The “MRMS aXelerate workflow”

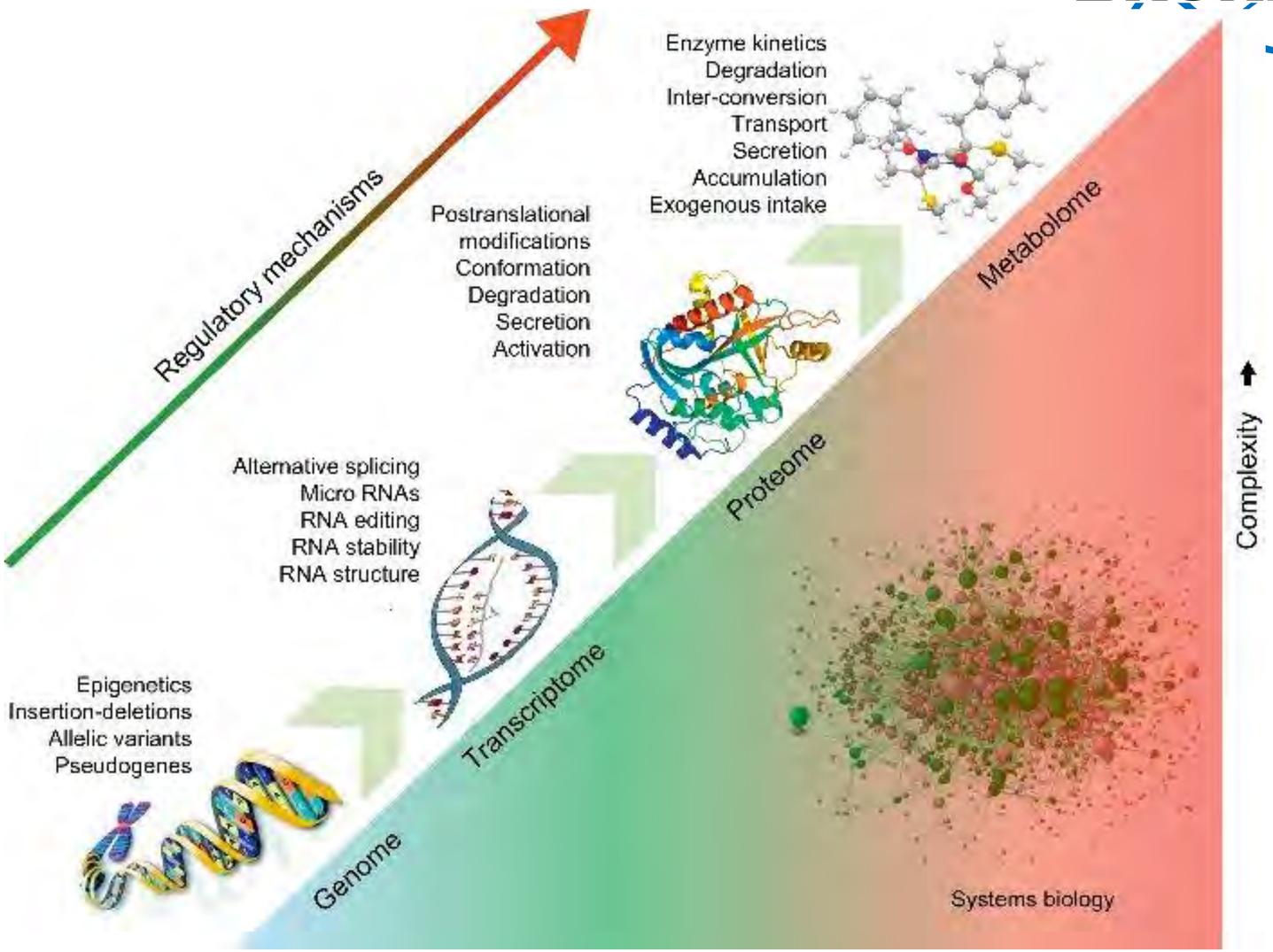
Matthias Witt, Bruker Daltonics GmbH & Co. KG, MRMS Solutions, Bremen, Germany

What is Metabolomics?

- Metabolomics is the systematic study of the small molecular metabolites in a cell, tissue, biofluid, or cell culture media that are the tangible result of cellular processes or responses to an environmental stress. The metabolome is the total complement of metabolites present in a biological sample under given genetic, nutritional or environmental conditions. Metabolomics technologies yield many insights into basic biological research in areas such as systems biology and metabolic modelling, pharmaceutical research, nutrition and toxicology.

- Applications of metabolomics are found within the pharmaceutical, healthcare, and agricultural industries, among others. There are two main approaches used in metabolomic studies: untargeted (global) and targeted (specific).

- *Nuclear magnetic resonance* (NMR) and *mass spectrometry* (MS) are two of the most commonly used analytical methods in metabolomic studies. Careful planning and design of experiments is of paramount importance in metabolomic studies.



