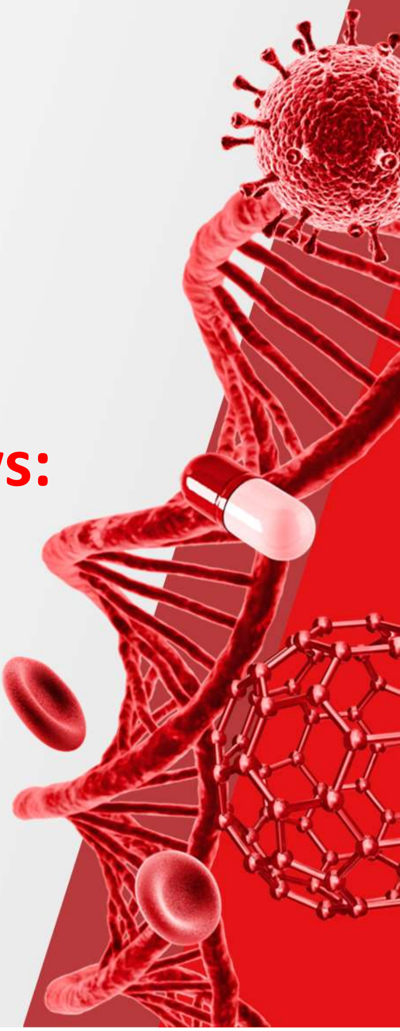
Intelligence-Driven Metabolomics & Lipidomics Workflows: Hardware and Software Innovations

Bashar Amer, Ph.D

Vertical Marketing – Metabolomics

The world leader in serving science



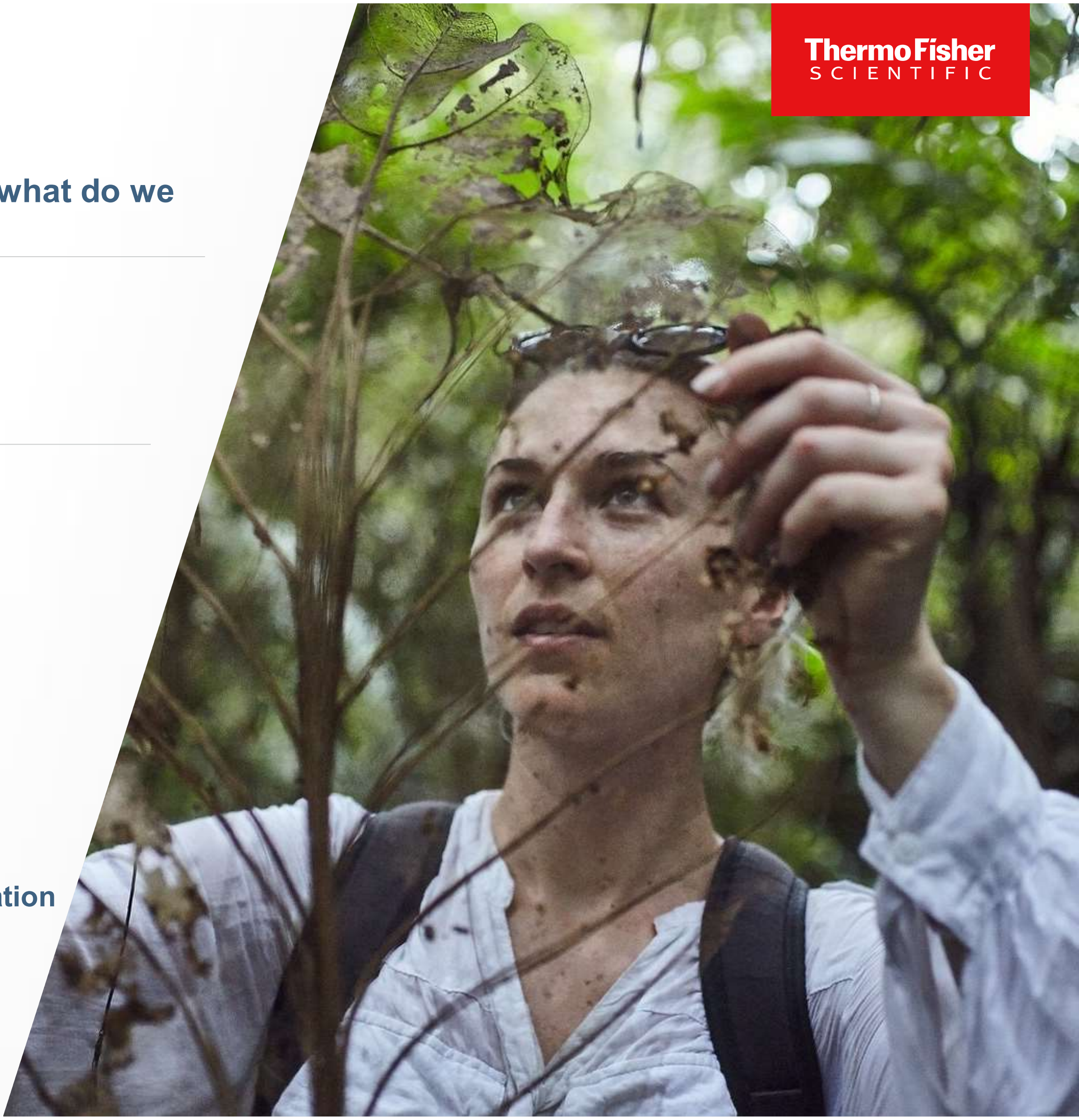
On the agenda

1 Who is the marketing metabolomics team and what do we do?

2 Metabolomics & lipidomics workflows

3 Hardware & software innovations

- i Sugar phosphates characterization by UVPD & HCD
- ii Lipid characterization by UVPD
- iii Real-Time Library Search for flavonoid characterization



Thermo Metabolomics and Lipidomics Marketing Team

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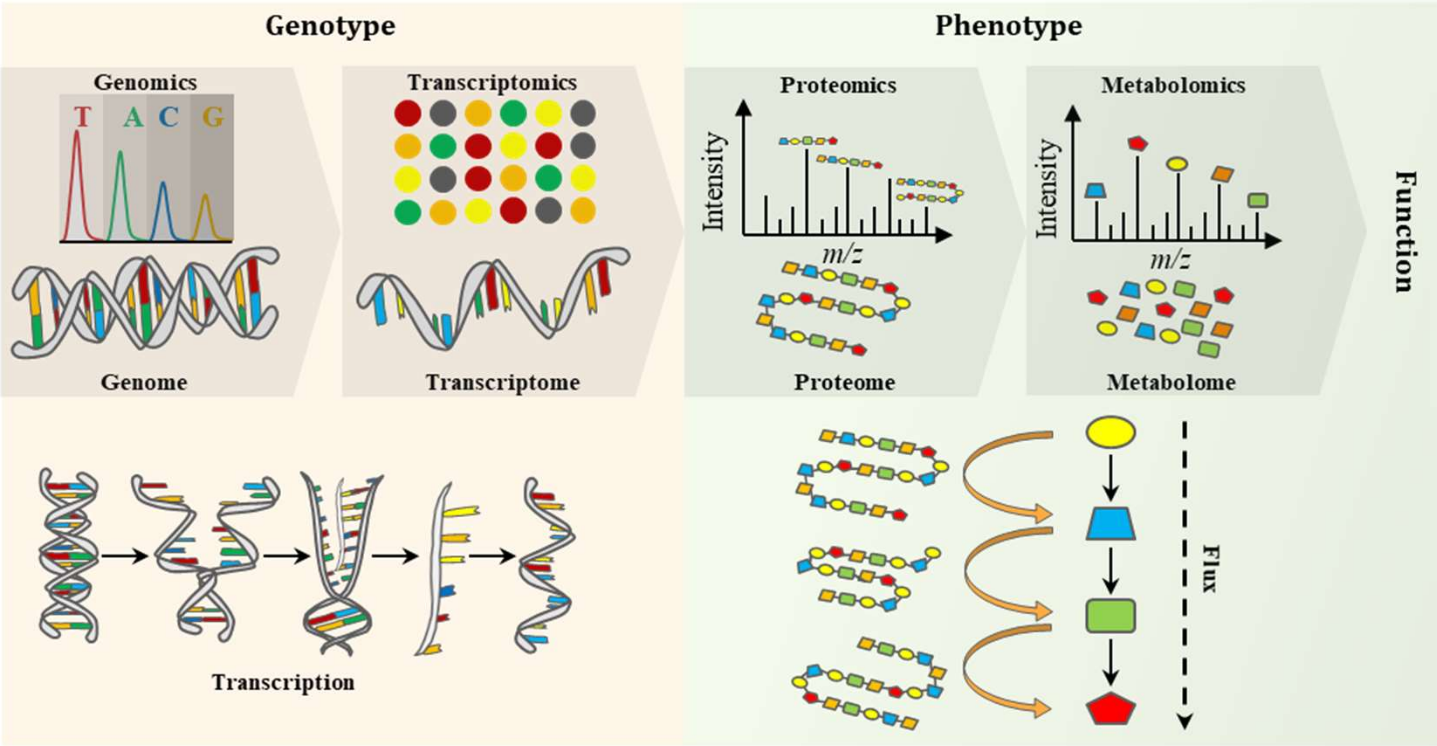
A new team with a fresh focus

- **Support, develop, and implement** metabolomics and lipidomics to help push the field to a new generation

- **Susan:** lipidomics and commercial experiences to translate customer voice directly into actionable workflows
- **Rahul:** lipidomics, stable isotope labeling, and flux experience
- **Bashar:** LCMS and GCMS metabolomics background

Omics - Insights into Biology

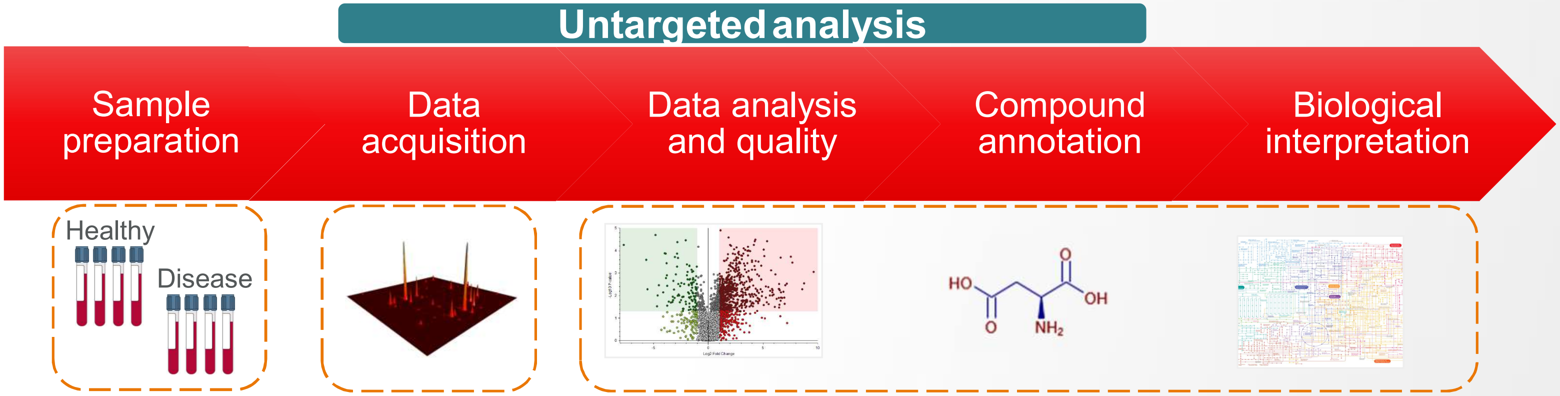


Genetic code carried in DNA → instructions copied into RNA→ translated into the proteins → alter metabolites and lipids

- Multi omics approach is necessary for the accurate prediction of phenotype from genotype but also for a deeper understanding of the principles of life
- Metabolomics and lipidomics provide information related to both function and phenotype

Adapted from: Amer & Baidoo, 2021

Discovery Metabolomics & Lipidomics

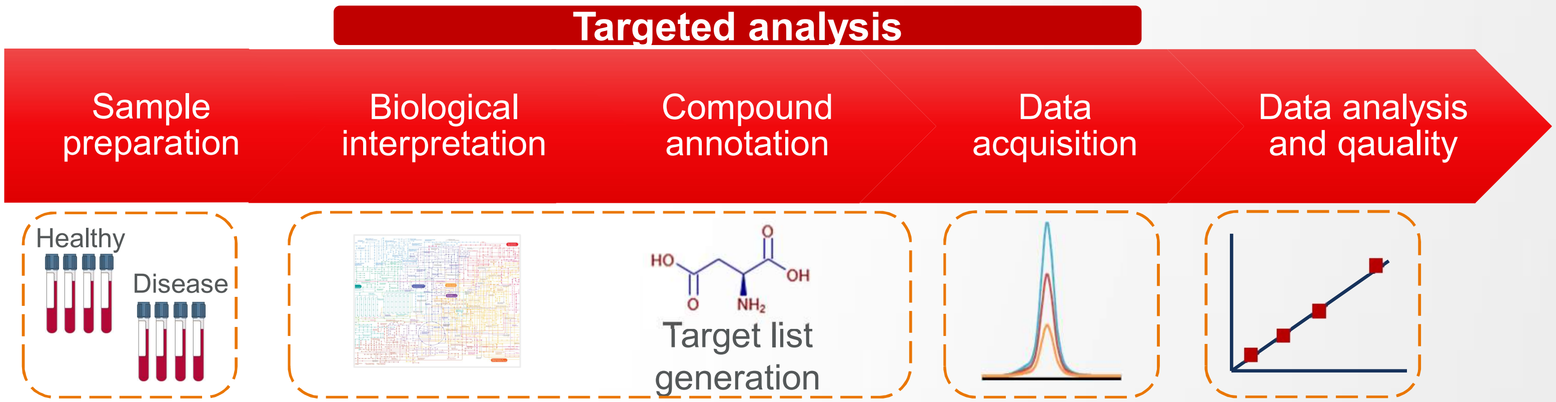


Sample preparation

Data acquisition

Data analysis

Hypothesis Driven Metabolomics & Lipidomics



Targeted analysis

Sample preparation

Biological interpretation

Compound annotation

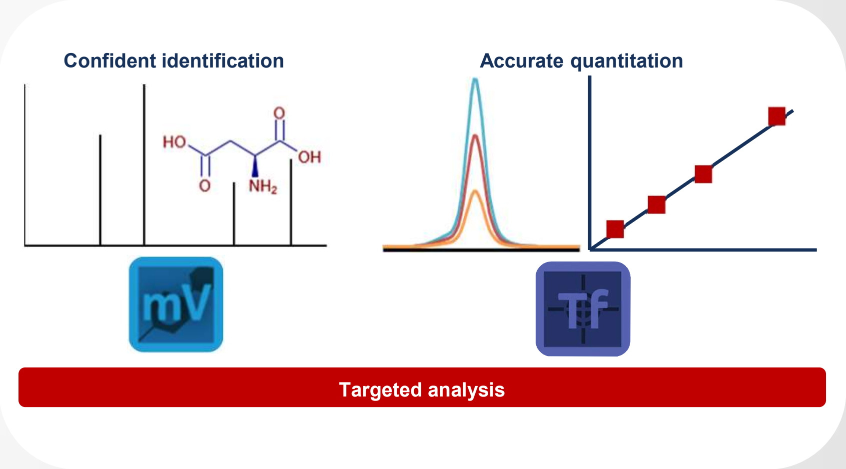
Data acquisition

Data analysis and qauality

Healthy

Disease

Target list generation



Sample preparation

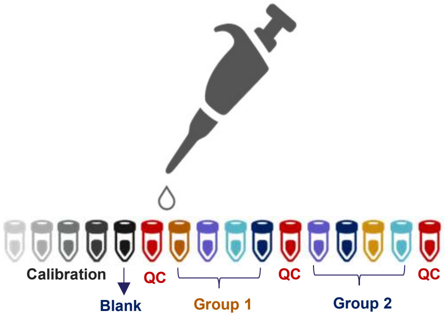
Data acquisition

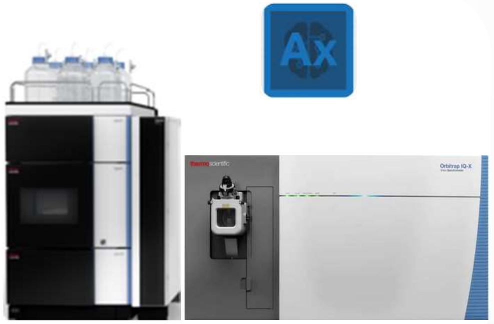
Not only LC-MS solutions but GC-MS and IC-MS

