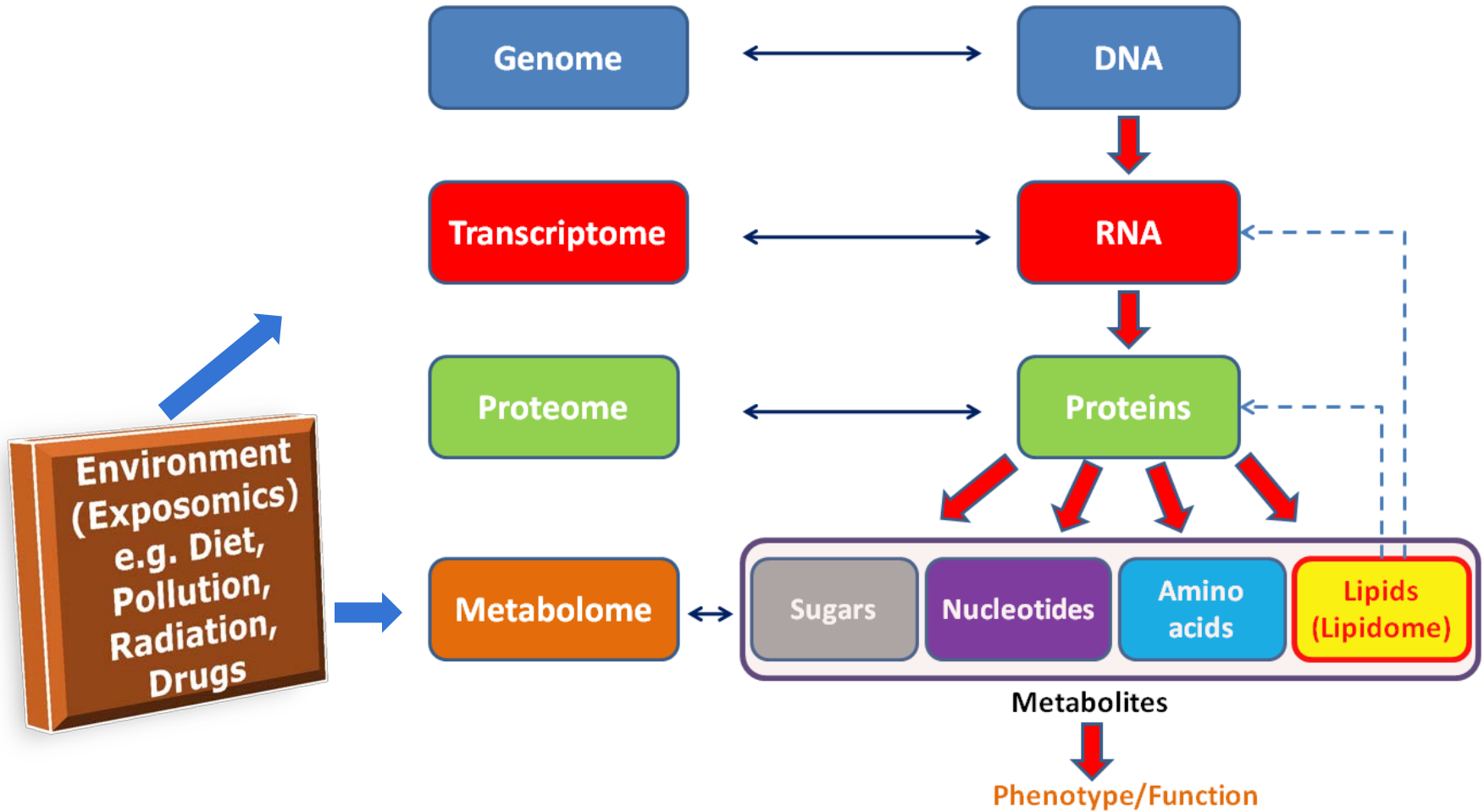


The routine application of Ion Mobility in Metabolomics

Combining separation strategies for maximum
depth of coverage and information content

Dave Heywood
Omics Business Development
david_heywood@waters.com

The Omics



Metabolomics Applications

■ Biomedical Sciences

- Biomarker discovery
- Drug Discovery and Development
- Microbiology
- Personalized medicine

■ Environmental Sciences

- Strain fingerprinting and ID
- Genetic modifications to improve phenotypes
- Pesticide Residue

■ Food Sciences

- Nutrients composition
- Purity

■ Metabolic engineering

- Improvement of metabolic pathways for the production of fuels and chemicals

■ Natural Products

- Traditional medicines



Reasons for failure in biomarker translation

- Small number of samples that are analysed
- Lack of information on the history of the samples
- Case and control specimens which are not matched with age and sex
- Limited metabolic and proteomic coverage
- The need to follow clear standard operating procedures for sample selection, collection, storage, handling, analysis and data interpretation

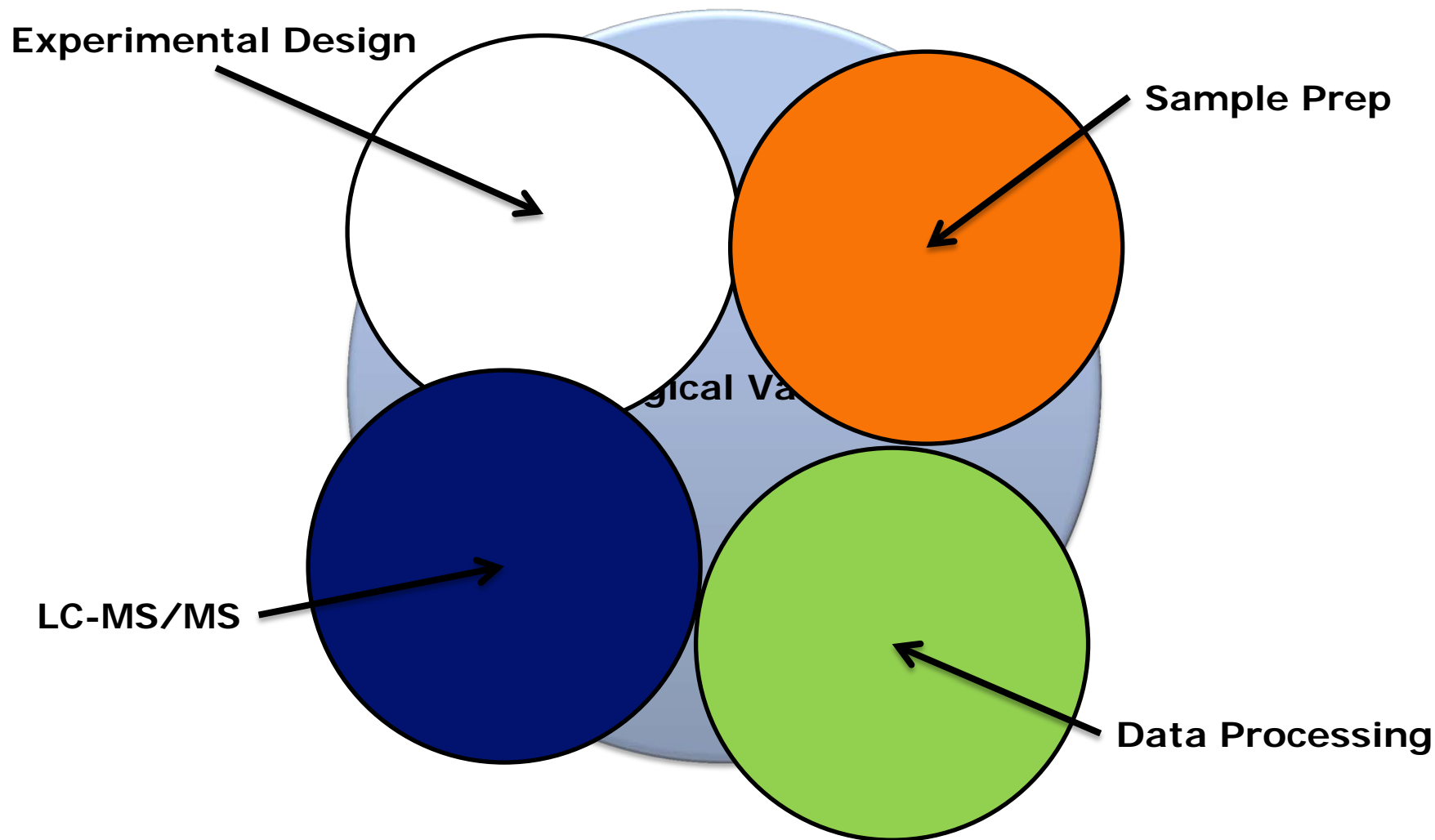
Experimental Design

Sample Handling

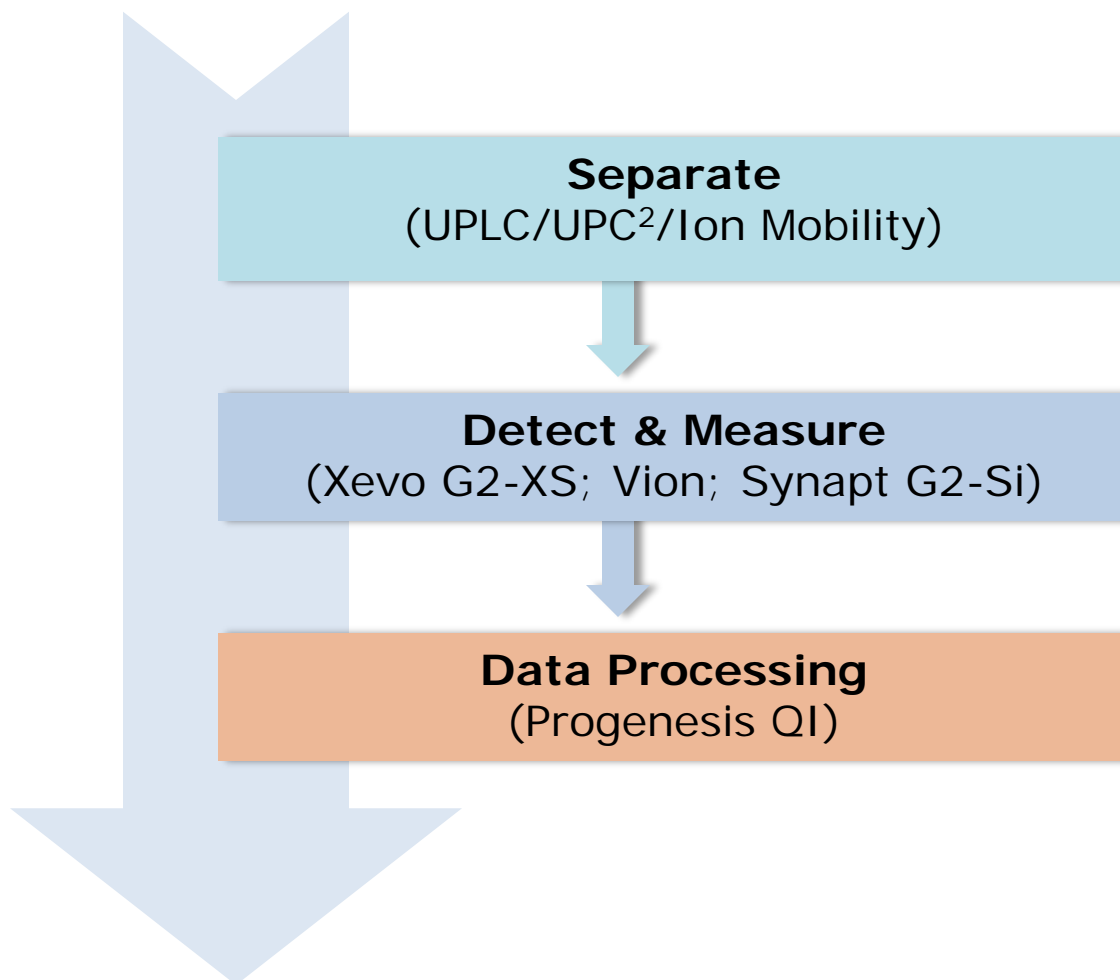
Analytical Approach

Pitfalls and limitations in translation from biomarker discovery to clinical utility in predictive and personalised medicine
Druker and Krapfenbauer The EPMA Journal 2013, 4: 7

Controlling Variance



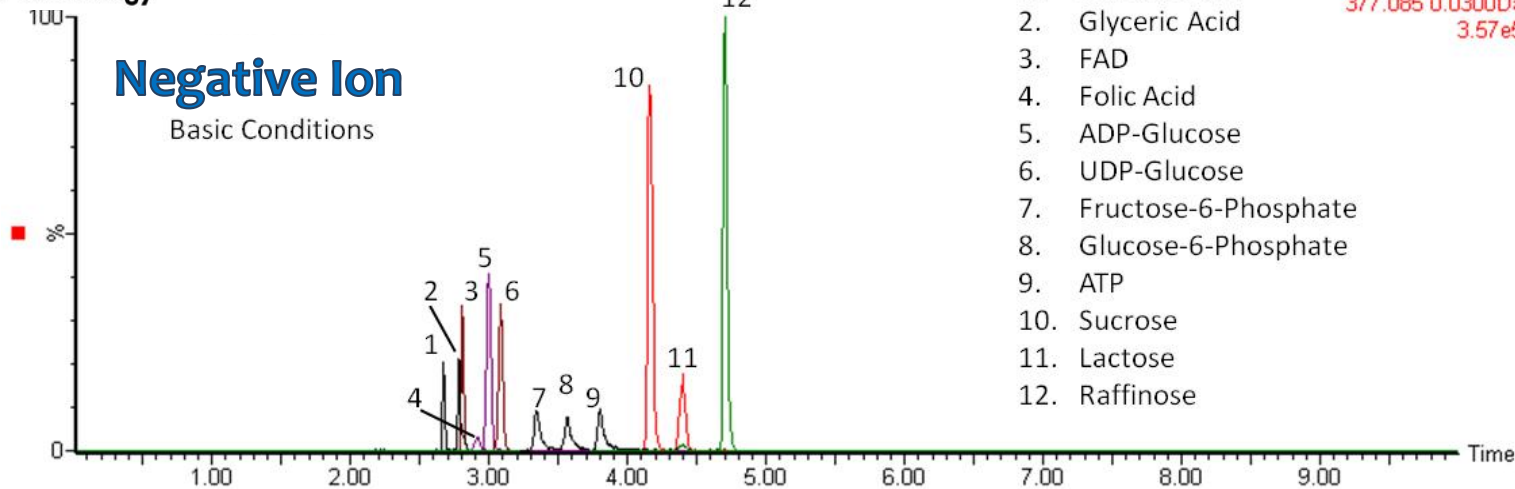
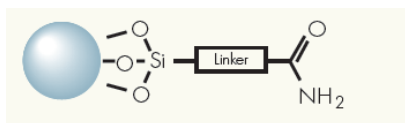
Non-Targeted Metabolomics/Lipidomics



Why Chromatographic Separation?

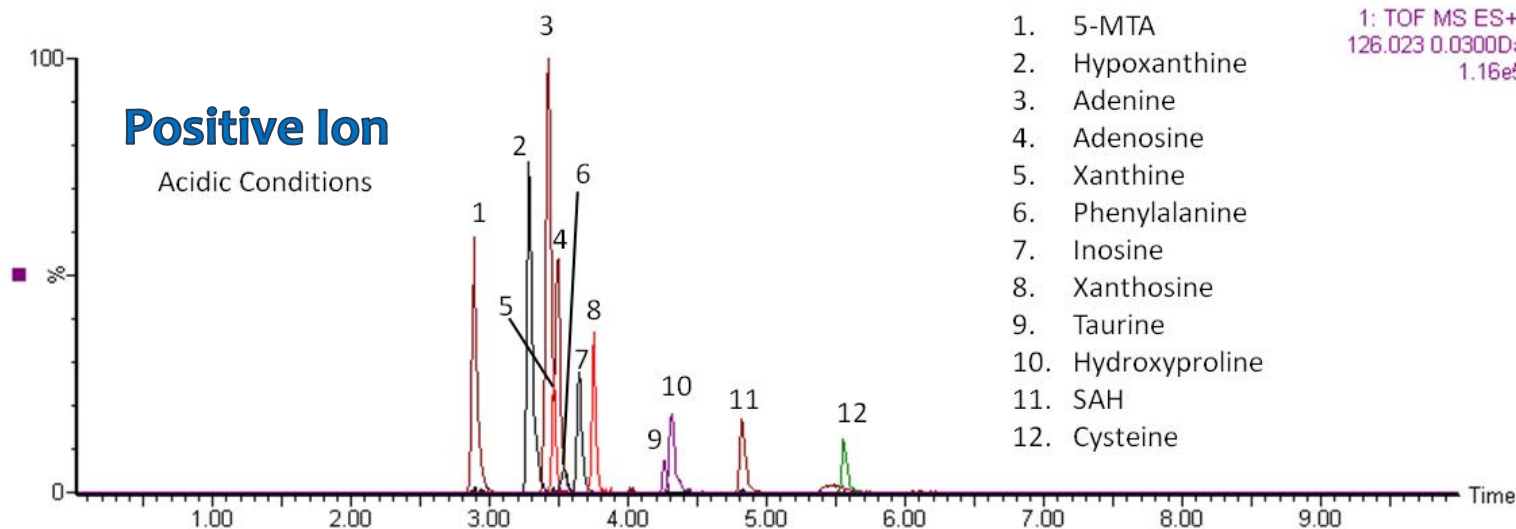
- Reduces ion suppression
- Separates many isobaric interferences
- Often separates isomers
- Better detection limits
 - Concentration effects
 - Reduced background noise
- Records a physiochemical property

Untargeted Metabolomics: Separate Polar Metabolites - HILIC



1. Succinic Acid
2. Glyceric Acid
3. FAD
4. Folic Acid
5. ADP-Glucose
6. UDP-Glucose
7. Fructose-6-Phosphate
8. Glucose-6-Phosphate
9. ATP
10. Sucrose
11. Lactose
12. Raffinose

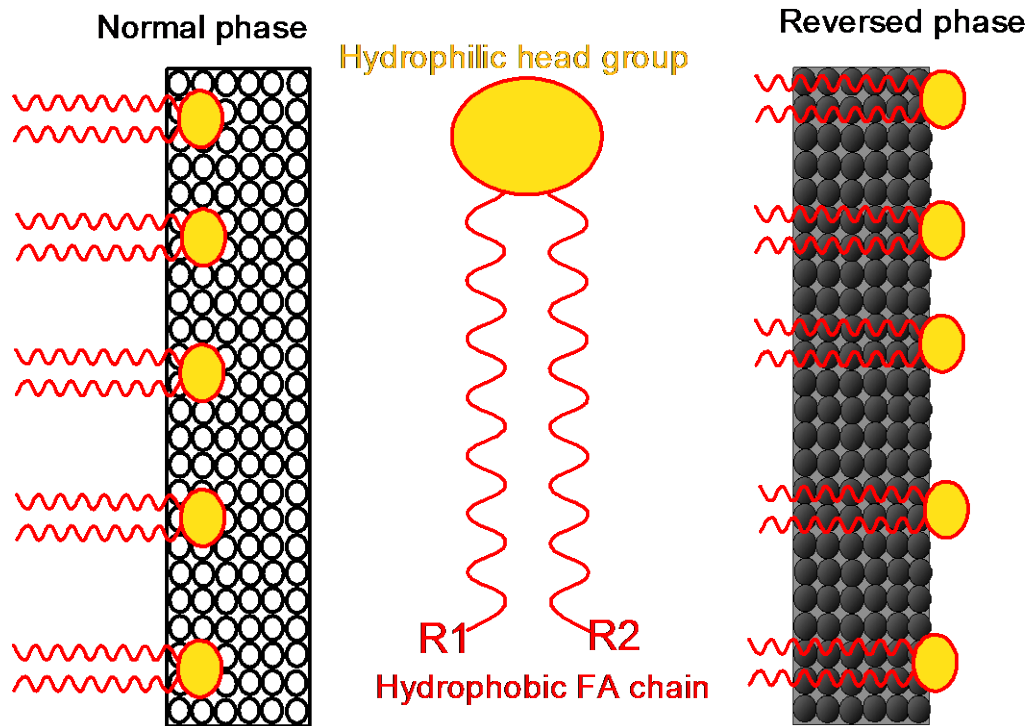
1: TOF MS ES-
377.085 0.0300Da
3.57e5



1. 5-MTA
2. Hypoxanthine
3. Adenine
4. Adenosine
5. Xanthine
6. Phenylalanine
7. Inosine
8. Xanthosine
9. Taurine
10. Hydroxyproline
11. SAH
12. Cysteine

1: TOF MS ES+
126.023 0.0300Da
1.16e5

Retention Mechanisms for Lipids

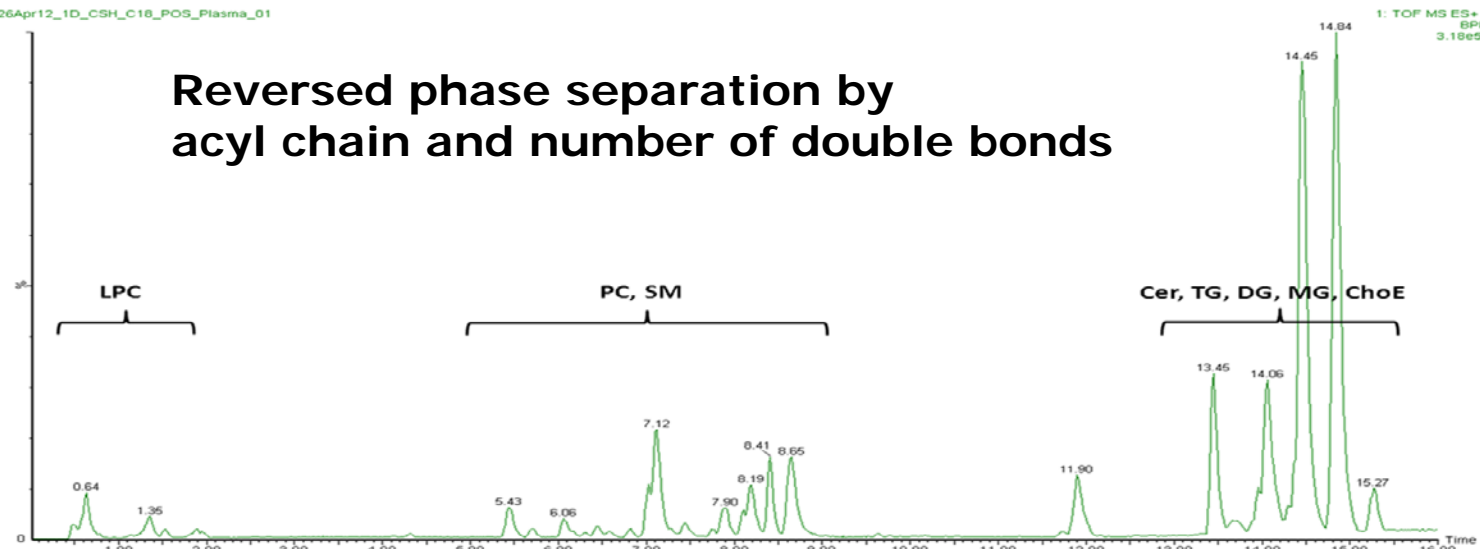


- ❑ Bare silica or silica bonded to polar group such as cyano, amino, PVA
- ❑ Non polar MP Hex, chloroform
- ❑ Separation based on **adsorption of the head group to the NP material** for lipid class separation.

- ❑ Silica bonded to nonpolar group such as C18, C8, C4
- ❑ Polar MP water, MeOH, ACN
- ❑ Separation based on **hydrophobic interaction of the FA chain and RP material** for lipid molecular species separation.

