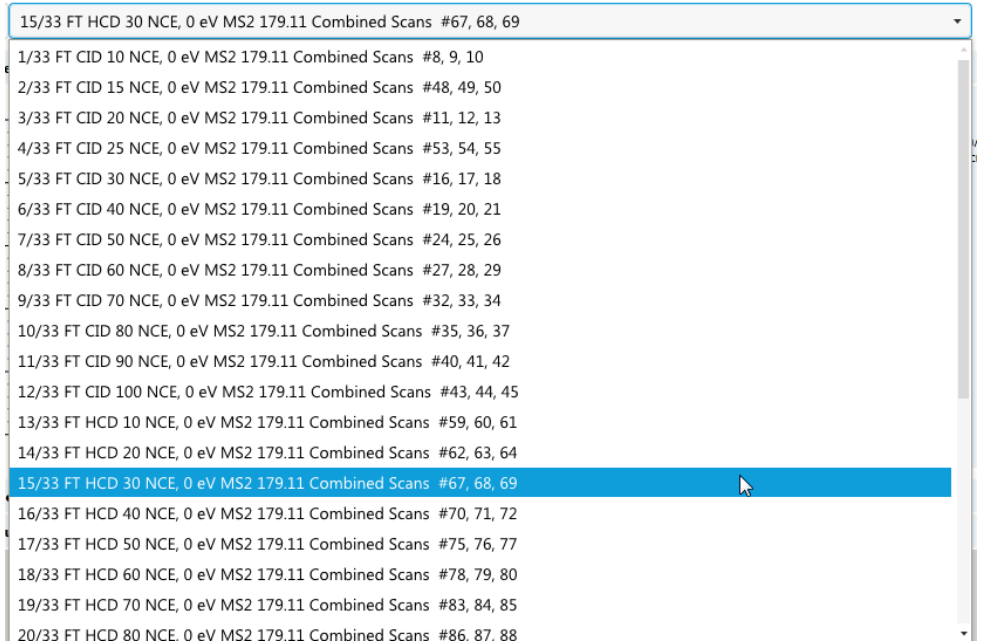
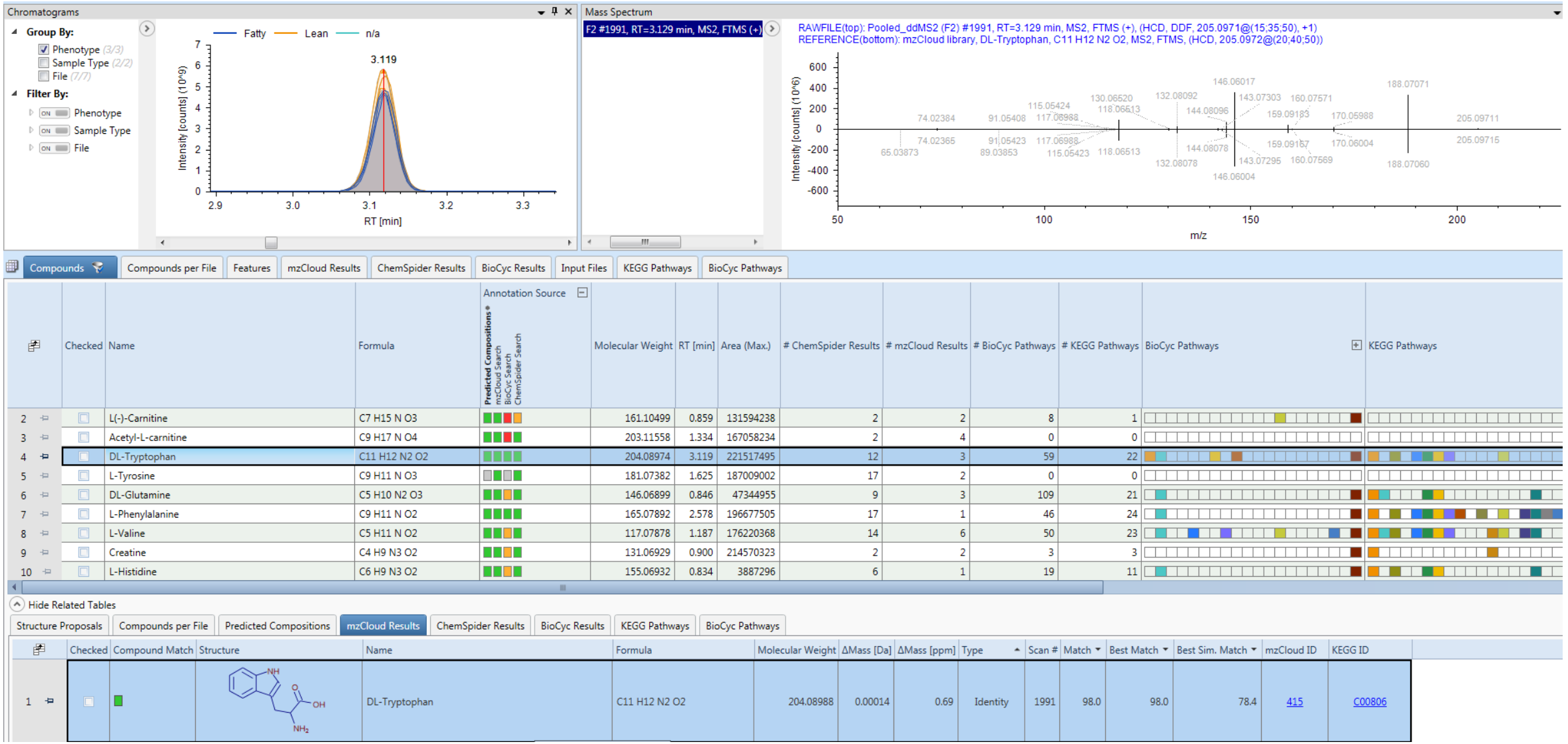
Compound Discoverer 2.1 Software – mzCloud Search Using Stepped Collision Energy