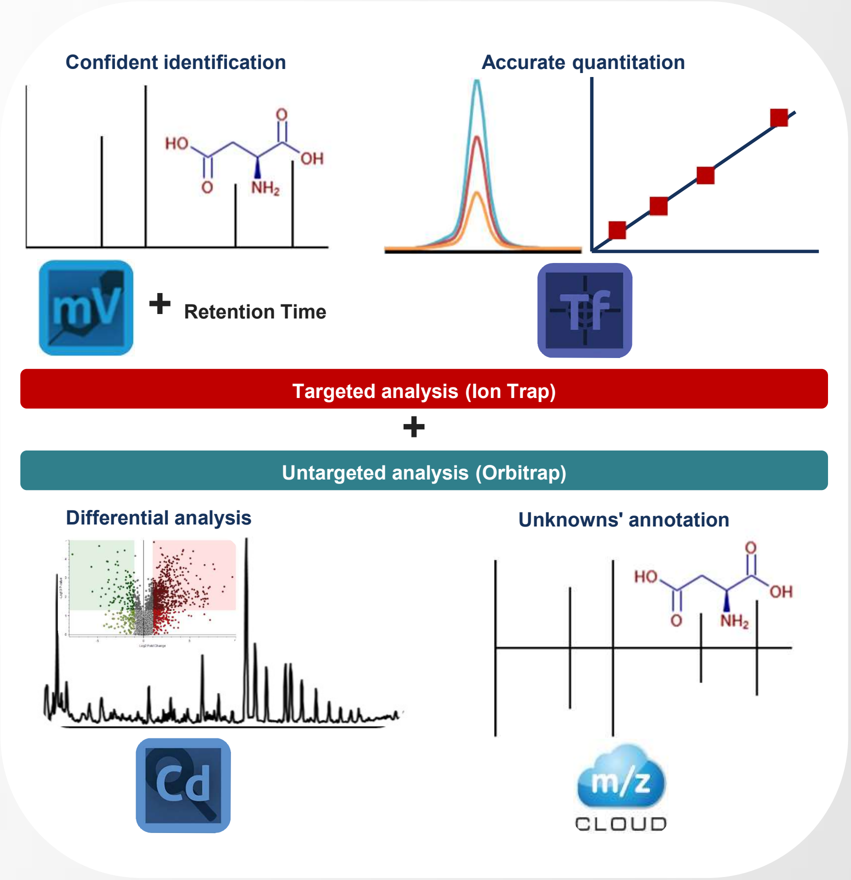
Data analysis

Simultaneous Quantitation and Discovery (SQUAD) Analysis – Tribrid

Single injection!!







Sample preparation

- Utilizing authentic standards and isotopically labeled internal standards for confident identification and absolute quantitation
- Incorporation of QC samples to ensure high-quality data

Data acquisition

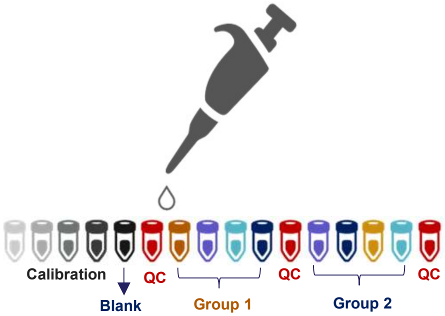
- Robust and high-throughput LC analysis
- High-resolution accurate mass spectrometry
- Sensitive and fast ion-trap mass spectrometry
- Intelligent data acquisition for deeper metabolome coverage

Data analysis

- Sophisticated and comprehensive software solutions that enable fast data processing, accurate quantification of metabolites, advanced differential analysis, confident metabolite annotation utilizing spectral libraries and databases, and biological interpretation

Simultaneous Quantitation and Discovery (SQUAD) Analysis - Exploris

Single injection!!



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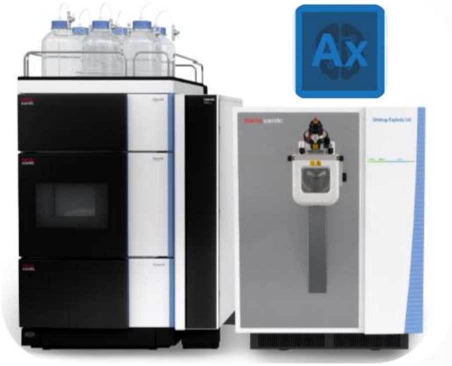

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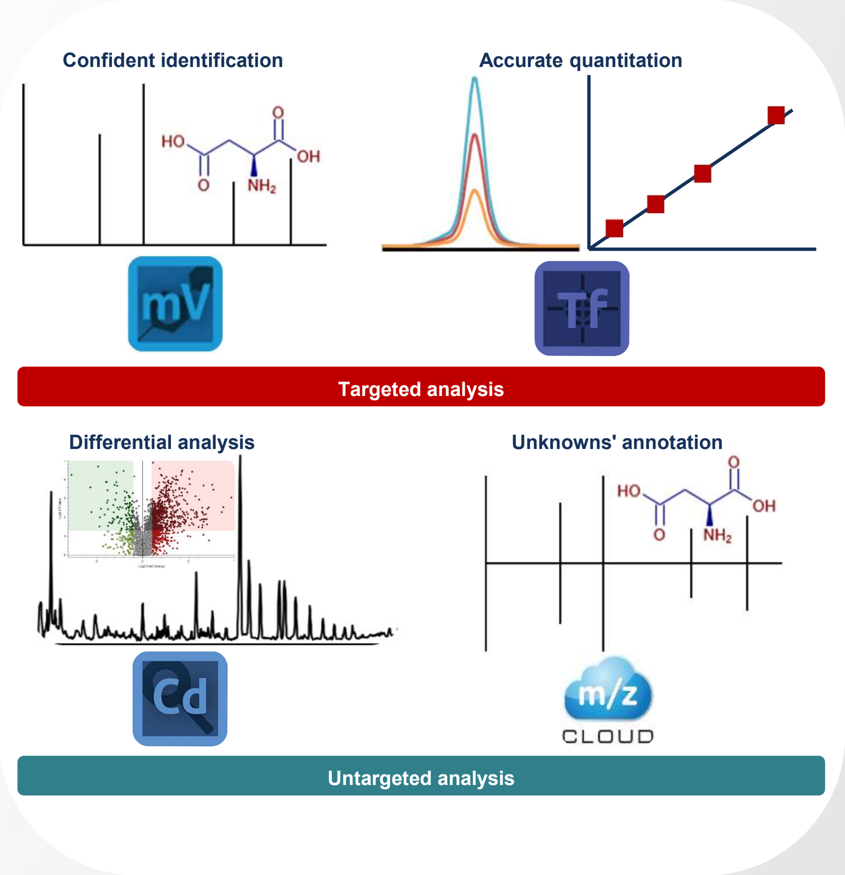

Data analysis

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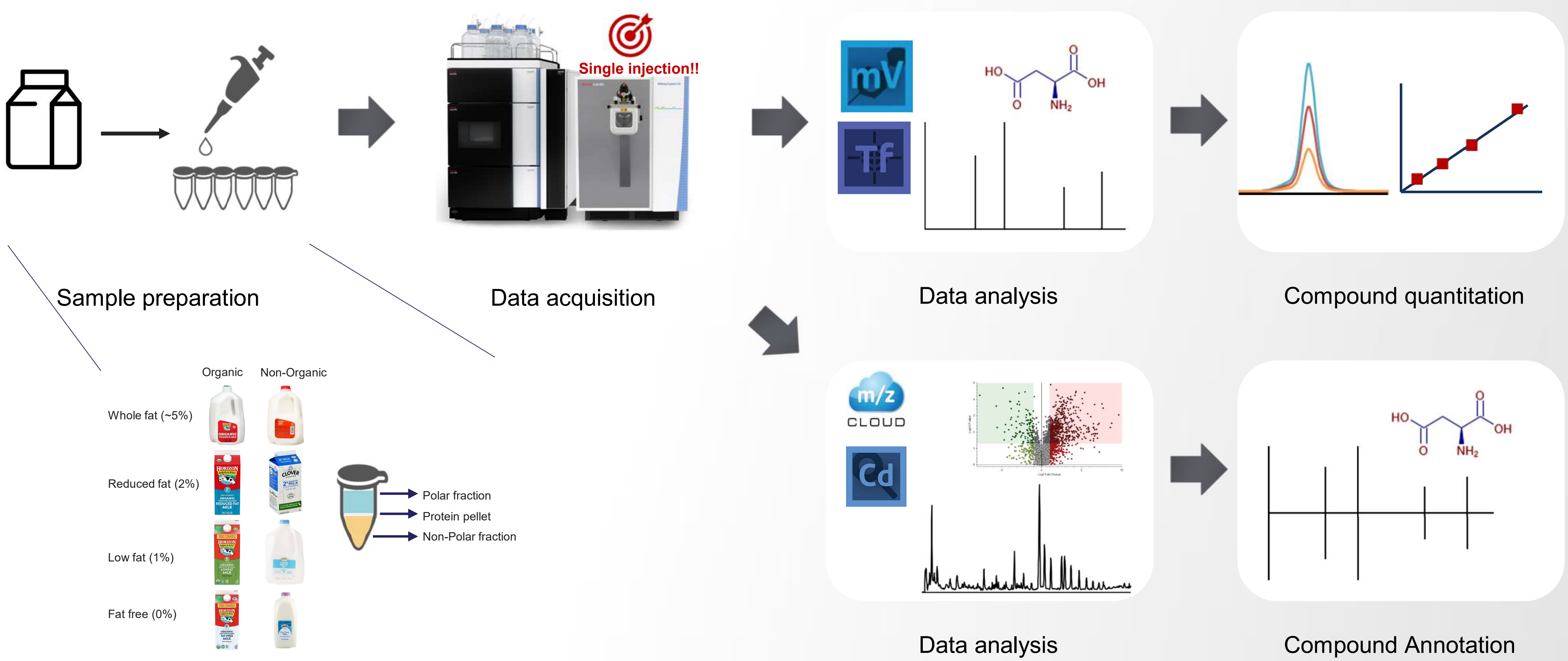
SQUAD workflow in milk



Single injection!!

Sample preparation

Data acquisition

Data analysis

Compound quantitation

Data analysis

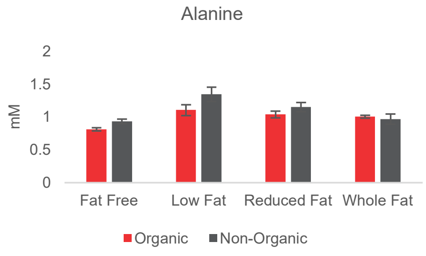
Compound Annotation

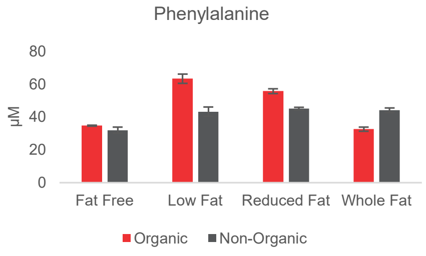
	Organic	Non-Organic
Whole fat (~5%)		
Reduced fat (2%)		
Low fat (1%)		
Fat free (0%)		

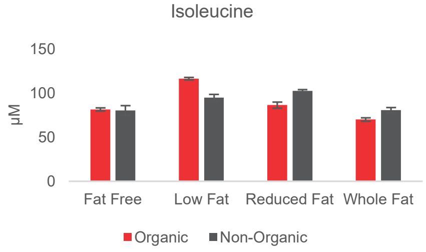
Polar fraction
Protein pellet
Non-Polar fraction