Rev Esp Cardiol. 2013;66:657

MRMS – Breakthrough Innovations

Not your father's FT-ICR MS

Magnetic Resonance Mass Spectrometry

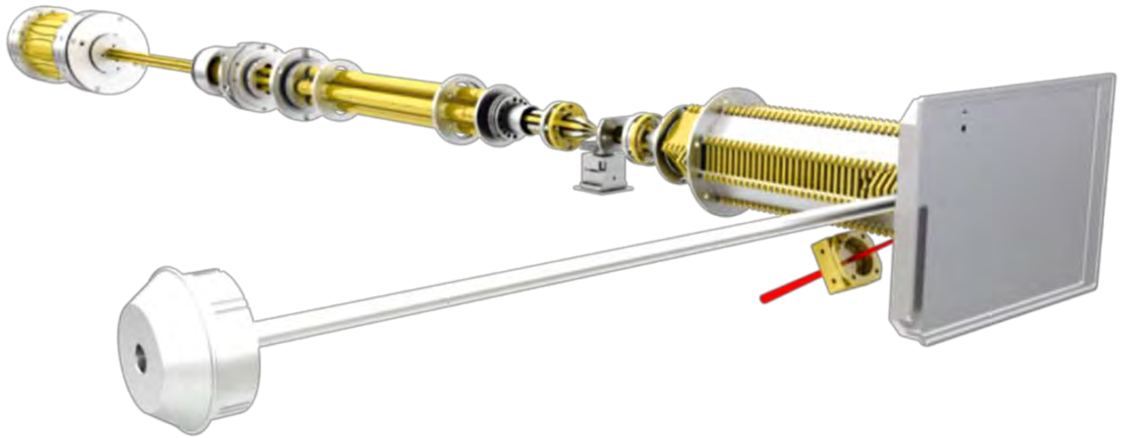
1973 → 1983 → 2009 → 2013 → 2016 → 2018

- Fourier transform ion cyclotron resonance mass spectrometry *FT-ICR MS* is a technique invented in **1973**.
- Commercially marketed as **FTMS** by Bruker starting in the **mid 80s**.
- Introduction of solariX in **2009**.
- The **ParaCell™** was introduced in **2013** with solariX XR and several generations later, the modern FT-ICR MS was nothing like the ones from 40 years ago.
- Introduction of solariX 2xR in **2016** with **quadrupolar detection**.
- Introduction of **scimaX** in **2018** with **Maxwell magnet technology**.
- Today **MRMS** defines the next generation of FTMS instrumentation.

Versatility, performance and accuracy all in one platform

scimaX delivers unmatched versatility and unparalleled performance to provide answers with confidence for your most challenging applications

- Molecular imaging of tissues
- In-depth analysis of petroleum products
- In-depth analysis of complex environmental samples
- **Comprehensive metabolomics and other life science studies**
- Intact protein analysis
- Glycan analysis



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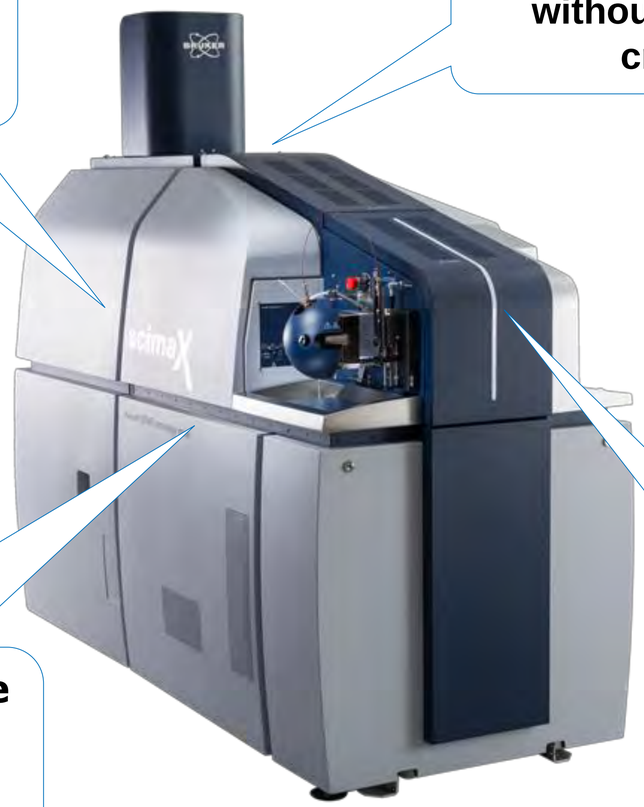
Versatility, performance and accuracy all in one platform

ParaCell™ for 20M resolution

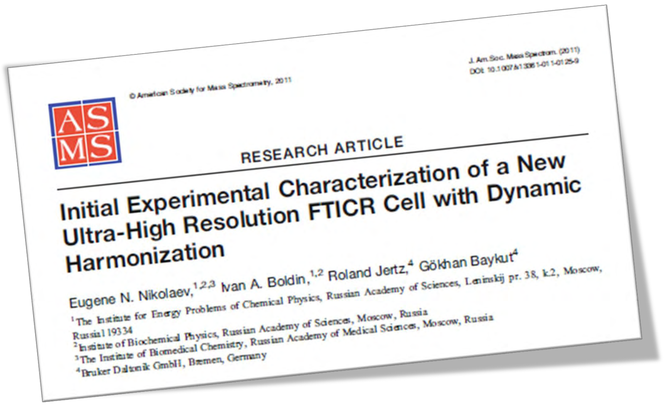
High field magnet without the need of cryogens



Front-end quadrupole CASI for enhanced sensitivity

ESI/MALDI dual ion source: 2kHz smartbeam II MALDI laser

- Extremely high mass resolution (access to isotopic fine structure)
- Ultimate mass accuracy for confident results (ppb range)
- Continuous Accumulation of Selected Ions (CASI) for enhanced sensitivity and enhanced dynamic
- 2ω detection enabling doubled acquisition speed



J. Am. Soc. Mass Spectrom. (2011)
DOI: 10.1007/s13361-011-0125-9

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RESEARCH ARTICLE

Initial Experimental Characterization of a New Ultra-High Resolution FTICR Cell with Dynamic Harmonization

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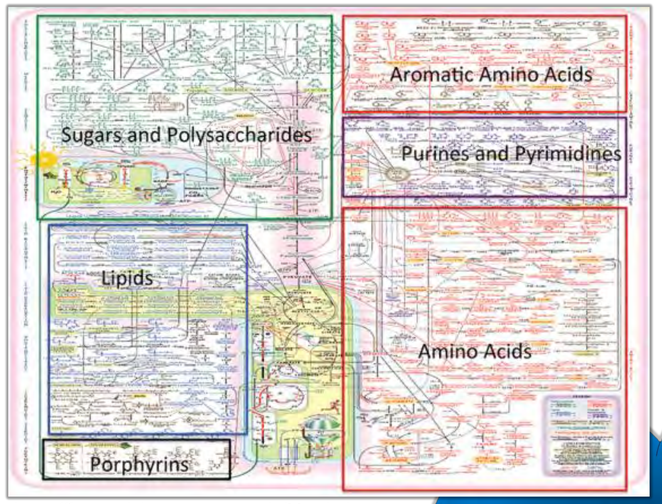
MS-based OMICs solutions



https://fornacelab.georgetown.edu/Metabolomics (Lab of Prof. A. J. Fornace Jr., U. Georgetown, Washington D.C.)