- CID 10, 15, 20, … , 100 NCE
- HCD 10, 20, 30, … , 200 NCE
- mzCloud search automatically matches your experimental conditions
- mzCloud automatically combines multiple scans during the search to match your experimental conditions for stepped collision energy





Compound Discoverer 2.1 Software – mzCloud Search Using Stepped Collision Energy



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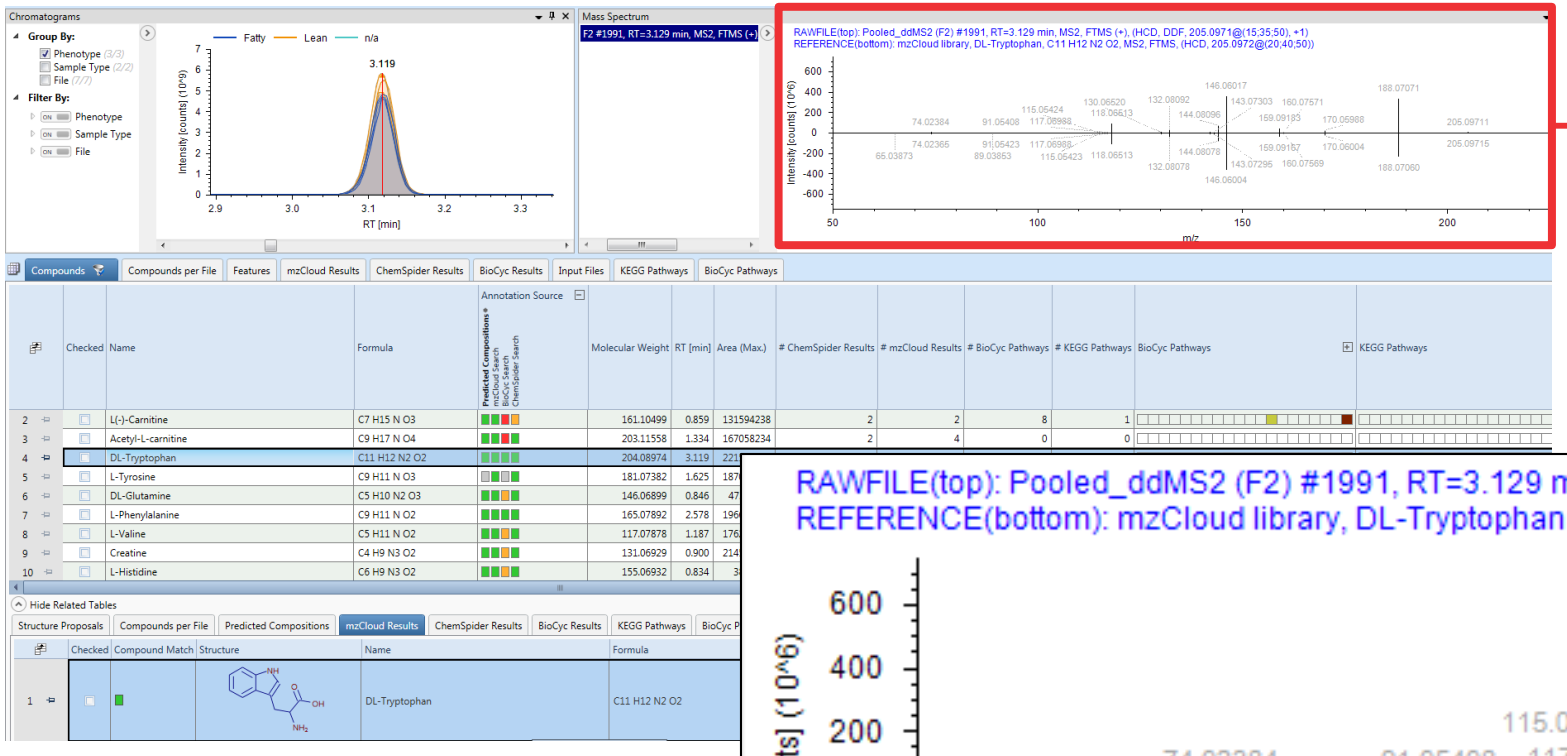
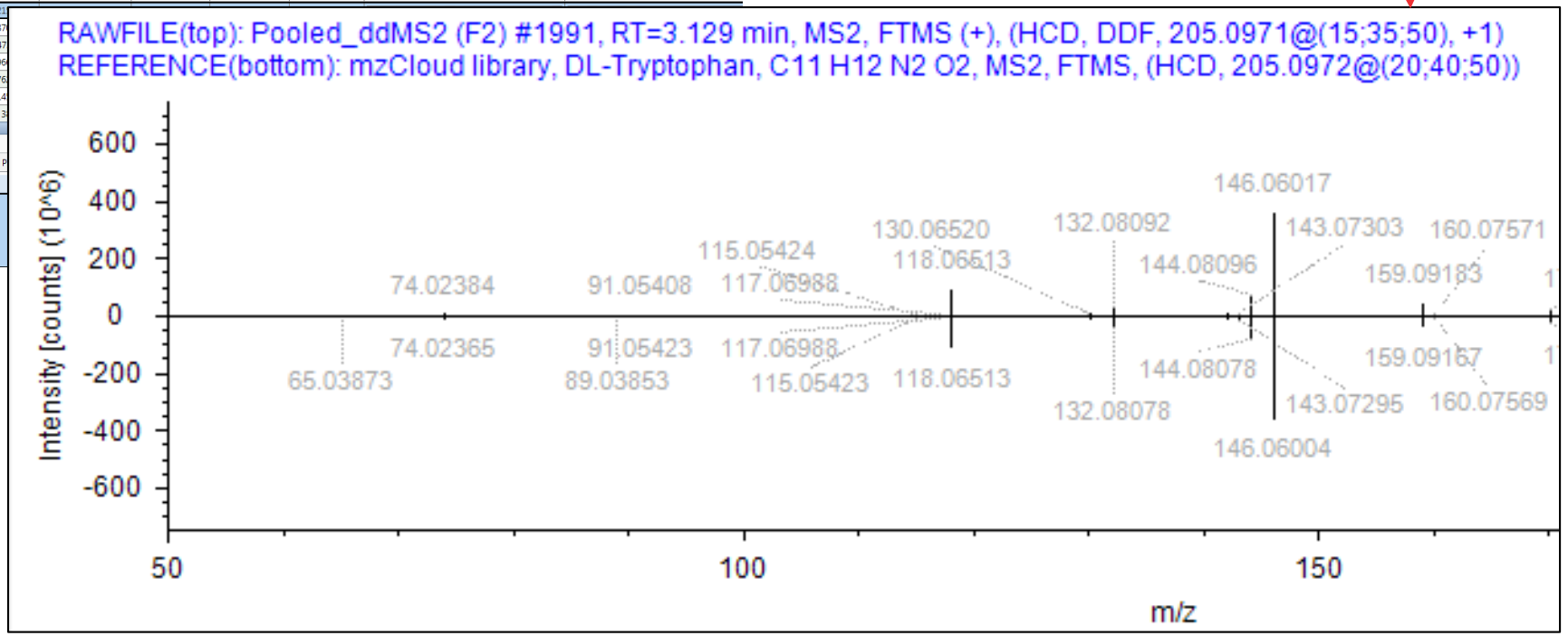
Compound Discoverer 2.1 Software – mzCloud Search Using Stepped Collision Energy