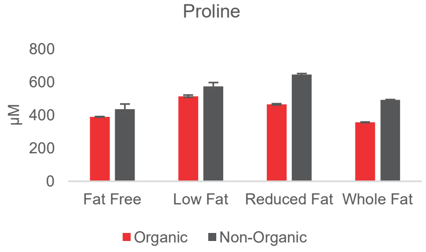
SQUAD workflow in milk

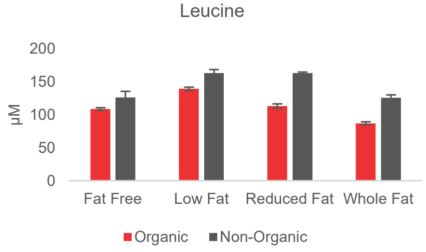
Targeted analysis

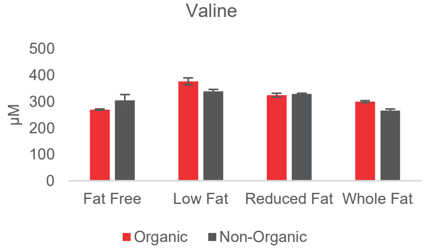




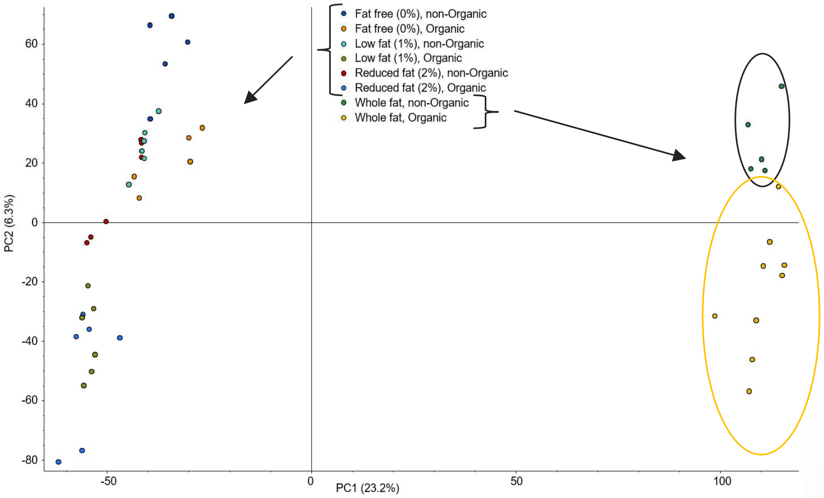


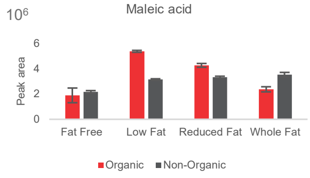


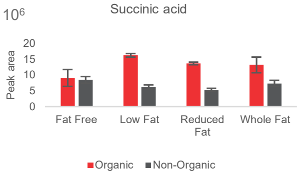


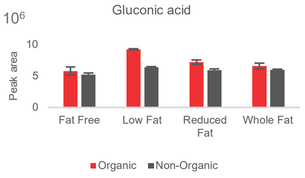


Untargeted analysis



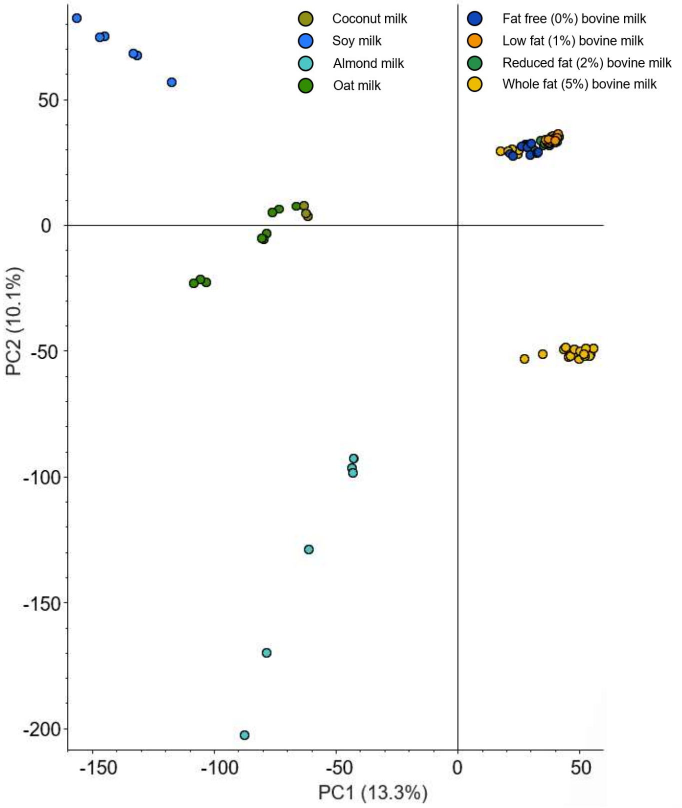


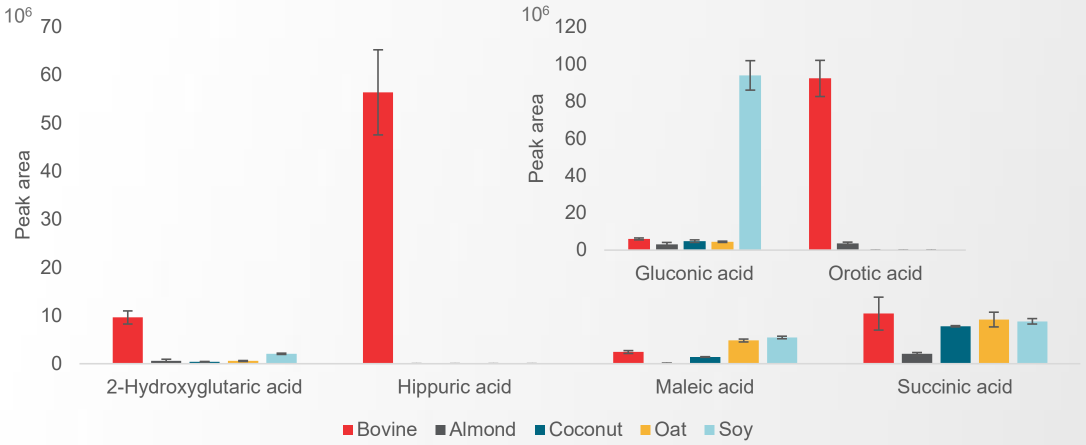




SQUAD workflow in milk

Untargeted analysis





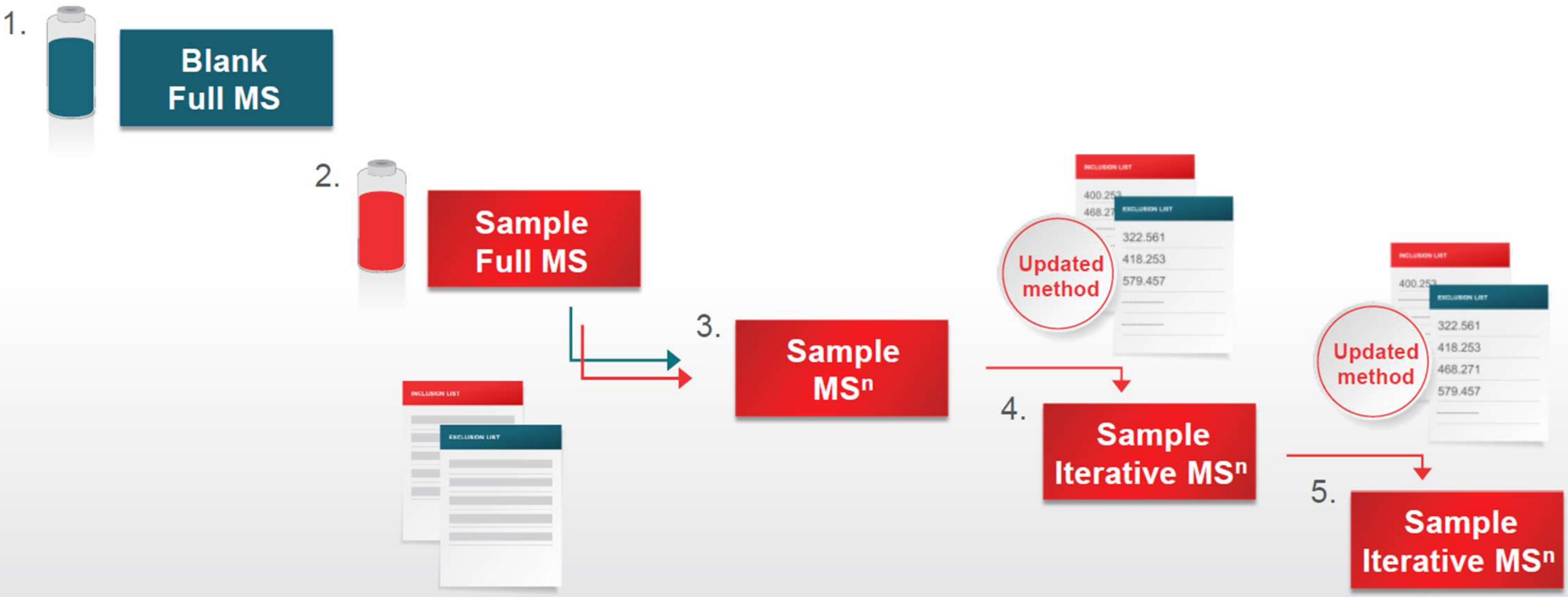
This could be used to assess the quality and authenticate milk for increased food security and consumer protection!

Hardware & Software Innovations

- **Intelligent data acquisition (i.e., Acquire-X™)** for deep and meaningful coverage
- **Multiple fragmentation methods** (i.e., HCD, CID, and UVPD) for confident structural characterization
- **Real-Time Library Search** for improved flavonoid structure elucidation

AcquireX Deep Scan Mode for Intelligent Data Acquisition

Real-time inclusion/exclusion lists



1. Blank Full MS

2. Sample Full MS

3. Sample MS^n

Updated method

4. Sample Iterative MS^n

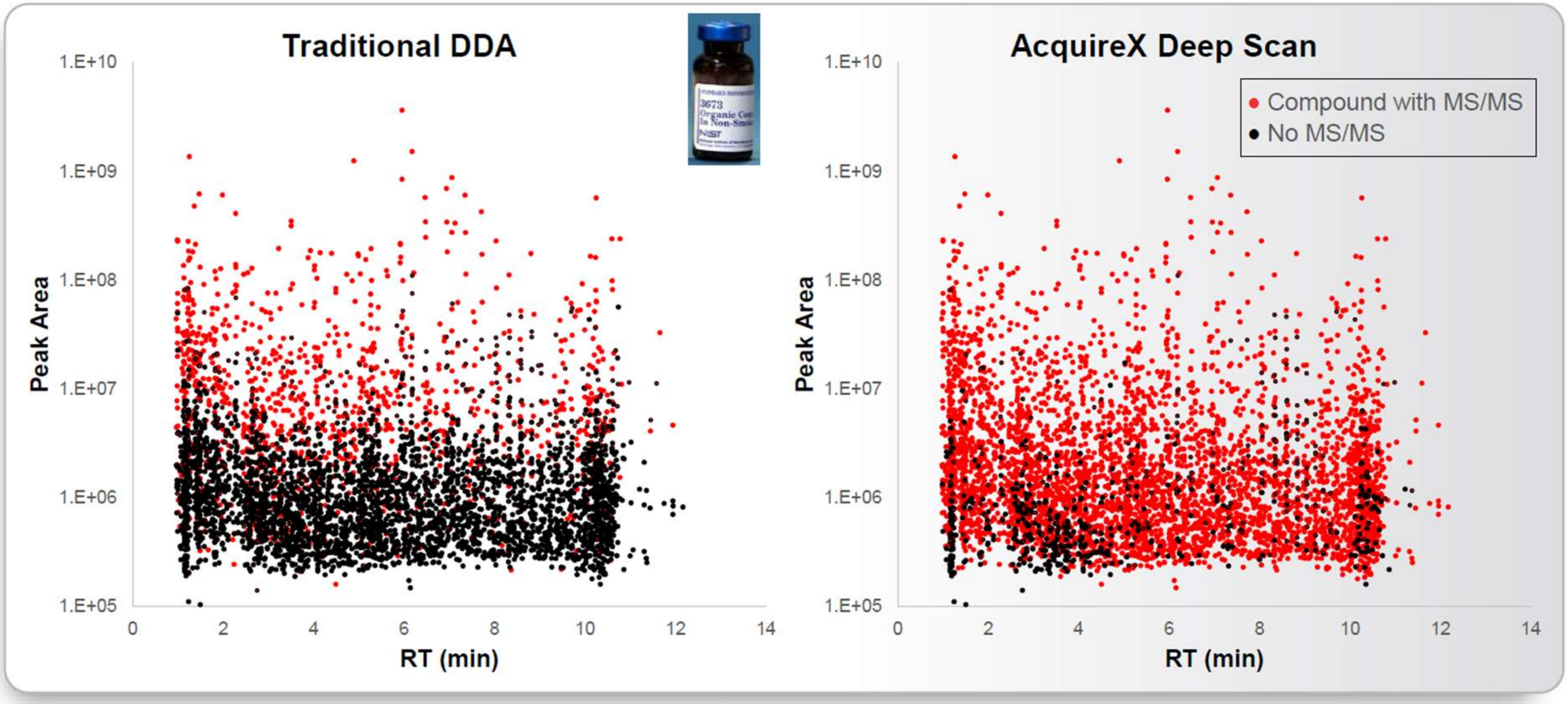
Updated method

5. Sample Iterative MS^n

Automatically generated
Inclusion and Exclusion Lists

Intelligent data acquisition – Acquire-X

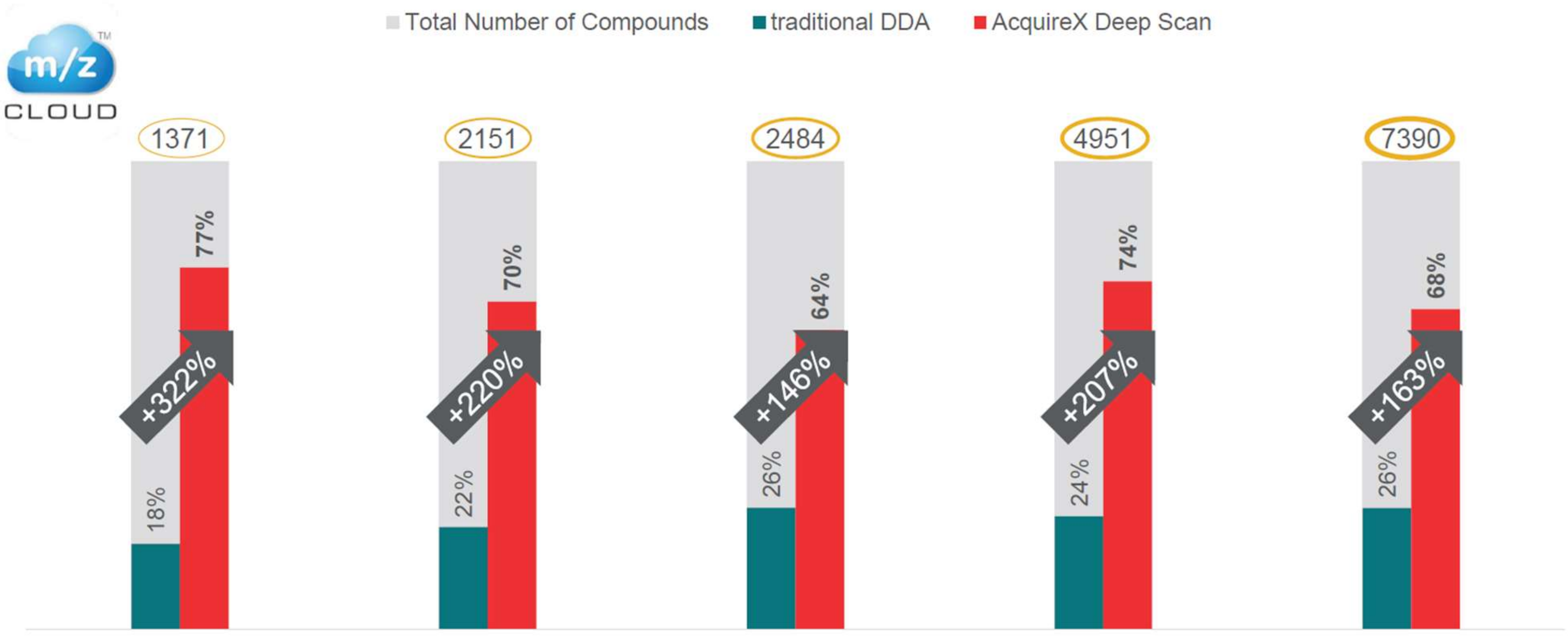
Efficient cycle time yields information on lower abundant compounds



Comparison of traditional DDA and AcquireX Deep Scan for **SRM3673 Non-smoker’s urine** (4951 detected compounds) shows increased depth of MS/MS fragmentation coverage with the AcquireX acquisitions, as illustrated after four injections.

Intelligent data acquisition – Acquire-X

AcquireX deep scan translates to spectral library matches

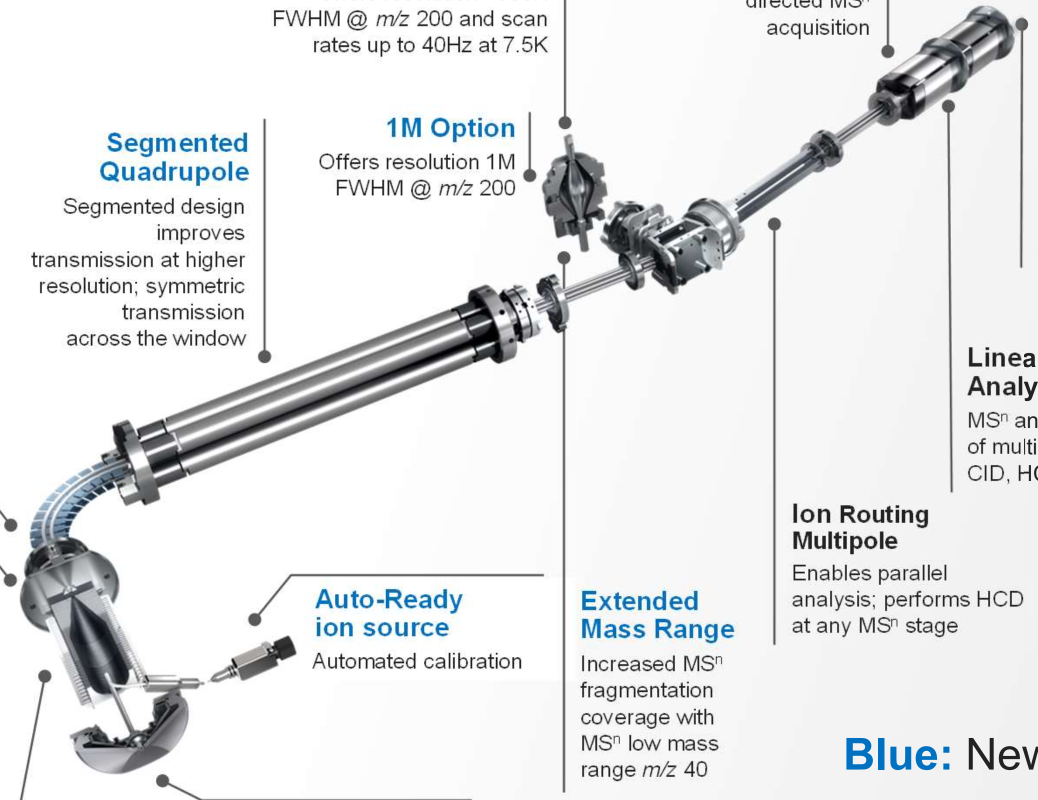


Percentage of compounds with Thermo Scientific™ mzCloud™ mass spectral library Exact+Similarity matches for $[M+H]^+$ by sample type after three cumulative injections

ThermoFisher
SCIENTIFIC

Hardware innovations

IQ-X™ Tribrid MS - Instrument schematic



Orbitrap Mass Analyzer
Offers resolution >500K FWHM @ *m/z* 200 and scan rates up to 40Hz at 7.5K

Real-Time Library Search
Spectral library directed MS^n acquisition

1M Option
Offers resolution 1M FWHM @ *m/z* 200

Segmented Quadrupole
Segmented design improves transmission at higher resolution; symmetric transmission across the window

Advanced Active Ion Beam Guide
Prevents neutrals and high velocity clusters from entering mass resolving quadrupole

UVPD Option
Unique fragmentation mode for structure elucidation

Linear Ion Trap Mass Analyzer
MS^n and sensitive mass analysis of multiple fragmentation types: CID, HCD