The "dark metabolome"

QTOF technology

- QTOF: workhorse for routine metabolomics projects

TIMS technology

- Advancing research by 4D-Lipidomcis and 4D-Metabolomics

MRMS solutions

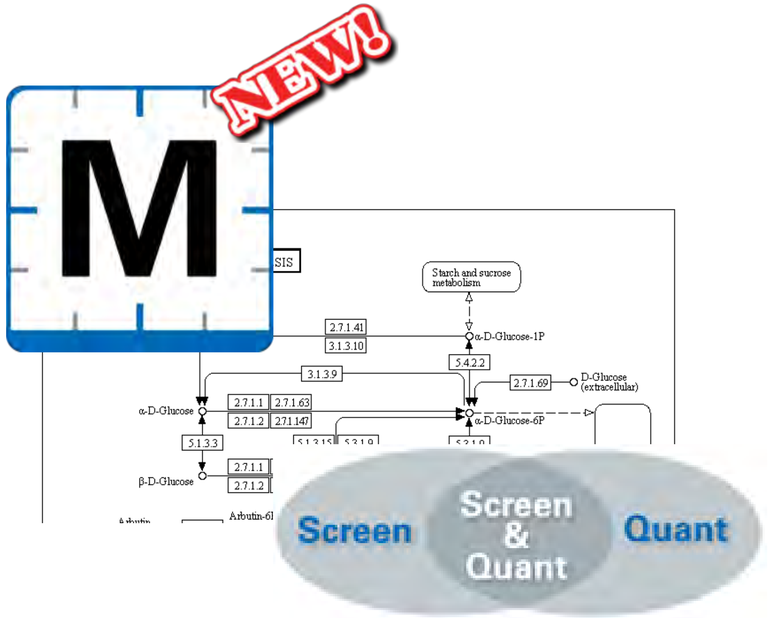
- skip time-consuming chromatography
- complementary MALDI data
- Pinpoint formulae with isotopic fine structures

A novel method, **FIA-MRMS** provides **high sample throughput** for complex samples (e.g. urine, plasma, tissue extracts, fecal extracts) in **phenomics** research with **~250 samples/day**.

MRMS metabolomics workflow

Hardware + Software + Content

scimaX



MetaboScape + TASQ

HMDB Metabolite Library

MetaboBASE Personal Library

MetaboBASE Plant Library

MRMS Technical innovations

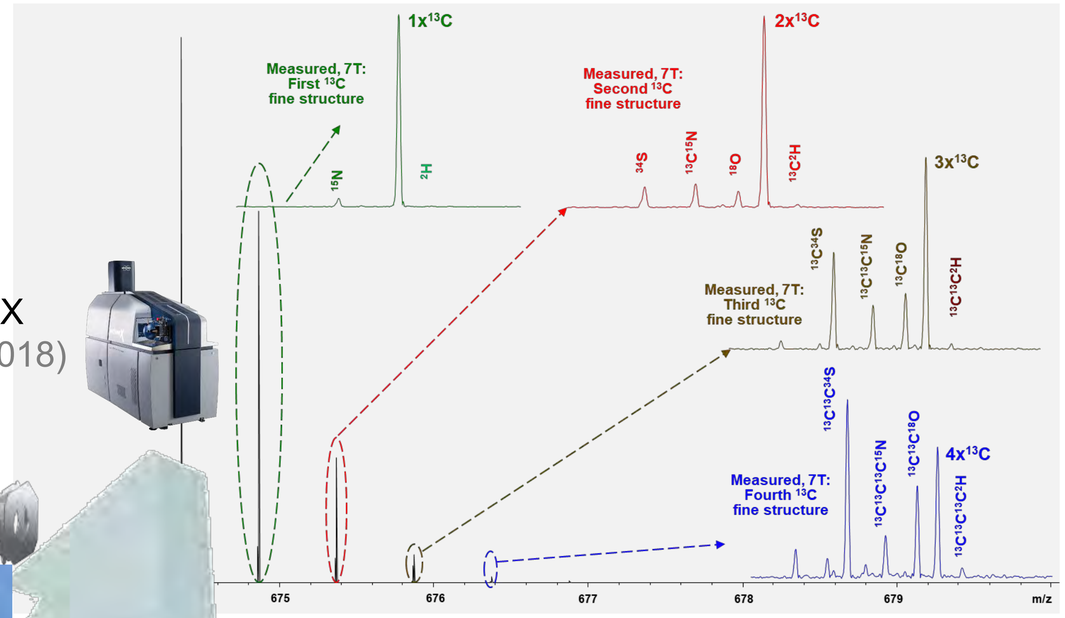
Evolution of innovation enables routine access to Isotopic Fine Structure (IFS) analysis

scimaX (7T, 2018)

solariX 2xR (7T, 2016)

solariX XR (2013)

solariX (2009)