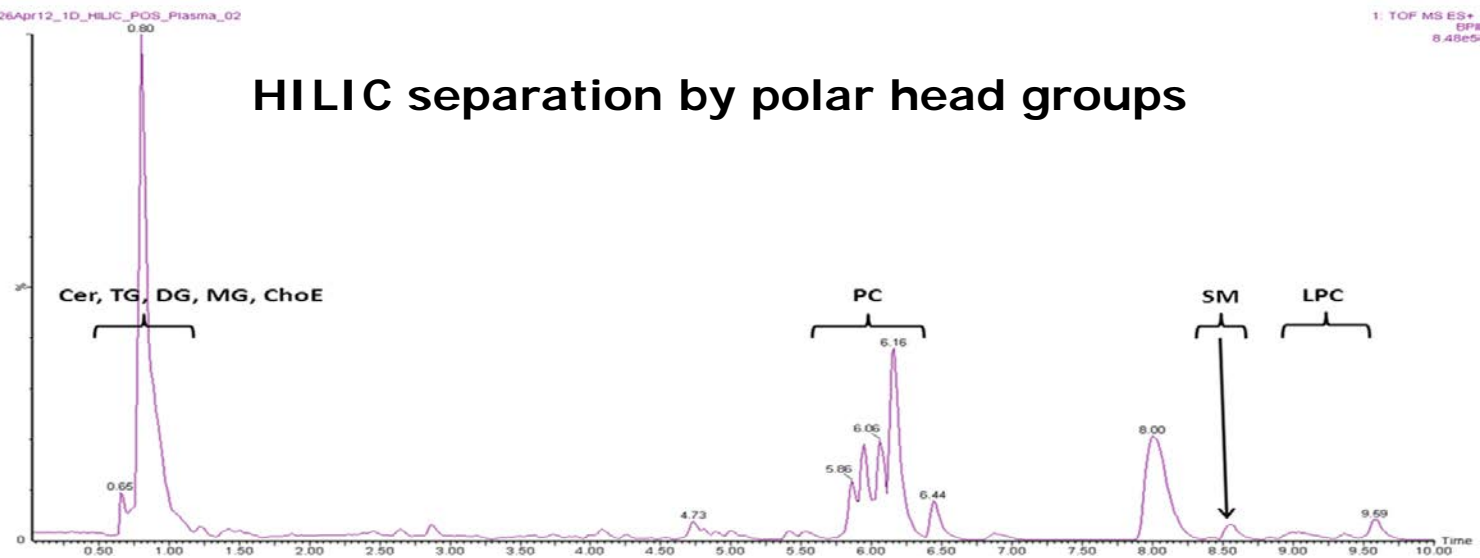
26Apr12_1D_CSH_C18_POS_Plasma_01

Reversed phase separation by
acyl chain and number of double bonds



26Apr12_1D_HILIC_POS_Plasma_02

HILIC separation by polar head groups

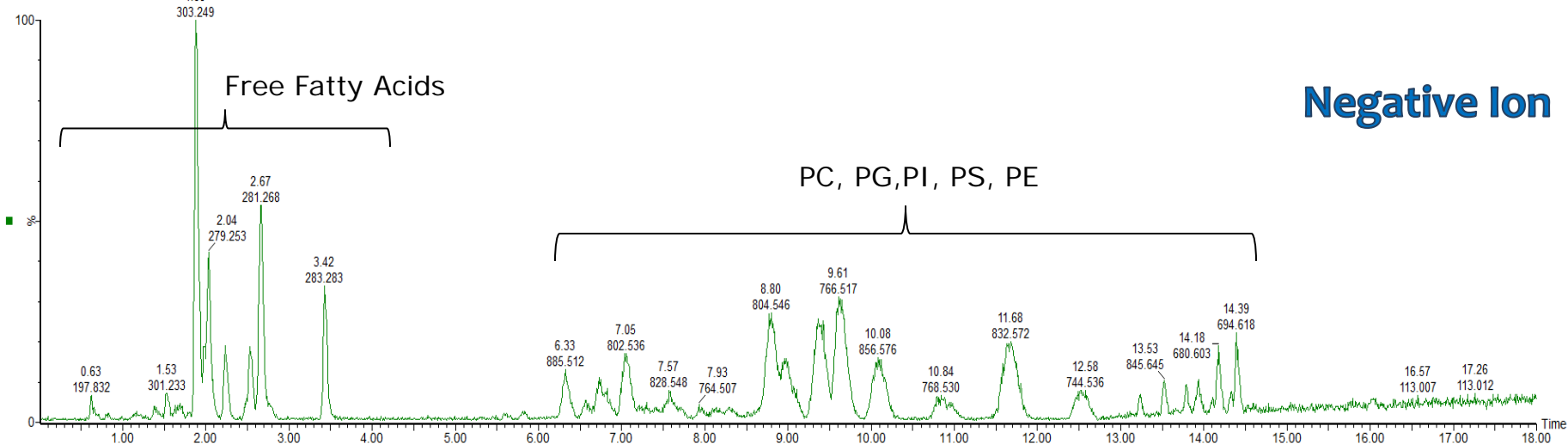
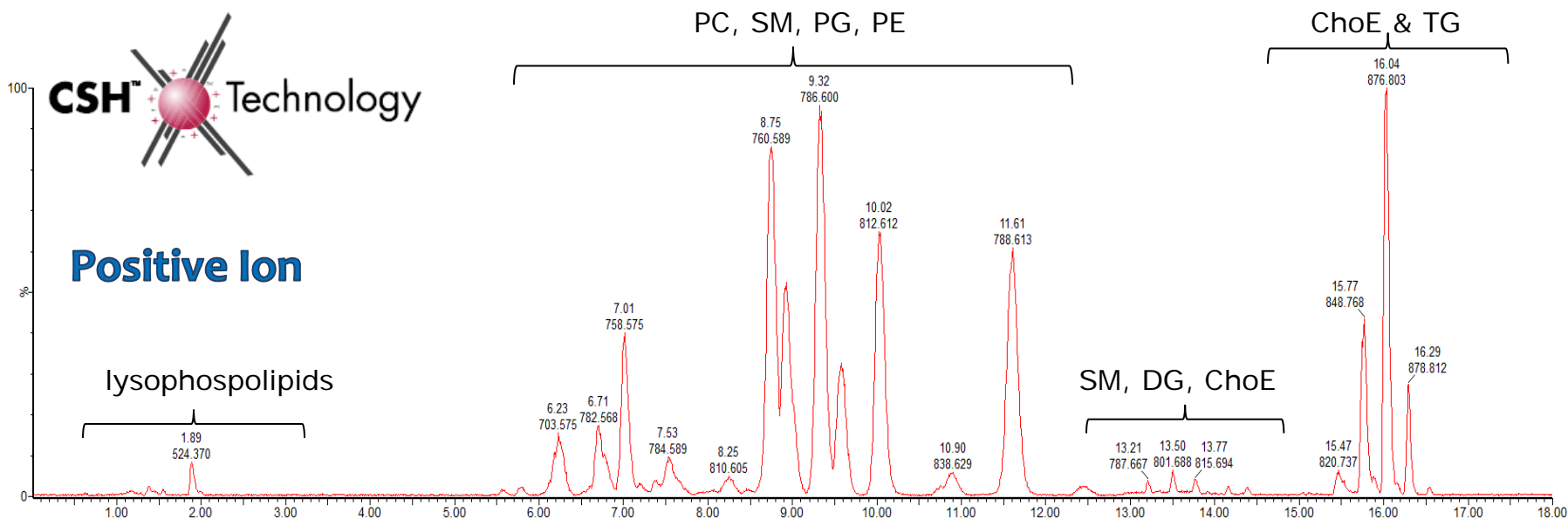


ACQUITY UPLC with *CSH C₁₈* liver extract

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Positive Ion

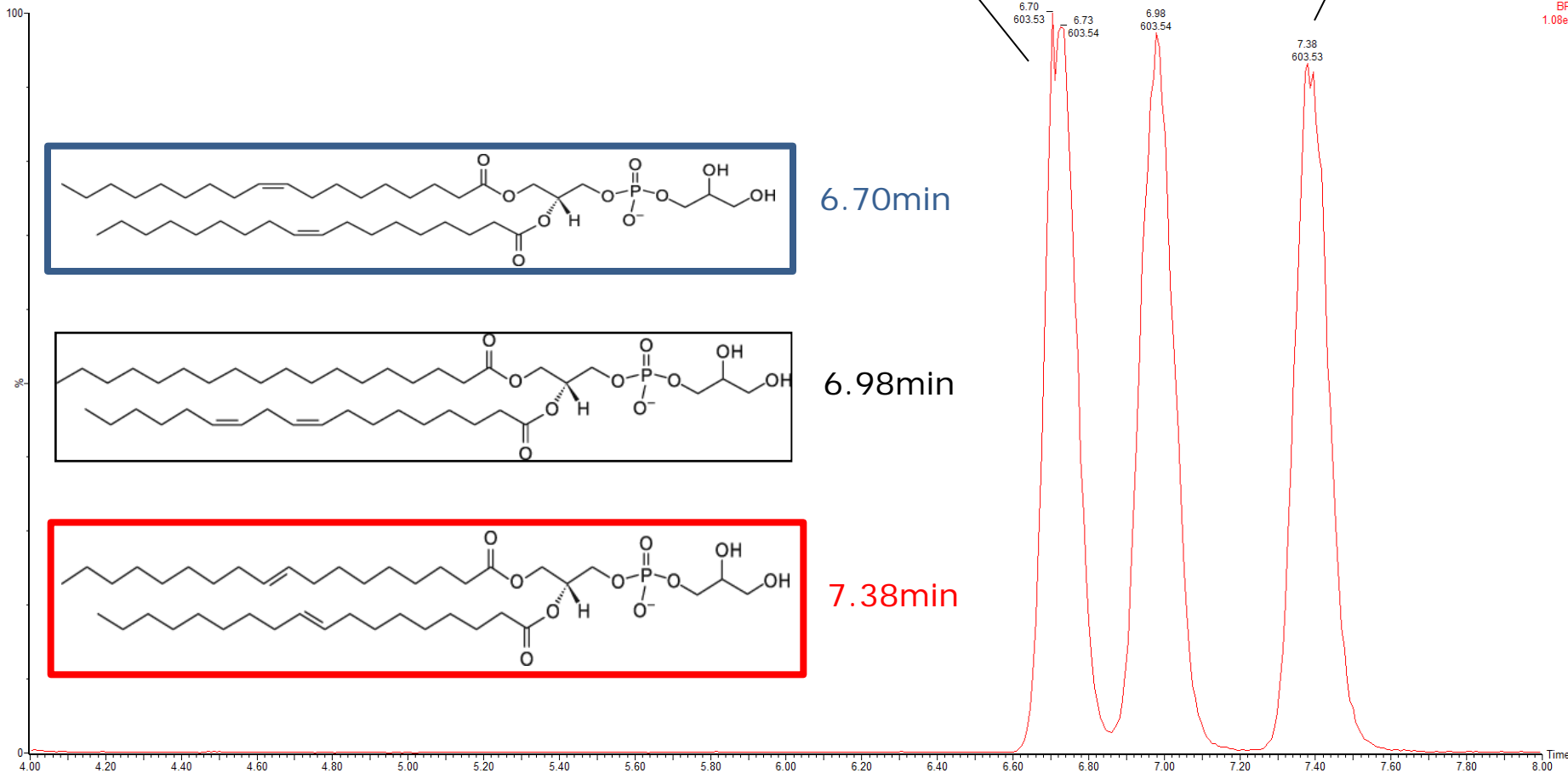


Negative Ion

Isomers Separation: CSH C₁₈



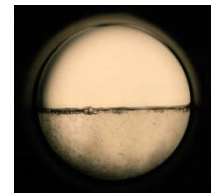
PG and LPG mix_0.01ug/uL_seeNB46
20110708_Pos_PG mix_CSH_MS_e_178



Convergence Chromatography

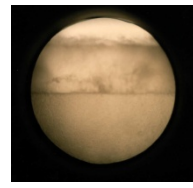


What is a Supercritical Fluid?



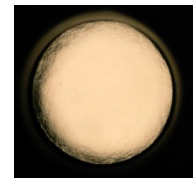
Liquid/gas

Increase temp and pressure



Critical point

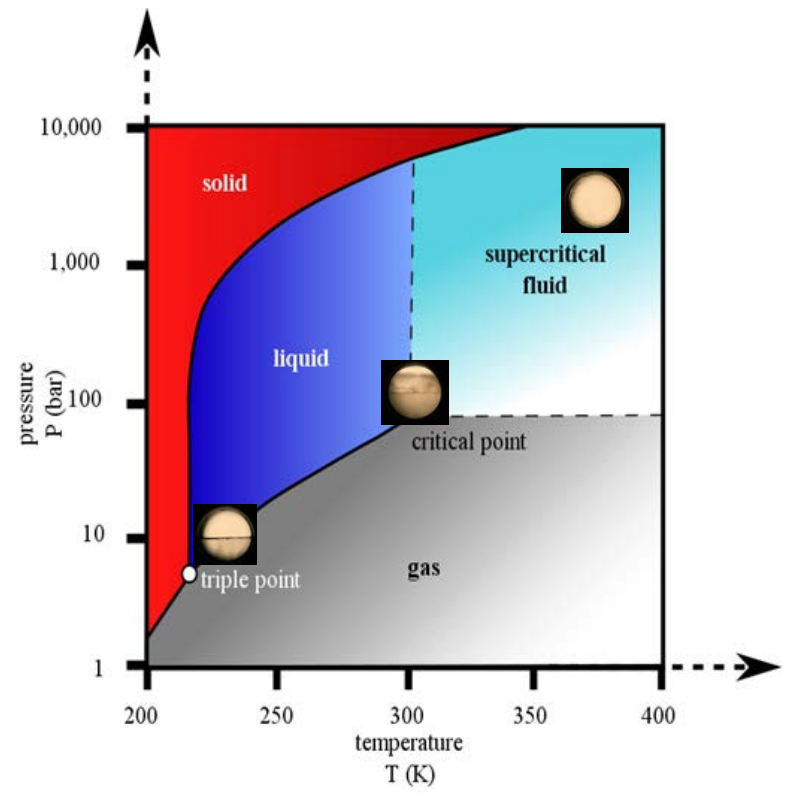
Increase temp and pressure



Supercritical fluid

	Diffusivity (cm ² /s)	Viscosity (g/cm x s)
Gas	10 ⁻¹	10 ⁻⁴
Supercritical Fluid	10 ⁻⁴ - 10 ⁻³ Liquid Like	10 ⁻⁴ - 10 ⁻³ Gas Like
Liquid	< 10 ⁻⁵	10 ⁻²

High diffusivity, and low viscosity result in **fast, efficient chromatography**



What is UPC²?

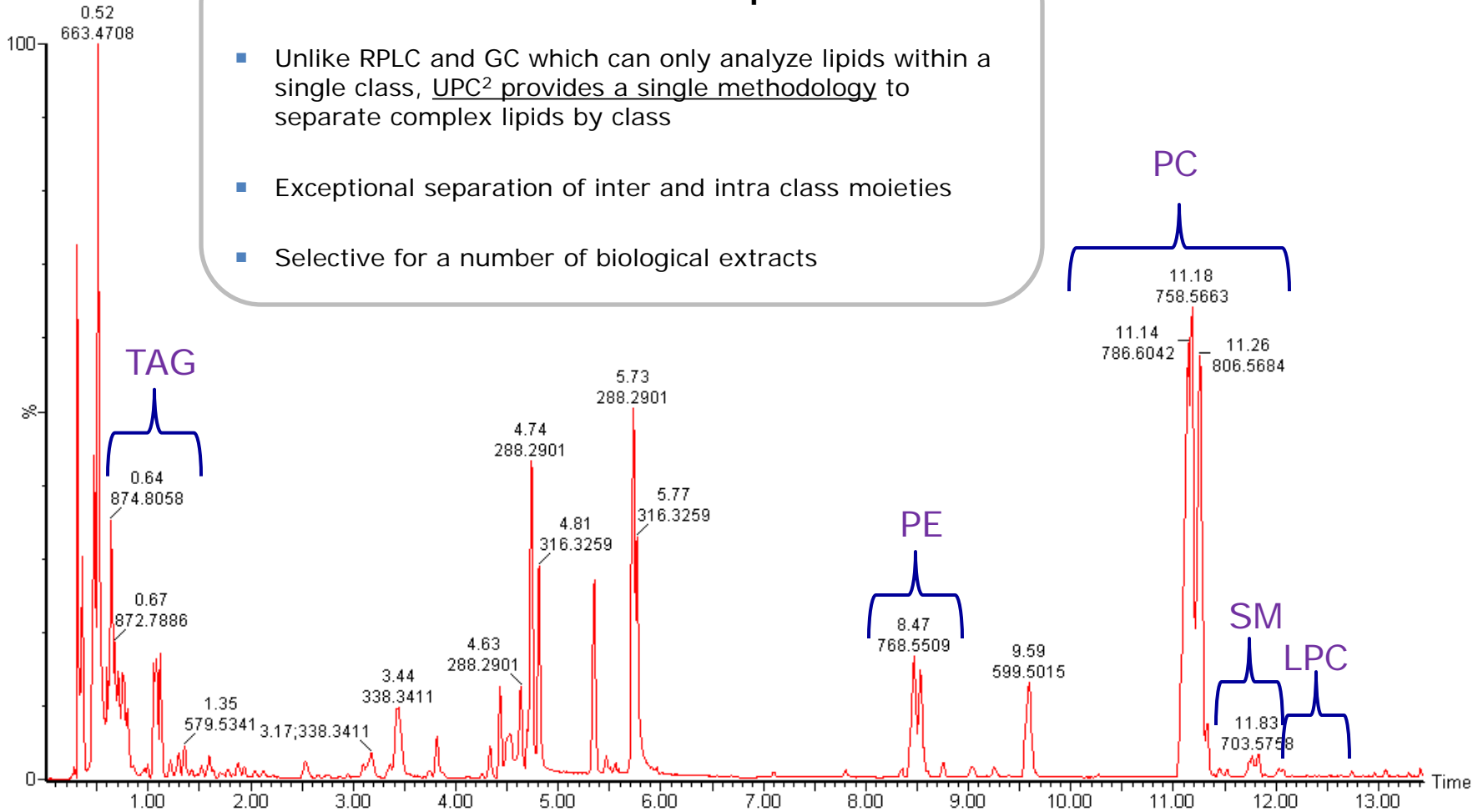
- Same as SFC but uses sub-2 μ m particle to increase chromatographic performance such as
 - Speed of separation
 - Peak capacity
 - Complements to MS due to its low solvent load
- Uses CO₂ as a major solvent and
- Uses co-solvents such as methanol to vary the mobile phase strength



Convergence Chromatography: Applied to Neutral and Polar Lipids

Neutral and Polar Lipids

- Unlike RPLC and GC which can only analyze lipids within a single class, UPC² provides a single methodology to separate complex lipids by class
- Exceptional separation of inter and intra class moieties
- Selective for a number of biological extracts