Ion Routing Multipole
Enables parallel analysis; performs HCD at any MS^n stage

EASY- IC
Based on Townsend discharge; reliable and easy to use

Auto-Ready ion source
Automated calibration

Extended Mass Range
Increased MS^n fragmentation coverage with MS^n low mass range *m/z* 40

Electrodynamic Ion Funnel
Efficient ion transfer
Broad tuning curves

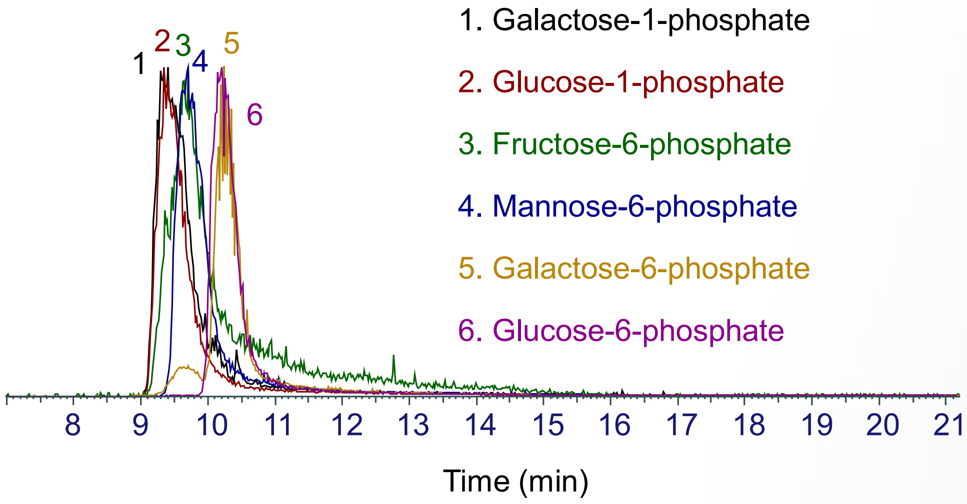
Round Bore Ion Transfer Tube
Optimized for labile compounds

Blue: New on this platform

OPTIONS UVPD | 1M

Sugar Phosphates Analysis

Structure elucidation with multiple fragmentation methods in the absence of chromatographic separation



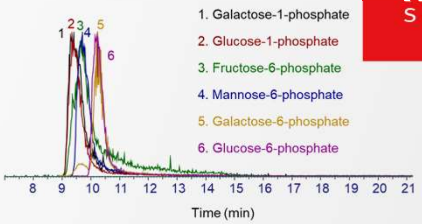
Six protonated sugar-phosphate standards

- Sugar phosphates are involved in metabolic regulation and signaling, but also involved in the synthesis of other phosphate compounds

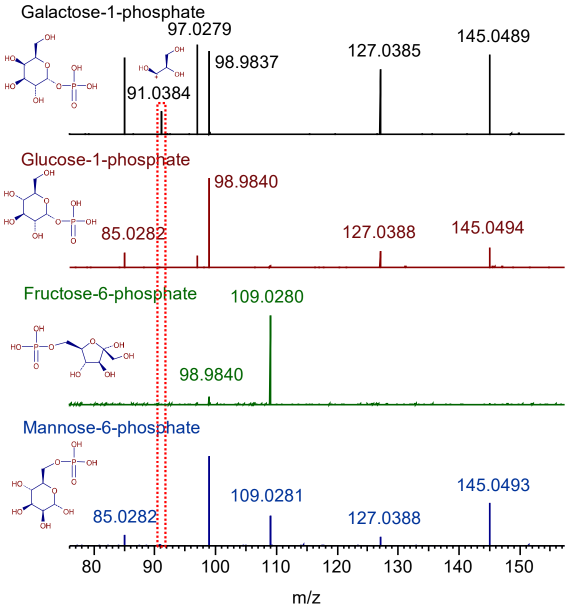
- They are often difficult to separate in standard untargeted methods

Sugar Phosphates Analysis

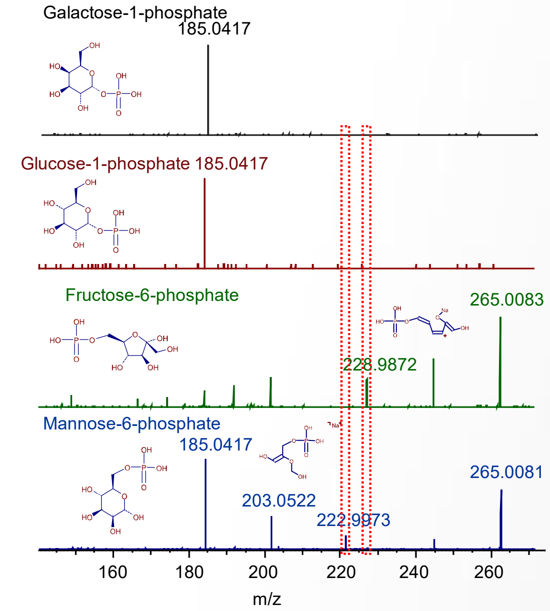
Structure elucidation with multiple fragmentation methods



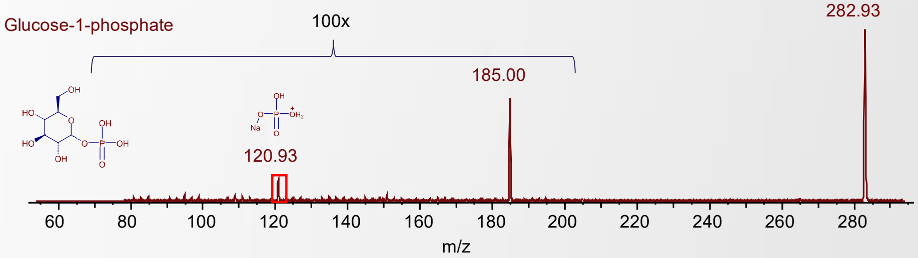
Diagnostic **UVPD** fragment for the protonated adduct of **galactose-1-phosphate**



Diagnostic **HCD** fragments for sodium adduct of **fructose-6-phosphate** and **mannose-6-phosphate**

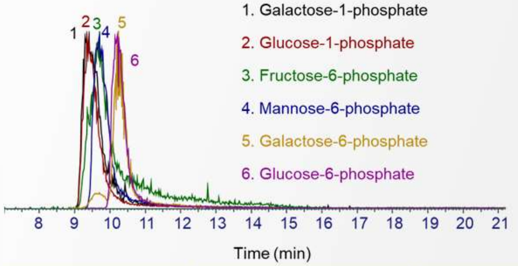


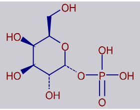
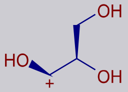
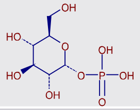
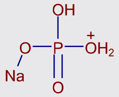
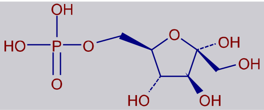
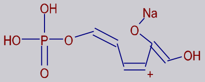
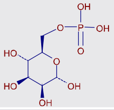
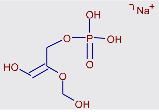
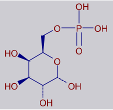
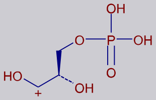
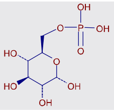
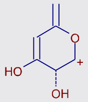
Diagnostic **UVPD** fragment collected in the **ion trap** for sodium adduct of **glucose-1-phosphate.**



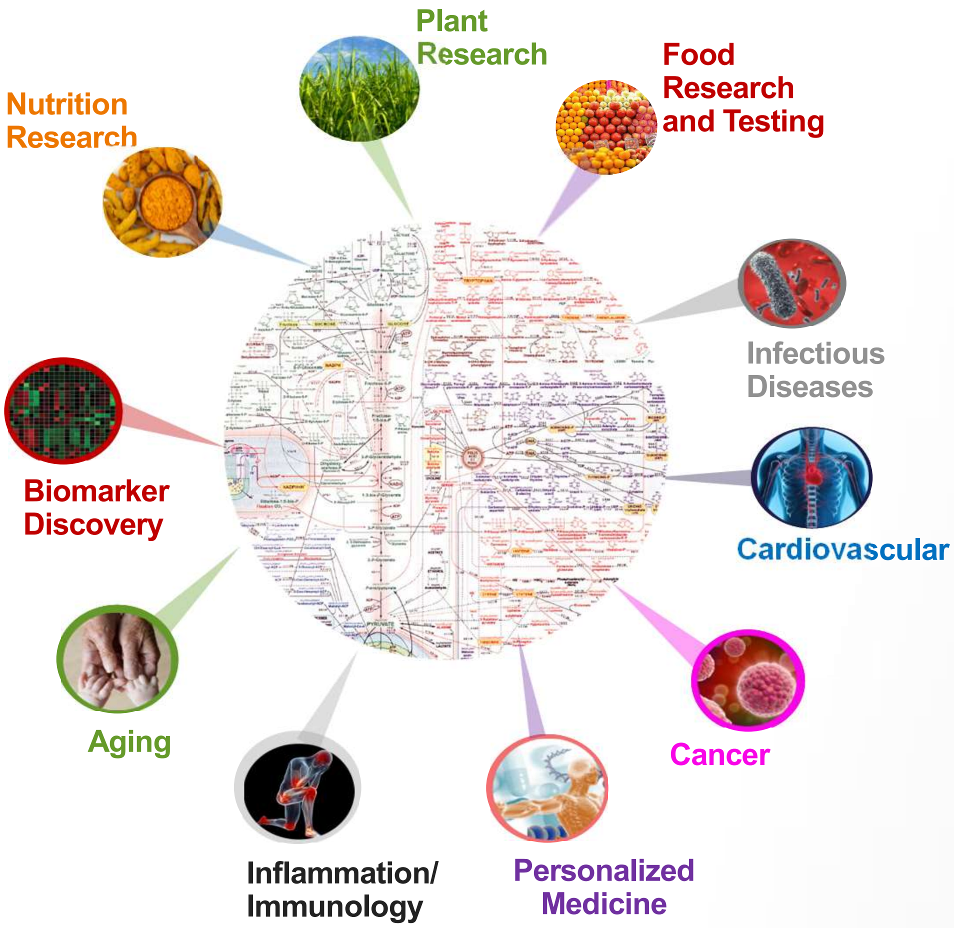
Sugar Phosphates Analysis

Structure elucidation with multiple fragmentation methods



Isomer	Structure	Molecular Formula	RT (min)	Diagnostic fragment ion (*m/z*)	Diagnostic fragment	Fragmentation method
Galactose-1-phosphate		$C_6H_{13}O_9P$	9.4	91.0384		UVPD
Glucose-1-phosphate		$C_6H_{13}O_9P$	9.4	120.9661		UVPD of sodium adduct* * Collected in the ion-trap
Fructose-6-phosphate		$C_6H_{13}O_9P$	9.6	228.9872		HCD of sodium adduct
Mannose-6-phosphate		$C_6H_{13}O_9P$	9.6	222.9973		HCD of sodium adduct
Galactose-6-phosphate		$C_6H_{13}O_9P$	10.4	171.0050		UVPD
Glucose-6-phosphate		$C_6H_{13}O_9P$	10.4	127.0386		UVPD

Lipids Annotation



Plant Research

Food Research and Testing

Nutrition Research

Infectious Diseases

Biomarker Discovery

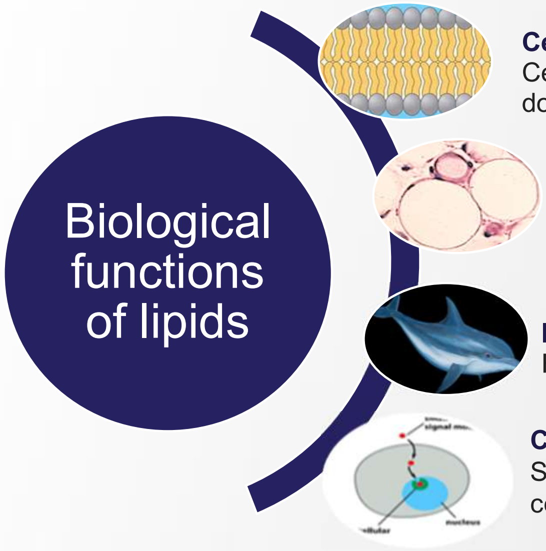
Cardiovascular

Aging

Cancer

Inflammation/ Immunology

Personalized Medicine



Biological functions of lipids

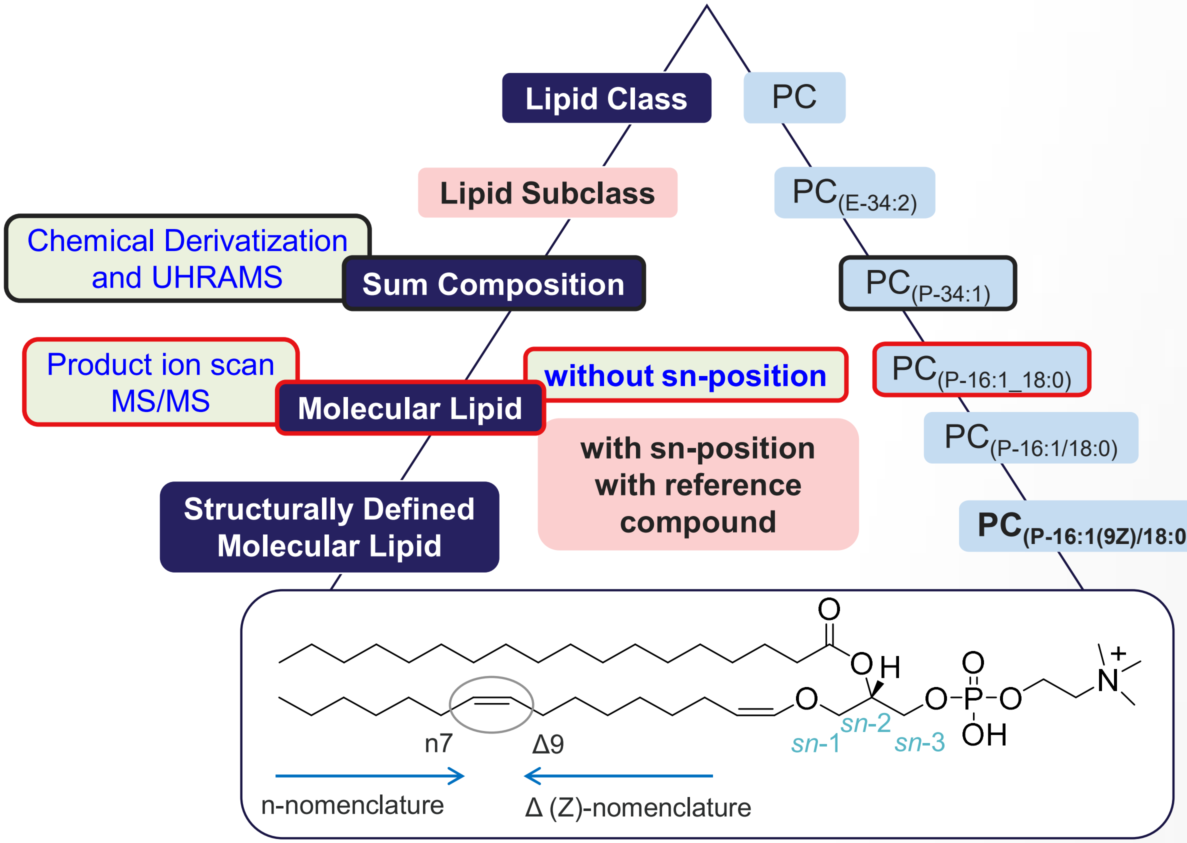
Cell Membranes
Cell membranes are made from a double layer of phospholipids

Energy Storage
Lipid play an important rule in storing energy

Insulation
Fats are important for heat insulation

Cell-cell communication
Steroids are a group of lipids involved in cellular communication

Hierarchal Scheme of Lipid Classification



Lipid Class — PC

Lipid Subclass — PC$_{(E-34:2)}$

Chemical Derivatization and UHRAMS — **Sum Composition** — PC$_{(P-34:1)}$

Product ion scan MS/MS — **Molecular Lipid** — **without sn-position** — PC$_{(P-16:1\_18:0)}$