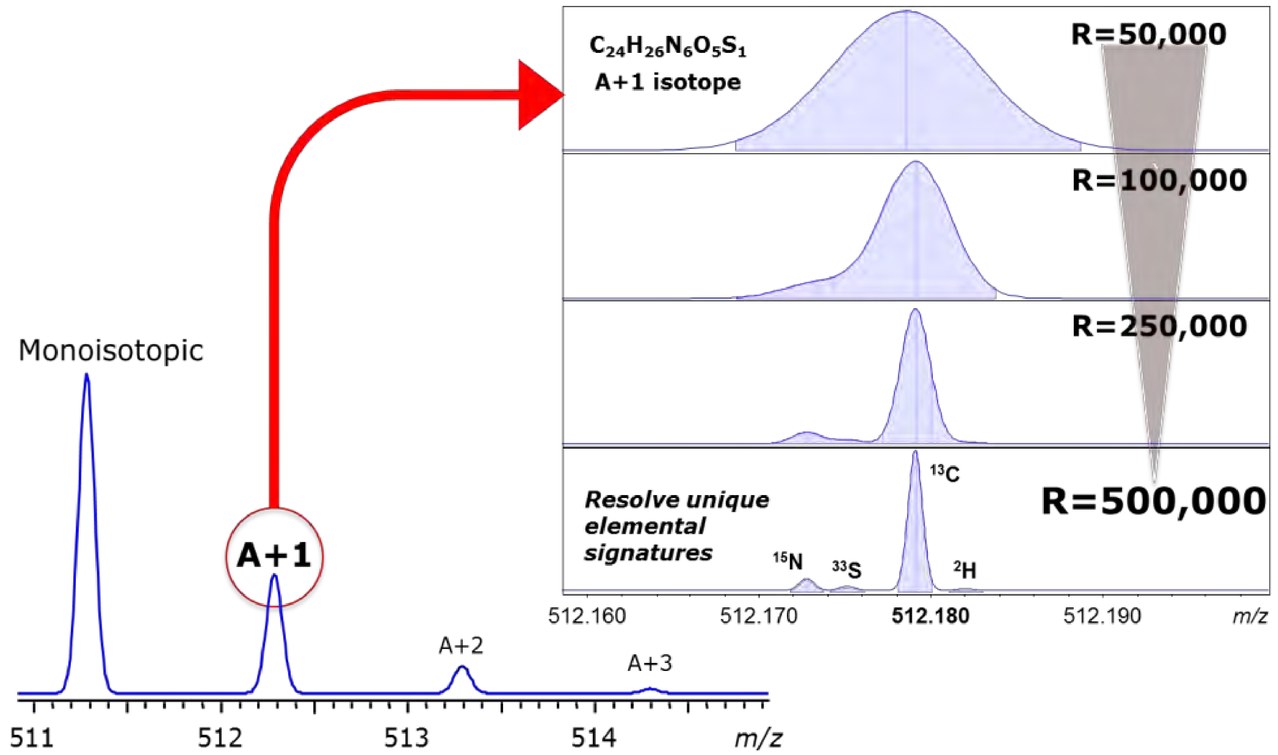


→ „counting“ heteroatoms reveals the molecules content

FIA-MRMS provides Isotopic Fine Structure

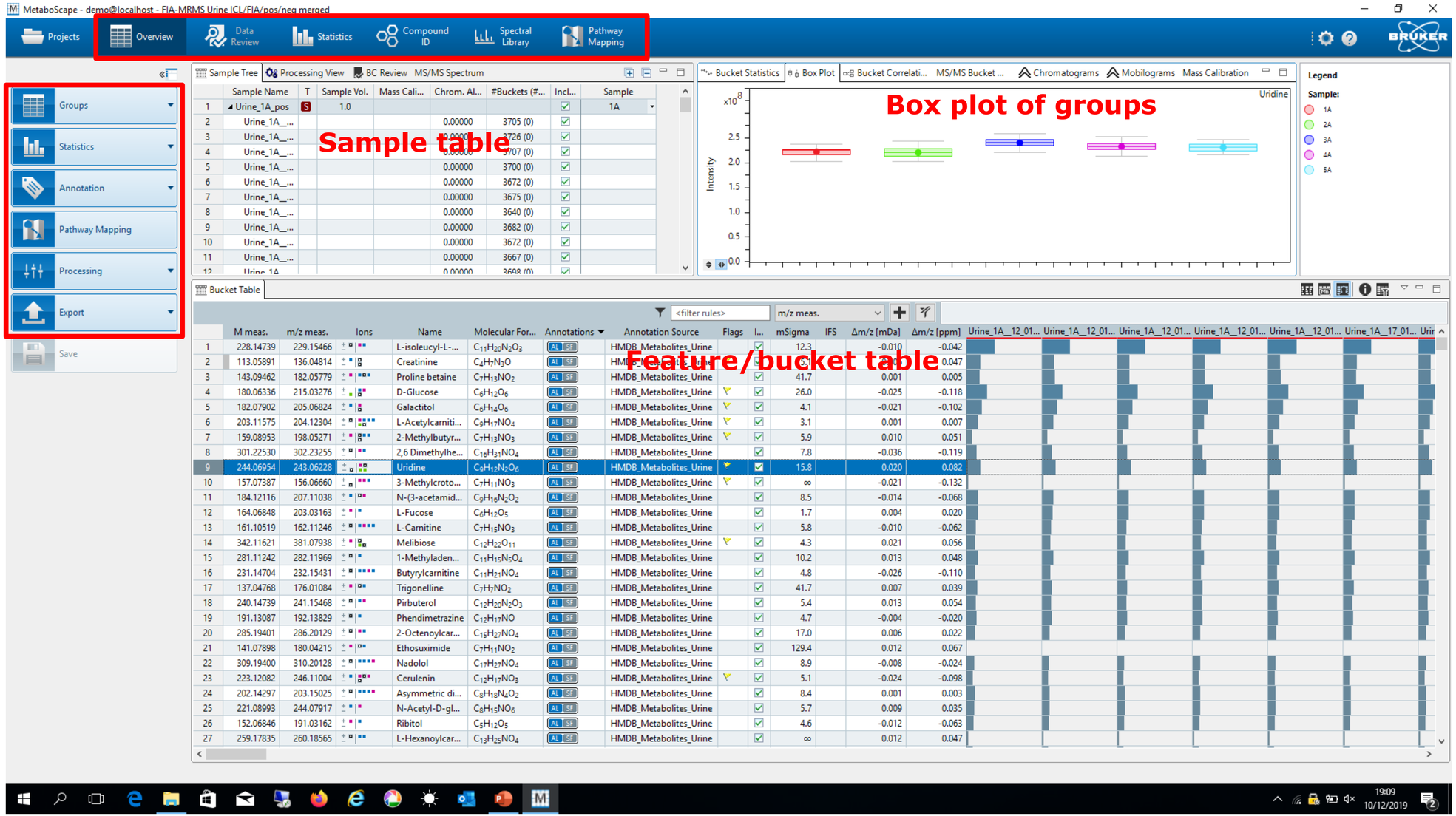
eXtreme Resolution enables **confident results**