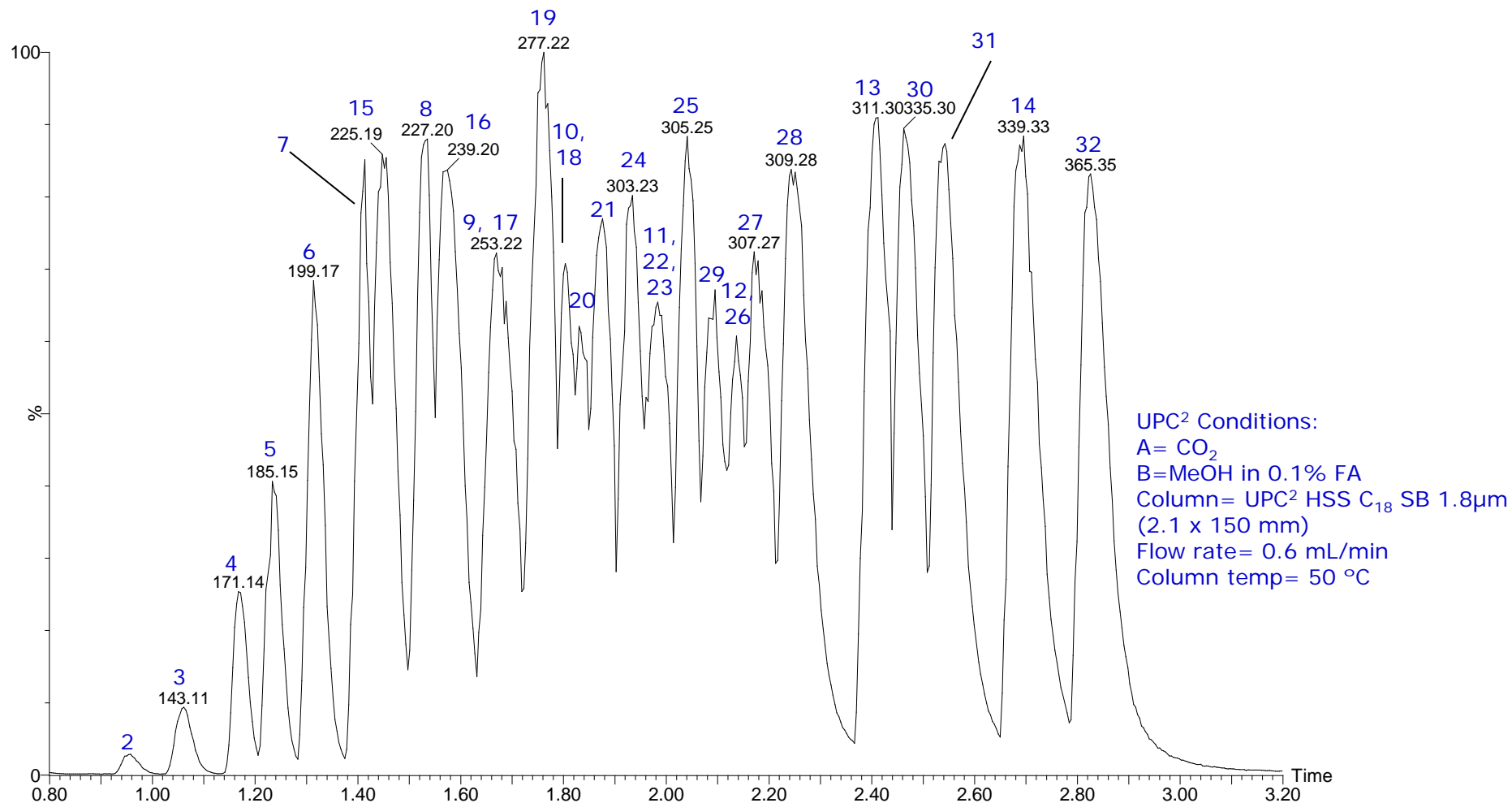


TAG: Triacylglycerides
SM: Sphingomyelin

PE: Phosphatidylethanolamine
LPC: Lysophosphatidylcholine

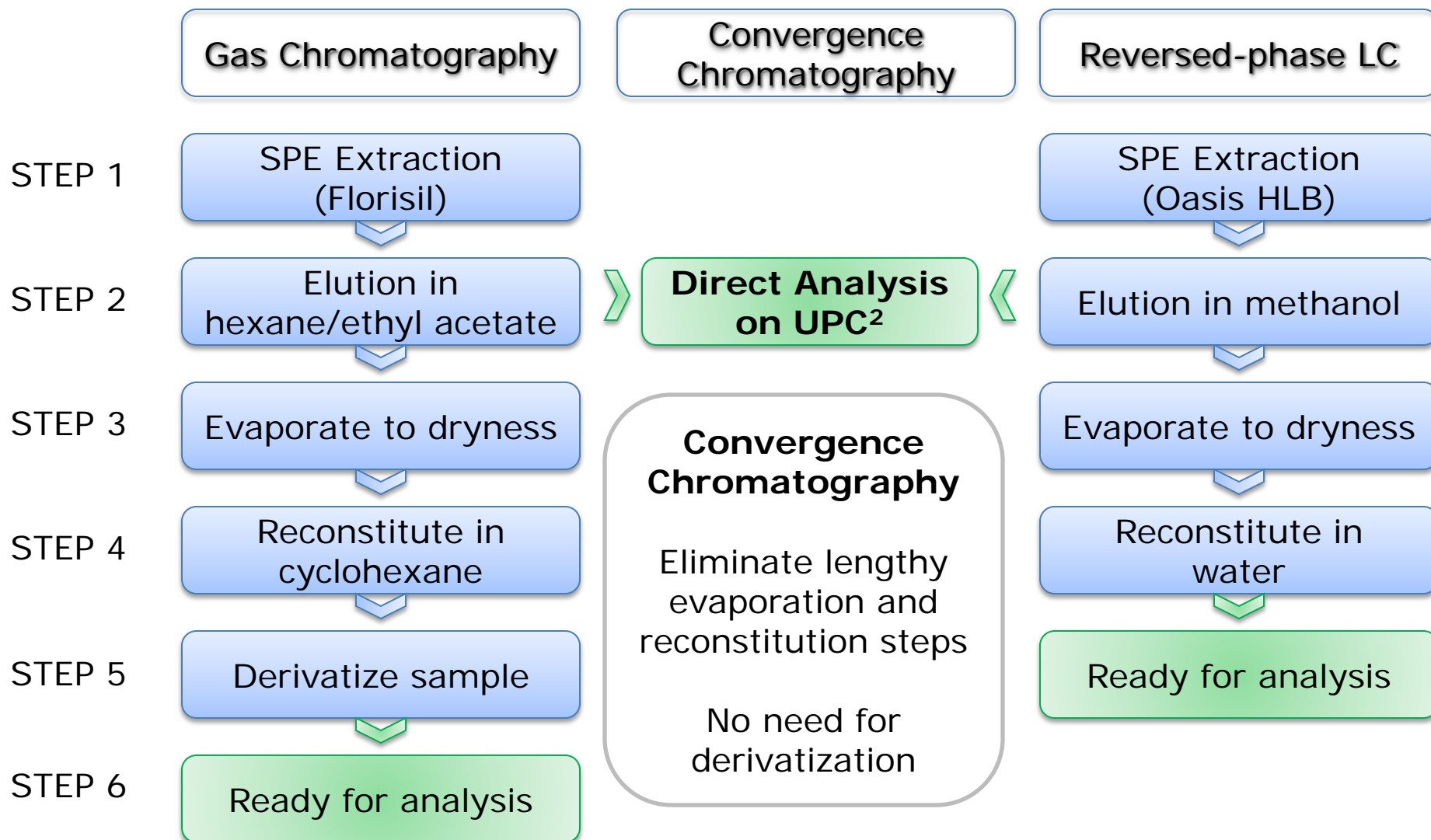
PC: Phosphatidylcholine

Fatty Acid Standard Mixture



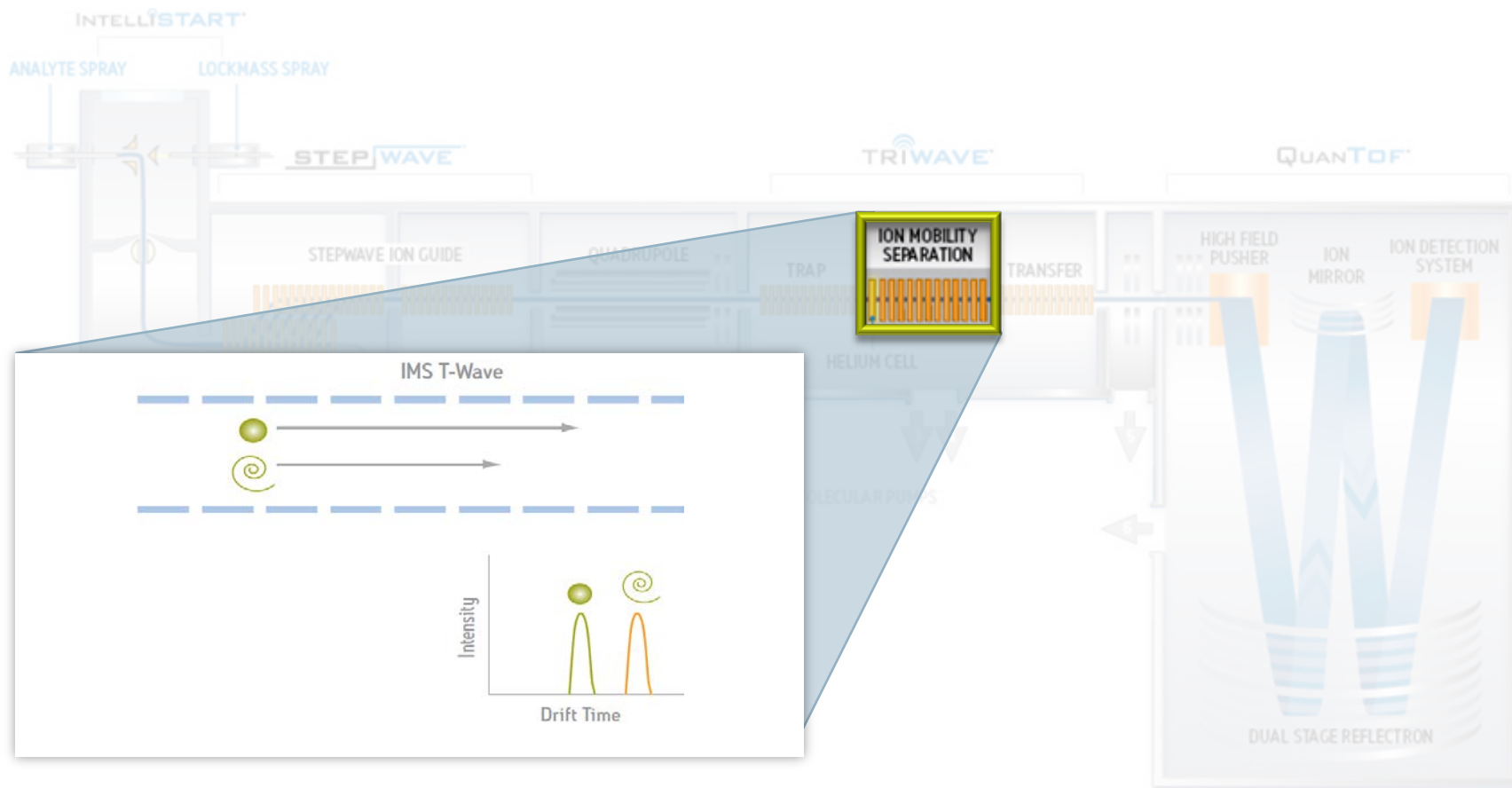
The separation of complex standard mixture that contain saturated, unsaturated, short and long chain FFA (32 different species).

Improving Workflow with Convergence Chromatography



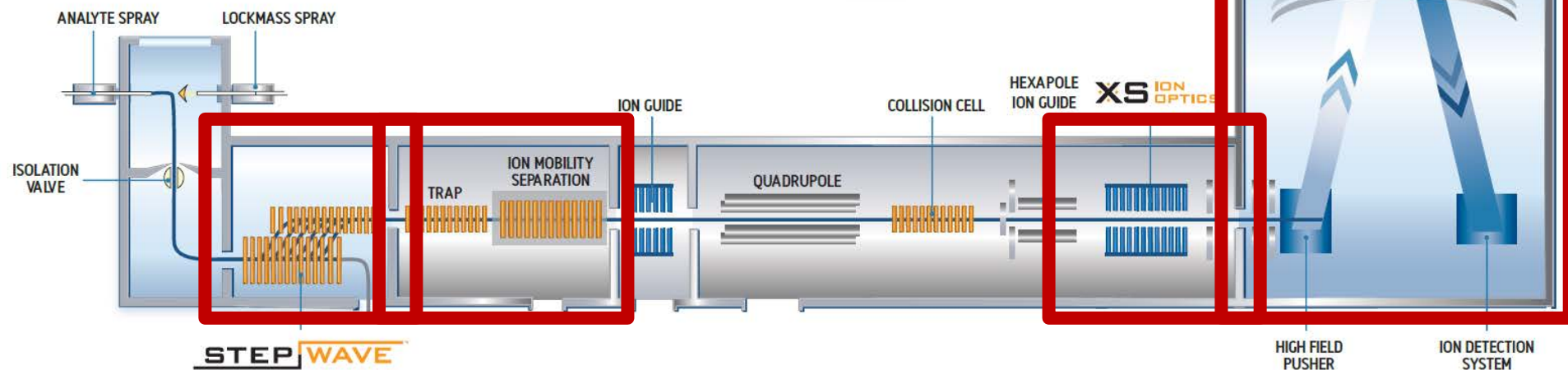
Mass Spectrometry

Synpat G2-Si



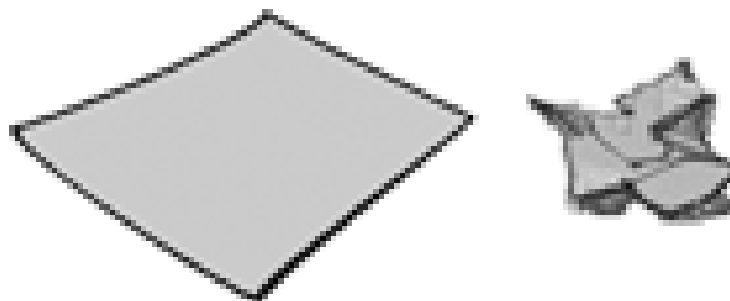
Vion™ IMS QTof

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What is Ion Mobility?

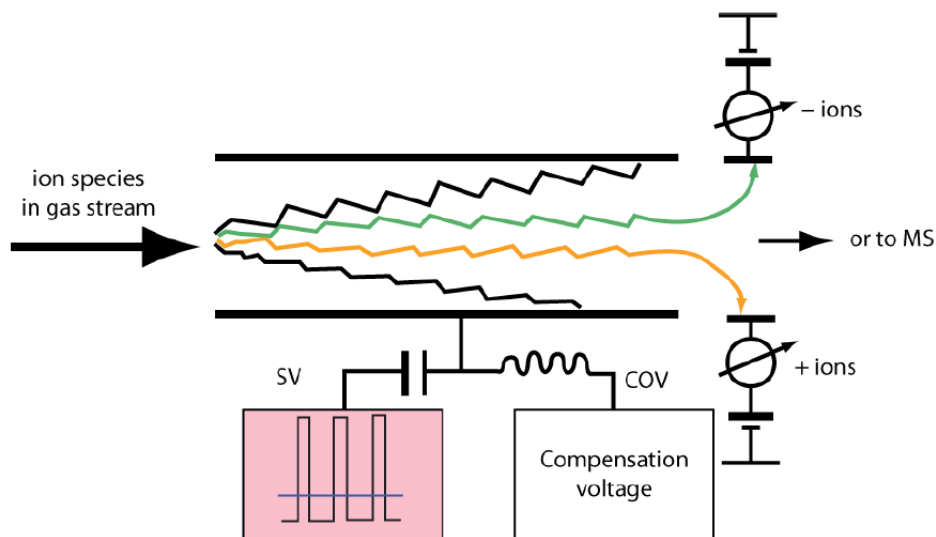
- Measuring the ion mobility of an ion;
 - Can yield information about its structure as small, compact, ions drift quicker than large extended ions
 - Introduce an additional dimension of sample separation to complex mixtures
- Similar to the effect that causes an extended paper towel to drift to the ground much more slowly under the influence of gravity and air resistance than a crushed towel of the same mass



Different types of Ion Mobility (integrated with MS)

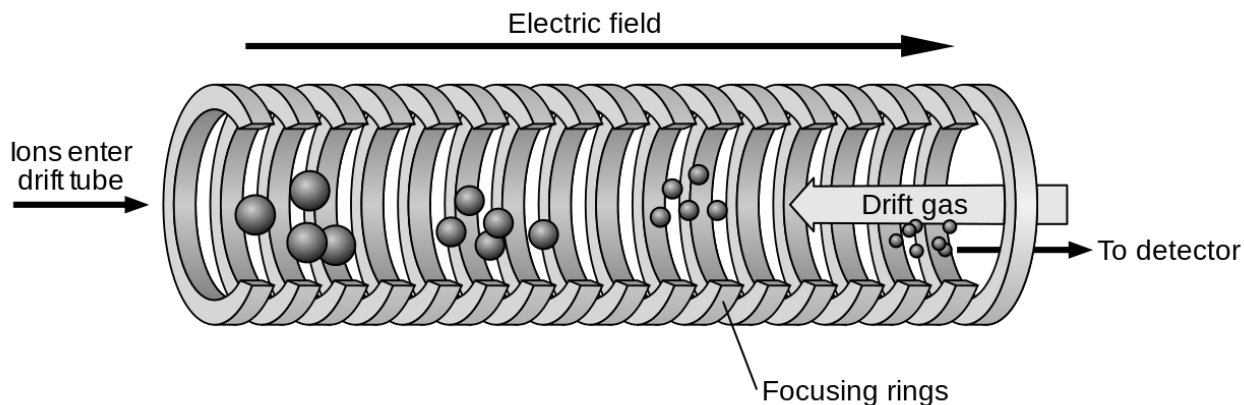
■ Filtering devices

- Differential Ion Mobility
- SelexIon (SCIEX)
- FAIMS (Thermo)
- Front end



■ Sorting devices

- Drift Tube Ion Mobility (Agilent, Bruker)
- Traveling Wave Ion Mobility (Waters)
- Integrated



Different types of Ion Mobility

■ Filtering devices

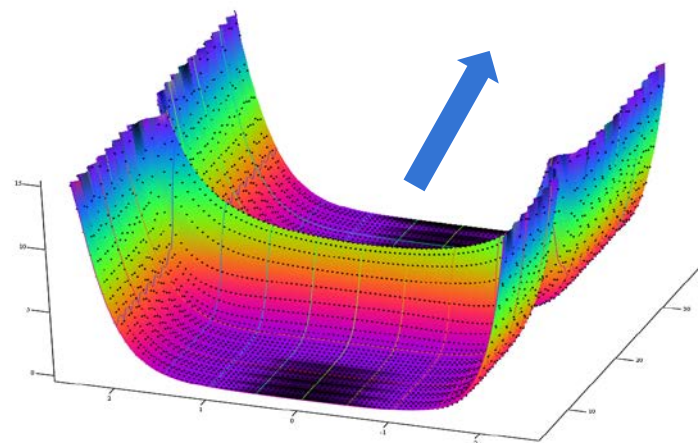
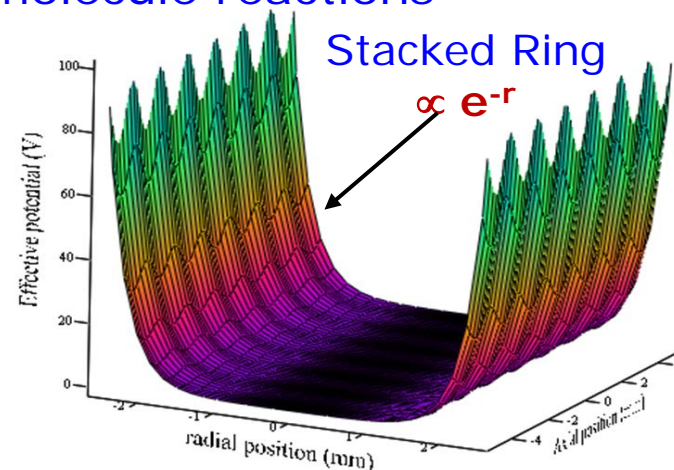
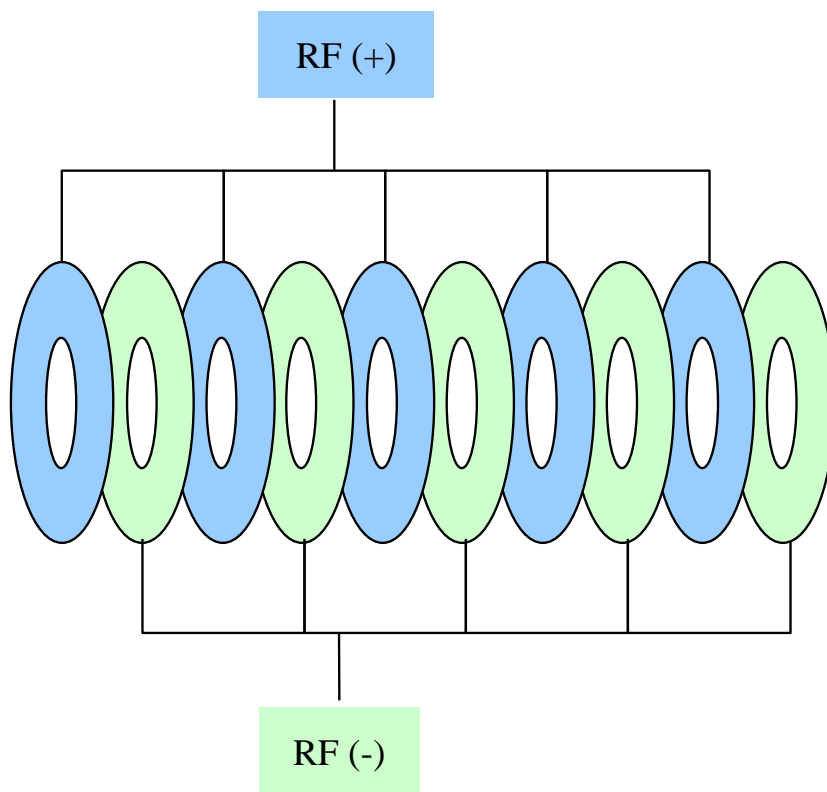
- Good for target analysis where a method (CV & modifier) can be selected.
- Good separation but only for specific compounds
- Not suitable for untargeted analysis of unknowns
- Sensitivity increases only through selectivity
- Duty cycle suited to low numbers of target compounds
- No CCS values

■ Sorting devices

- Good for untargeted analysis of unknowns
- Increases overall system peak capacity
- Duty cycle suited to full scan analysis
- Measures CCS

An RF-Only Stacked Ring Device

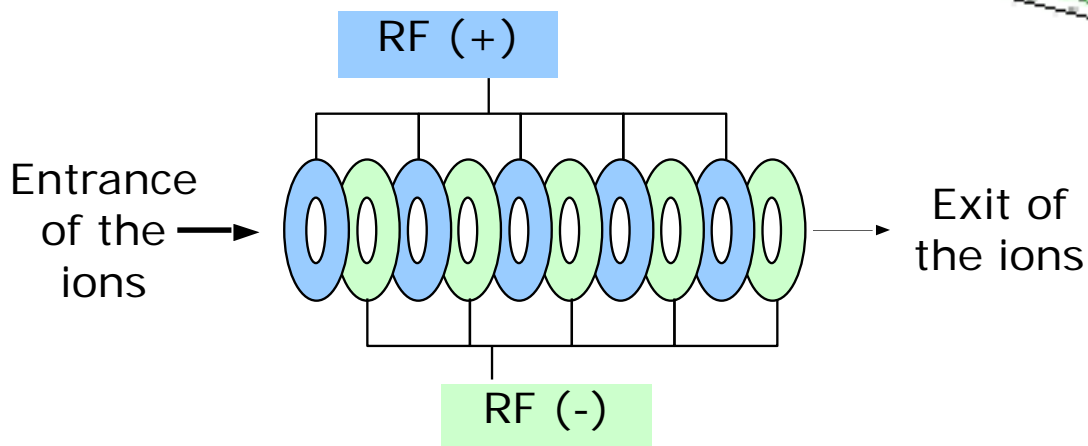
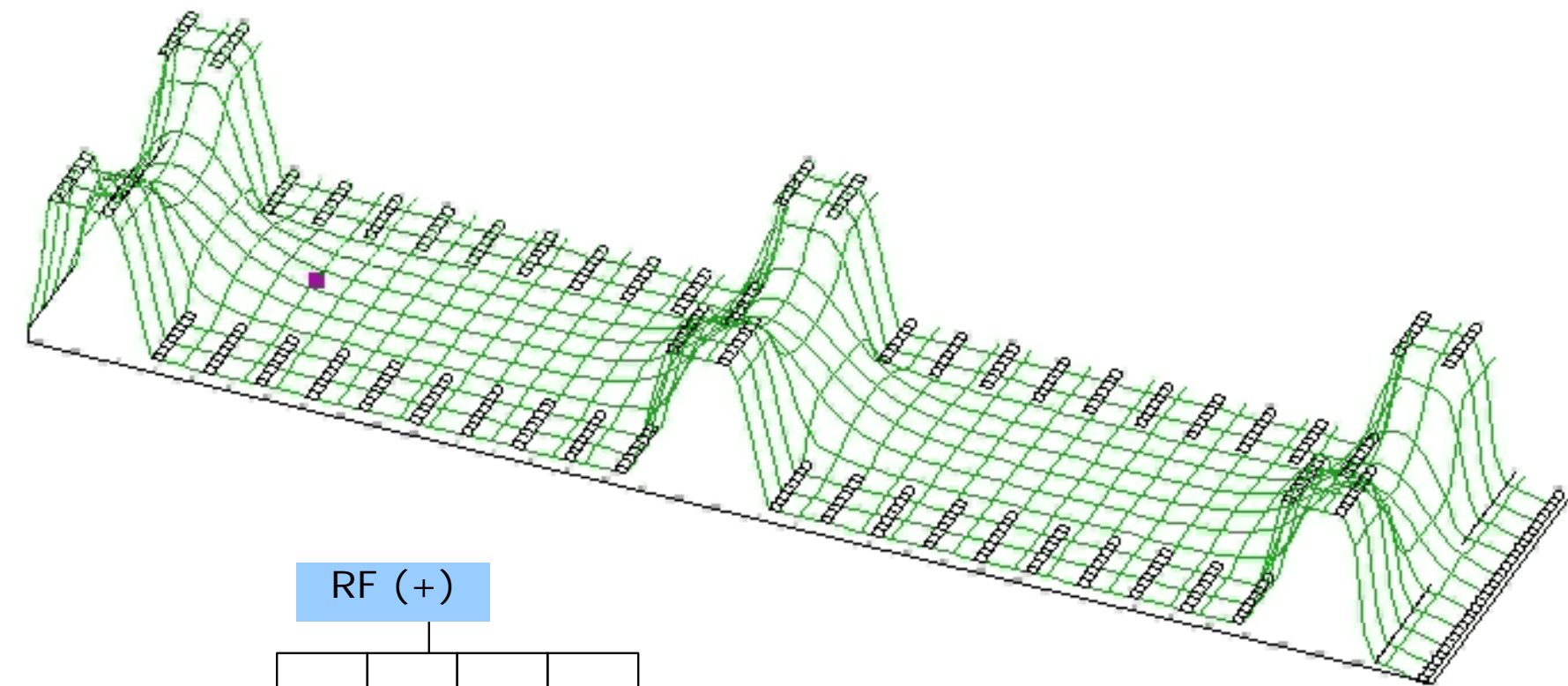
- Bahr, Gerlich and Telyo in 1969¹ and onward²
 - ion trapping device for studying ion-molecule reactions



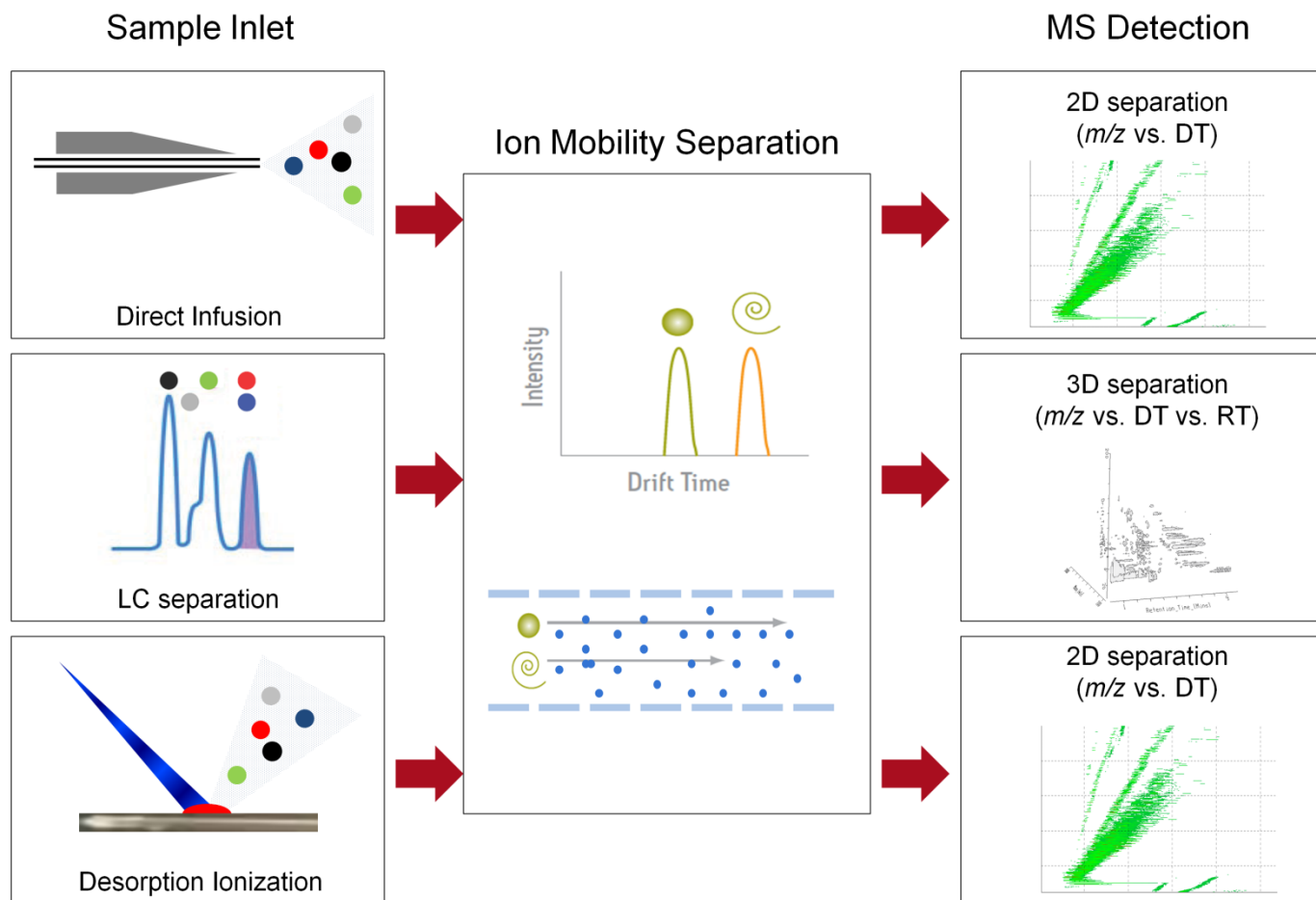
T-WAVE

1. Bahr R, Gerlich D, Telyo E. *Verhandl. DPG (VI)* 1969; **5**: 131
2. Gerlich D. *in State-Selected and State-to-State Ion-Molecule Reaction Dynamics, Part 1: Experiment*, Wiley: 1992