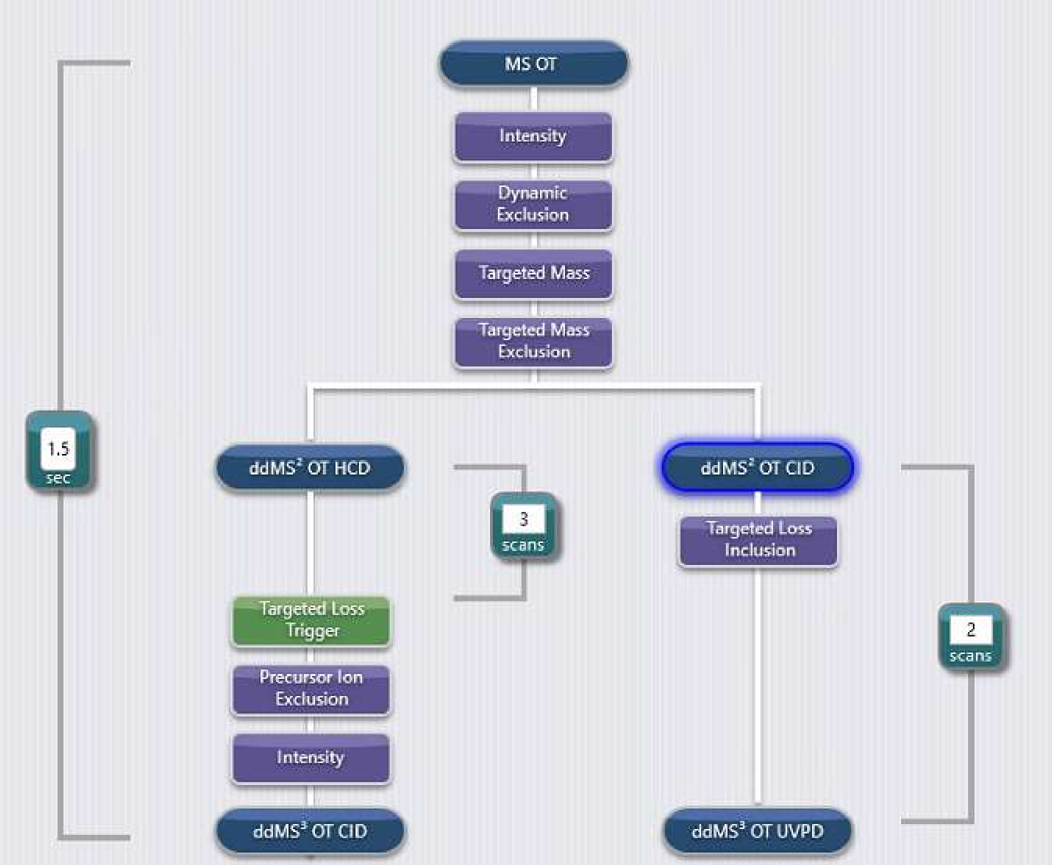
with sn-position with reference compound — PC$_{(P-16:1/18:0)}$

Structurally Defined Molecular Lipid — **PC$_{(P-16:1(9Z)/18:0)}$**

- This diagram indicates the type of MS information leading to different levels of lipid annotation
  - Nominal mass is not sufficient to identify lipids without for example specific MS/MS precursor or neutral loss scans
  - Accurate mass & derivatization give elemental composition or "sum composition"
  - Accurate mass & MS/MS give molecular lipids without sn-position
  - Specialized methods may allow the assignment of double bonds and stereochemistry

Adapted from: Ekroos, K. In Lipidomics, Ch 1. 2012 Wiley-VCH Verlag GmbH & Co

UVPD Information in a Biological Matrix



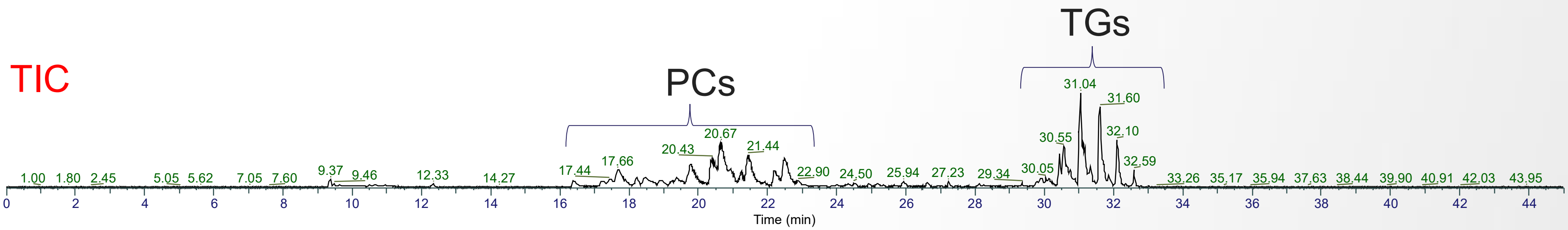
Fatty acid composition of PC

SN position of the fatty acids

- Bovine liver lipid extract
- MS^2 fragmentation of PC using HCD and CID
- MS^3 fragmentation using CID for characterization of PC: MS^3 is triggered for lipids with loss of fatty acids
- MS^3 fragmentation using UVPD for SN position: MS^3 is triggered for ions with a loss of 183 (Sodium Adduct)

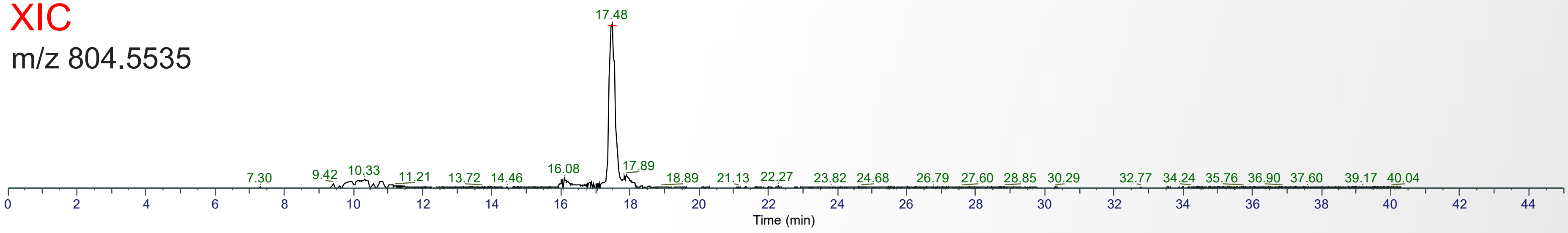
Identification of PC 36:4

TIC

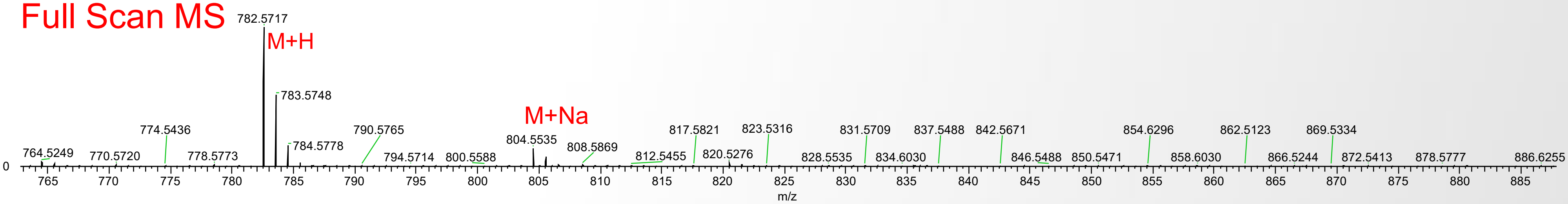


XIC

m/z 804.5535



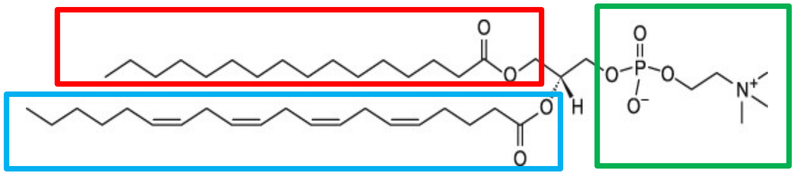
Full Scan MS



Structural Characterization of PC 16:0_20:4

HCD MS^2 of M+H m/z 782.57 – Fatty acids composition

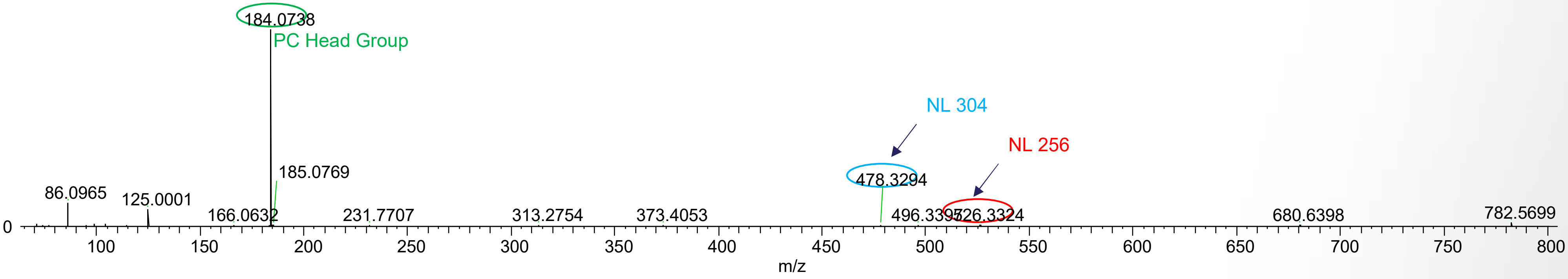
NL 256 : FA 16:0



NL 304 : FA 20:4

m/z 184 : Phosphocholine

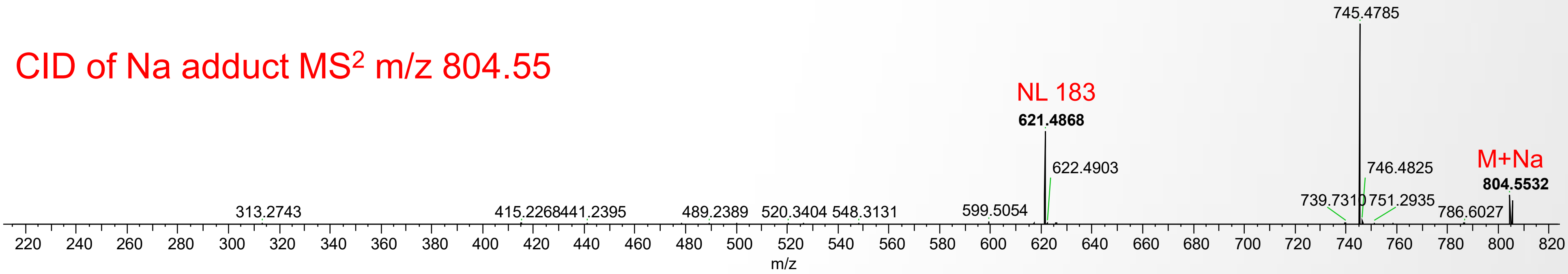
HCD MS^2 of M+H m/z 782.57



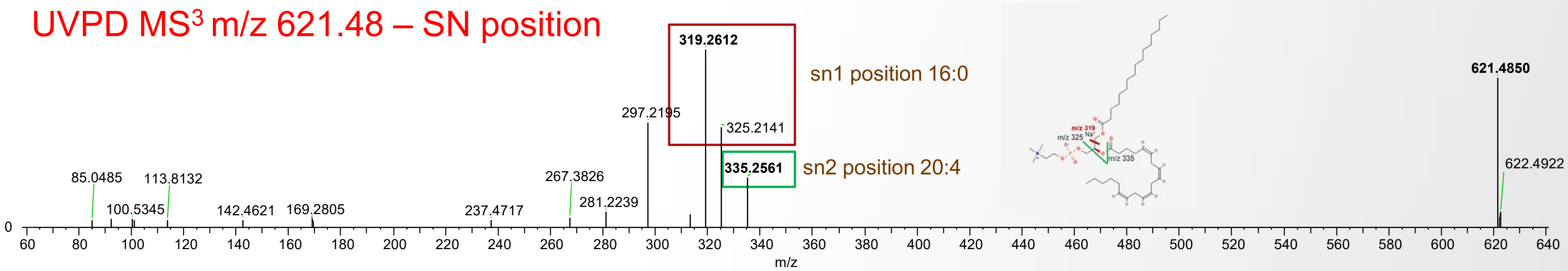
Structural Characterization of PC 16:0/20:4