Example:



Example: A+1 peak of $C_{24}H_{26}N_6O_5S_1$

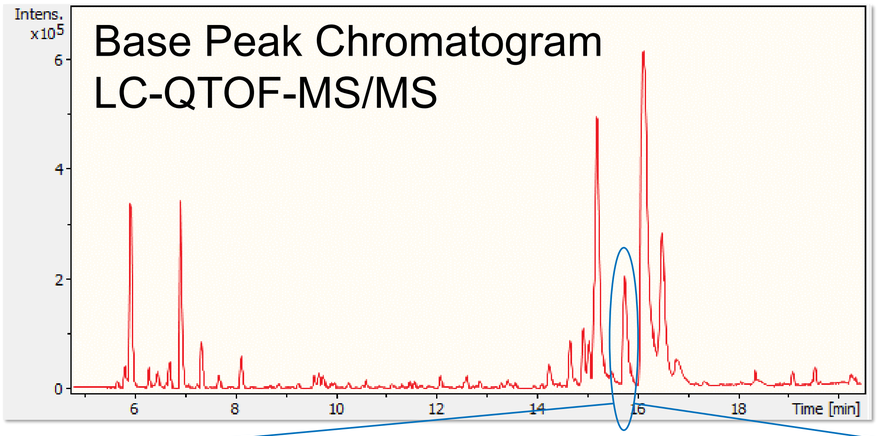
MetaboScape



FIA-MRMS solution

Can MRMS support the ID of unknowns in Phenomics research by generating unique molecular formulae?

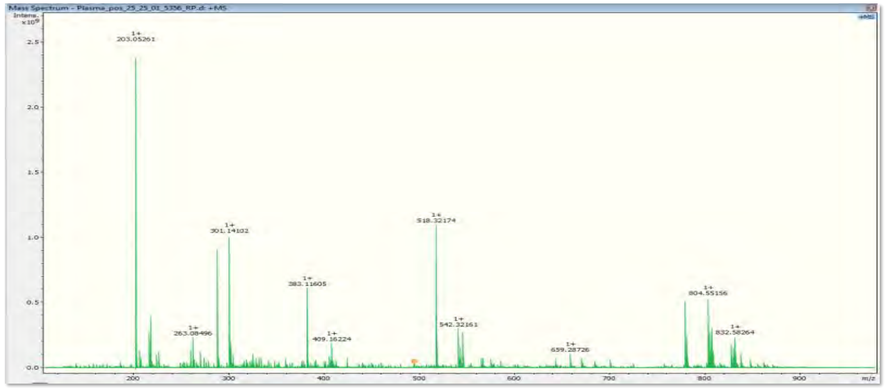
LC-QTOF-MS (~25 min turnover time)

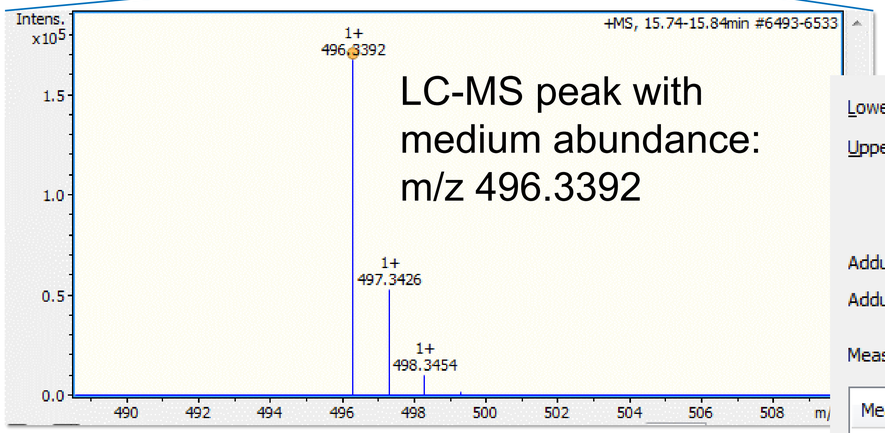


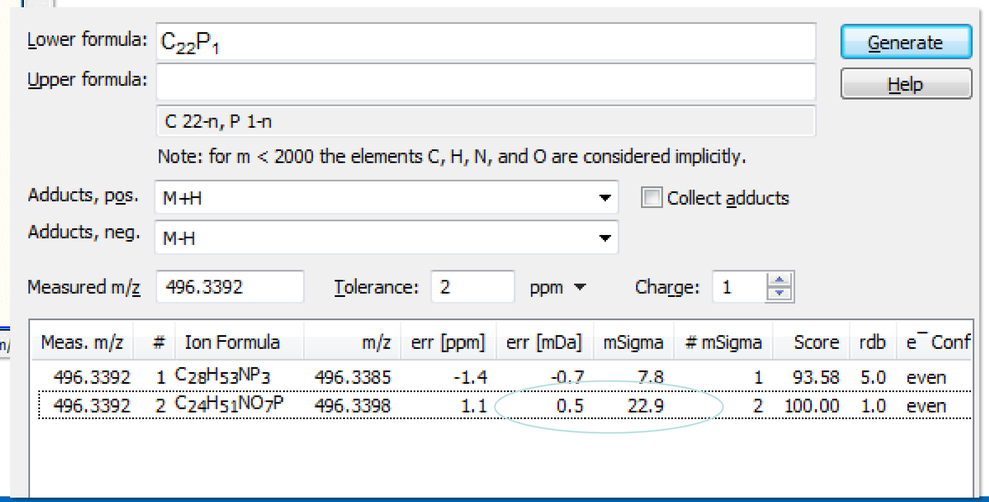
A human Plasma extract analyzed by
<- *LC-QTOF-MS/MS* (impact II)

and *FIA-MRMS* (solariX XR 7T)
->

FIA-MRMS (single spectrum)
(~2.5 min turnover time)