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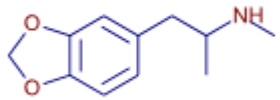
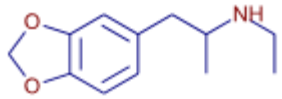
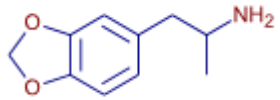
Compound Class Scoring

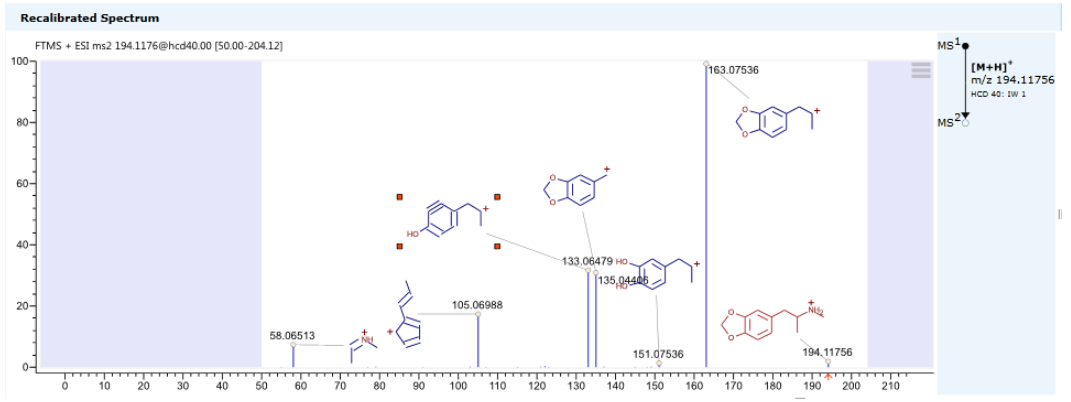
- Detects and scores similarities based on common fragments
- forensic applications
- e.g., detecting new designer drugs