CID of Na adduct MS^2 m/z 804.55 followed by UVPD MS^3 m/z 621.48 – SN position

CID of Na adduct MS^2 m/z 804.55

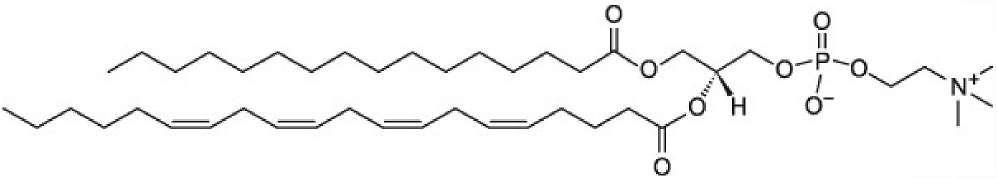


UVPD MS^3 m/z 621.48 – SN position

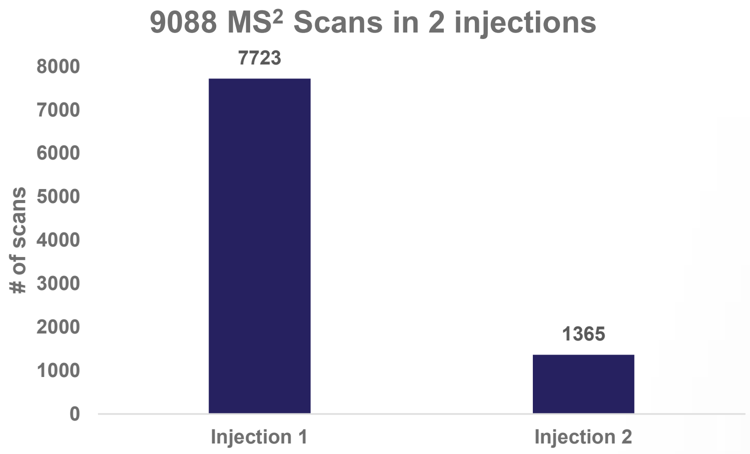


sn1 position 16:0

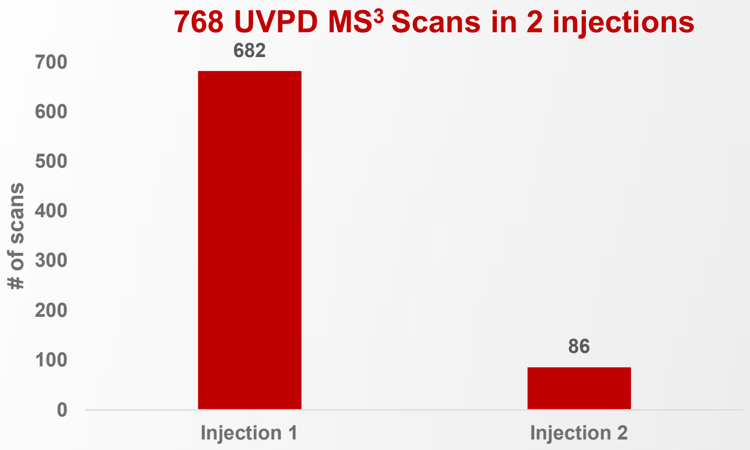
sn2 position 20:4



UVPD Analysis on LC Timescale with Acquire-X



9088 MS^2 Scans in 2 injections

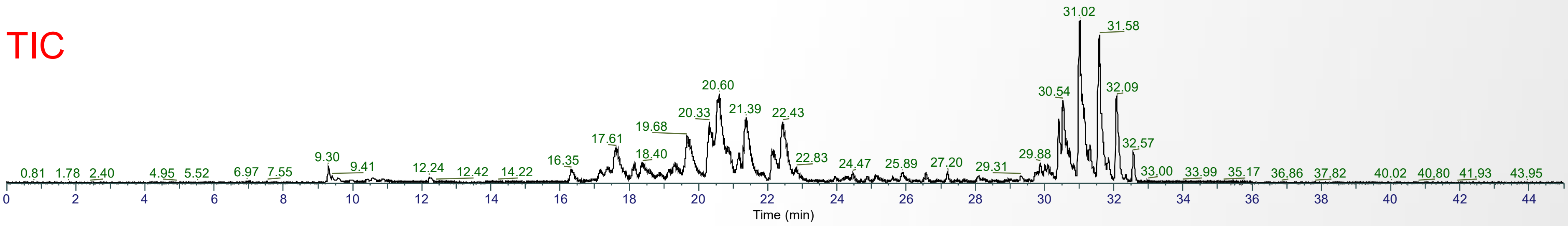


768 UVPD MS^3 Scans in 2 injections

Acquire-X for UVPD of Low Abundant PC

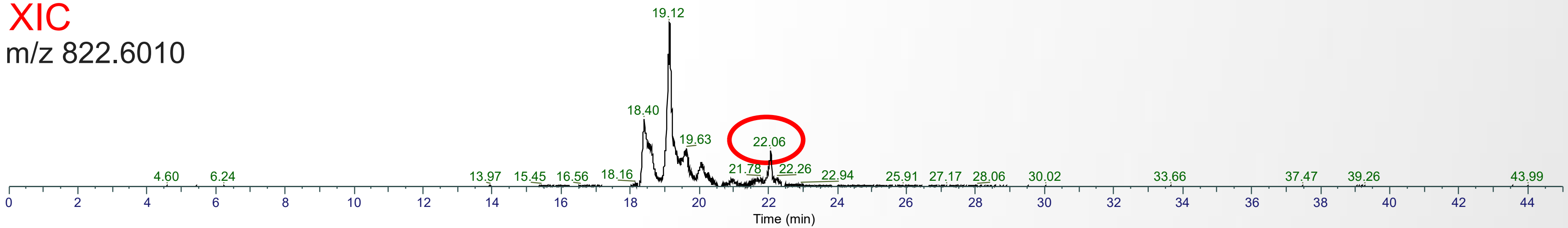
m/z 822.6010

TIC

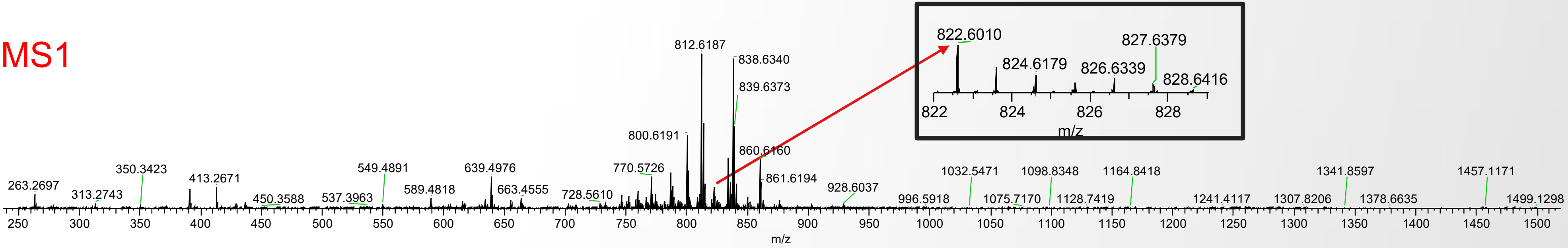


XIC

m/z 822.6010



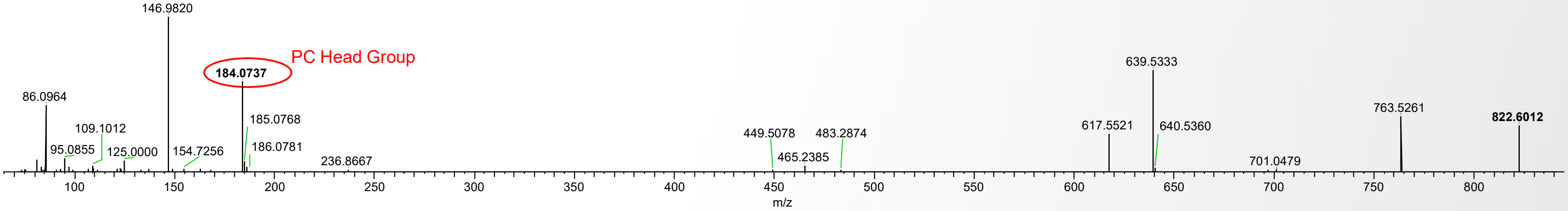
MS1



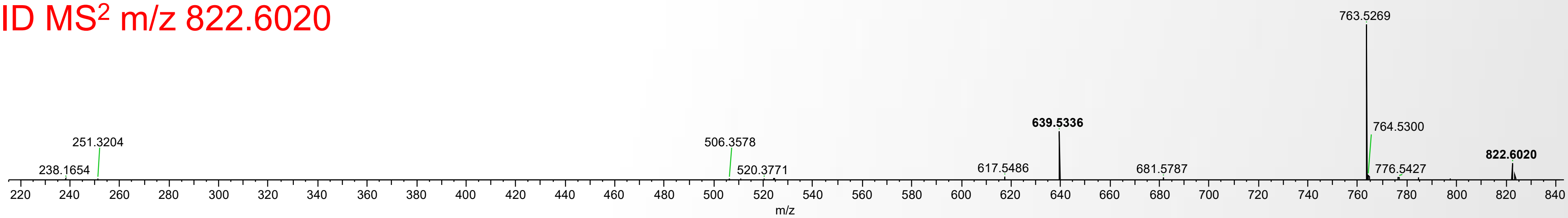
Acquire-X for UVPD of Low Abundant PC

PC m/z 822.6010

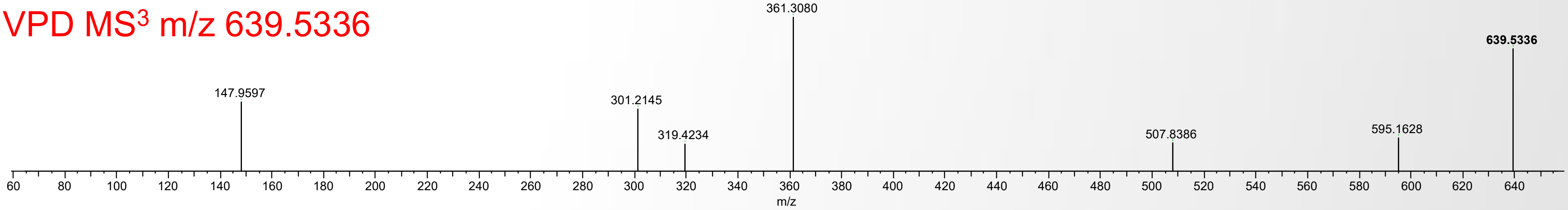
HCD MS^2 m/z 822.6012



CID MS^2 m/z 822.6020



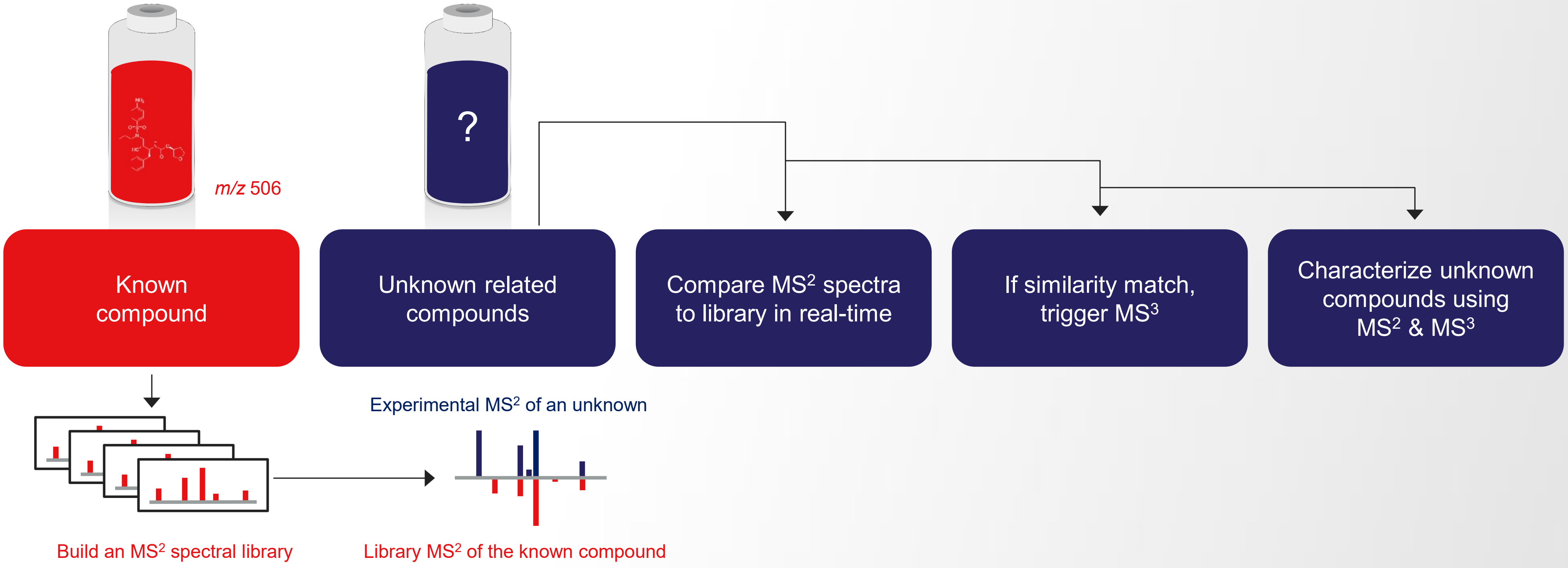
UVPD MS^3 m/z 639.5336



Real-Time Library Search for Improved Structure Elucidation

Challenge: Identification of unknown metabolites, degradants, or transformation products

Solution: Intelligent MS^3 data acquisition enables annotation and characterization of unknown compounds



m/z 506

?

Known compound

Unknown related compounds

Compare MS^2 spectra to library in real-time

If similarity match, trigger MS^3

Characterize unknown compounds using MS^2 & MS^3

Experimental MS^2 of an unknown

Build an MS^2 spectral library

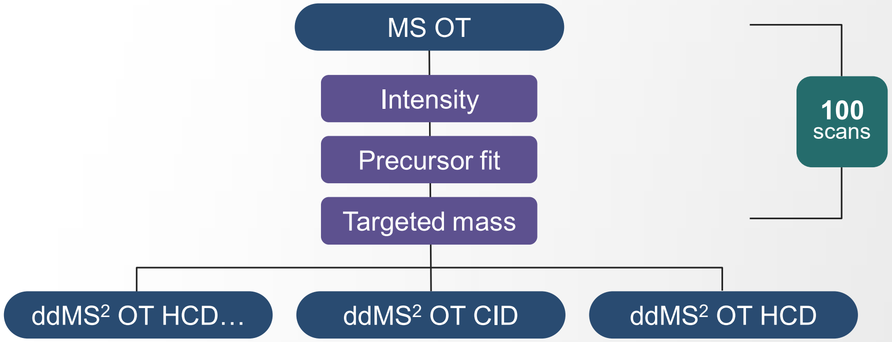
Library MS^2 of the known compound

STEP 1

Create a spectral library
for the compounds of interest.

Orbitrap IQ-X Tribrid MS

Library builder method

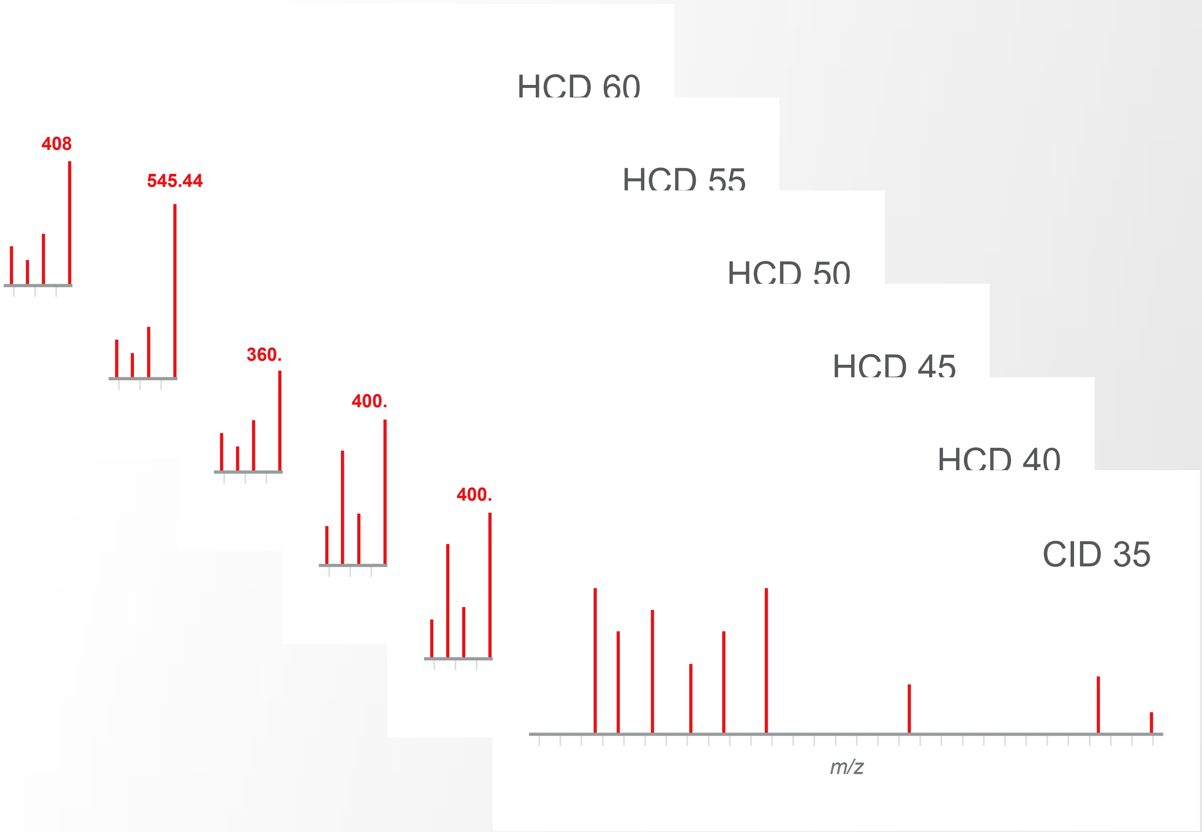

MS OT
Intensity
Precursor fit
Targeted mass
ddMS² OT HCD…
ddMS² OT CID
ddMS² OT HCD
100 scans

STEP 1

Create a spectral library
for the compound of interest.

Spectral libraries are created in using the library builder method template in the IQ-X instrument control software.

MS^2 Spectra

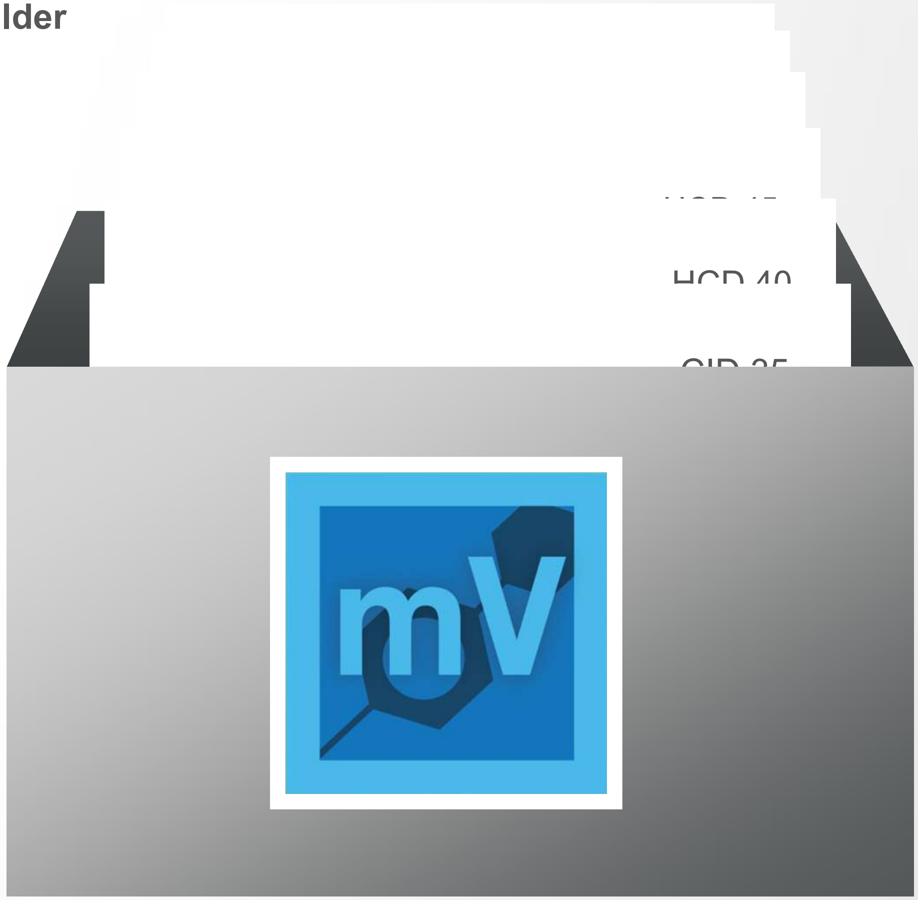


STEP 1

Create a spectral library
for the compound of interest.