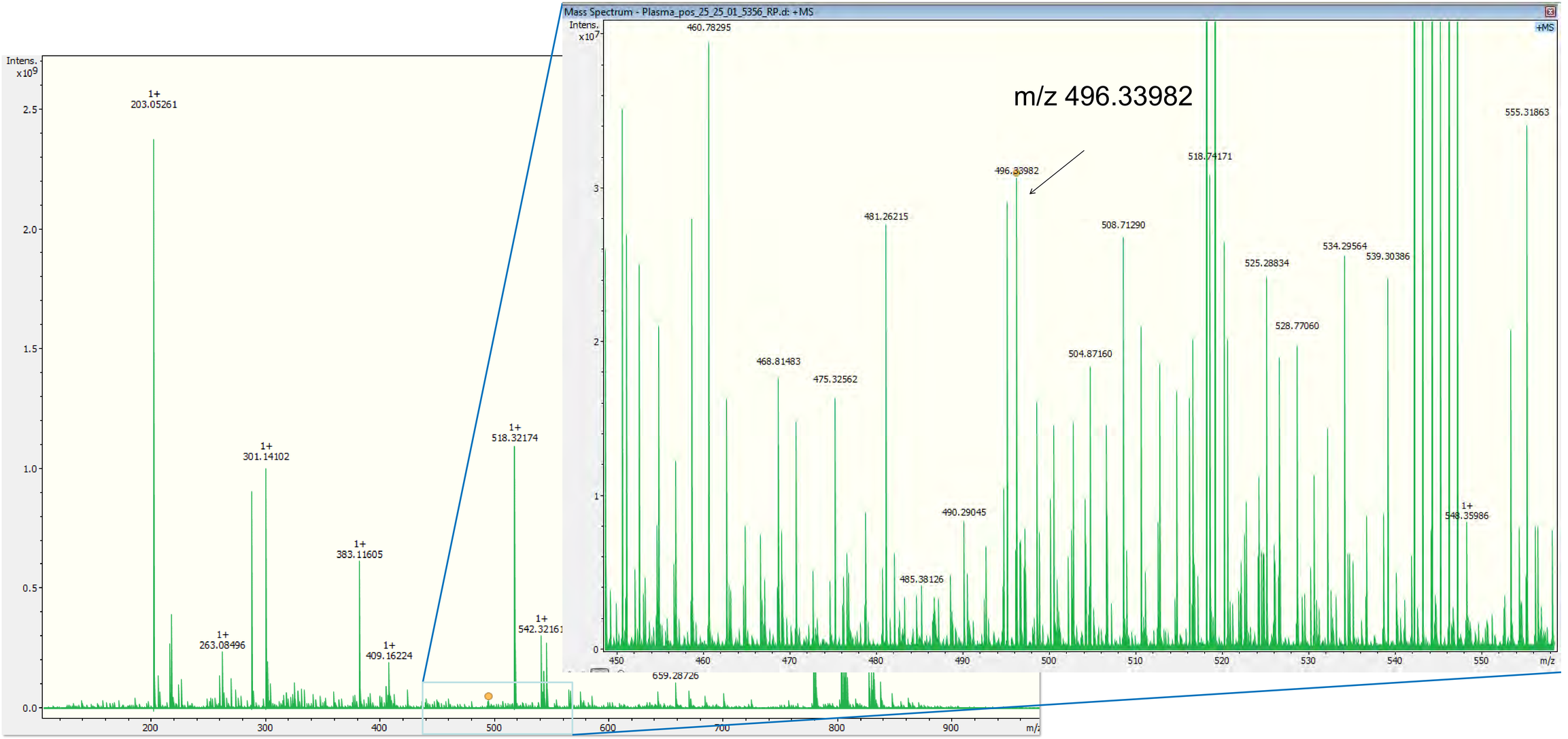






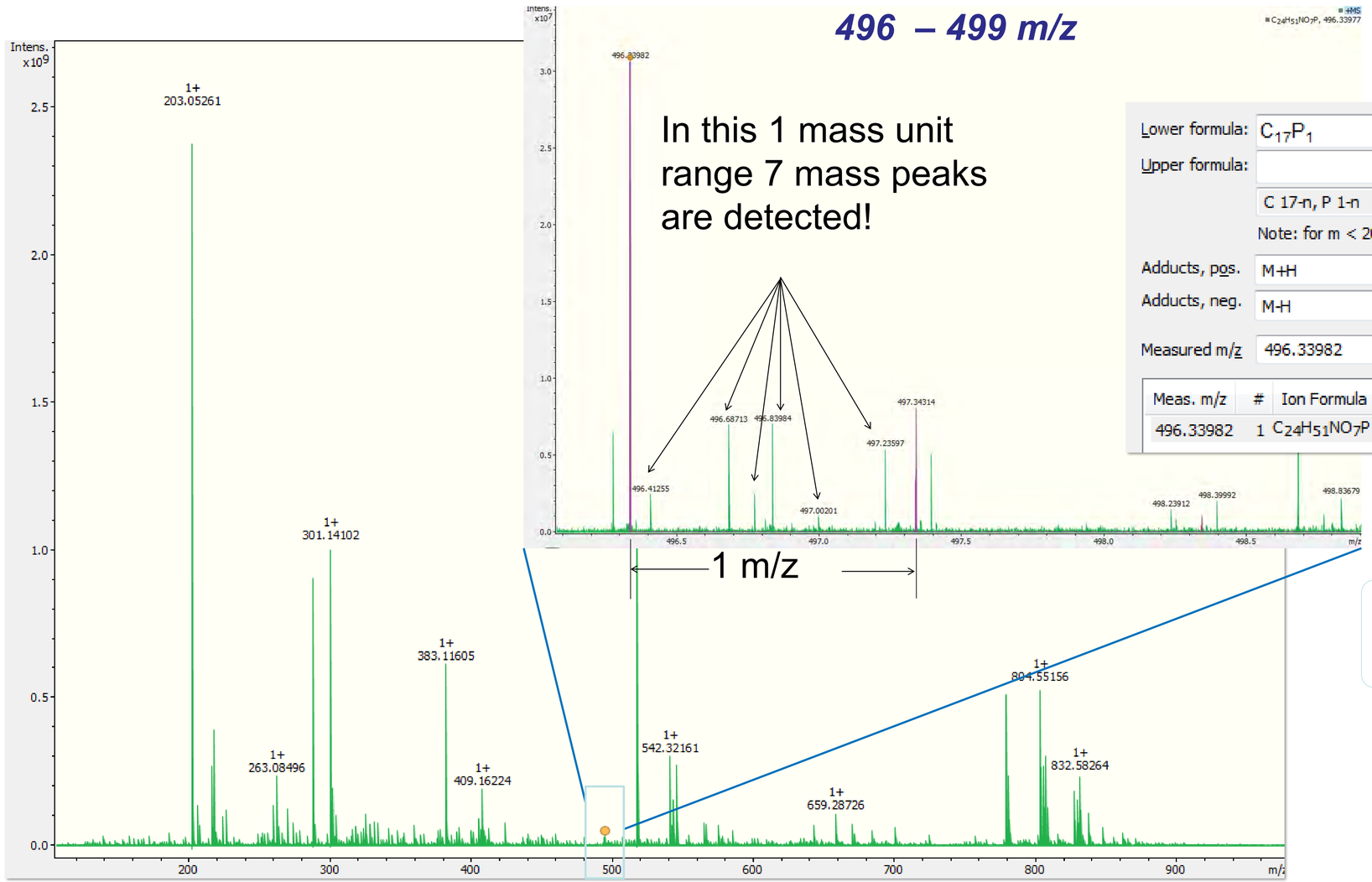
FIA-MRMS single spectra analysis

Zoom-in reveals data richness