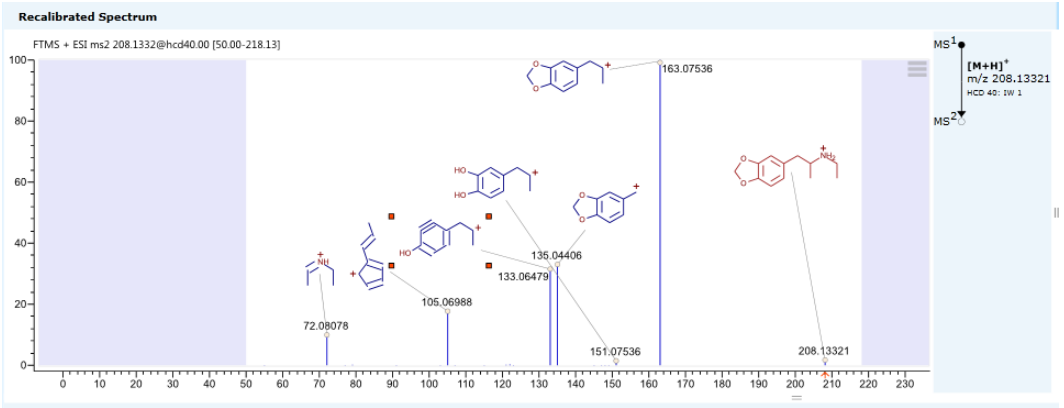


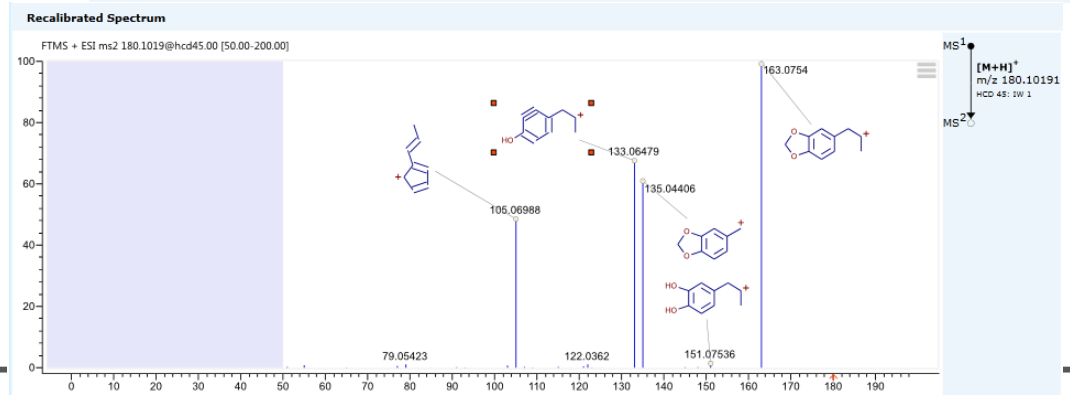




Substituted methylenedioxy-phenethylamines (MDxx)







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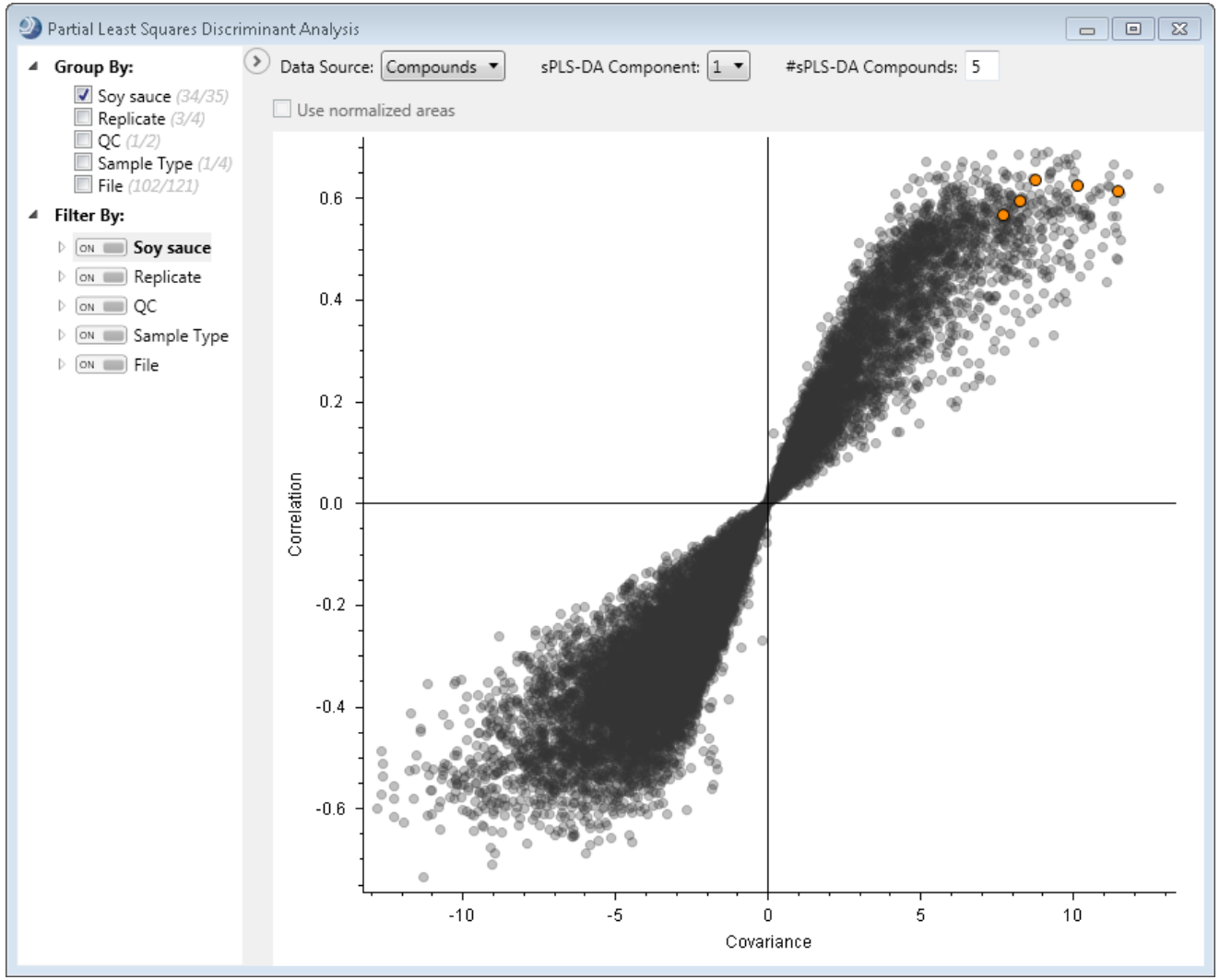
Compound Discoverer 2.1 Software - Statistics

- PLS – DA
  - Partial Least Squares Discriminant Analysis
  - What are the *compounds* that best describe the differences between the groups?
  - Biomarker discovery