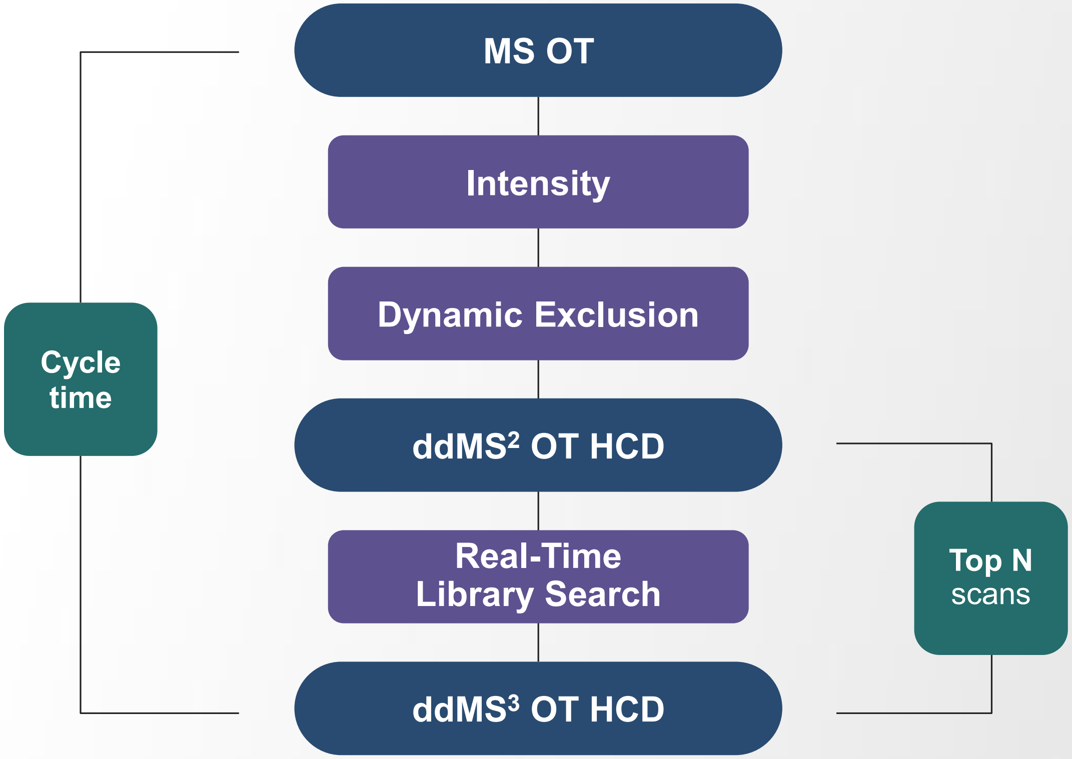
Spectral libraries are curated in
mzVault software.

Library Builder



STEP 2

Build a new acquisition method using RTLS method template to interrogate your samples of interest



MS OT

Intensity

Dynamic Exclusion

ddMS² OT HCD

Real-Time Library Search

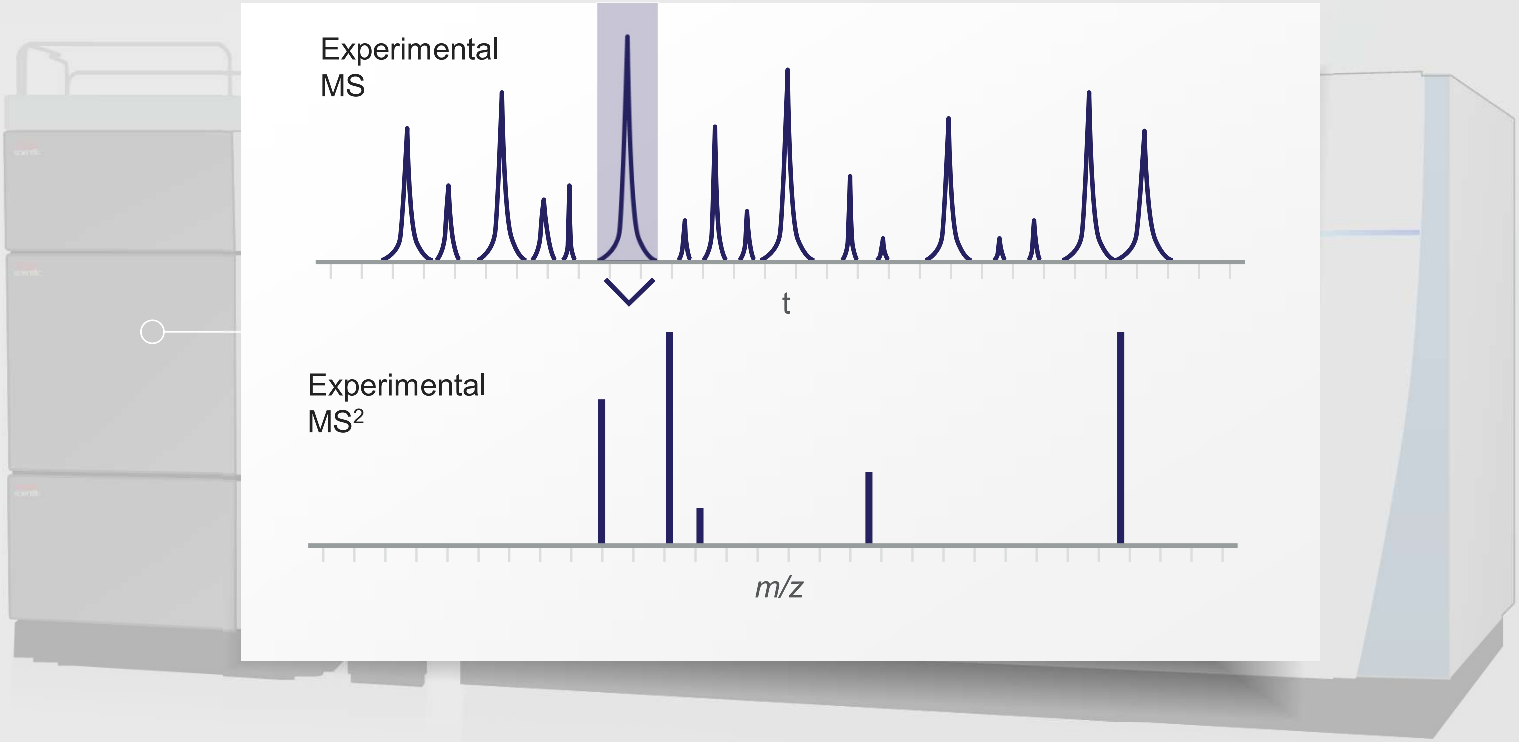
ddMS³ OT HCD

Cycle time

Top N scans

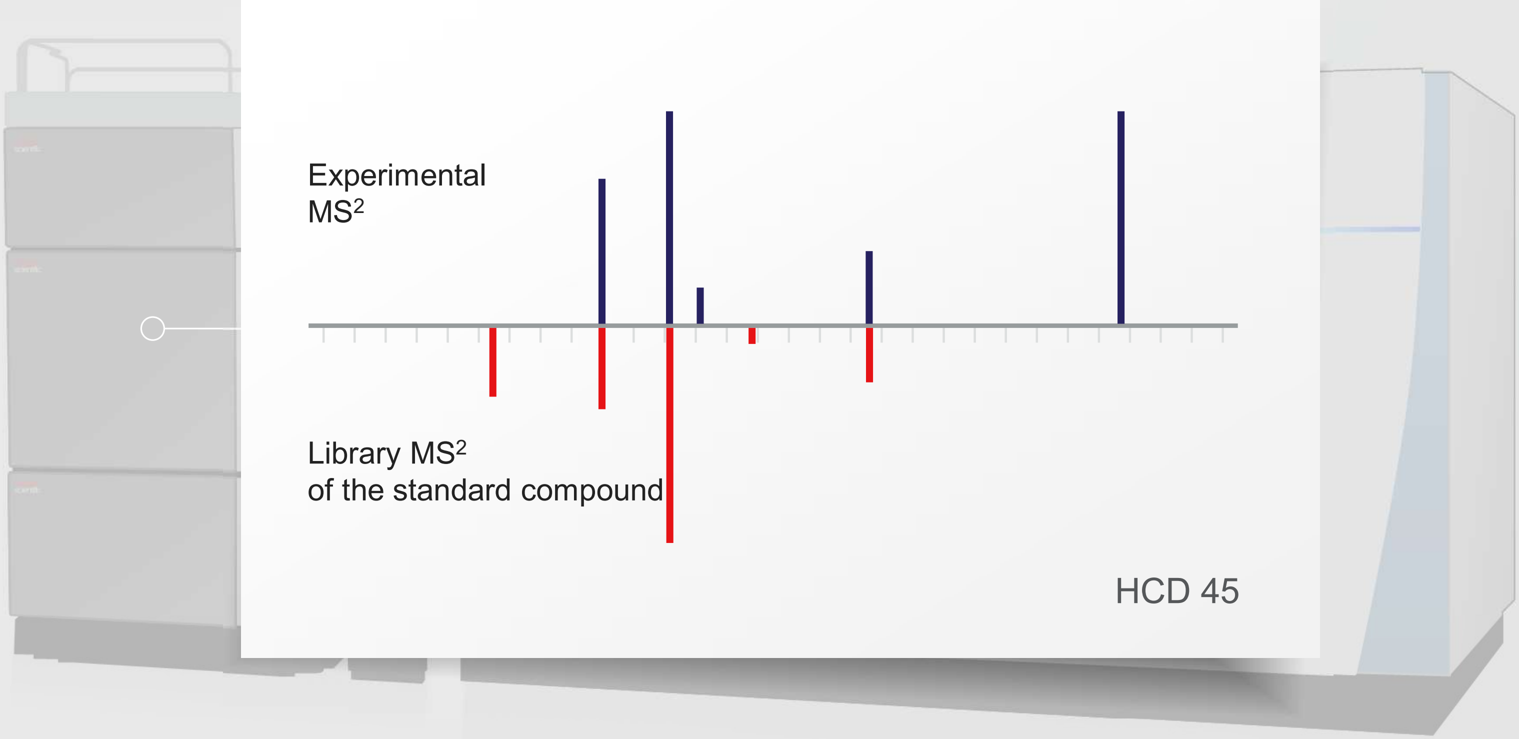
STEP 3

LC-MS analysis of my sample



STEP 4

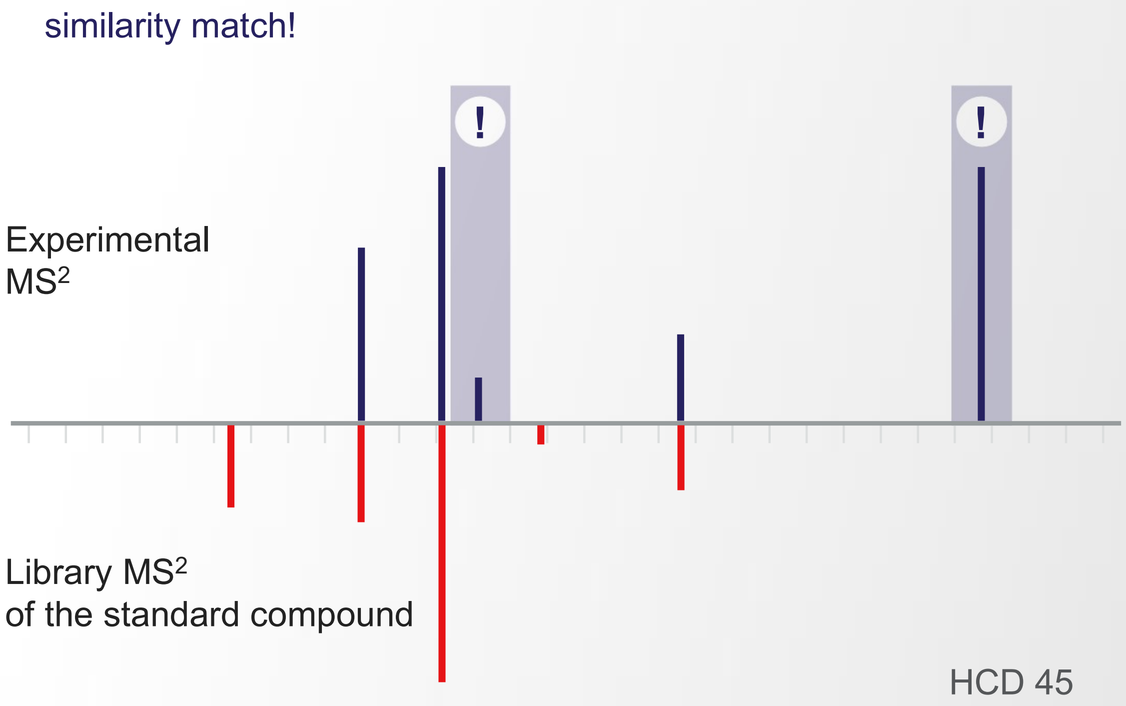
Real-Time Library Search of the MS^2 spectra



STEP 4

Real-time Library Search of the MS^2 spectra

This suggests a flavonoid compound based on spectral similarity.

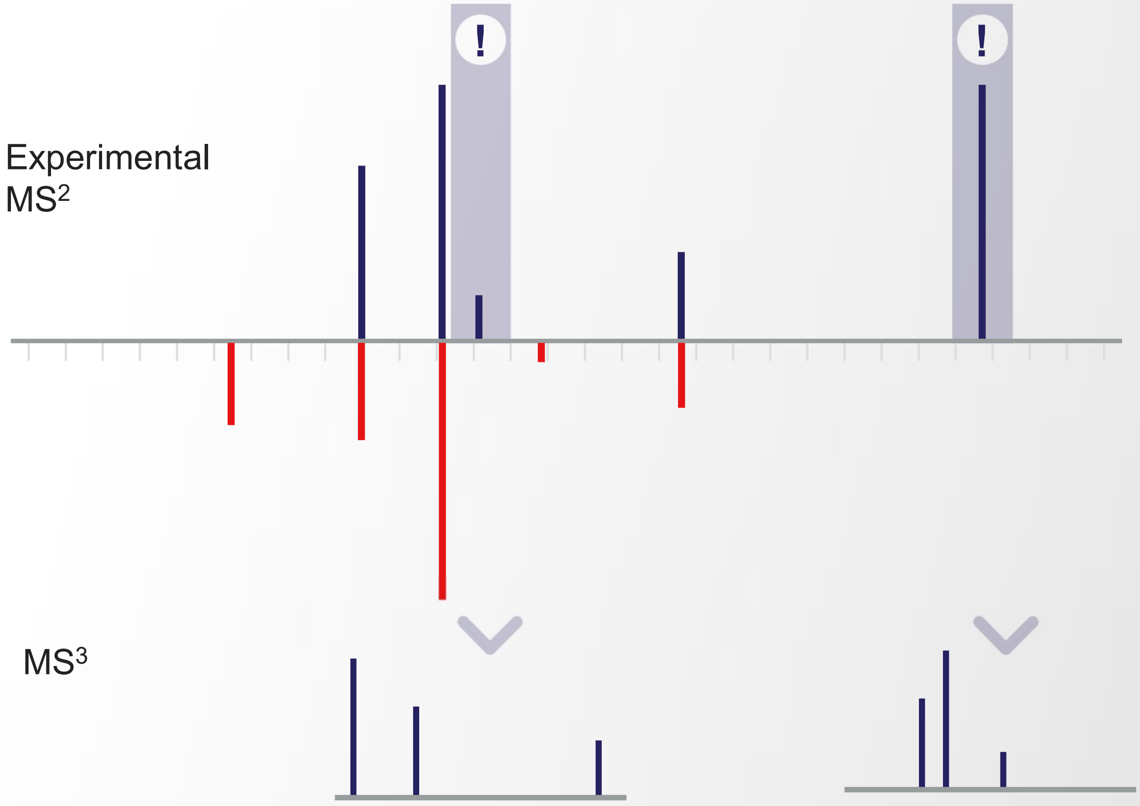


STEP 5

Gain additional information with MS^3 fragmentation of the unmatched peaks

Every MS^3 trigger would only occur if there is a good similarity match to compound of interest.