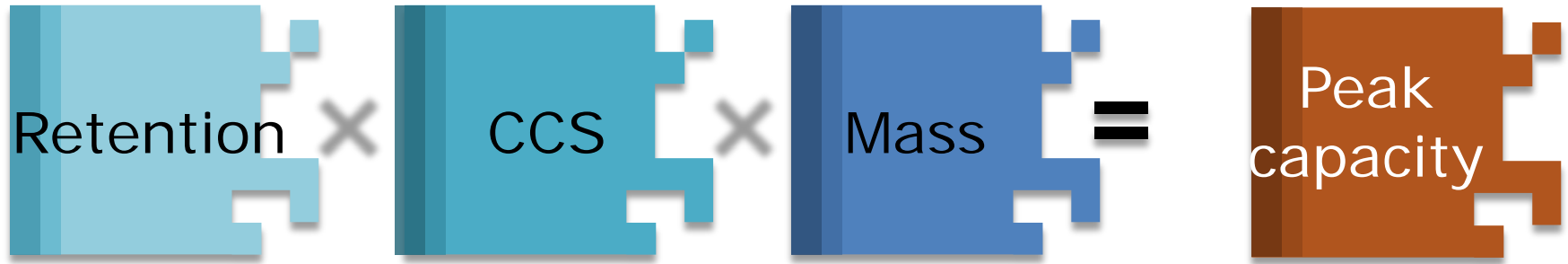
T-wave technology in SYNAPT mass spectrometer



Ion Mobility approaches in Metabolomics



System Peak Capacity



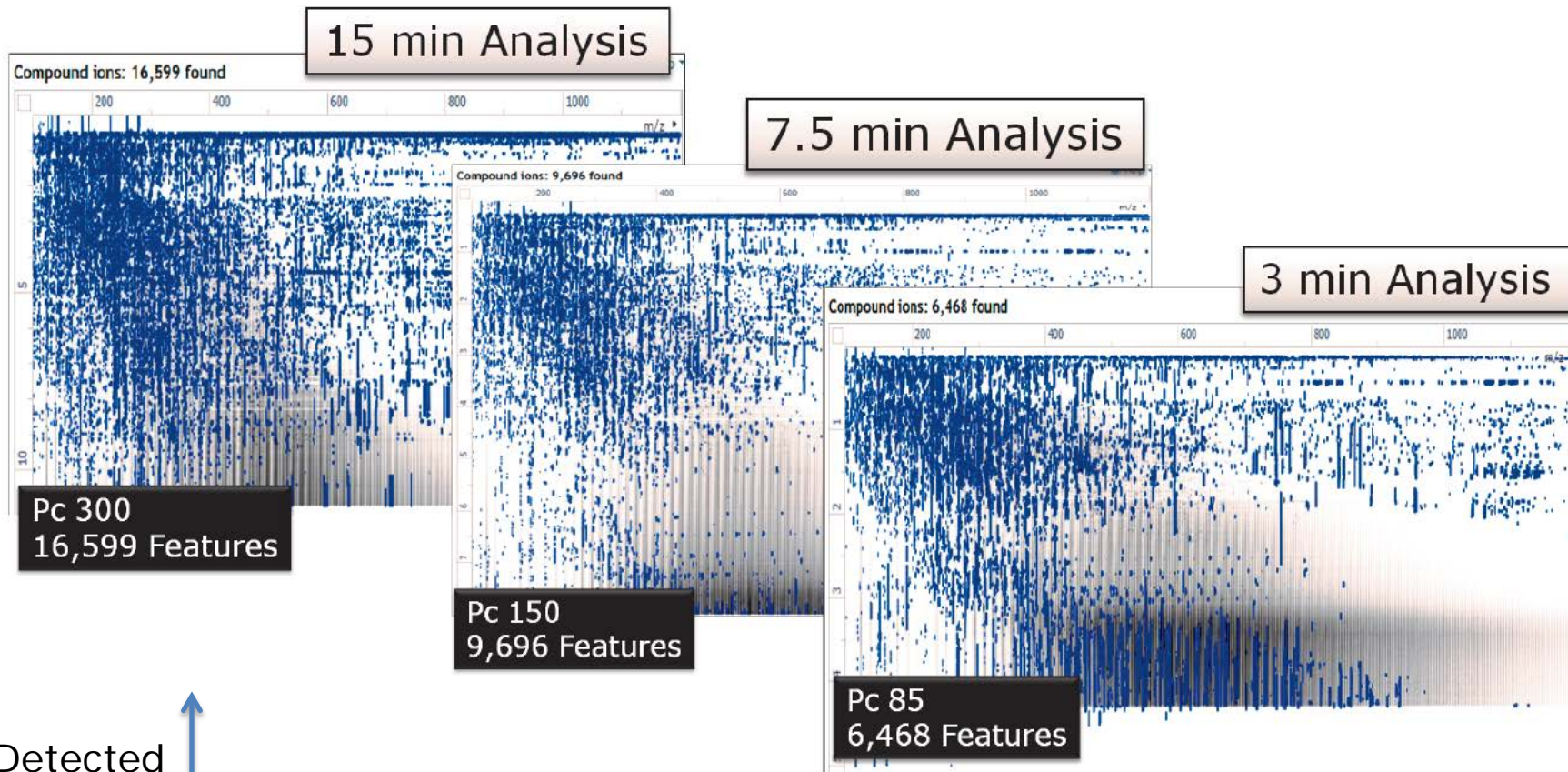
Acquity
UPLC®

T-WAVE™
ION MOBILITY
POWERED

QuanTof

UPLC-MS/MS

Separation Power

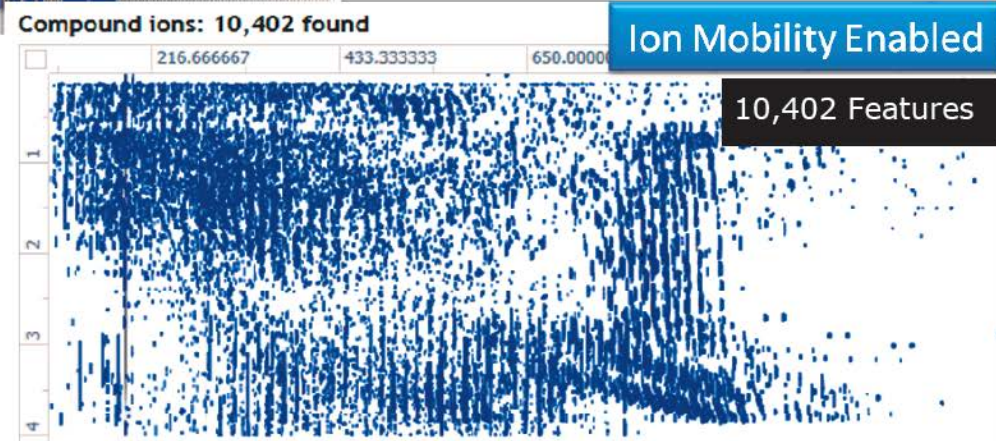
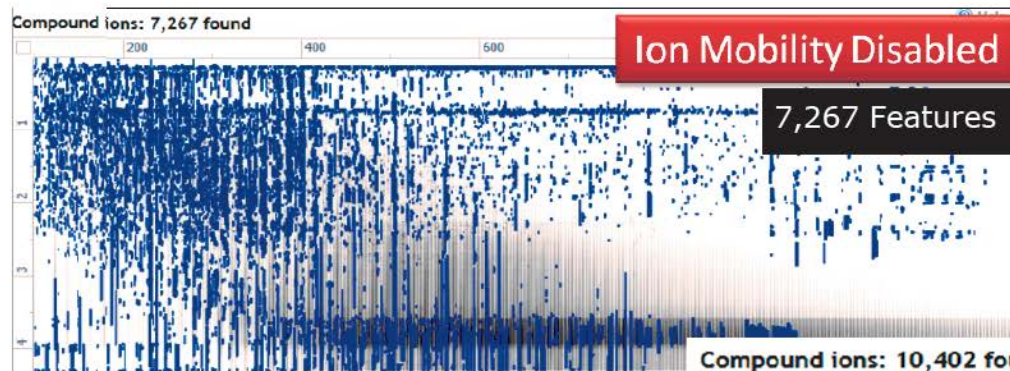


Detected Features

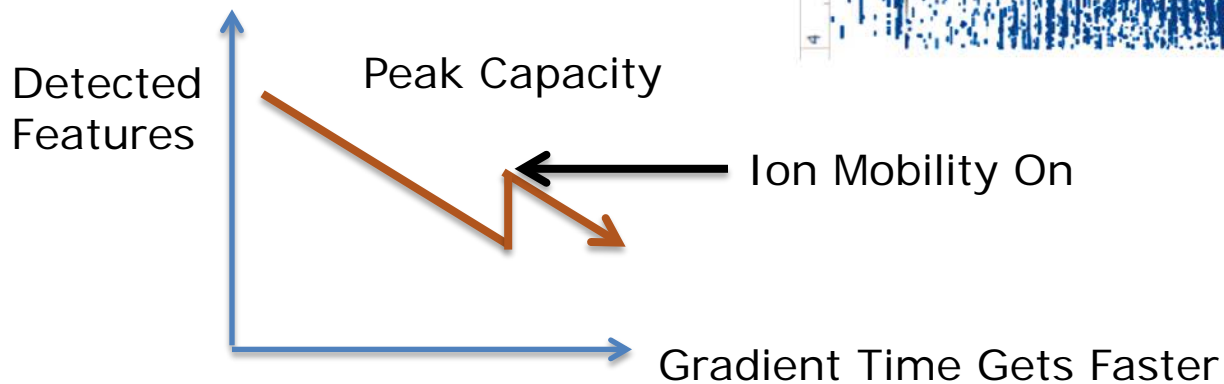
Peak Capacity

Gradient Time Gets Faster

Separation Power

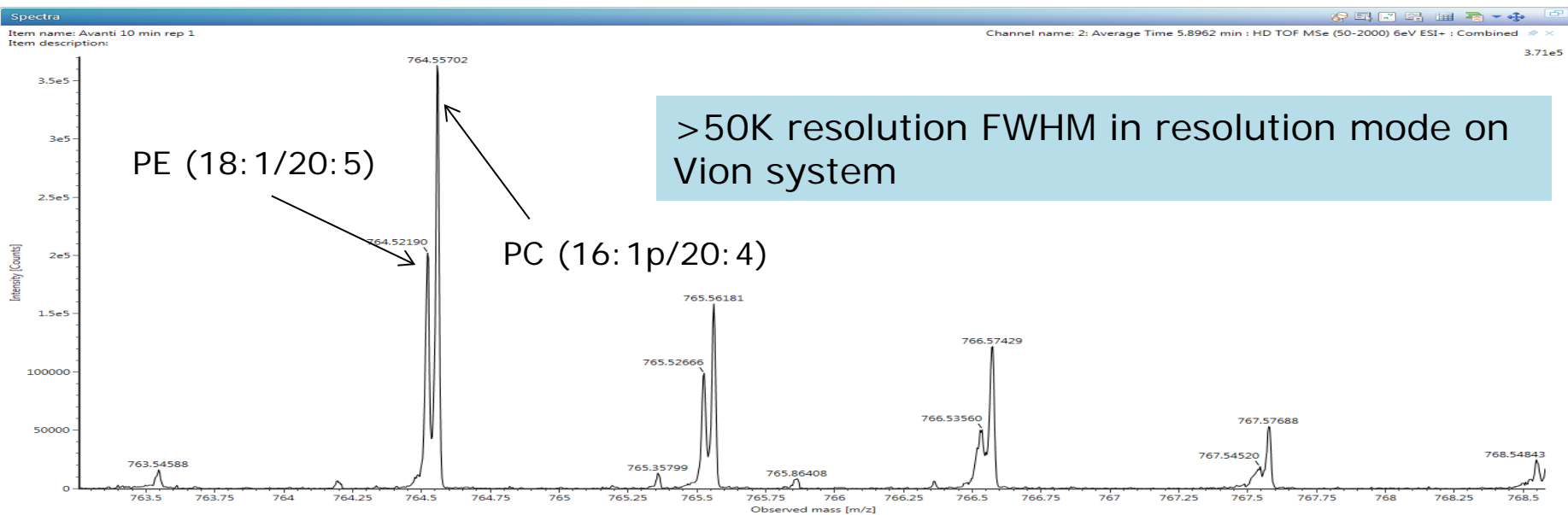
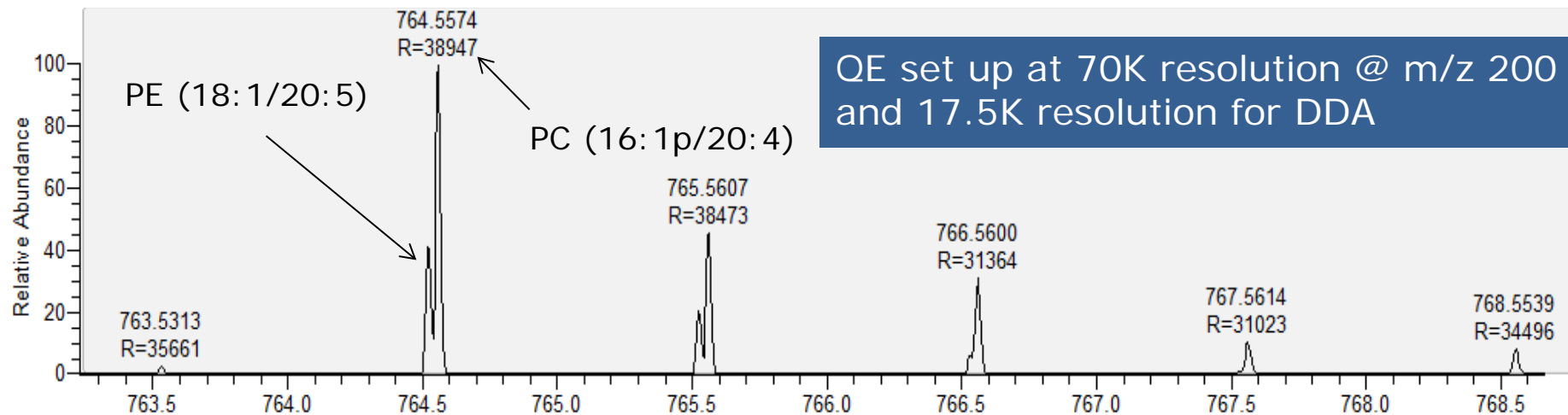


*3 minute UPLC/MS analysis
with 7.5cm column &
elevated linear velocity*



Ion Mobility Enhanced DIA Increases Information Content

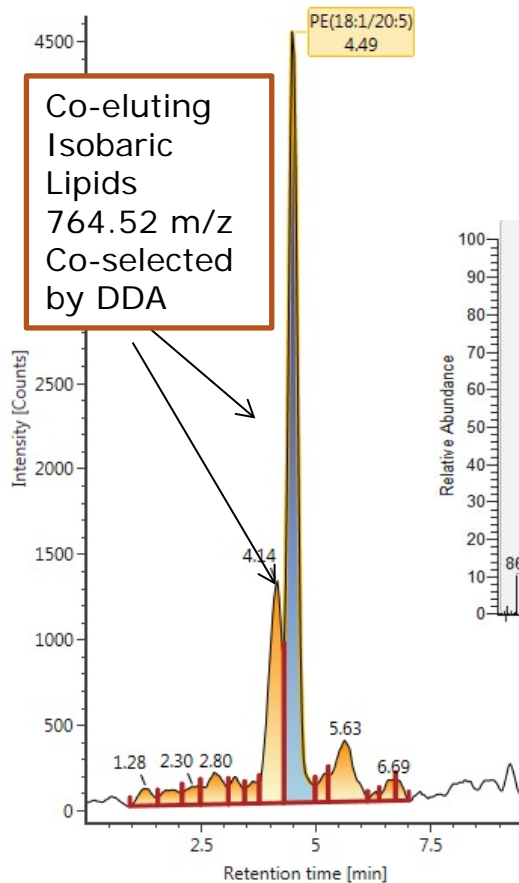
Spectra for closely co-eluting lipids from Liver Extract



Separation Power

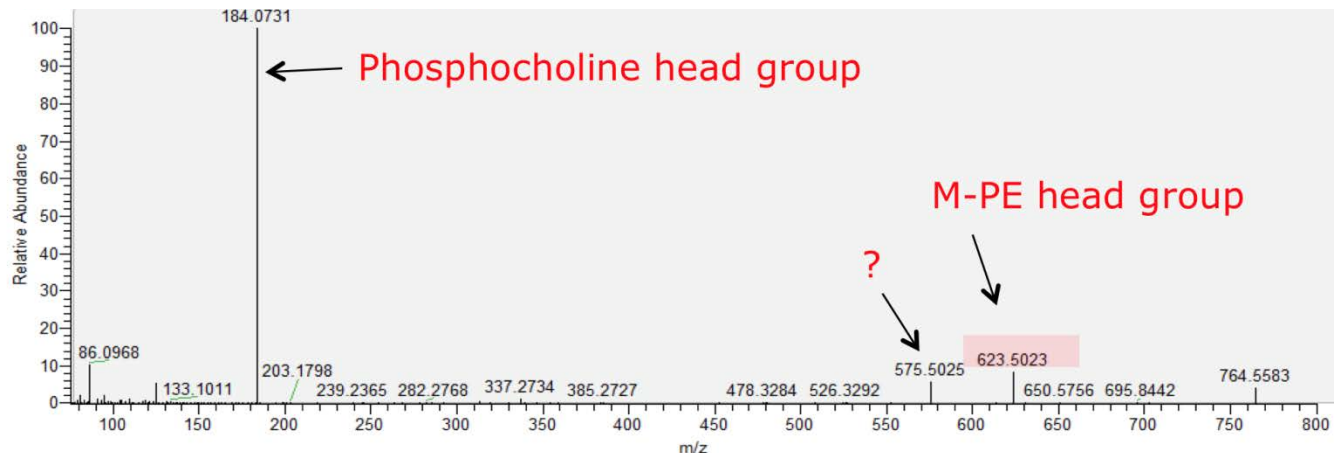
- HDMS^E Maximizing MS/MS content

Channel name: PE(18:1/20:5) [+H]: (17.8 PPM) 764.5214 ...

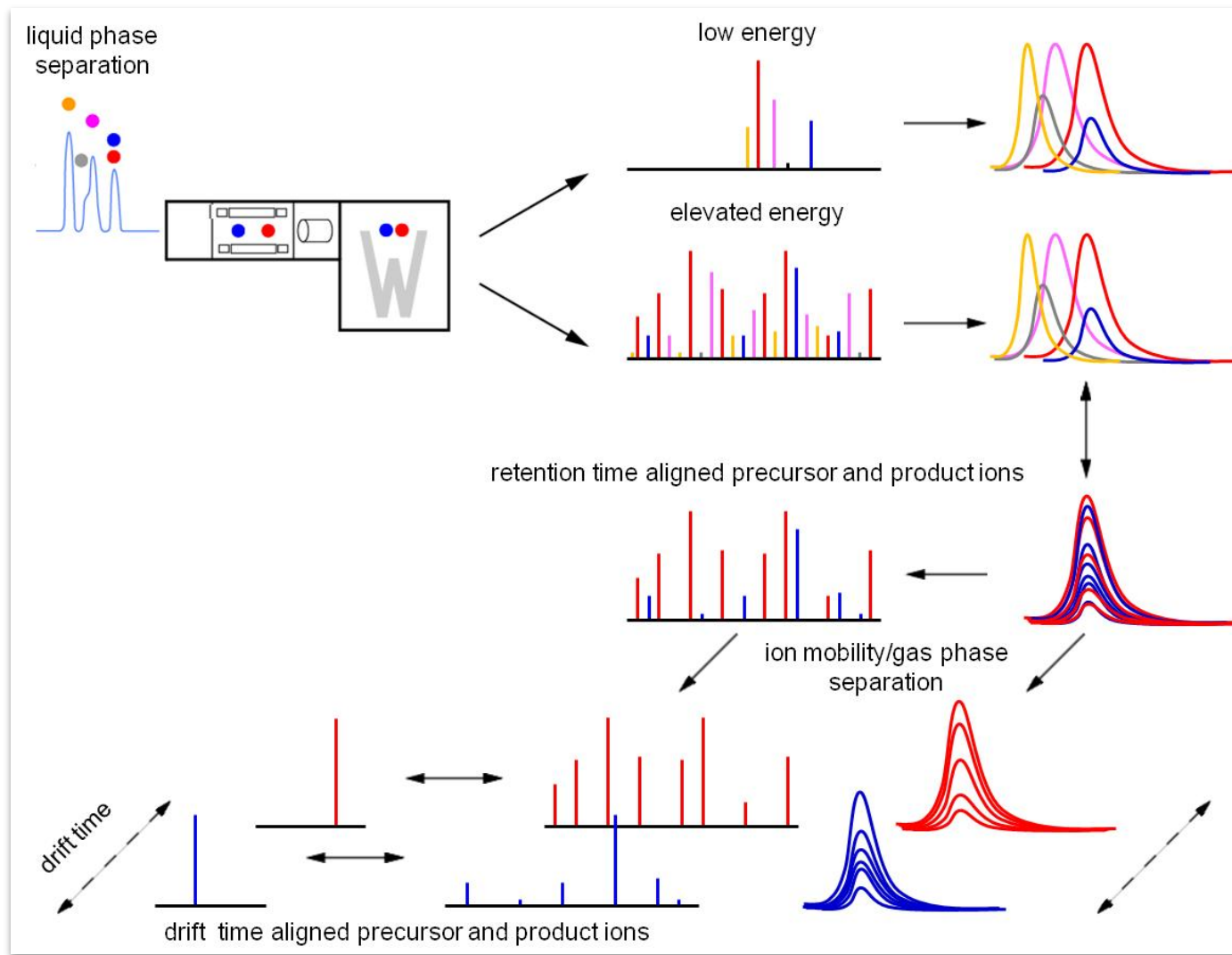


Co-eluting
Isobaric
Lipids
764.52 m/z
Co-selected
by DDA

Q-Exacte DDA Data – One MS/MS Spectrum



Retention and drift time separation ... LC-DIA-IM-MS (HDMSE^E)



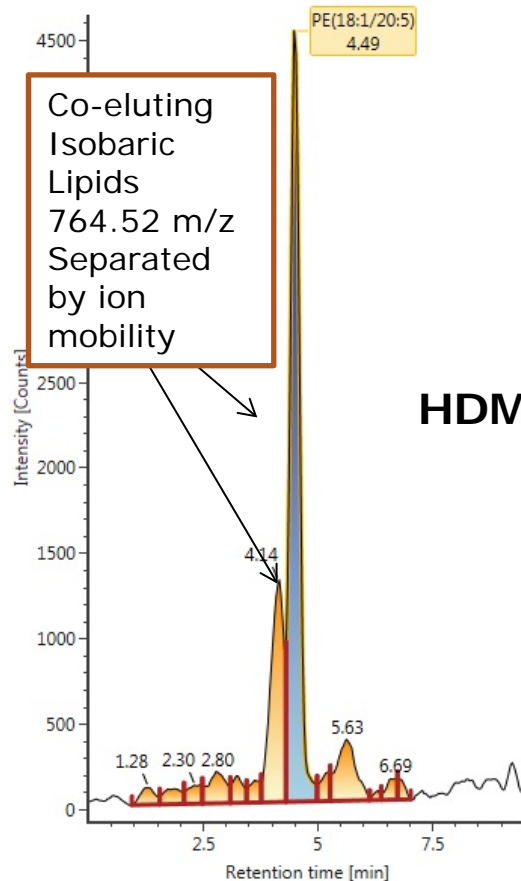
Separation Power

- HDMS^E Maximizing MS/MS content

Combination of Ion Mobility and HDMS^E extracts more discrete spectra than traditional MS/MS

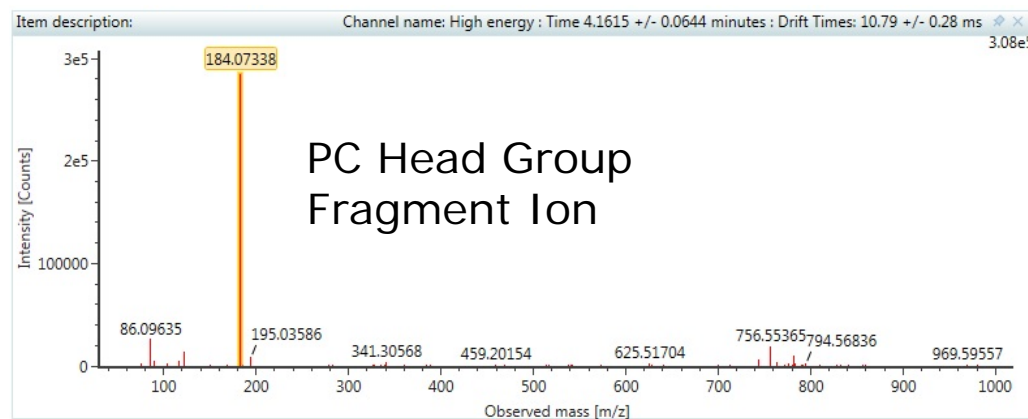
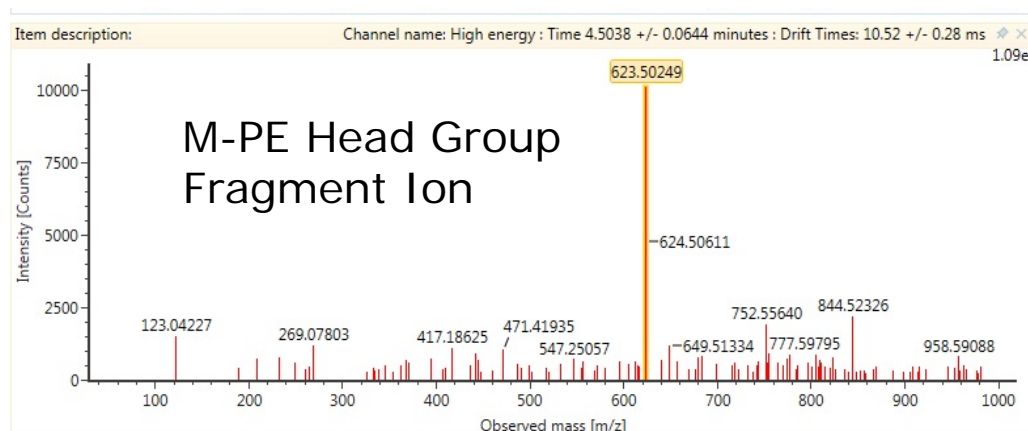
More interpretable data helps identification and quantitation

Channel name: PE(18:1/20:5) [+H]: (17.8 PPM) 764.5214 ...