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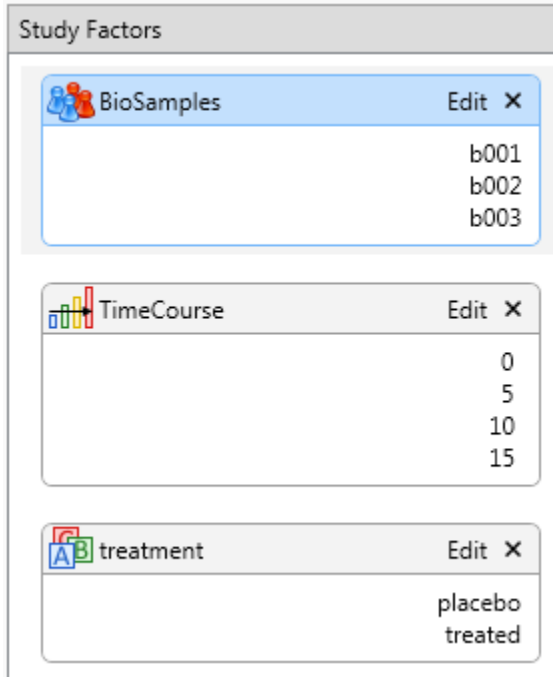
Compound Discoverer 2.1 Software - Statistics

- Support for biological vs. technical replicates
  - New type of study factor
- Support for nested study design
  - Paired tests



Compound Discoverer 2.1 Software - Statistics

- Support for biological vs. technical replicates
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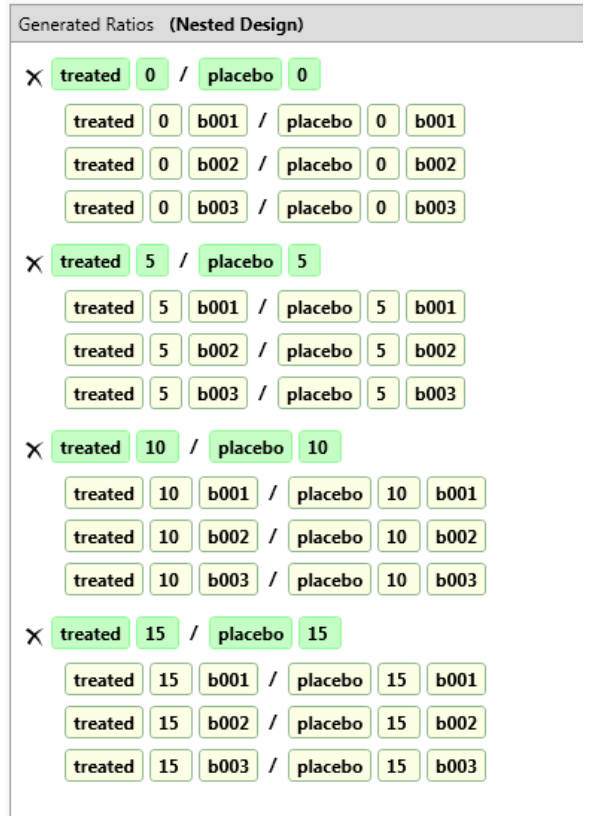

Group CV [%]								Ratio				Bio. Rep. Ratio													
0 , placebo *	0 , treated	5 , placebo	5 , treated	10 , placebo	10 , treated	15 , placebo	15 , treated	(0 , treated) / (0 , placebo) *	(5 , treated) / (5 , placebo)	(10 , treated) / (10 , placebo)	(15 , treated) / (15 , placebo)	(0 , treated,b001) / (0 , placebo,b001) *	(0 , treated,b002) / (0 , placebo,b002)	(0 , treated,b003) / (0 , placebo,b003)	(5 , treated,b001) / (5 , placebo,b001)	(5 , treated,b002) / (5 , placebo,b002)	(5 , treated,b003) / (5 , placebo,b003)	(10 , treated,b001) / (10 , placebo,b001)	(10 , treated,b002) / (10 , placebo,b002)	(10 , treated,b003) / (10 , placebo,b003)	(15 , treated,b001) / (15 , placebo,b001)	(15 , treated,b002) / (15 , placebo,b002)	(15 , treated,b003) / (15 , placebo,b003)
3	6	41	4	12	6	21	27	0.982	0.823	0.406	0.074	0.982	1.026	0.925	1.364	0.520	0.823	0.406	0.437	0.318	0.034	0.074	0.074
3	6	41	4	12	7	21	27	0.982	0.822	0.442	0.075	0.982	1.027	0.924	1.365	0.521	0.822	0.442	0.470	0.337	0.034	0.075	0.075
3	4	96	4	12	5	21	13	1.023	0.909	0.296	0.075	1.023	1.008	1.032	1.385	0.162	0.909	0.308	0.296	0.237	0.044	0.077	0.075
6	4	55	2	12	10	40	11	1.005	0.739	0.286	0.095	1.041	0.957	1.005	1.712	0.425	0.739	0.286	0.319	0.222	0.039	0.095	0.099