MS^3 triggers pinpoint the precursor ions of likely similar compounds, greatly simplifying data analysis compared to the traditional workflow.



Unique MS^3 fragment ions distinguishes similar compounds

Real-Time Library Search for Improved Structure Elucidation

Flavonoids chemical structures usually have spectral similarities

40 molecules included

MetaSci

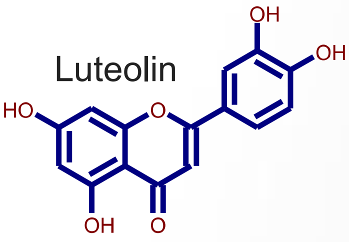
Providing high quality metabolite library

Polyphenols Standards Mix

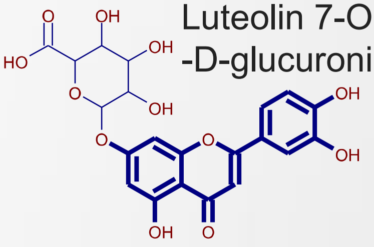
Flavonoids Standard Mixture

For HPLC and Mass Spectrometry

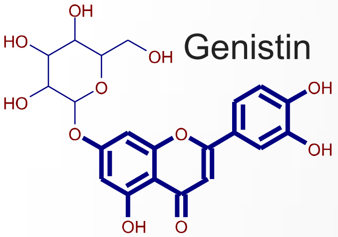
Luteolin



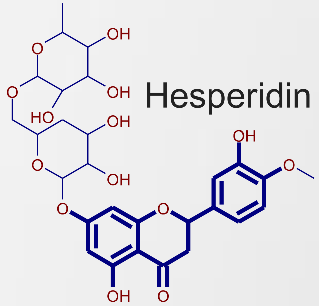
Luteolin 7-O-beta -D-glucuronide



Genistin

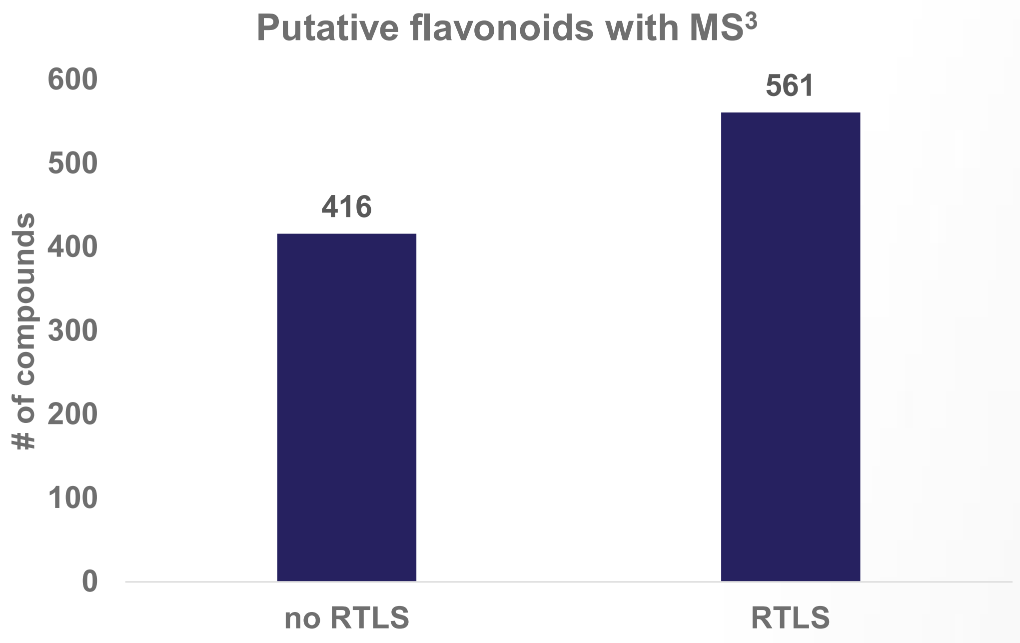


Hesperidin



Case Study: Tea Samples

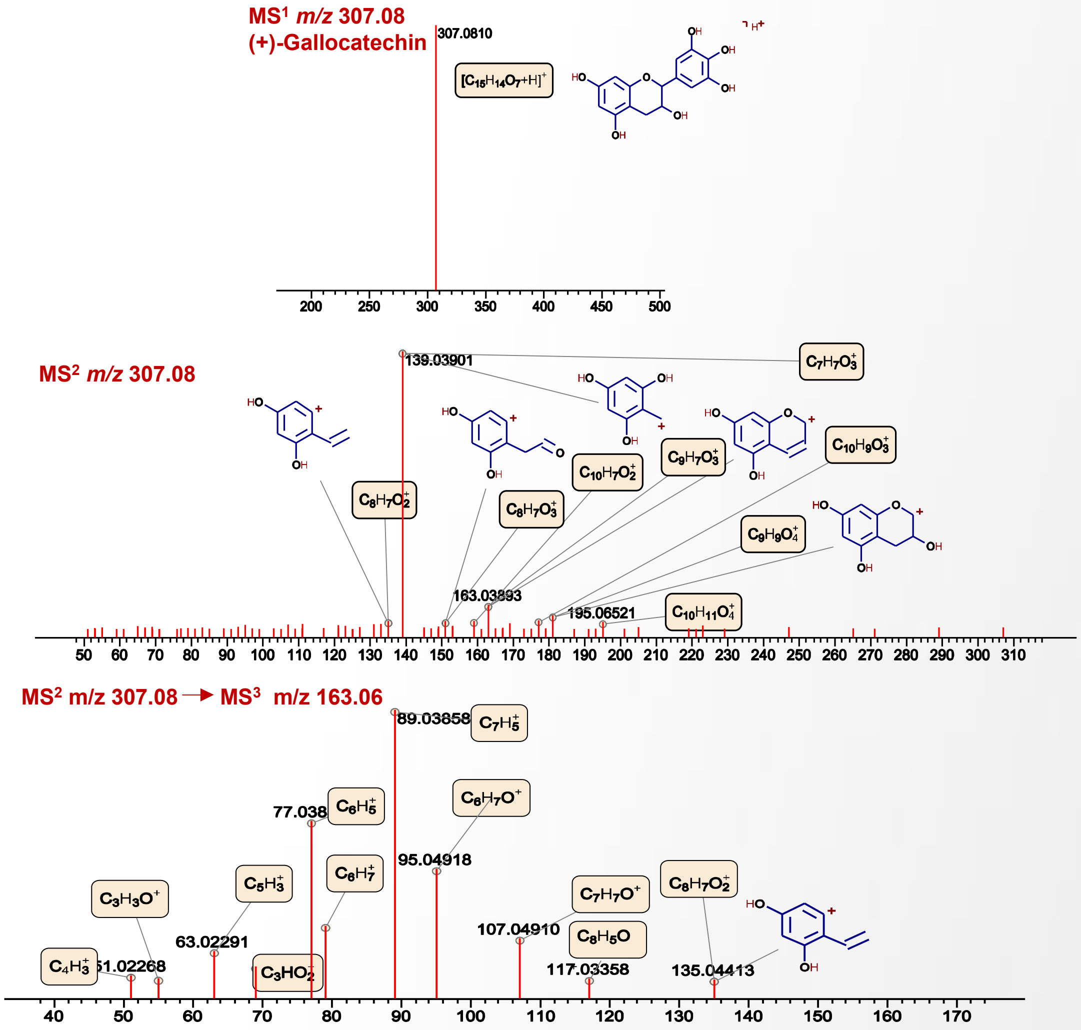
RTLS with DDA triggered MS^3 for **33% more scans** than the traditional DDA experiment



- Data obtained for pooled tea samples (Green, Black and Herbal)
- LC-MS data acquired with 1) RTLS with DDA MS^3 top 3 product ions; 2) Traditional DDA MS^3 top 3 product ions
- Results were filtered using CD 3.3 for putative flavonoids using mass list.
- MS^3 was triggered for **561** flavonoid related peaks in the RTLS with DDA experiment vs. **416** metabolites in the traditional DDA experiment

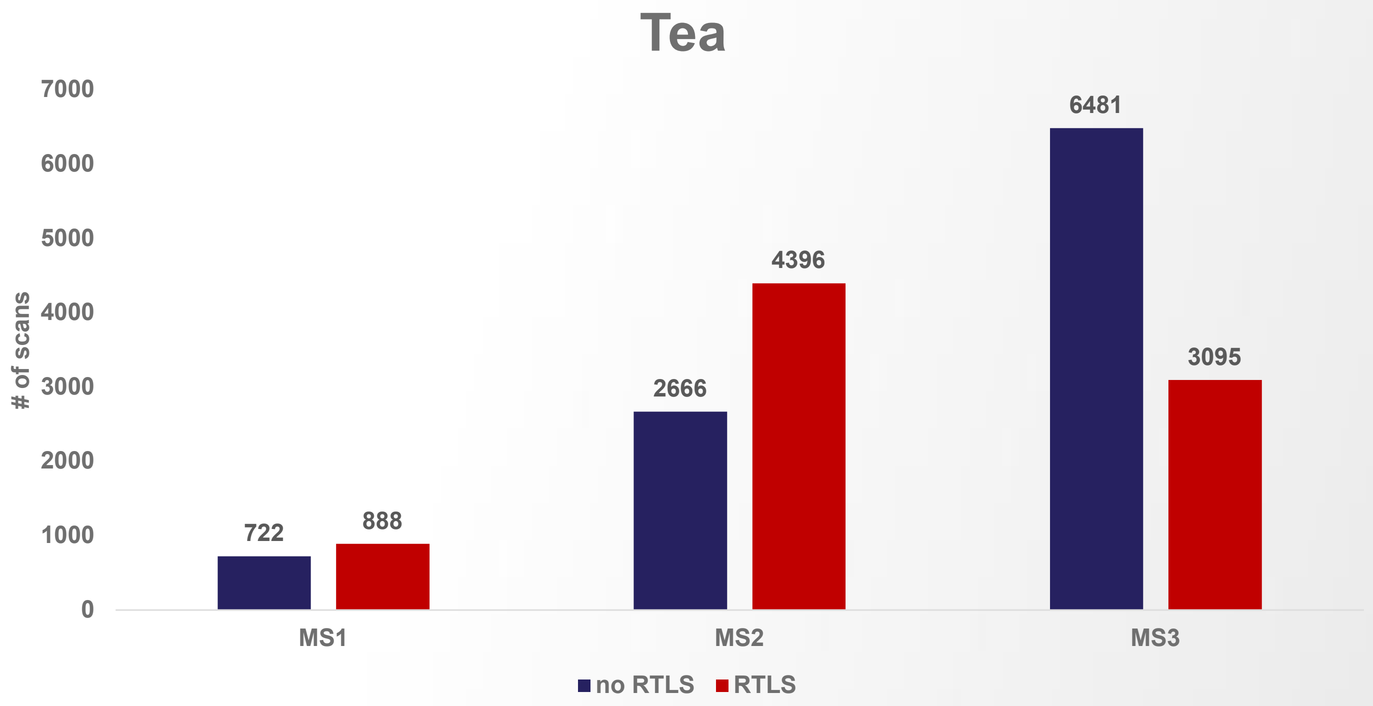
RTLS: High confidence metabolite structure annotation on the fly

Annotation of MS^2 and MS^3 fragment ions for structure elucidation using parent structural similarity in RTLS where traditional DDA did not trigger for MS^3 fragmentation



Case Study: Tea Samples

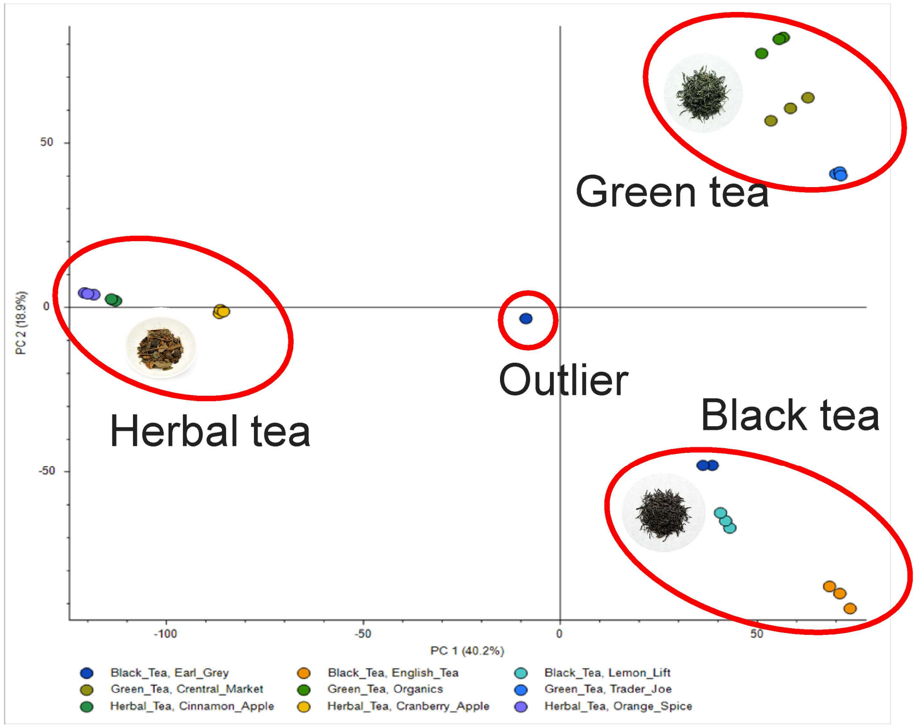
Intelligent MS3 fragmentation with RTLS



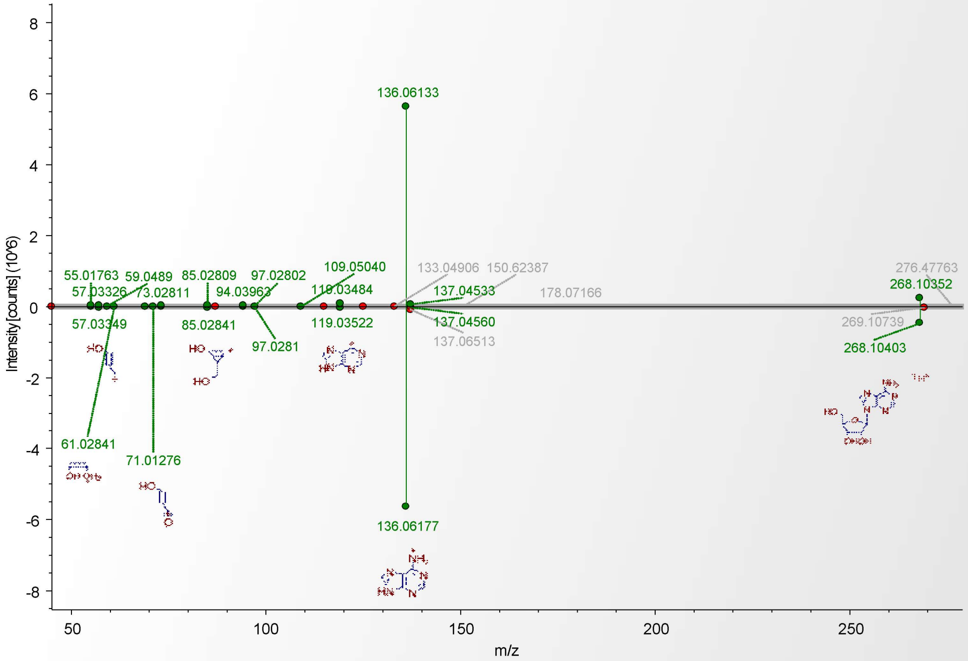
Case Study: Tea Samples

Intelligent MS3 fragmentation with RTLS

PCA plot showing differentiation between tea types



Adenosine found in tea: non-flavonoid compound



Thank you