Co-eluting
Isobaric
Lipids
764.52 m/z
Separated
by ion
mobility

HDMS^E



Collision Cross Section

Increasing analytical perspective

Representative analytical measurement

Property of molecule



rt



K_D



m/z



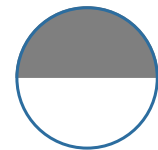
mass



dt



CCS



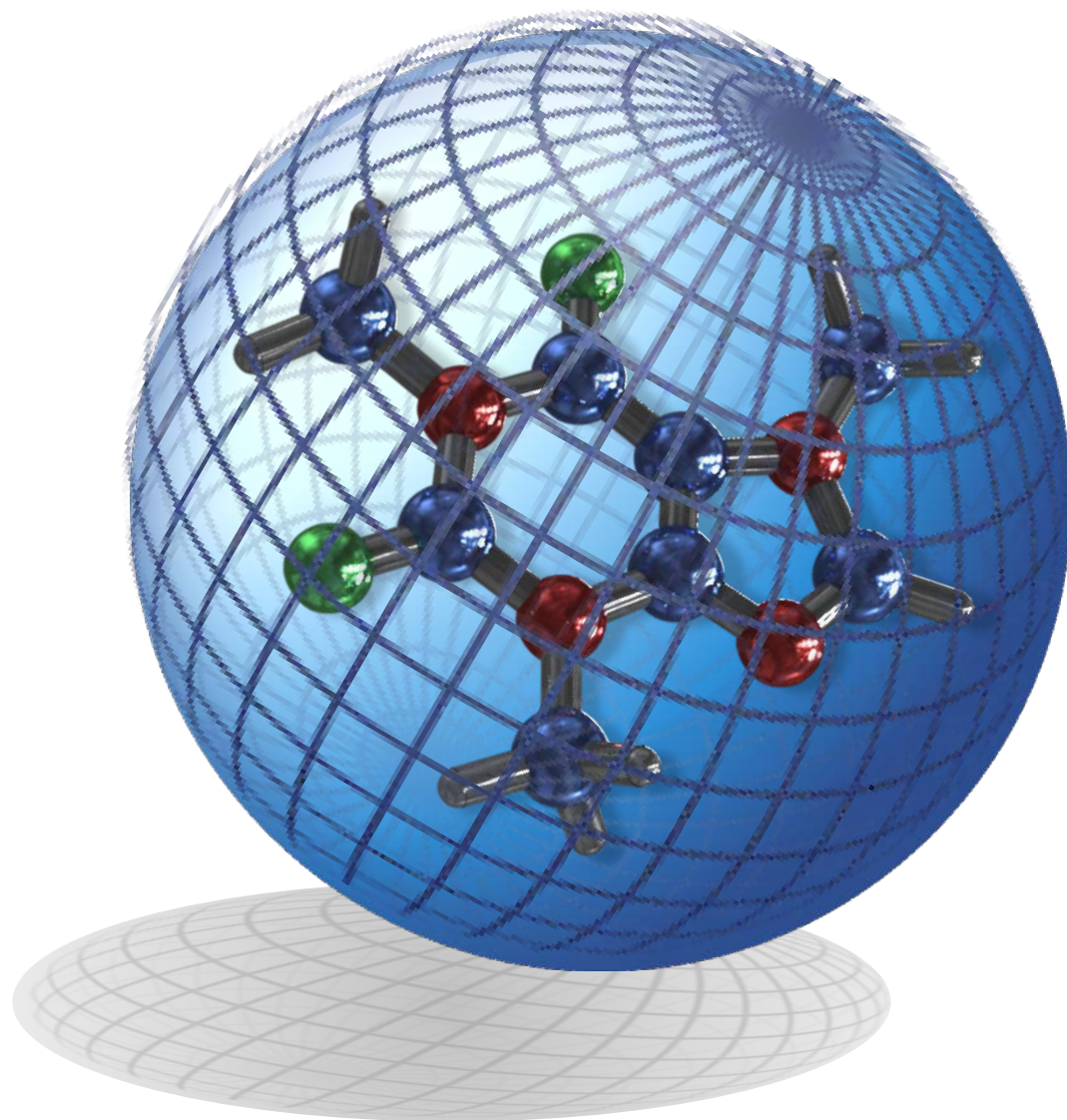
What is CCS?

**Important
differentiating
characteristic of
an ion**

- Chemical Structure (mass, size)

- 3-dimensional Conformation (shape)

**Precise
Physicochemical
Property of an ion**



What are the Benefits of Ion Mobility in Metabolomics?

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analytical
chemistry

Article

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Ion Mobility Derived Collision Cross Sections to Support Metabolomics Applications

Giuseppe Paglia,^{*,†} Jonathan P. Williams,[‡] Lochana Menikarachchi,[§] J. Will Thompson,^{||} Richard Tyldesley-Worster,[‡] Skarphédinn Halldórsson,[†] Ottar Rolfsson,[†] Arthur Moseley,^{||} David Grant,[§] James Langridge,[‡] Bernhard O. Palsson,^{†,⊥} and Giuseppe Astarita^{*,#}

[†]Center for Systems Biology, University of Iceland, IS 101, Reykjavik, Iceland

^{*}Waters Corporation, Manchester M23 9LZ, U.K.

[§]Department of Pharmaceutical Sciences, University of Connecticut, Storrs, Connecticut 06269, United States

^{||}Duke Proteomics Core Facility, Durham, North Carolina 27710, United States

[⊥]Systems Biology Research Group, University of California San Diego, La Jolla, California 92093, United States

[#]Waters Corporation, Milford, Massachusetts 01757, United States

[○]Georgetown University, Washington, District of Columbia 20057, United States

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analytical
chemistry

Ion Mobility-Derived Collision Cross Section As an Additional Measure for Lipid Fingerprinting and Identification

Giuseppe Paglia,^{†,‡} Peggì Angel,[§] Jonathan P. Williams,^{||} Keith Richardson,^{||} Hernando J. Olivos,^{||} J. Will Thompson,[⊥] Lochana Menikarachchi,[#] Steven Lai,^{||} Callee Walsh,[§] Arthur Moseley,[⊥] Robert S. Plumb,^{||,∇} David F. Grant,[#] Bernhard O. Palsson,[∇] James Langridge,^{||} Scott Geromanos,^{||} and Giuseppe Astarita^{*,||,○}

[†]Istituto Zooprofilattico Sperimentale della Puglia e Della Basilicata, Foggia, Italy

[‡]Center for Systems Biology, University of Iceland, Reykjavik, Iceland

[§]Protea Biosciences Group, Inc., Morgantown, West Virginia 26505, United States

^{||}Waters Corporation, Milford, Massachusetts 01757, United States

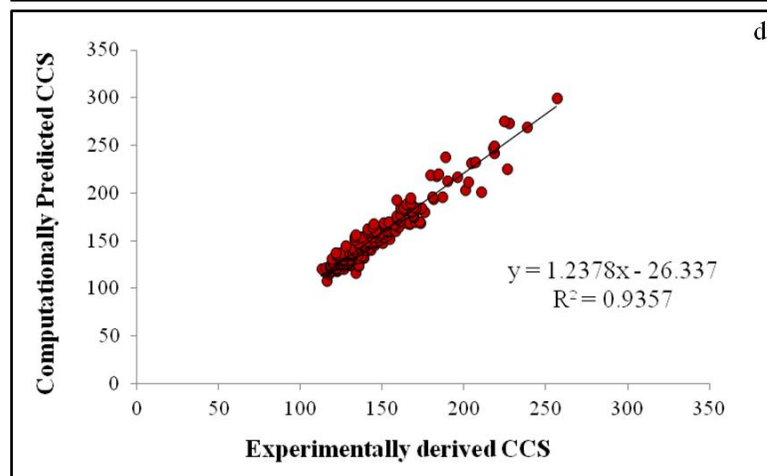
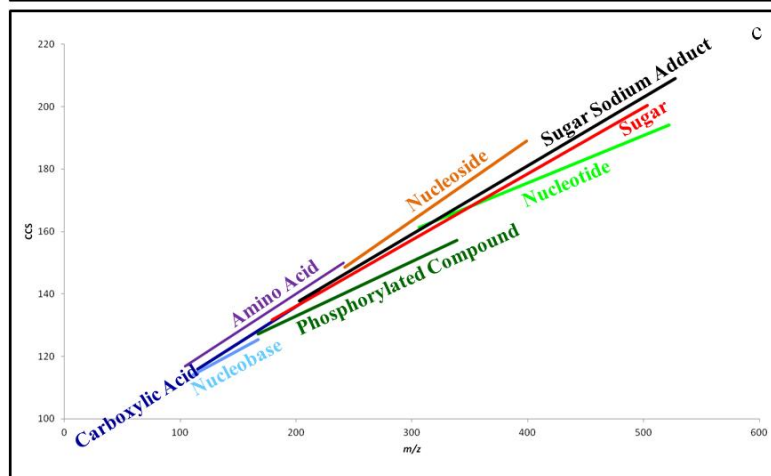
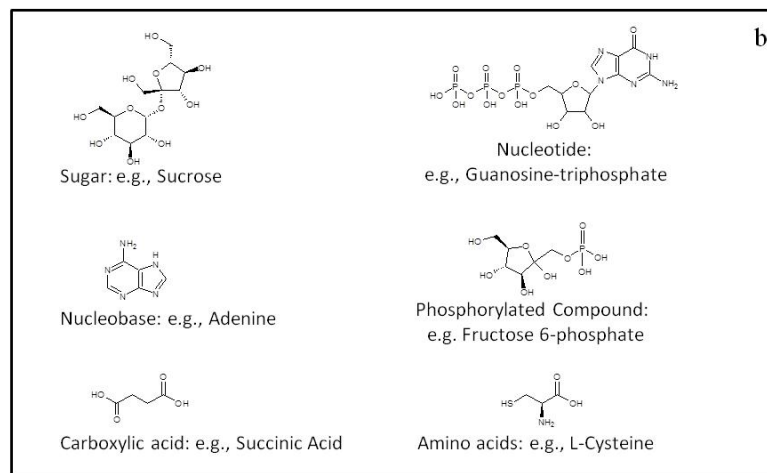
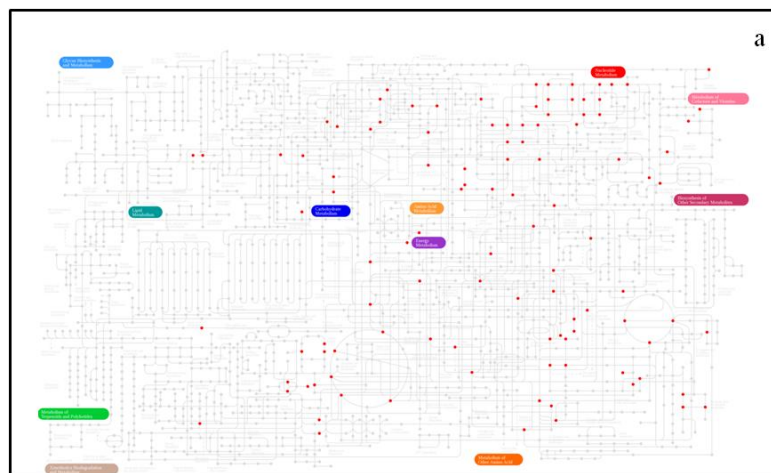
[⊥]Duke Proteomics Core Facility, Durham, North Carolina 27708, United States

[#]Department of Pharmaceutical Sciences, University of Connecticut, Storrs, Connecticut 06268, United States

[∇]Computational and Systems Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London, United Kingdom

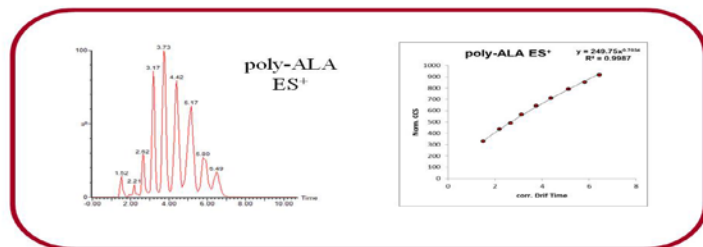
[○]Department of Biochemistry and Molecular & Cellular Biology, Georgetown University, Washington, DC 20057, United States

CCS Measurements for Common Metabolites

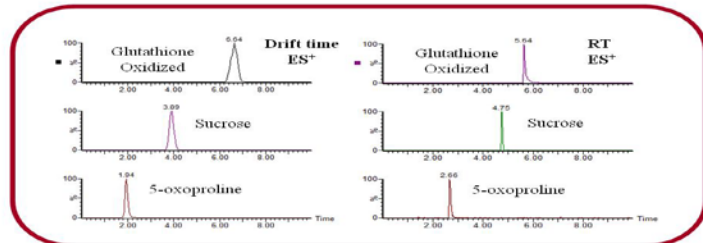


Inter-Lab Reproducibility of CCS Measurements

- Drift time calibration with known CCSs for polyAla

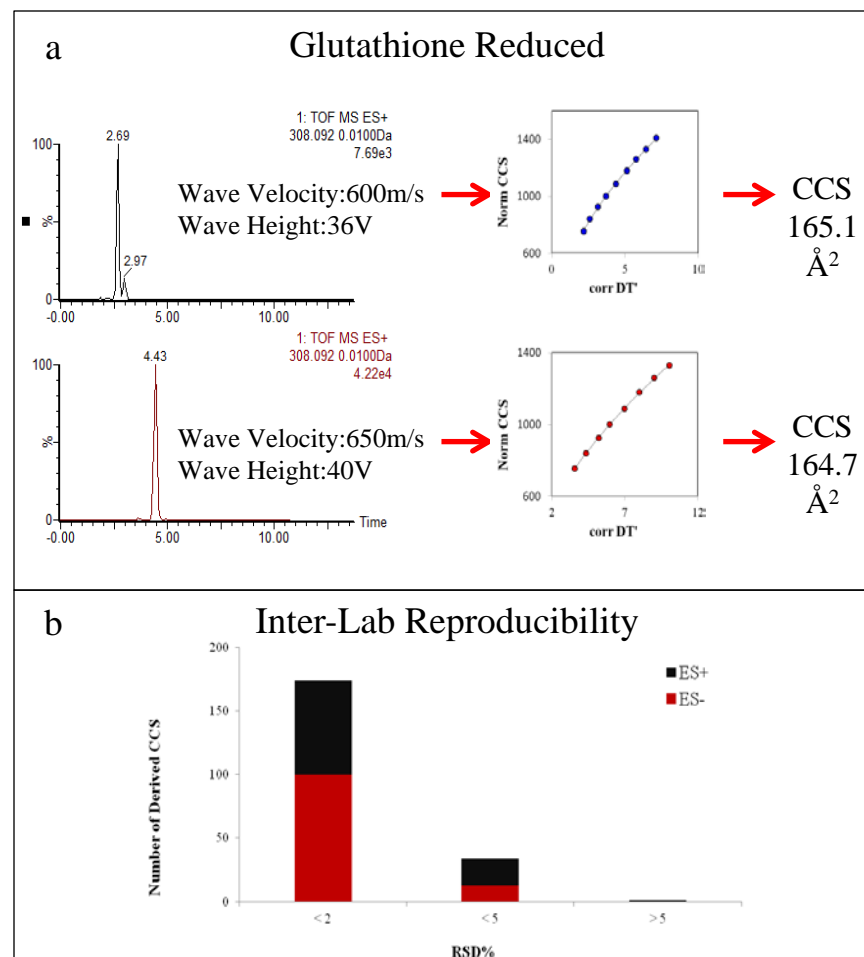


- Annotation of drift times and retention times (RT) of standard metabolites

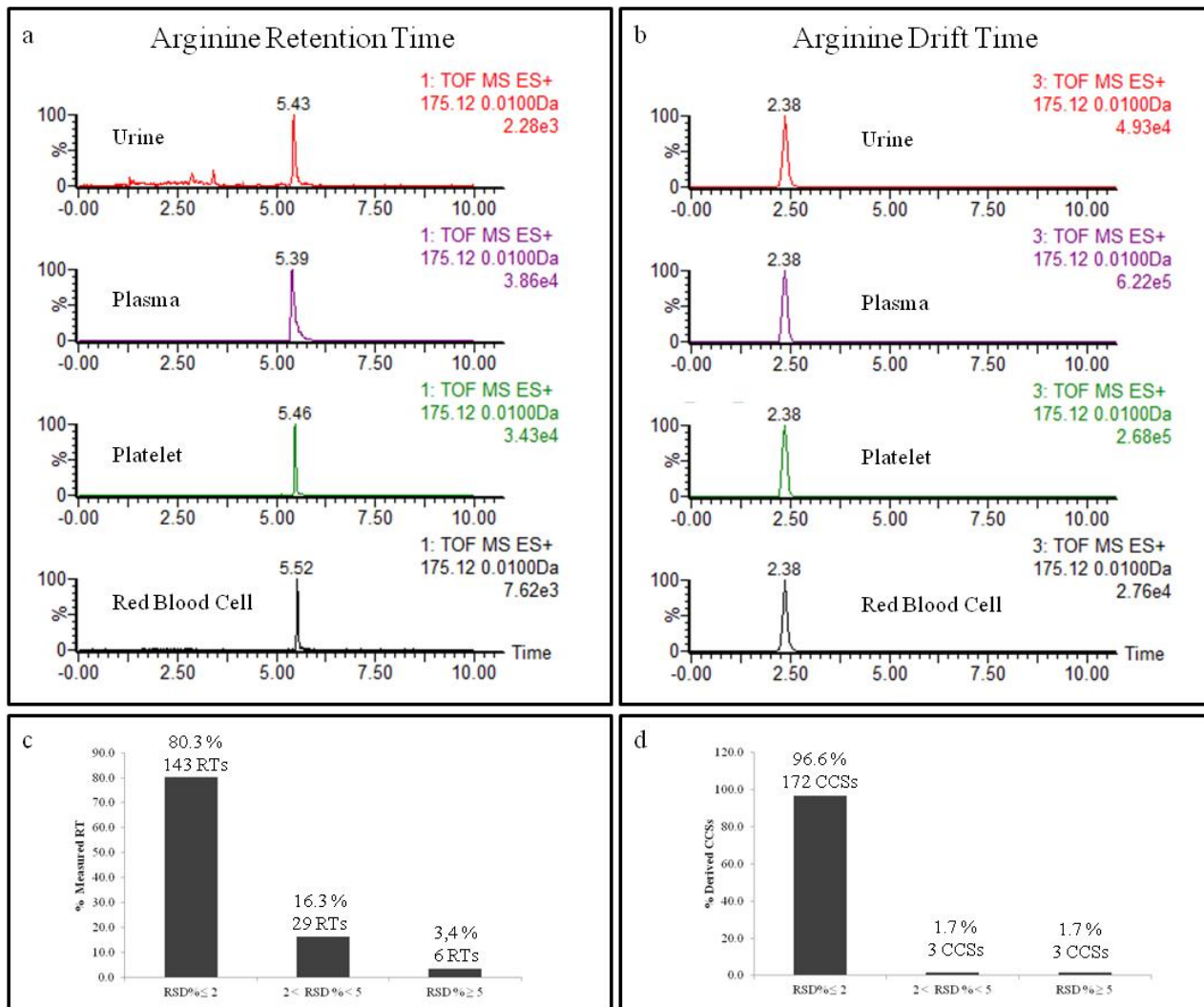
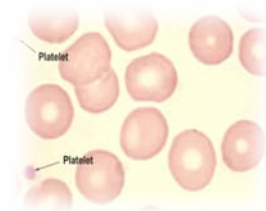


- Drift time to CCS conversion with polyAla calibration
- Database generation for m/z, CCS and RT

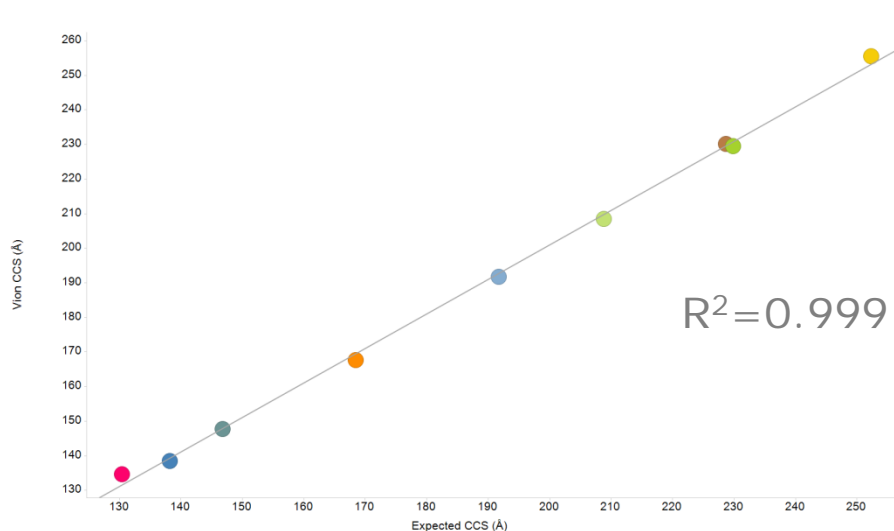
Metabolite	ES+			
	Ion	m/z	CCS (Å ²)	RT (min)
5-Oxoproline	[M+H] ⁺	130.0504	121	2.68
Sucrose	[M+Na] ⁺	365.1060	170	4.75
Glutathione Oxidized	[M+H] ⁺	613.1598	227	5.70