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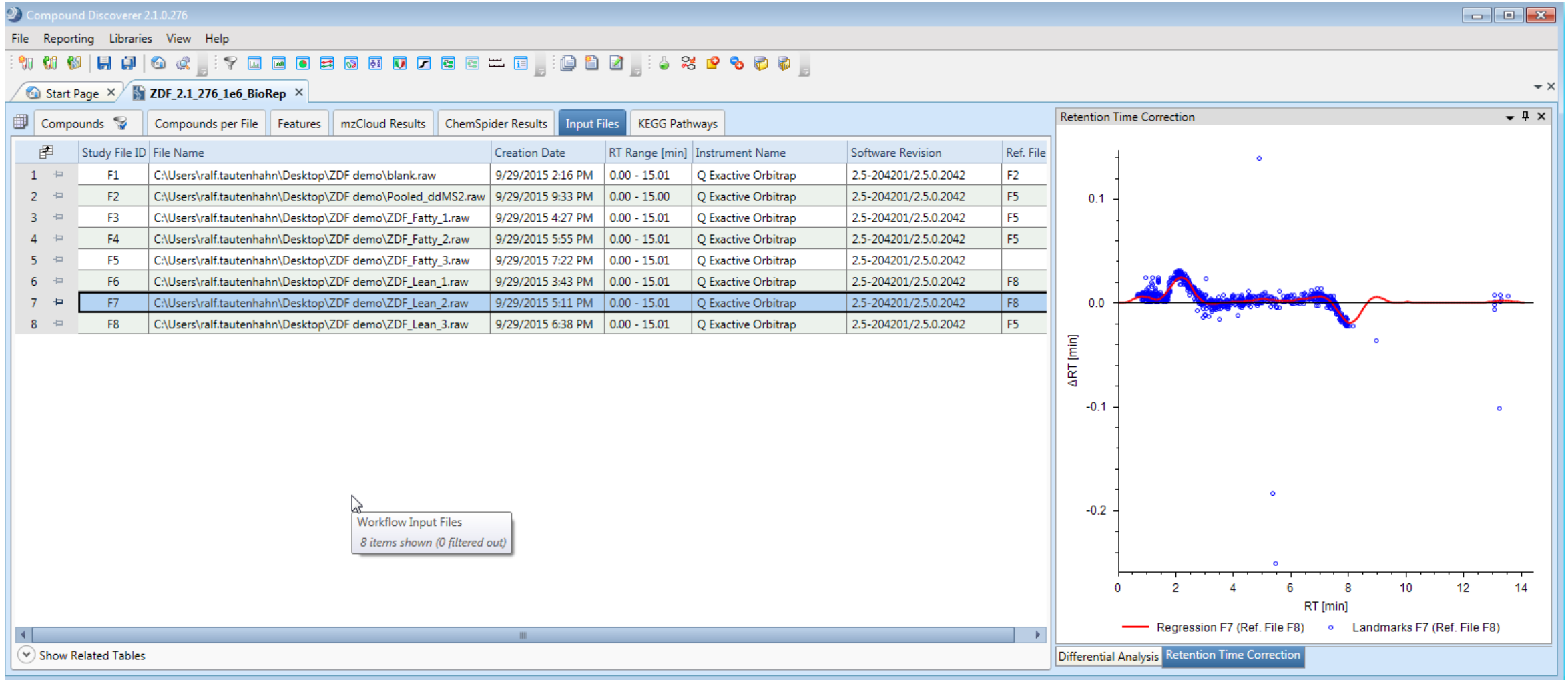
Improved Alignment and New RT Correction Plot



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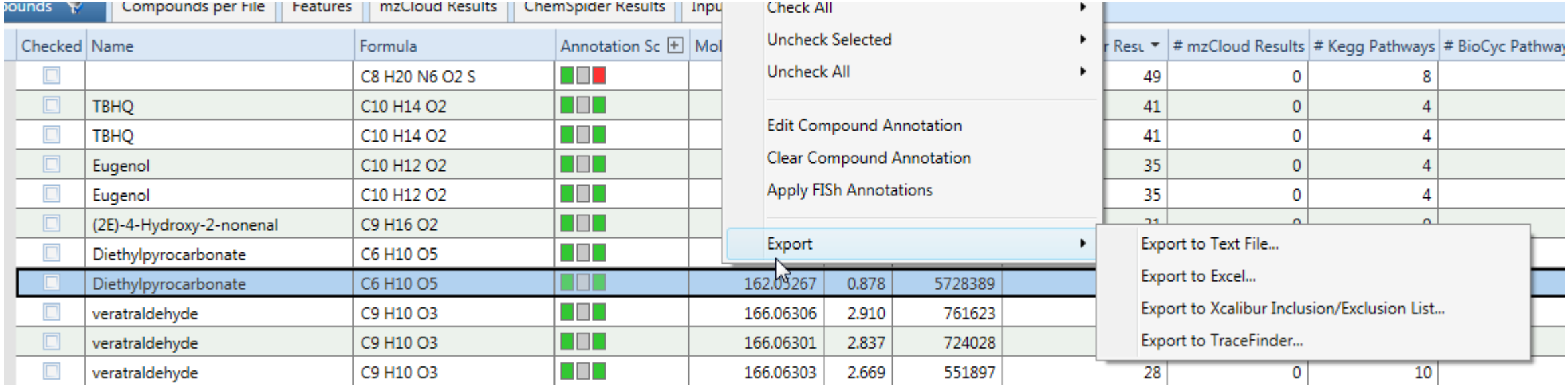
Export to Thermo Scientific Xcalibur and TraceFinder Software