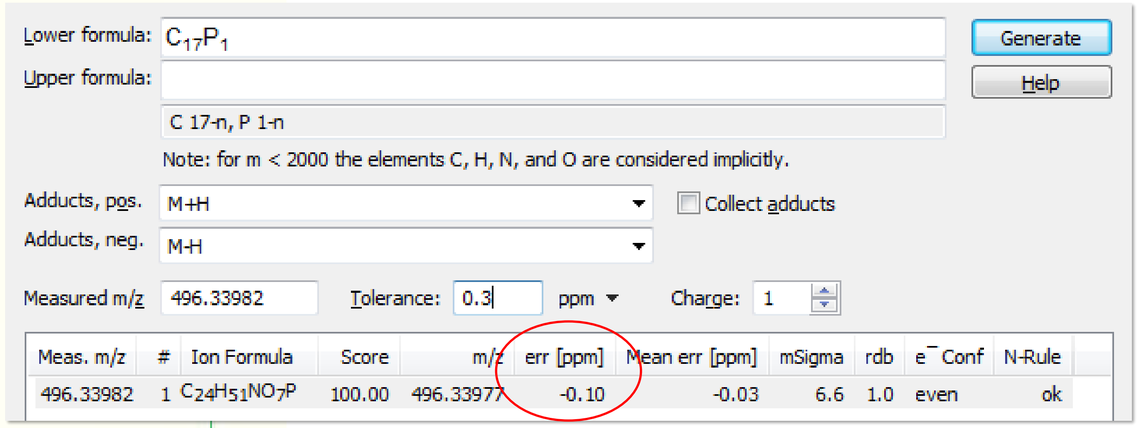
FIA-MRMS single spectra analysis

Example: target mass 496.3392 m/z



496 – 499 m/z

In this 1 mass unit range 7 mass peaks are detected!