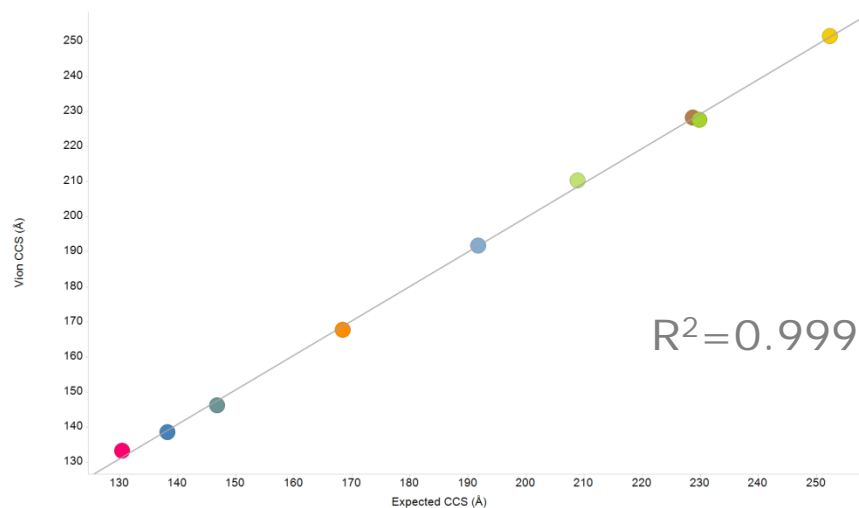
Matrix effect: Retention Times vs. CCS



CCS correlation between gradients

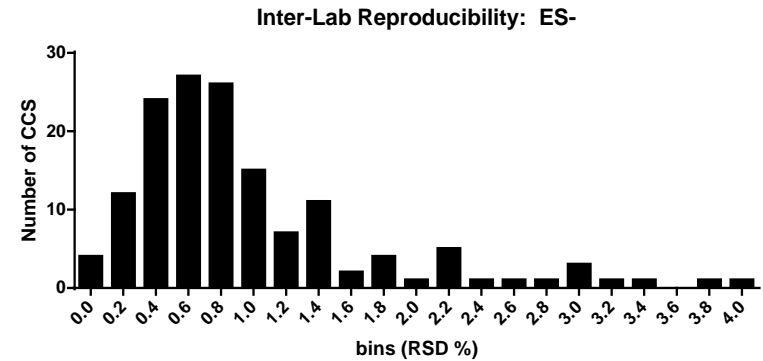
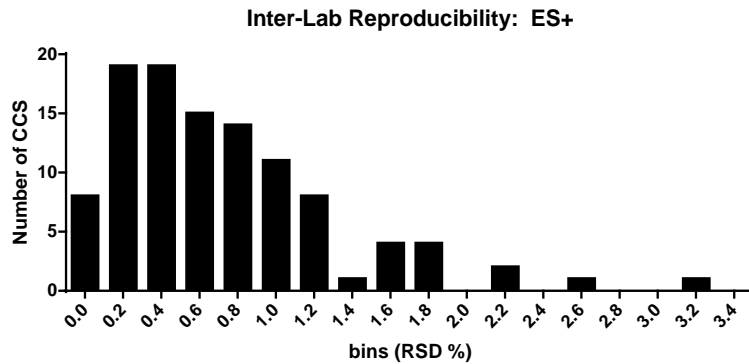
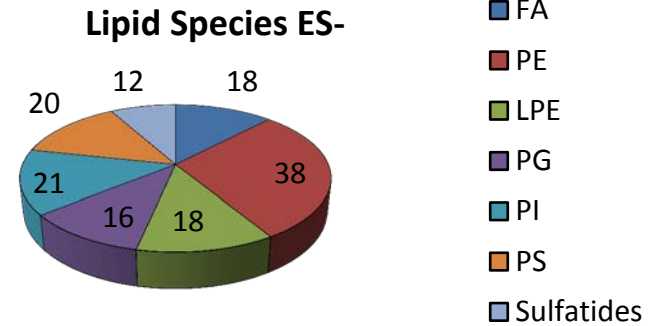
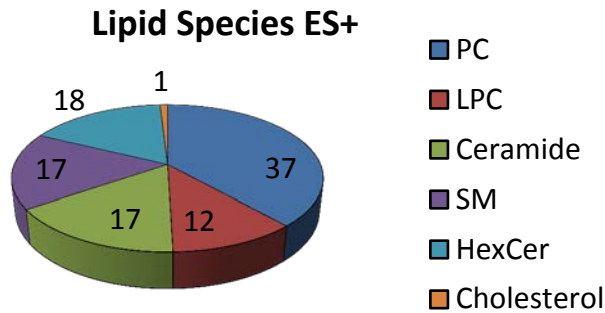


- Acetaminophen
- Caffeine
- Leu Enk
- Reserpine
- Sulfadimethoxine
- Sulfaguanidine
- Terfenadine
- Val-Tyr-Val
- Verapamil

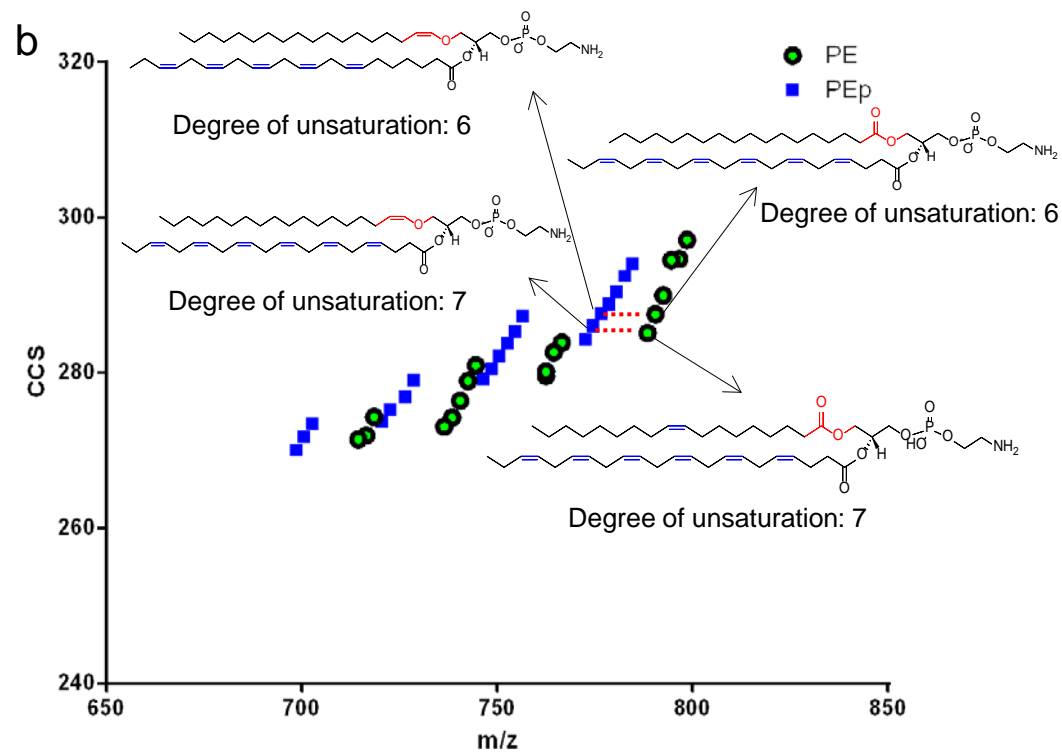
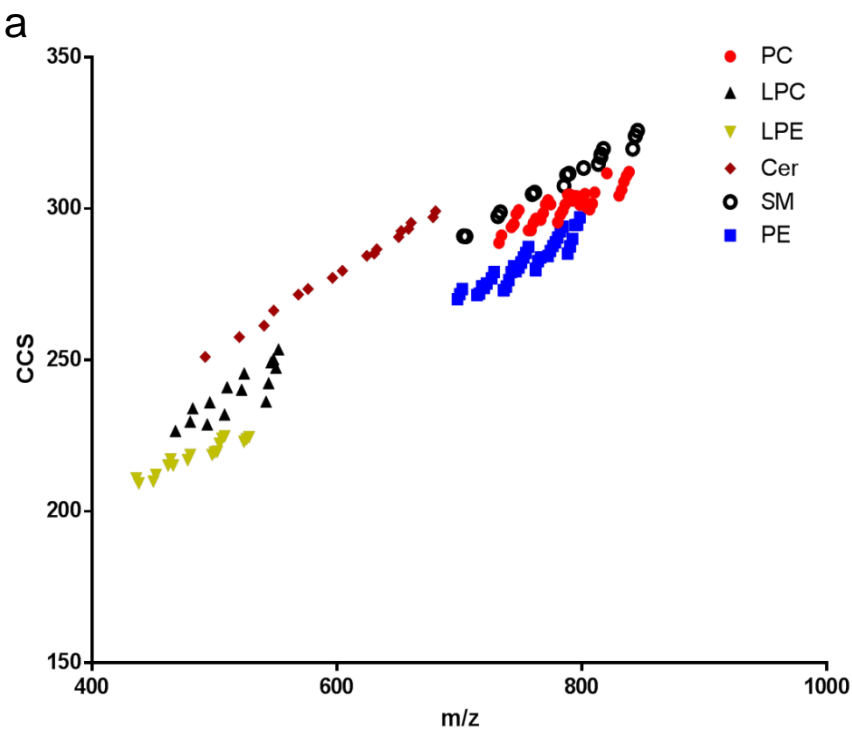


- Acetaminophen
- Caffeine
- Leu Enk
- Reserpine
- Sulfadimethoxine
- Sulfaguanidine
- Terfenadine
- Val-Tyr-Val
- Verapamil

Inter-Lab Reproducibility of Lipids CCS

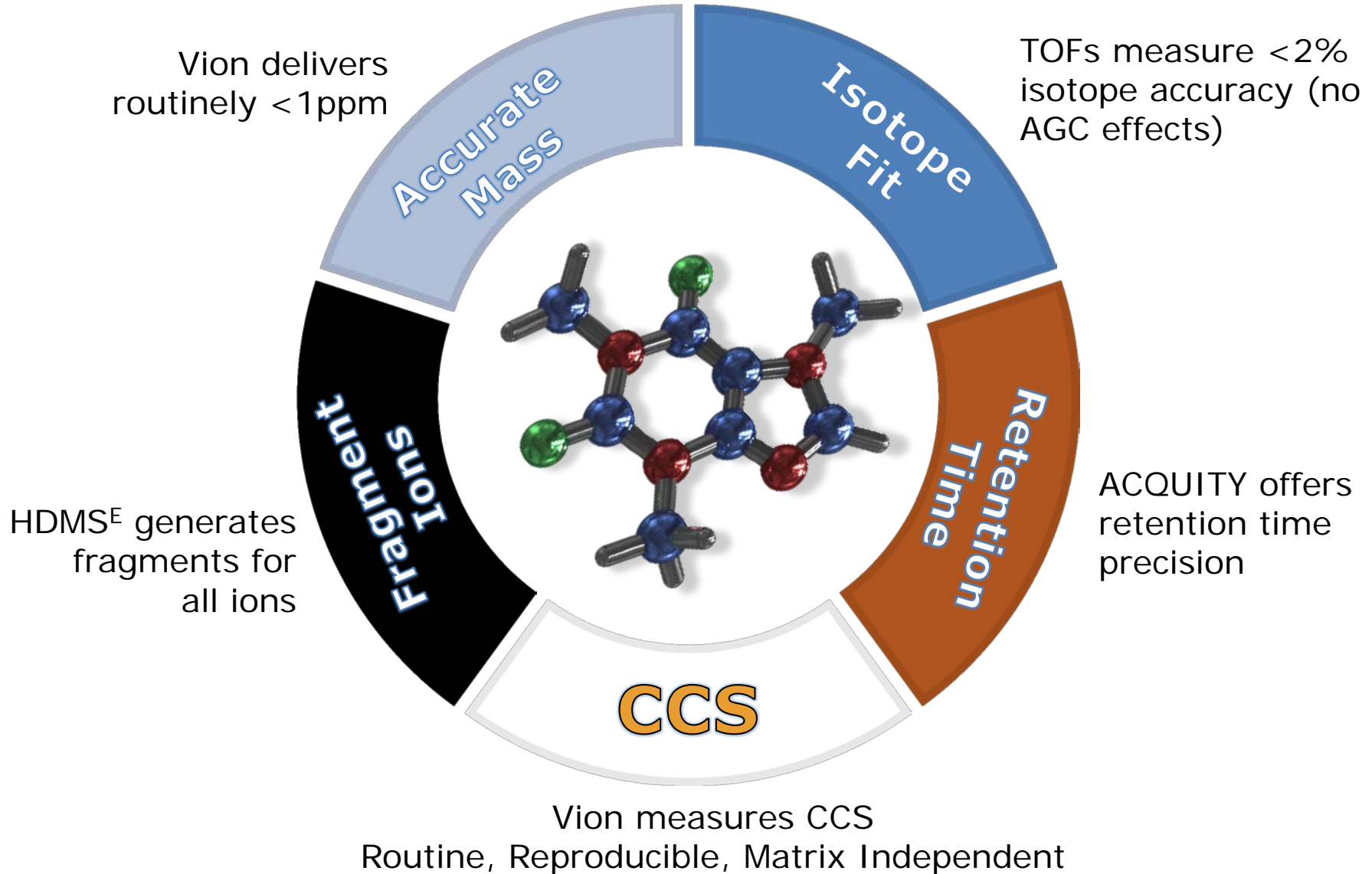


Mobility-Mass Correlations



Phosphatidylethanolamine (PE, green dots)
Plasmalogen PE (PEp, blue squares)

Search Criteria for Maximum Confidence



MS/MS Library Search

LibraryPrototype - Progenesis QI

File | Review | Experiment | Peak Picking | Review | Identify | Review | Compound

Import Data | Alignment | Design Setup | Deconvolution | Compounds | Compounds | Statistics

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Identify Compounds

Select your identification method:
Progenesis MetaScope

[About this method](#) | [Download others](#)

- Filter the compounds**
Using the list below, filter the compounds to show only those you want to identify.
- Choose search parameters**
Select your MetaScope search parameters or create a new parameter set:
METS | Edit
- Search for identifications**
Identifications will be assigned to the relevant compounds automatically.
Search for identifications

No filter applied | Create...

Compound	Accepted ID	Tag
8.68_487.1094m/z	13804	
2.63_283.0673m/z	64959	
4.93_172.0957m/z	70912	
3.75_275.0209m/z	91493	
4.38_146.0604m/z	150923	
11.19_342.2634m/z	168381	
9.09_407.2799m/z	221493	
7.44_407.2793m/z	221493	
9.33_407.2792m/z	221493	
8.50_407.2796m/z	221493	
7.87_407.2787m/z	221493	
8.02_407.2778m/z	221493	
8.30_407.2795m/z	221493	
9.43_407.2804m/z	221493	
9.14_391.2855m/z	222528	
9.36_359.1894m/z	222786	
9.24_359.1897m/z	222786	
9.45_359.1895m/z	222786	
9.19_359.1894m/z	222786	
4.70_121.0647m/z	443135	

68 of 1531 compounds have been identified.

Clear compound identifications

Compound 9.43_407.2804m/z ((4R)-4-[(3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl]pentanoic acid)

Legend: ■ Matched fragment ■ Unmatched fragment

Possible identifications: 1

Compound ID	Description	Adducts	Formula	Retention time	Score	Fragmentation score	Mass error (ppm)	Retention time error (mins)	Isotope similarity	Link
221493	(4R)-4-[(3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl]pentanoic acid	M-H	C ₃₄ H ₅₀ O ₇	280.1	51.3	59	0.21		97.88	

cholic acid

Section Complete

Automatic CCS measurement and search integration

↓ CCS Measured on all components

1.

Compound	Neutral mass	m/z	z	Retention time	CCS	Peak Width	Tag	Accepted ID	Identifications	Anova (p)	Max fold change
○ 11.35_905.6611m/z	<unknown>	905.6611	1	11.35	331.94	0.09			0	0.364	1.7
○ 11.35_1019.7836m/z	<unknown>	1019.7836	1	11.35	354.11	0.15			0	0.0564	2.1
○ 11.35_1370.3534n	1370.3534	1353.3501	1	11.35	379.21	0.11			0	9.89E-09	Infinity
○ 11.35_1401.2646m/z	<unknown>	1401.2646	1	11.35	359.79	0.04			0	0.00058	Infinity
○ 11.36_563.4414m/z	<unknown>	563.4414	1	11.36	280.81	0.07			0	0.715	1.98
● 11.36_660.6633m/z	<unknown>	660.6633	1	11.36	298.70	0.08			3	0.00865	2.24
○ 11.36_714.6024m/z	<unknown>	714.6024	1	11.36	306.23	0.11			0	0.755	1.22
○ 11.36_756.6448m/z	<unknown>	756.6448	1	11.36	317.19	0.09			0	0.739	1.98
○ 11.36_908.7803m/z	<unknown>	908.7803	1	11.36	341.04	0.04			0	0.382	1.17
○ 11.36_928.7398m/z	<unknown>	928.7398	1	11.36	337.55	0.07			0	0.912	10.3
○ 11.36_1054.2973m/z	<unknown>	1054.2973	1	11.36	310.90	0.15			0	0.377	15.9
○ 11.36_1054.5987m/z	<unknown>	1054.5987	1	11.36	310.89	0.08			0	0.327	Infinity
○ 11.36_1081.3066m/z	<unknown>	1081.3066	1	11.36	319.41	0.11			0	0.194	Infinity
○ 11.36_1095.3230m/z	<unknown>	1095.3230	1	11.36	319.15	0.09			0	0.261	Infinity
○ 11.36_1440.1966m/z	<unknown>	1440.1966	1	11.36	484.10	0.03			0	0.066	1.44
○ 11.38_467.4076m/z	<unknown>	467.4076	1	11.38	302.07	0.09			0	0.111	1.71
○ 11.38_828.7054m/z	<unknown>	828.7054	1	11.38	327.58	0.14			0	0.296	1.23
○ 11.38_947.7099m/z	<unknown>	947.7099	1	11.38	340.21	0.38			0	0.191	1.14

Progenesis[®] QI

2.

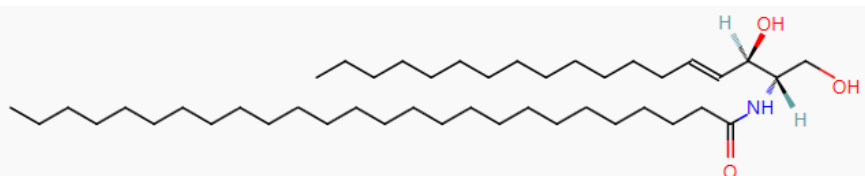
Expected CCS

↓ Difference in CCS (1.2%)

Possible identifications: 3

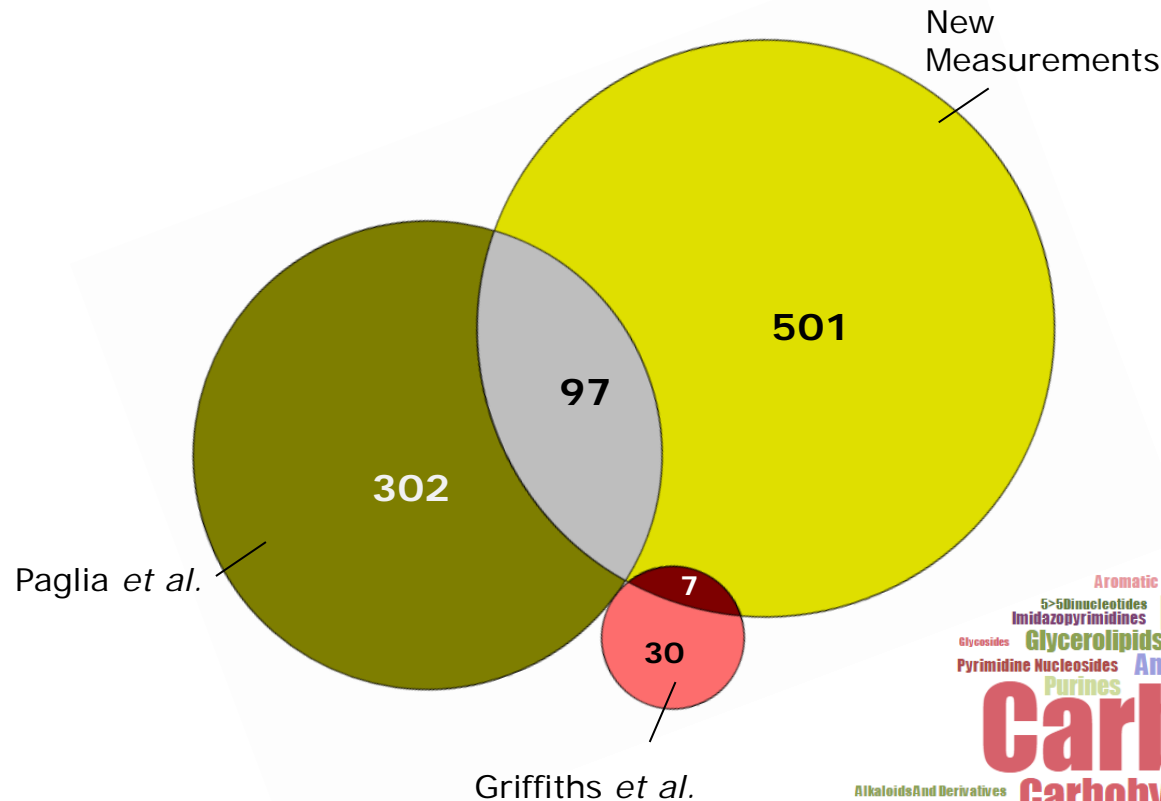
☆	Compound ID	Description	Adducts	Formula	Retention time	CCS	Score	Fragmentation score	Mass error (ppm)	Retention time error (mins)	ΔCCS (Å ²)	Isotope similarity
☆	7850629	Cer(d18:1/26:0)	M+H-H...	C ₄₄ H ₈₇ NO ₃	11.36	295.00	41	6.53	-2.90	1.33e-004	3.70	77.12
☆	173737523	Cer(m18:1(4E)/26:1(17Z))	M+H	C ₄₄ H ₈₅ NO ₂			36.1	6.59	-2.98			77.33
☆	7850637	Cer(d18:0/26:1(17Z))	M+H-H...	C ₄₄ H ₈₇ NO ₃			36	6.53	-2.90			77.12

3.

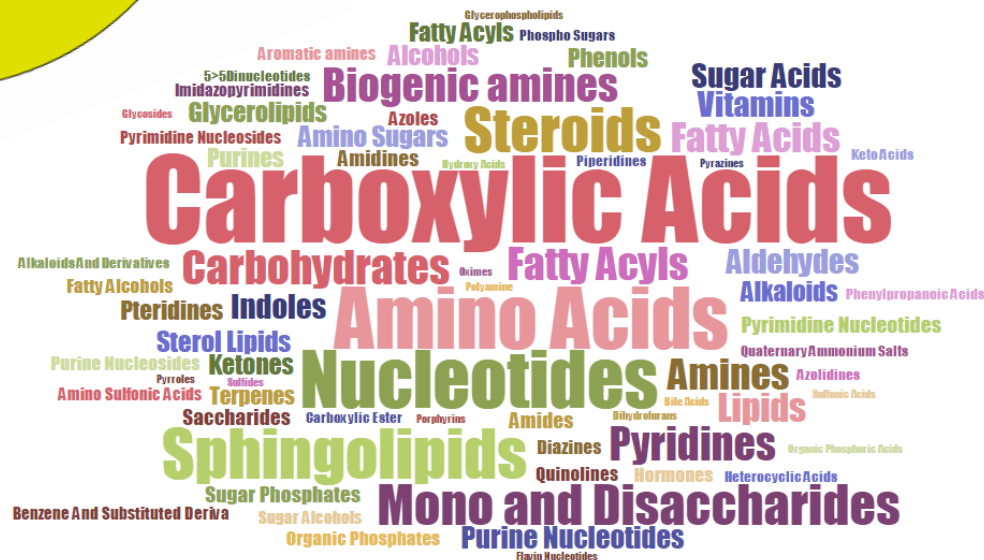


Cer(d18:1/26:0)

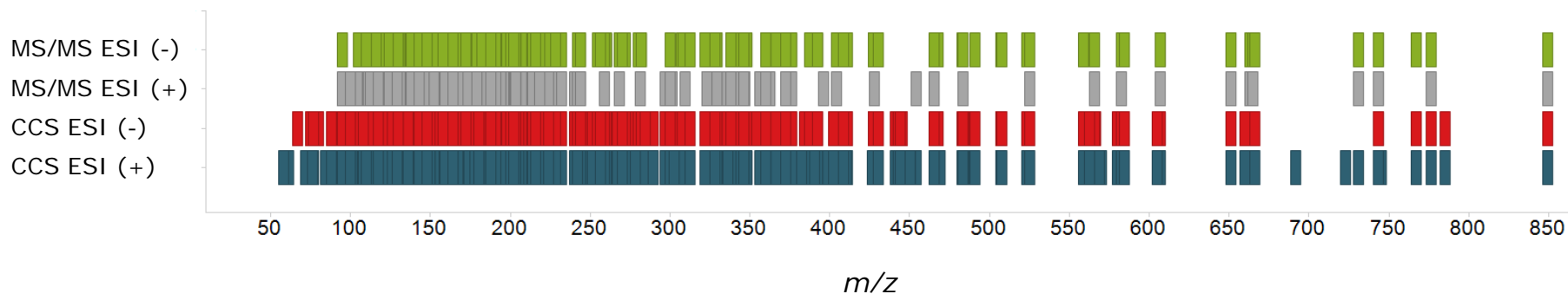
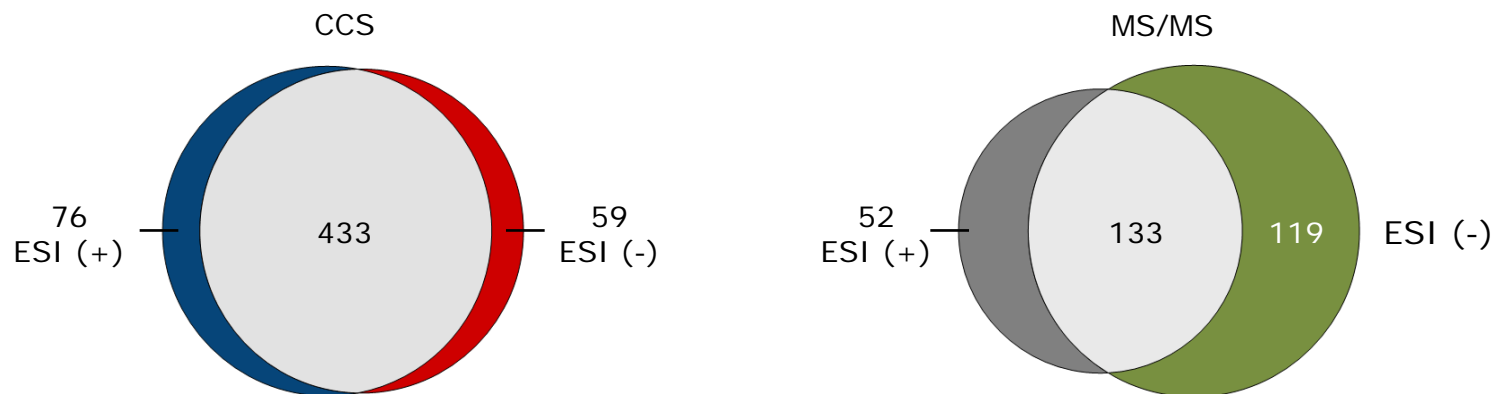
Metabolic Profiling CCS & MS/MS Library