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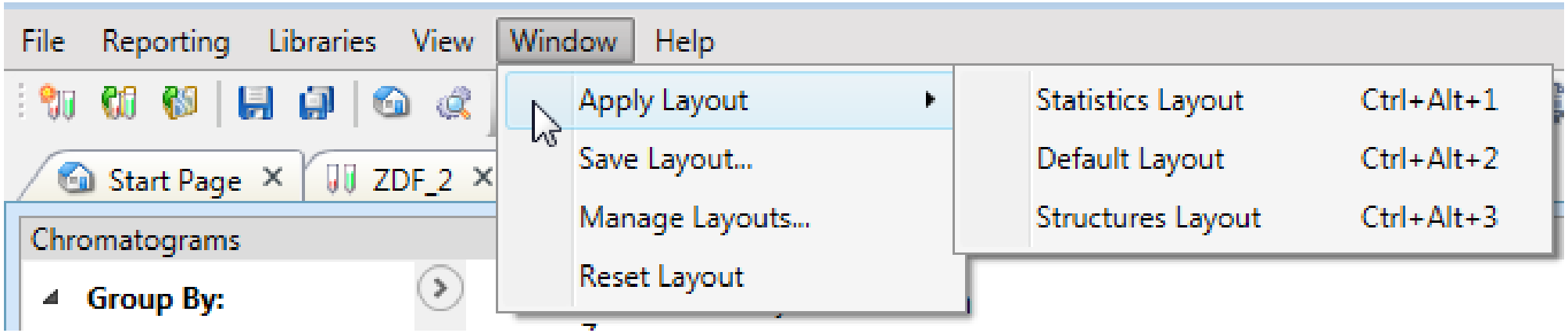
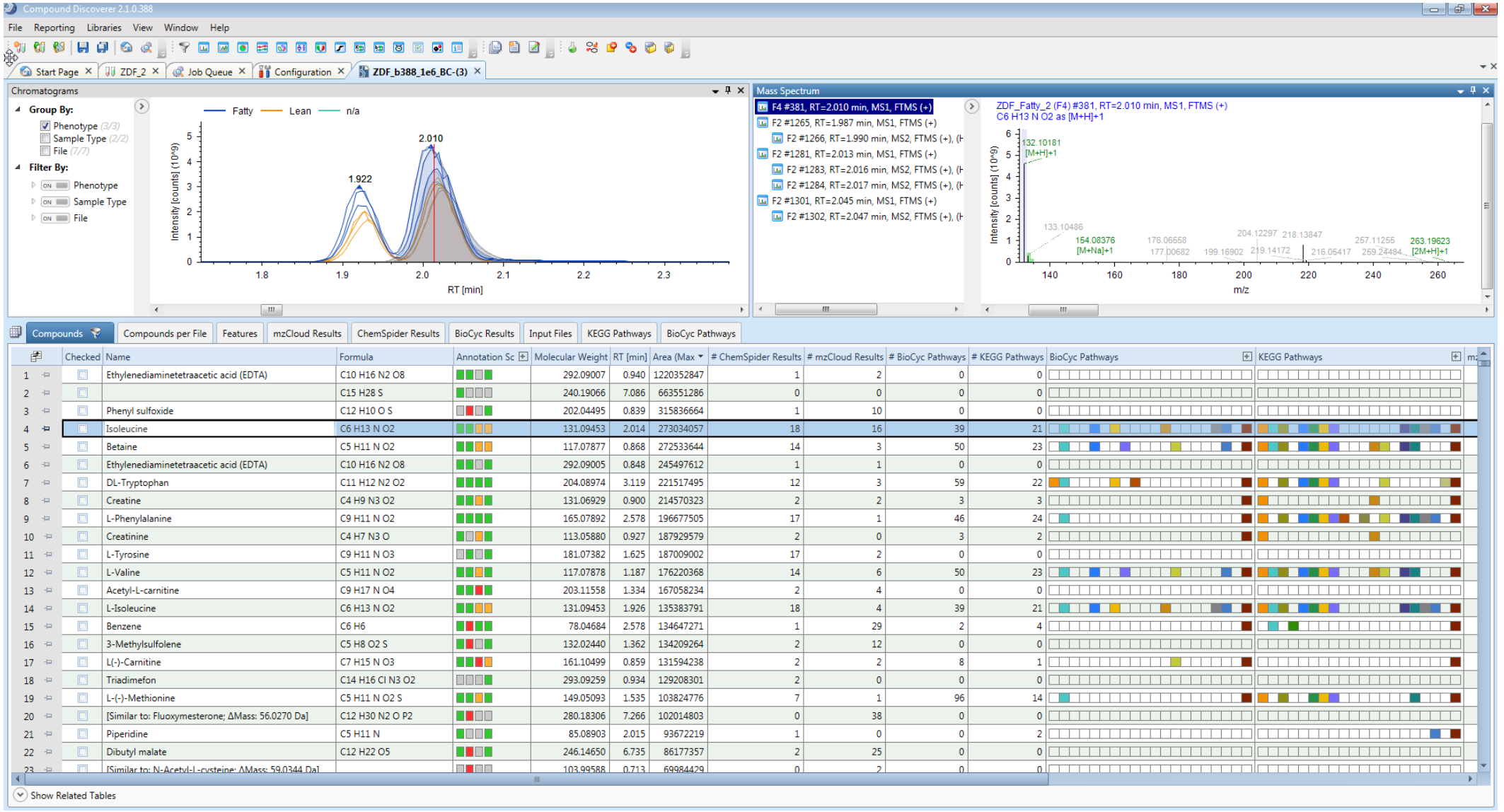
Compound Discoverer 2.1 Software – Layouts



Compound Discoverer 2.1 Software – Layouts

Default Layout



	Checked	Name	Formula	Annotation Sc	Molecular Weight	RT [min]	Area (Max	# ChemSpider Results	# mzCloud Results	# BioCyc Pathways	# KEGG Pathways	BioCyc Pathways	KEGG Pathways
1		Ethylenediaminetetraacetic acid (EDTA)	C10 H16 N2 O8		292.09007	0.940	1220352847	1	2	0	0		
2			C15 H28 S		240.19066	7.086	663551286	0	0	0	0		
3		Phenyl sulfoxide	C12 H10 O S		202.04495	0.839	315836664	1	10	0	0		
4		Isoleucine	C6 H13 N O2		131.09453	2.014	273034057	18	16	39	21		
5		Betaine	C5 H11 N O2		117.07877	0.868	272533644	14	3	50	23		
6		Ethylenediaminetetraacetic acid (EDTA)	C10 H16 N2 O8		292.09005	0.848	245497612	1	1	0	0		
7		DL-Tryptophan	C11 H12 N2 O2		204.08974	3.119	221517495	12	3	59	22		
8		Creatine	C4 H9 N3 O2		131.06929	0.900	214570323	2	2	3	3		
9		L-Phenylalanine	C9 H11 N O2		165.07892	2.578	196677505	17	1	46	24		
10		Creatinine	C4 H7 N3 O		113.05880	0.927	187929579	2	0	3	2		
11		L-Tyrosine	C9 H11 N O3		181.07382	1.625	187009002	17	2	0	0		
12		L-Valine	C5 H11 N O2		117.07878	1.187	176220368	14	6	50	23		
13		Acetyl-L-carnitine	C9 H17 N O4		203.11558	1.334	167058234	2	4	0	0		
14		L-Isoleucine	C6 H13 N O2		131.09453	1.926	135383791	18	4	39	21		
15		Benzene	C6 H6		78.04684	2.578	134647271	1	29	2	4		
16		3-Methylsulfolene	C5 H8 O2 S		132.02440	1.362	134209264	2	12	0	0		
17		L(-)-Carnitine	C7 H15 N O3		161.10499	0.859	131594238	2	2	8	1		
18		Triadimefon	C14 H16 Cl N3 O2		293.09259	0.934	129208301	2	0	0	0		
19		L-(-)-Methionine	C5 H11 N O2 S		149.05093	1.535	103824776	7	1	96	14		
20		[Similar to: Fluoxymesterone; ΔMass: 56.0270 Da]	C12 H30 N2 O P2		280.18306	7.266	102014803	0	38	0	0		
21		Piperidine	C5 H11 N		85.08903	2.015	93672219	1	0	0	2		
22		Dibutyl malate	C12 H22 O5		246.14650	6.735	86177357	2	25	0	0		
23		[Similar to: N-Acetyl-L-cysteine; ΔMass: 59.0344 Da]			103.99588	0.713	69984429	0	2	0	0		

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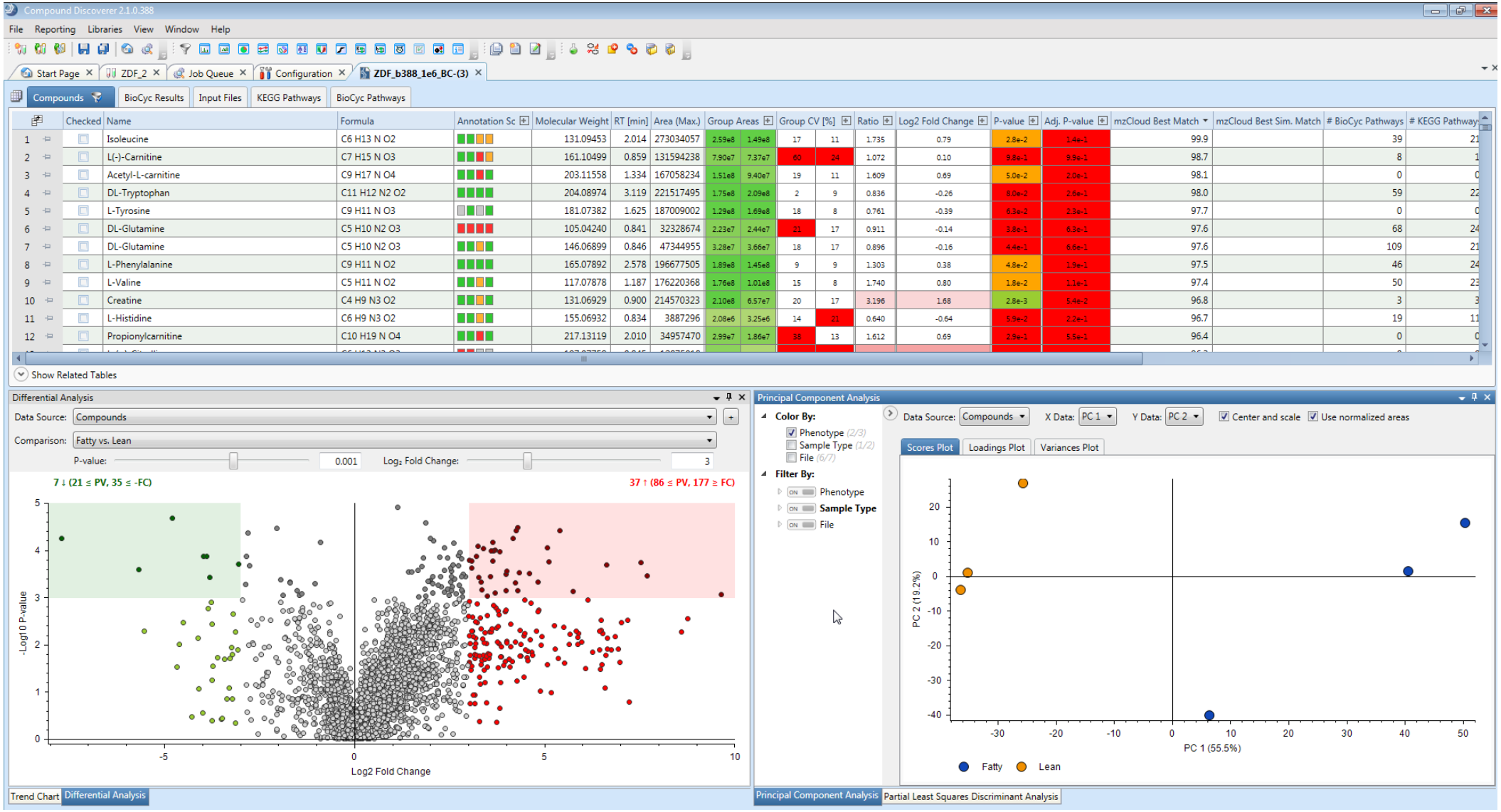
Compound Discoverer 2.1 Software – Layouts

Statistics Layout



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