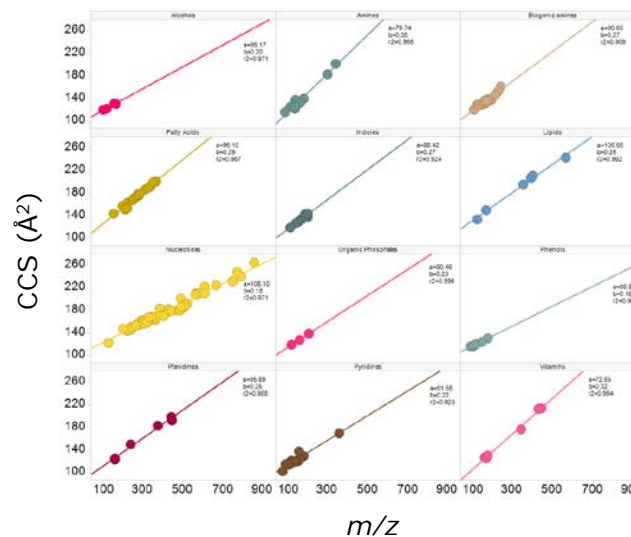
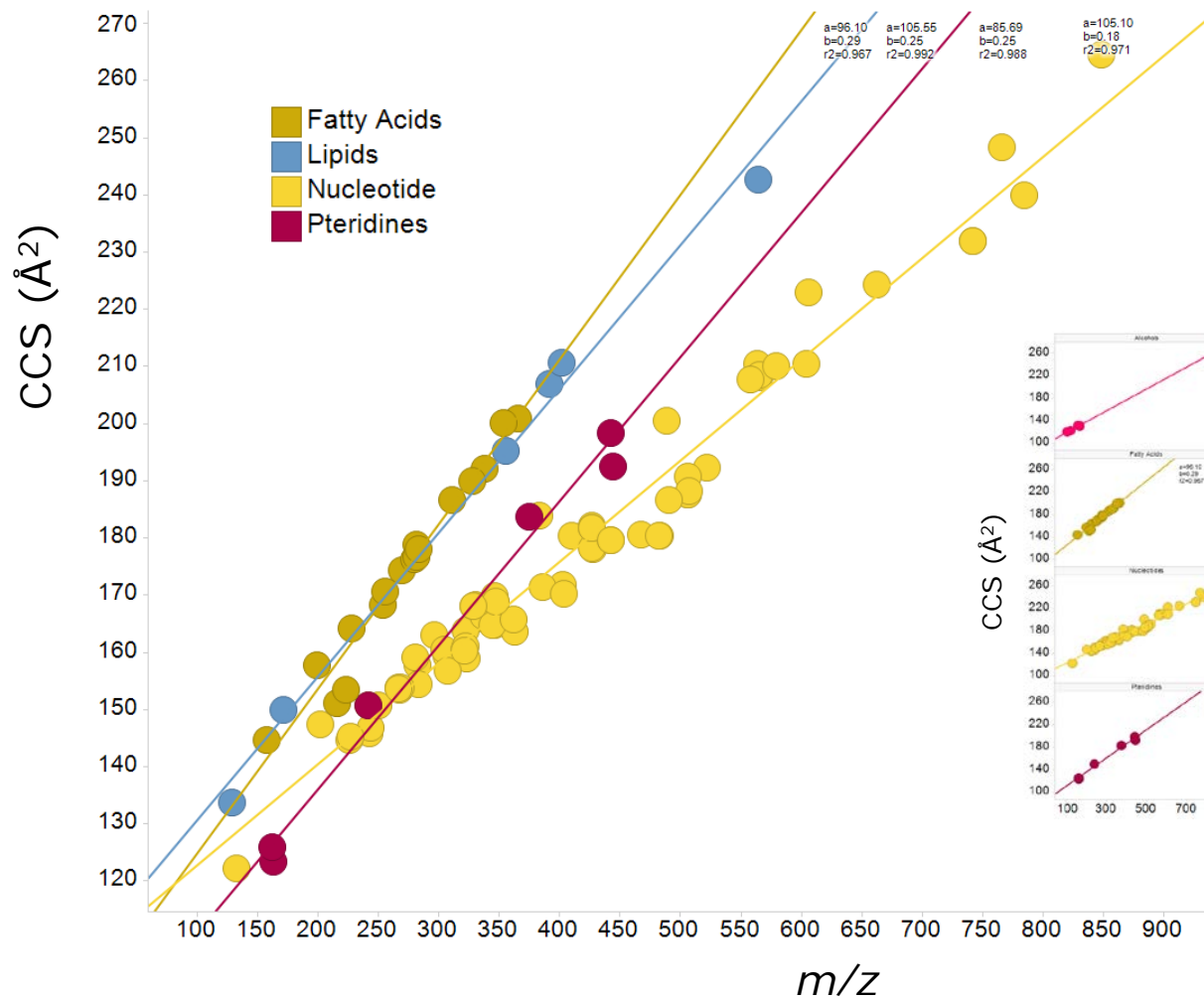
> 900 CCS Measurements
> 600 MS/MS Spectra



CCS & MS/MS Coverage by ionisation mode



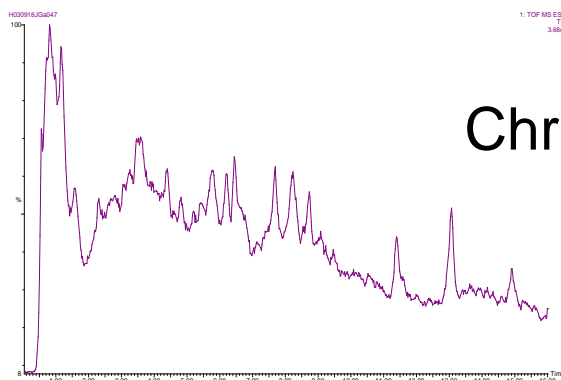
Molecule class relationship to m/z & CCS



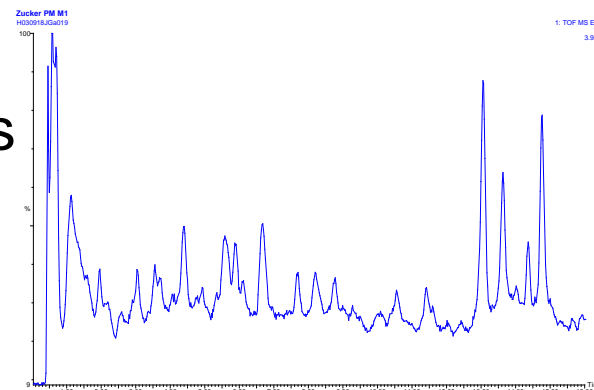
Data Processing



Metabolomics



Urine From Group 1



Urine From Group 2



Exact Mass
Chromatographic Peaks

Extract variables

Normalize

Align like variables

Populate Markers Table

Multivariate Data Array

Retention Time *m/z* *Drift Time* →

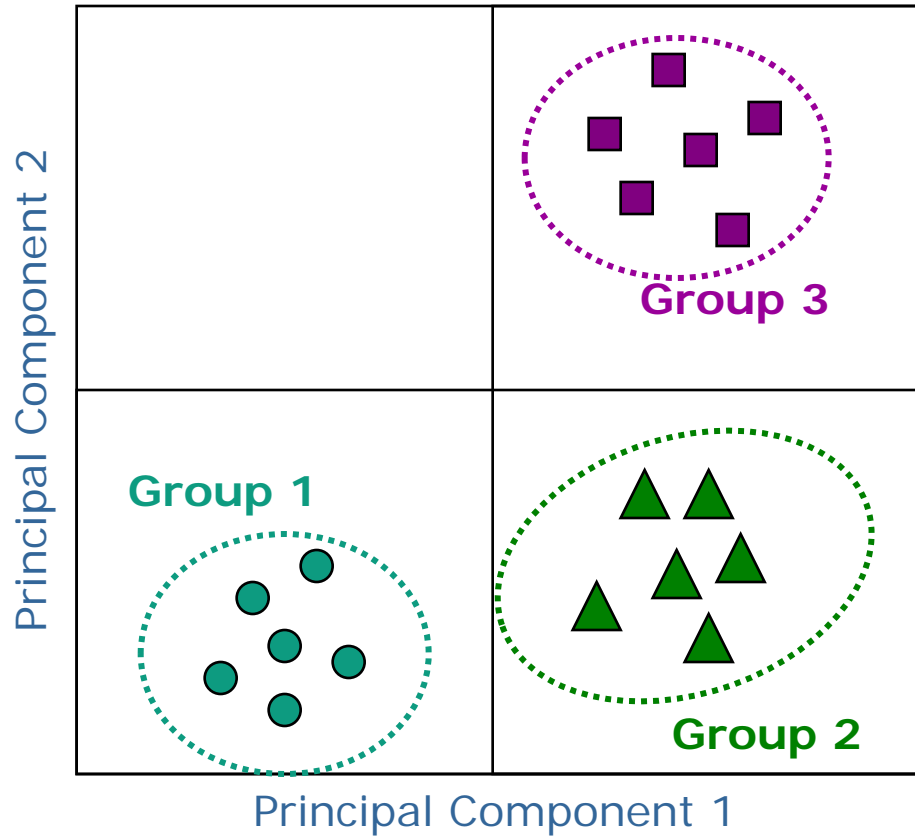
Samples ↓

	2.24_318.0634_12.3	5.46_317.1806_11.7	6.05_317.1722_11.1	2.97_317.1714_10.9	...
RAT 1	0	0	0	2.15351	...
RAT 2	0	0	0	2.10822	...
RAT 3	1.63034	0	0	0	...
RAT 4	1.62986	0	0	0	...
RAT 5	4.70965	0	0	0.730389	...
RAT 6	0	1.03318	0	1.83726	...
RAT 7	0	0	0	0	...
RAT 8	2.83714	0.947788	0	0.919644	...
RAT 9	5.23023	0	0	0.956396	...
RAT 10	0	0.843124	0	0	...

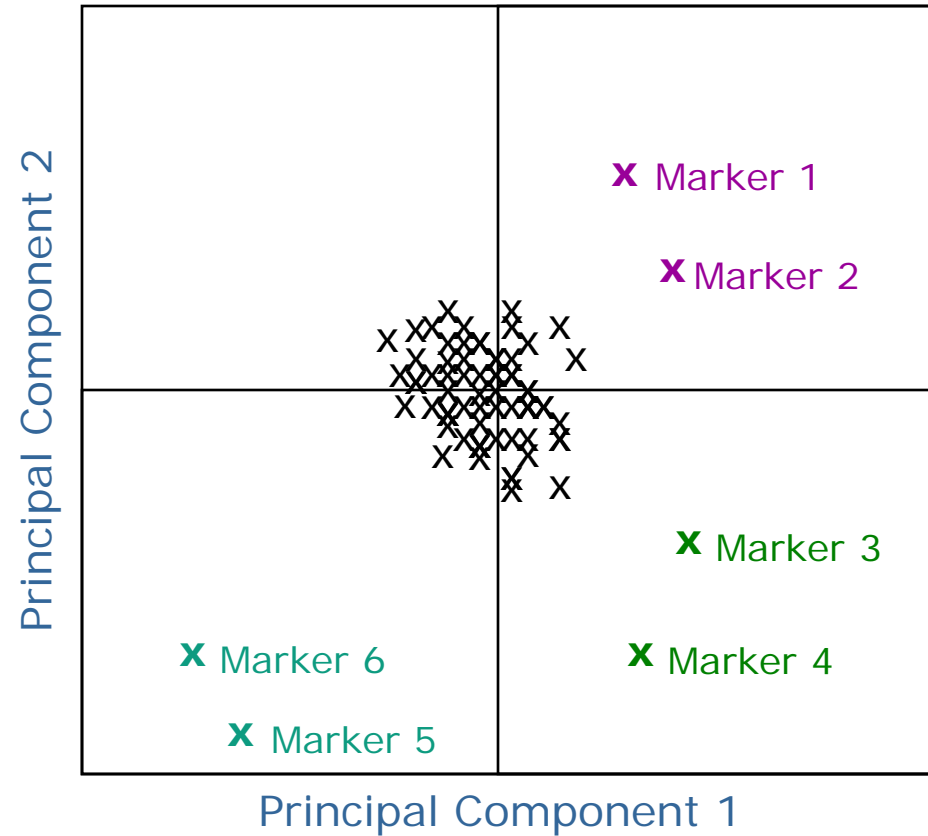
⋮ ⋮ ⋮ ⋮ ⋮ ⋮ ⋮

Understanding PCA Results

Scores Plot



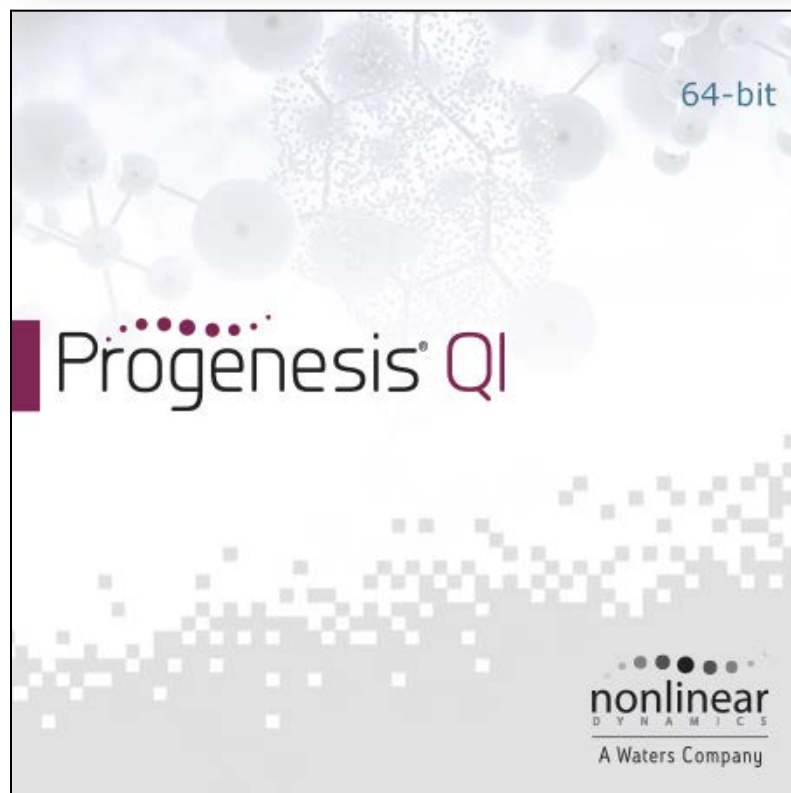
Loadings Plot



Progenesis QI

The logo for Progenesis QI, featuring the word "Progenesis" in a dark grey sans-serif font, followed by "QI" in a larger, purple sans-serif font. Above the "Progenesis" text is a horizontal row of seven purple dots of varying sizes, decreasing from left to right.

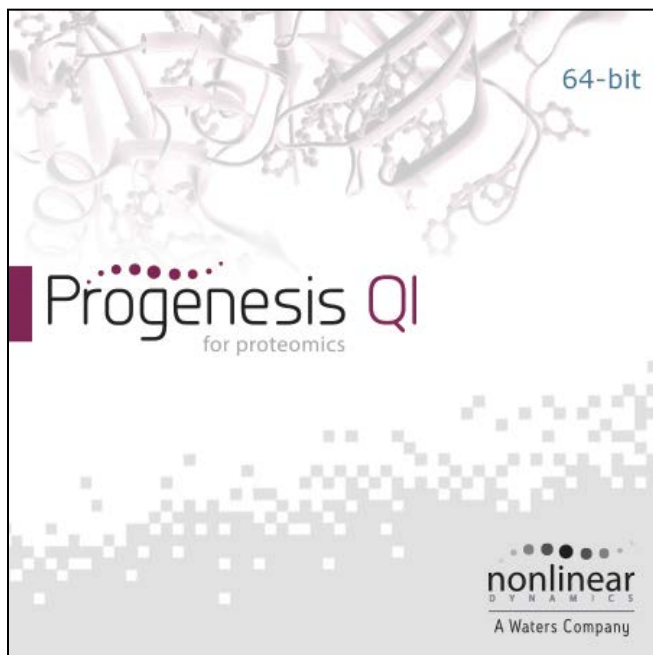
Quantify
Identify



- For 'small molecules'
- Wide applicability
- Metabolomics
- Biomarkers
- Food
- Wine
- Toxicology
- Environmental
- Pesticides
- Etc

Data Import

Multi-vendor support



Waters (.raw)

Waters (.raw)

Version: 1.0.5024.44574

AB SCIEX (.wiff)

Version: 1.0.5031.27094

Agilent (.d)

Version: 2.0.5031.27063

Bruker Daltonics (.d)

Version: 1.0.5031.27077

mzML Files

Version: 1.0.5031.27086

mzXML files

Version: 1.0.5031.27080

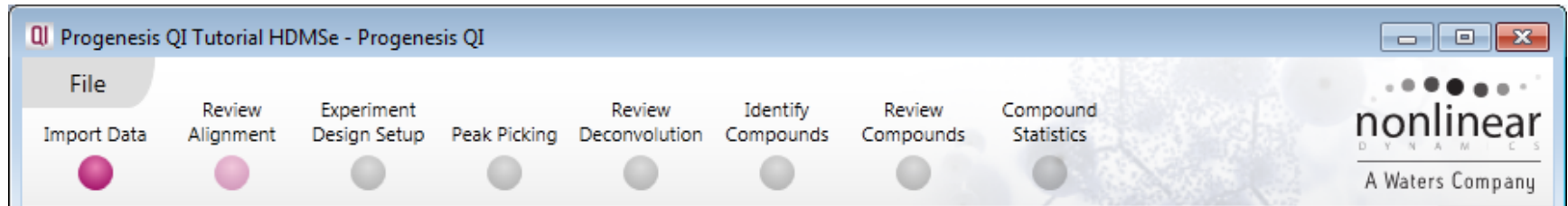
Thermo (.raw)

Version: 1.0.5031.27110

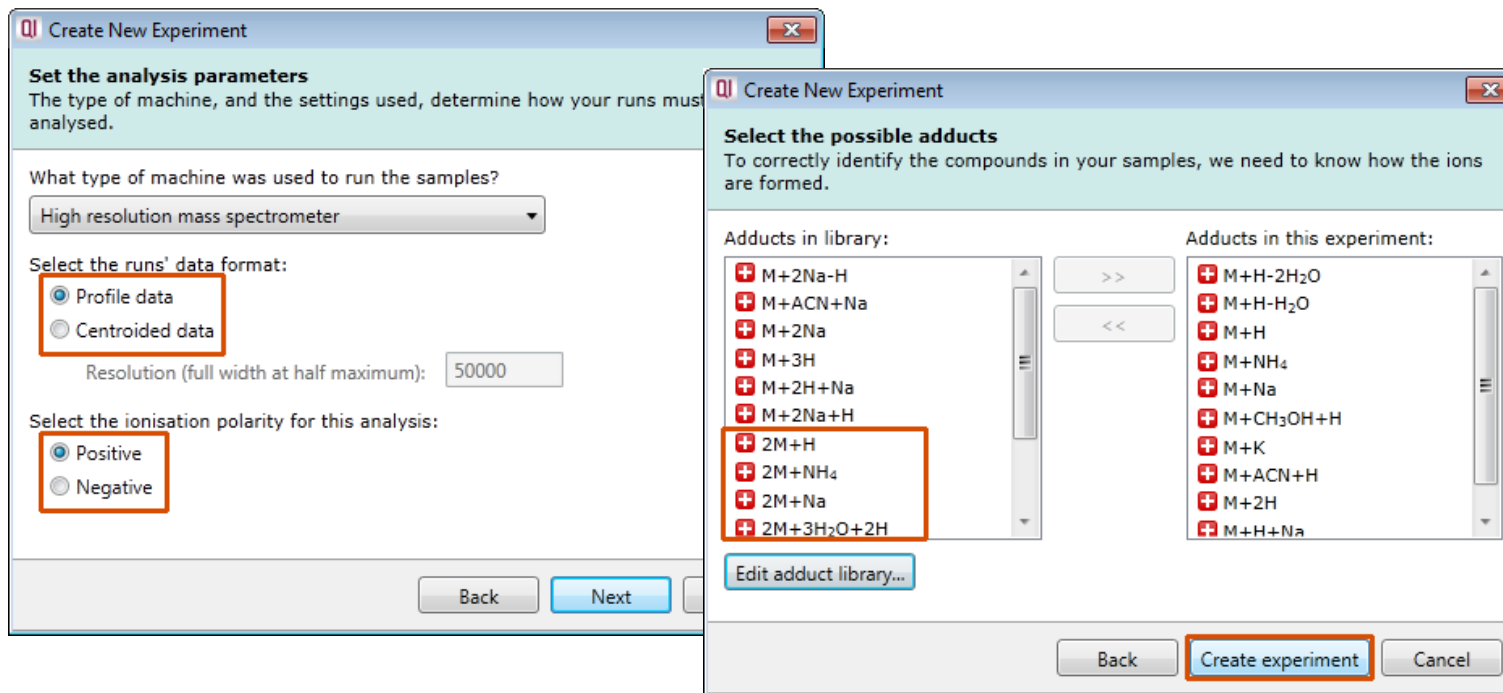
Thermo FT-ICR (.raw)

Version: 1.0.5031.27110

Progenesis QI guided workflow and data import / adduct selection



Details of data file format and a list of 'expected' adducts are entered to facilitate the handling of data import



Data Import

Low and High energy views

The screenshot displays the Progenesis QI software interface. The top menu bar includes File, Review Alignment, Experiment Design Setup, Peak Picking, Review Deconvolution, Identify Compounds, Review Compounds, and Compound Statistics. The 'Import Data' section is active, showing three numbered steps: 1. Import your run data, 2. Start the alignment process, and 3. Review the chromatography. Below these steps is a grid of imported runs labeled A_LD_1 through A_LD_6. Two mass spectra are shown: 'A_LD_1 (low energy)' and 'A_LD_1 (high energy)', both with m/z on the x-axis (100-900) and Retention Time (min) on the y-axis (1-9). A 'Section Complete' button is visible in the bottom right corner.

Import Data

1 Import your run data
Select one of the available data formats then click the Import button:
Format: Waters (.raw) Import...
[About this data format](#) | [Download others](#)

2 Start the alignment process
While your runs are importing, click the button below to:

- Select [alignment reference](#) candidates
- Determine the best of the candidates
- Align all runs to that reference run

[Start alignment process](#)

3 Review the chromatography
Look at all of the runs in the list below, checking for any [sample-running problems](#) that might affect analysis. Right-click to remove any runs that have significant problems.

Imported runs:

A_LD_1 A_LD_2 A_LD_3
A_LD_4 A_LD_5 A_LD_6

A_LD_1 (low energy)

A_LD_1 (high energy)

About this run

- Low energy peak count: 270,242
- High energy peak count: 131,562
- Total ion intensity: 2.426e+006
- Masked areas : none

Data import details

- Lock mass calibrated
 - Lock mass m/z: 556.2771

Section Complete

Review alignment (zoom) Un-aligned

QIP Tutorial_B_Aligned HDMSe - Progenesis QI for proteomics

File Import Data Review Alignment Filtering Experiment Design Setup Review Peak Picking Peptide Statistics Identify Peptides Refine Identifications Resolve Conflicts Review Proteins Protein Statistics Report

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Review Alignment

Sample ions are aligned to compensate for drifts in retention time between runs.

[Learn about the visualisations shown here](#)

- Align retention times automatically**
For maximum reproducibility, the software can automatically align your runs.
- Review the alignment**
Using the quality control measures, review and edit the runs' alignment:
 - Order the runs by alignment score and start by selecting the first run
 - Within each run, inspect and edit any areas rated as Needs Review

[Learn about the review and editing process](#)

Run	Include?	Vectors	Score
A_01	<input checked="" type="checkbox"/>	Ref	
A_02	<input checked="" type="checkbox"/>	2025	97.3%
A_03	<input checked="" type="checkbox"/>	1931	97.3%
B_01	<input checked="" type="checkbox"/>	1951	96.3%
B_02	<input checked="" type="checkbox"/>	1839	95.9%
B_03	<input checked="" type="checkbox"/>	1873	96.1%
C_01	<input checked="" type="checkbox"/>	1923	94.9%
C_02	<input checked="" type="checkbox"/>	1940	95.8%
C_03	<input checked="" type="checkbox"/>	1903	93.7%

Ion maps: ■ Alignment target ■ Run being aligned

Alignment quality: ■ Good ■ OK ■ Needs review

Section Complete

Show Aligned Show Unaligned Remove Vectors

Vector editing

Transition

Ion intensity map

Total ion chromatogram

Review alignment (zoom)

Aligned

QIP Tutorial_B_Aligned HDMSe - Progenesis QI for proteomics

File Import Data Review Alignment Filtering Experiment Design Setup Review Peak Picking Peptide Statistics Identify Peptides Refine Identifications Resolve Conflicts Review Proteins Protein Statistics Report

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Review Alignment

Sample ions are aligned to compensate for drifts in retention time between runs.

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Run	Include?	Vectors	Score
A_01	<input checked="" type="checkbox"/>	Ref	
A_02	<input checked="" type="checkbox"/>	2025	97.3%
A_03	<input checked="" type="checkbox"/>	1931	97.3%
B_01	<input checked="" type="checkbox"/>	1951	96.3%
B_02	<input checked="" type="checkbox"/>	1839	95.9%
B_03	<input checked="" type="checkbox"/>	1873	96.1%
C_01	<input checked="" type="checkbox"/>	1923	94.9%
C_02	<input checked="" type="checkbox"/>	1940	95.8%
C_03	<input checked="" type="checkbox"/>	1903	93.7%

Vector editing

Transition

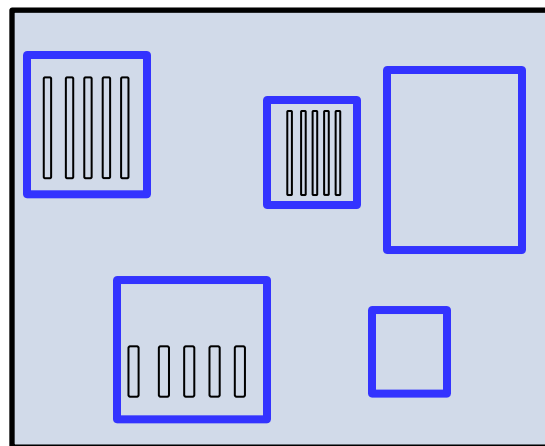
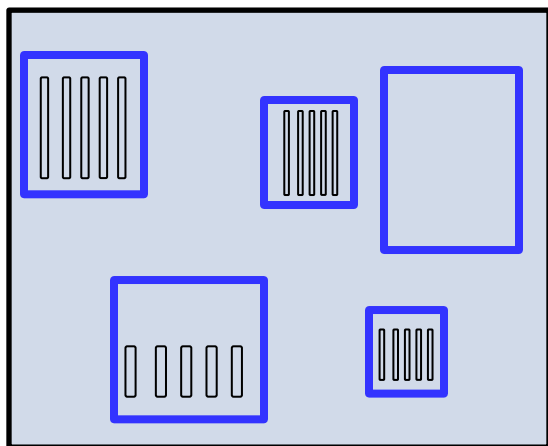
Ion intensity map

Total ion chromatogram

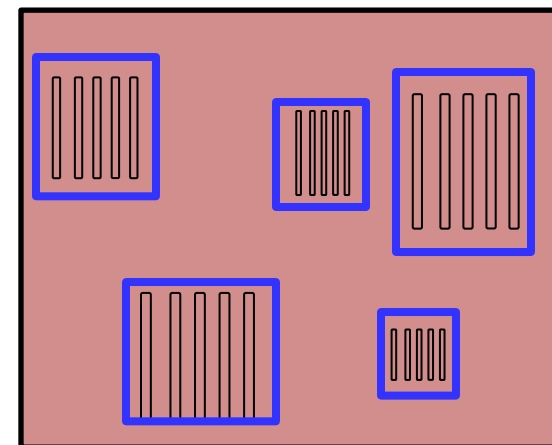
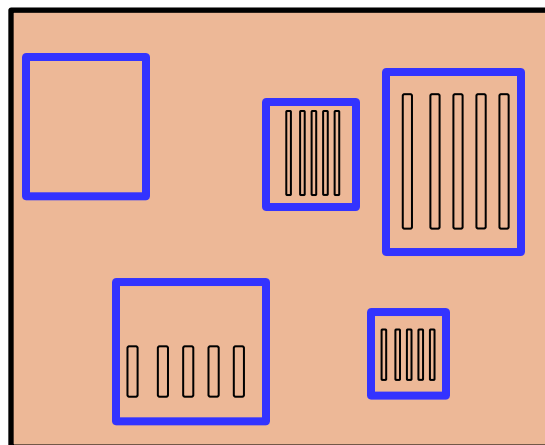
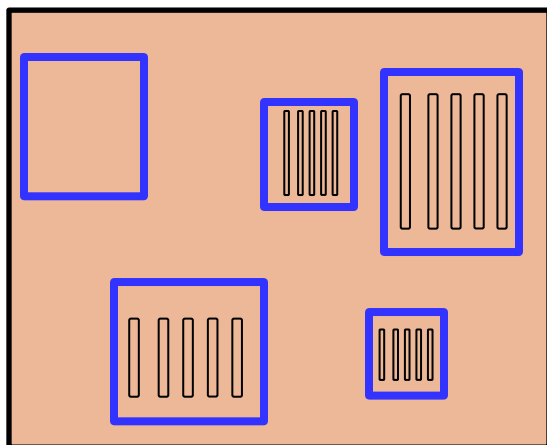
Alignment quality: ■ Good ■ OK ■ Needs review

Ion maps: ■ Alignment target ■ Run being aligned

Peak picking and co-detection



Mapping the detection to all runs avoiding missing data



Aggregate co-detection

Review adduct deconvolution

Progenesis QI Tutorial HDMSe - Progenesis QI

File | Import Data | Review Alignment | Experiment Design Setup | Peak Picking | Review Deconvolution | **Identify Compounds** | Review Compounds | Compound Statistics

Review Deconvolution
After peak picking, ions are grouped by compound in a process called deconvolution.
[How does deconvolution work?](#)

1 Review the compounds
Select compounds below to view:

- areas of the ion map showing the different adduct forms
- the mass and retention time profiles of their ions

These views can be used to confirm the validity of your interesting compounds.
[How do I use this screen?](#)

2 Optimise for your samples
If any compounds have an ion whose profile doesn't match the majority in the compound, it can be removed. Likewise, missing compound ions can be added.

No filter applied

Compound	Accepted ID	Tag
<input type="radio"/> 8.54_470.3260n		
<input type="radio"/> 9.02_574.0954n		
<input type="radio"/> 4.49_560.3061n		
<input type="radio"/> 4.30_542.2949n		
<input type="radio"/> 4.35_556.2742n		
<input checked="" type="radio"/> 4.24_458.2754n		
<input type="radio"/> 3.95_344.2050n		
<input type="radio"/> 7.33_370.3199n		●
<input type="radio"/> 7.64_484.2687n		
<input type="radio"/> 9.62_518.1326n		
<input type="radio"/> 8.44_354.2176n		●
<input type="radio"/> 9.95_710.5520n		●
<input type="radio"/> 9.22_362.3033n		●

Compound 4.24_458.2754n

M+H | M+NH₄⁺ | M+Na⁺ | M+CH₃OH+H | M+K⁺

About this compound
Compound 4.24_458.2754n has the following properties:

- Retention time: 4.237 mins
- Neutral mass: 458.2754
- Adducts: 4 (M+H, M+NH₄⁺, M+Na⁺, M+K⁺)

This compound's neutral mass and adducts are based on mass differences between its adduct forms.

Reviewing assistance
The grid at the left shows all potential ion locations for this compound.
Can you see any ions that could be another adduct form of this compound? Or any that don't belong here?

Mass Spectra
Relative Intensity vs. Neutral Mass (Da)

Chromatograms
Relative Intensity vs. Retention time (min)

Peak

Section Complete →

Example of the deconvoluted detection for a compound's adducts.
Deconvolution is performed during Peak Detection

Tagging – simple filtering tool

Eg. Use to limit number of compounds searched

The screenshot displays the Progenesis Q1 software interface during the 'Review Deconvolution' step. The main window title is 'Progenesis Q1 Tutorial HDMSe - Progenesis Q1'. The menu bar includes 'File', 'Import Data', 'Review Alignment', 'Experiment Design Setup', 'Peak Picking', 'Review Deconvolution' (highlighted with a red box), 'Identify Compounds', 'Review Compounds', and 'Compound Statistics'. The central panel shows 'Compound 4.24_458.2754n' with five mass spectra views: M+H, M+NH₄, M+Na, M+CH₃OH+H, and M+K. Below these are 'Mass Spectra' and 'Chromatograms' plots. A 'Peak' list is visible on the right. A 'Quick Tags' dropdown menu is open, showing various statistical and identification filters. The 'Section Complete' button is at the bottom right.

Review Deconvolution
After peak picking, ions are grouped by compound in a process called deconvolution.

1 Review the compounds
Select compounds below to view:

- areas of the ion map showing the different adduct forms
- the mass and retention time profiles of their ions

These views can be used to confirm the validity of your interesting compounds.

2 Optimise for your samples
If any compounds have an ion whose profile doesn't match the majority in the compound, it can be removed. Likewise, missing compound ions can be added.

Compound 4.24_458.2754n

About this compound
Compound 4.24_458.2754n has the following properties:

- Retention time: 4.237 mins
- Neutral mass: 458.2754
- Adducts: 4 (M+H, M+NH₄, M+Na, M+K)

This compound's neutral mass and adducts are based on mass differences between its adduct forms.

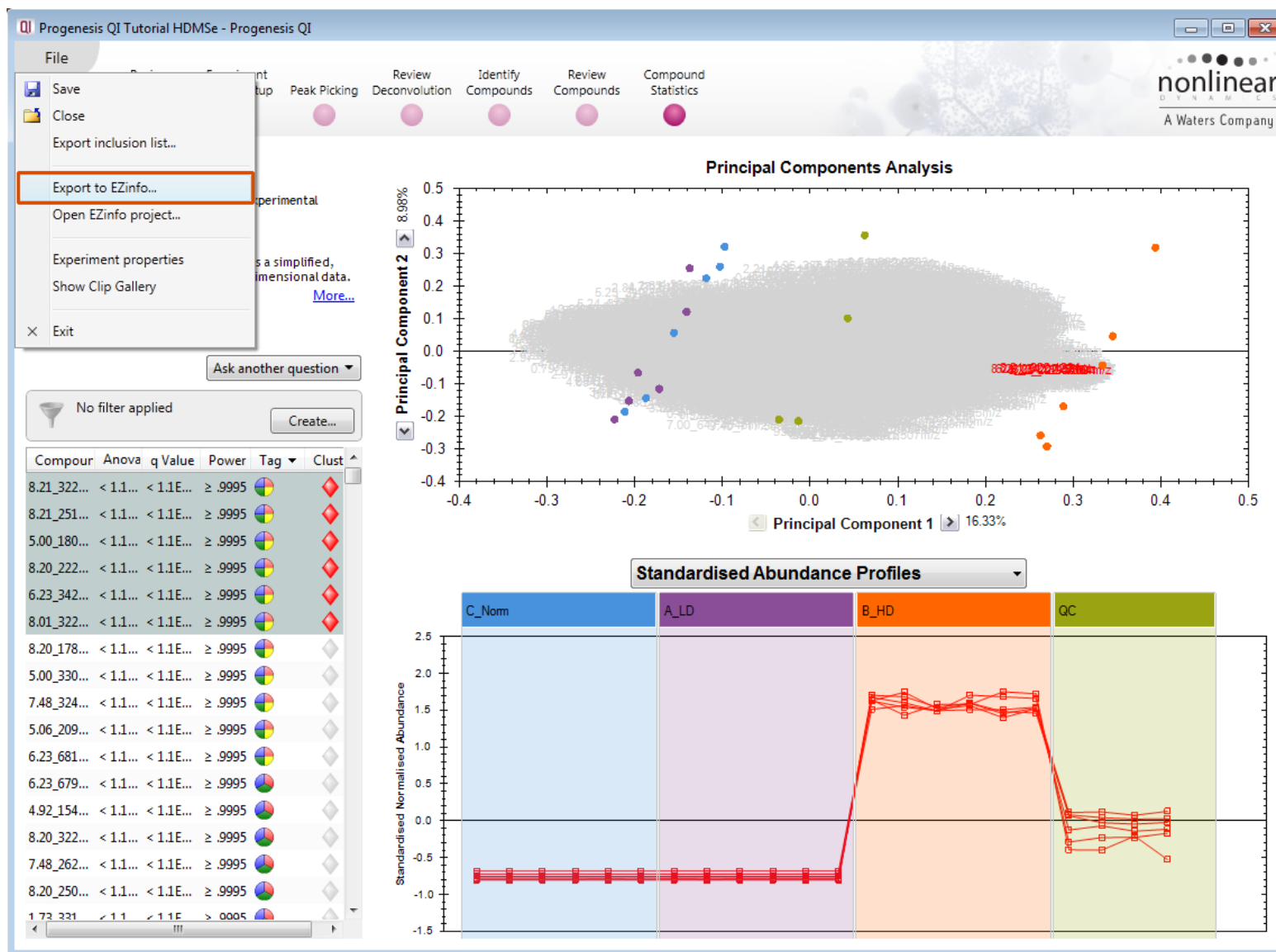
Reviewing assistance
The grid at the left shows all potential ion locations for this compound.
Can you see any ions that could be another adduct form of this compound? Or any that don't belong here?

Quick Tags

- Anova p-value ≤ 0.05
- Max fold change ≥ 2
- New tag...
- Edit tags
- Add to Clip Gallery...
- Anova p-value...
- Max fold change...
- Minimum CV...
- Not identified
- Not fragmented
- Matched in spectral database
- Separated by drift time
- Identified and separated by drift time

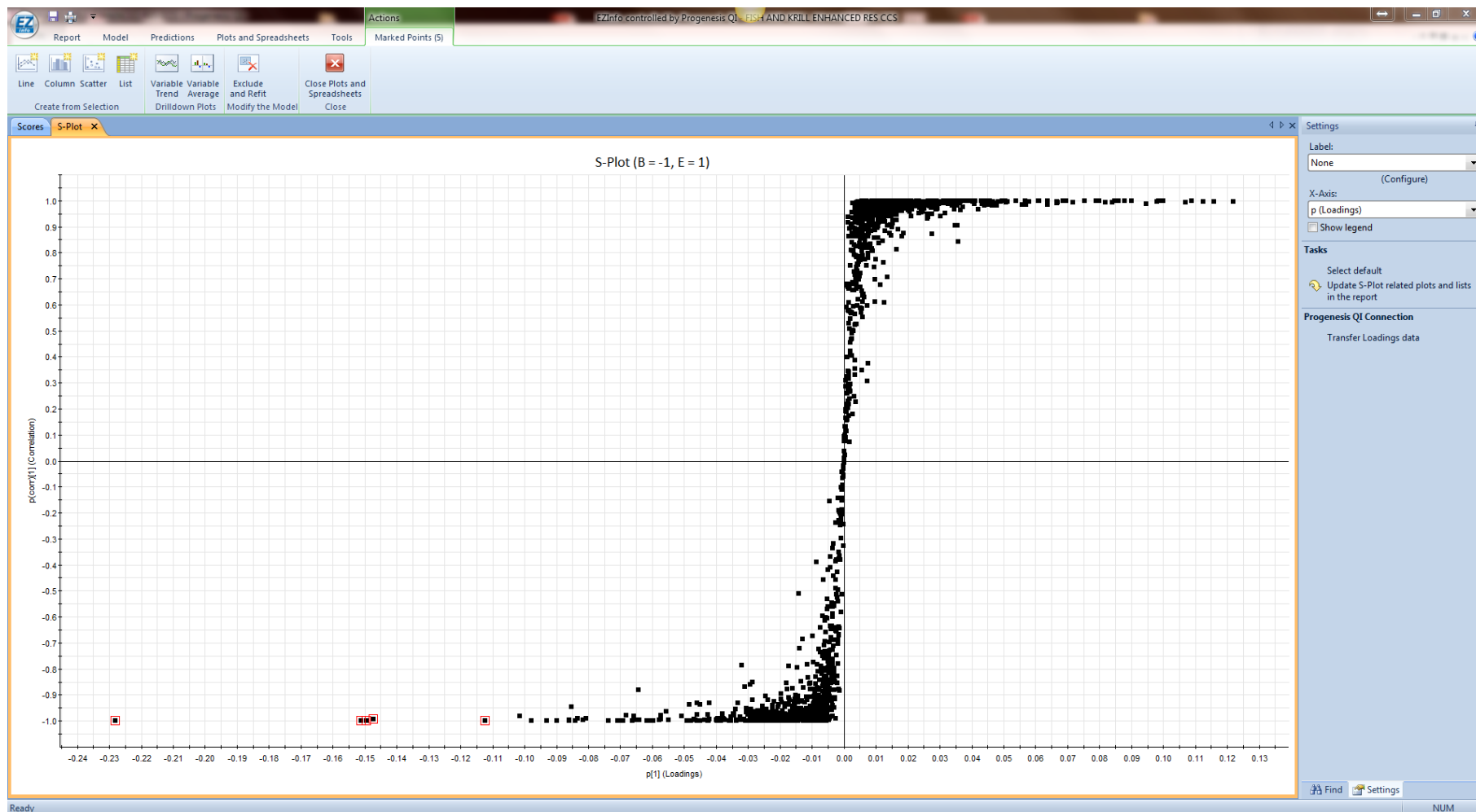
Tagging groups of Compounds based on Statistical attributes is available from Review Deconvolution onwards in the workflow

Compound Statistics PCA and Export to EZinfo



Compound Statistics EZinfo

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Multivariate Statistical Models: PCA, PLS, OPLS, OPLS-DA

Compound Statistics Tag-set Back from EZInfo

The screenshot displays the Progenesis QI interface with a PCA plot and two dialog boxes. The PCA plot shows 'Component 1' (16.33%) on the x-axis and 'Component 2' (10.33%) on the y-axis. A table of compounds is visible on the left, and a 'Profiles' section shows 'B_HD' and 'QC' data.

Question:
Are there any outliers in my data?
Does my data cluster according to my experimental conditions?

What's this?
[Principal Components Analysis](#) produces a simple graphical representation of your multidimensional data.

Collecting data from EZInfo

Select the compounds you are interested in on the EZInfo Loadings plot, then click the 'Transfer Loadings data' link to return a batch of compounds to Progenesis QI.

The compound batch will appear below where you can tag all the compounds in it ready for identification or further investigation.

Right-click on a batch to assign a tag to them.

Batch	Compounds (count)	Tag
1	212	

Close connection to EZInfo?

Close connection to EZInfo?

Importing these tags will close the connection to EZInfo.

To import additional tags after closing the connection, close the existing EZInfo instance, and reopen the EZInfo project using the "Open EZInfo project..." option in the File menu.

Buttons: Import tags and close connection, Keep connection open

Compound identification

MetaScope search, new features

Compounds are identified using the integral MetaScope search engine and a compound database in Structure Data File (SDF)

Edit Search Parameters

MetaScope search parameters
Define a set of MetaScope parameters that can be saved for later reuse. Learn more in the [online reference](#).

Name:
Tutorial No Fragmentation

Compound database
C:\Program Files (x86)\Nonlinear Dynamics\Prog
Data format: Auto-detect

Search parameters
Mass within: 12 ppm
 Retention time within: 0.1 minutes
 CCS within: 2.5 %

Additional compound properties source
 Read additional compound properties from this file
<no database selected>

Fragment search method
 Do not use fragmentation data
 Perform theoretical fragmentation
Relative mass error: 12 ppm
 Perform fragment database search
<no database selected>
Mass within: 12 ppm

- More search parameters **for increased specificity** for ID
 - Dt/CCS, fragment ion data

Theoretical fragmentation search result

Progenesis QI Tutorial HDMSe - Progenesis QI

File Import Data Review Alignment Experiment Design Setup Peak Picking Review Deconvolution Identify Compounds Review Compounds Compound Statistics

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Identify Compounds

Select your identification method:
Progenesis MetaScope

- Filter the compounds**
Using the list below, [filter the compounds](#) to show only those you want to identify.
- Choose search parameters**
Select your MetaScope search parameters or create a new parameter set:
Tutorial Theoretical Fragmentat Edit
- Search for identifications**
Identifications will be assigned to the relevant compounds automatically.
Search for identifications

Tag filter applied
compounds may be hidden Edit...

Compound	Accepted ID	Tag
7.11_308.1070n	4702	
7.11_121.0290m/z		
6.50_270.1043n	9357	
6.22_308.0859n	7847292	
5.18_179.0950n	49854487	
5.18_151.0639n		
4.62_152.0702m/z		
4.42_136.0759m/z	14708992	
4.34_176.0712m/z		
4.21_135.0444m/z		
4.08_235.1824m/z	855682	

All 19 filtered compounds have been identified.

Clear all compound identifications

Compound 5.18_179.0950n (Phenacetin)

CaH₁₃NO+H

Fragment m/z: 138.0914
Peak m/z: 138.0923
Error (ppm): 6.46
Charge: 1
Neutral change: +H

Possible identifications: 4

Compound ID	Description	Adducts	Formula	Retention time	Score	Fragmentation s
49854487	Phenacetin	M+H+H...	C ₁₀ H ₁₃ NO ₂	5.16	B 68.1 B 93.6	
HMDB31811	3,5-Dimethylphenyl methy	M+H+H...	C ₁₀ H ₁₃ NO ₂		B 53.9 B 73.9	
HMDB40021	2,3-Dihydro-5-(3-hydroxyyl	M+H+H...	C ₁₀ H ₁₃ NO ₂		B 50.3 B 56	
HMDB41931	3,4-Methylenedioxyamphic	M+H+H...	C ₁₀ H ₁₃ NO ₂		B 49.8 B 53.2	

Search configuration: B

Database: tutorial fragmentation.sdf
Mass within: 12 ppm
Retention time within: 0.1 minutes
Theoretical fragmentation within: 12 ppm

Section Complete →

Fragment database search result

Mirror plot

Progenesis Q1 Tutorial HDMSe - Progenesis Q1

File Import Data Review Alignment Experiment Design Setup Peak Picking Review Deconvolution Identify Compounds Review Compounds Compound Statistics

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Identify Compounds

Select your identification method:
Progenesis MetaScope

- Filter the compounds**
Using the list below, [filter the compounds](#) to show only those you want to identify.
- Choose search parameters**
Select your MetaScope search parameters or create a new parameter set:
Tutorial Theoretical Fragmentat Edit
- Search for identifications**
Identifications will be assigned to the relevant compounds automatically.
Search for identifications

Tag filter applied
compounds may be hidden Edit...

Compound	Accepted ID	Tag
9.48_332.1162m/z		
7.13_163.0405m/z		
7.11_308.1070n	4702	
7.11_121.0290m/z		
6.50_270.1043n		
6.22_308.0859n	7847292	
5.18_179.0950n	49854487	
5.18_151.0639n		
4.62_152.0702m/z		
4.42_136.0759m/z		
4.34_176.0712m/z		

All 19 filtered compounds have been identified.
Clear all compound identifications

Compound 5.18_179.0950n (Phenacetin)

Possible identifications: 4

Compound ID	Description	Adducts	Formula	Retention time	Score	Fragmentation s
★ 49854487	Phenacetin	M+H-H...	C ₁₀ H ₁₃ NO ₂	5.16	C 69.3	C 100
★ HMDB31811	3,5-Dimethylphenyl methy	M+H-H...	C ₁₀ H ₁₃ NO ₂		B 52.0	B 72.0
★ HMDB40021	2,3-Dihydro-5-(3-hydroxy)	M+H-H...	C ₁₀ H ₁₃ NO ₂		B	
★ HMDB41931	3,4-Methylenedioxyamph	M+H-H...	C ₁₀ H ₁₃ NO ₂		B	

Search configuration: C
Database: tutorial fragmentation.sdf
Mass within: 12 ppm
Retention time within: 0.1 minutes
Fragment database: Q1_HDMSe fragment Database.msp
Fragment mass within: 12 ppm

CC(=O)Nc1ccc(C)cc1

Section Complete



Conclusion

- The “best” analytical system is worth nothing if the experiment is flawed.
- Good chromatography benefits mass analysis and gives you information as well.
- Find the right balance between coverage and method robustness.
- Ion mobility coupled with TOF gives more depth of coverage and an important useful measurement.
- DIA strategies such as HDMSE allow more information content to be extracted from each injection.
- Solutions are available today to routinely apply LC-IMS-MS approaches to Metabolomics.