eXtreme Resolution enables **confident results**

Confidence in annotation by very **high mass accuracy**

FIA-MRMS single spectra analysis

Example: target mass 496.3392 m/z

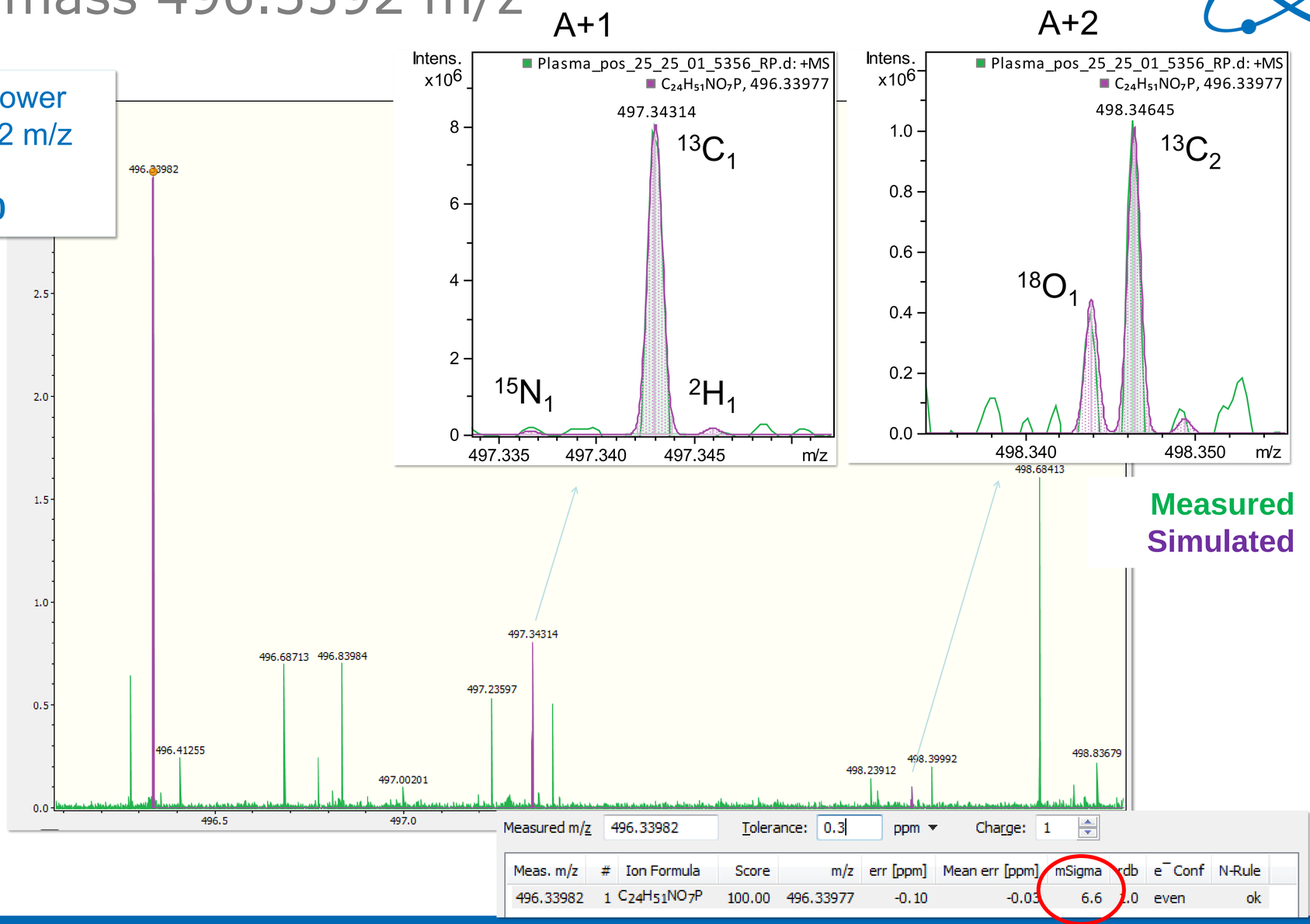
Resolving Power
@ 496.33982 m/z

575.000

eXtreme Resolution enables **confident results**

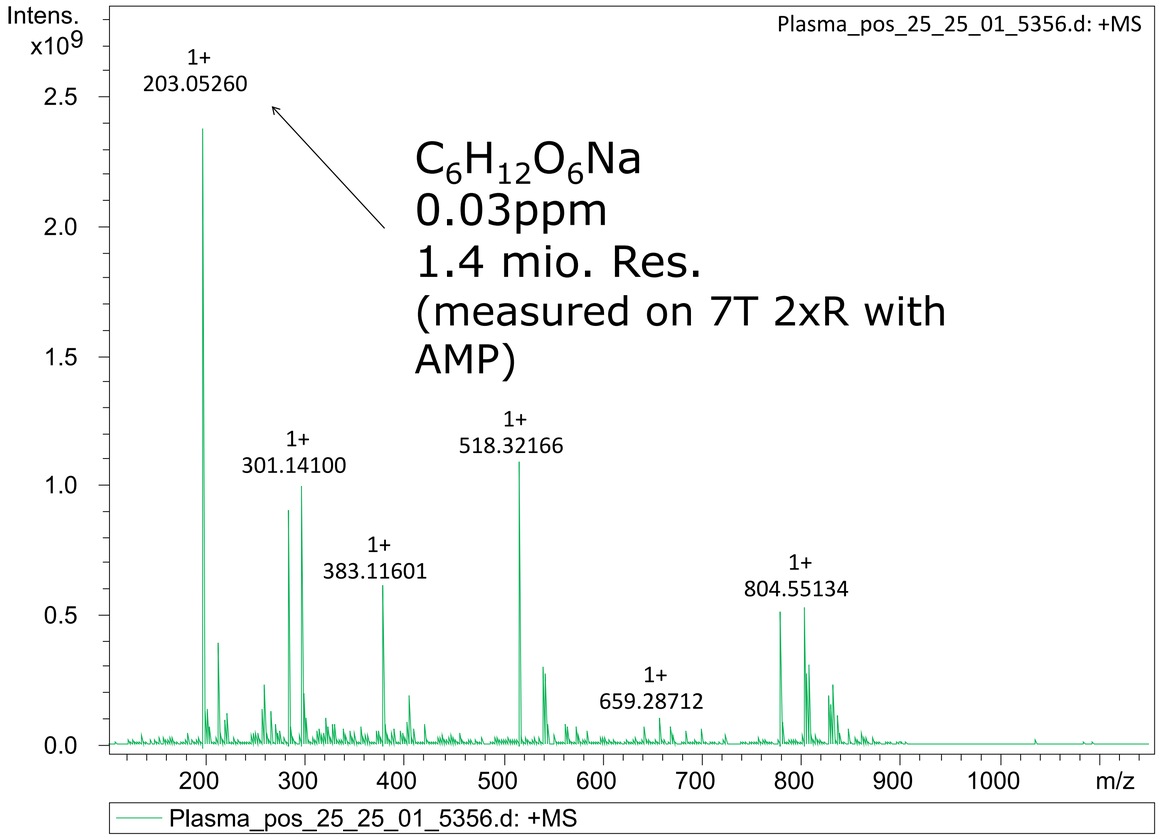
Isotopic Fine Structure information allows elemental **formula** to be **READ** directly from extreme resolution mass spectra.

mSigma value:
Expresses the matching of measured and theoretical isotopic pattern in a numerical value – the lower value the better the fit (Scale 0-1000).

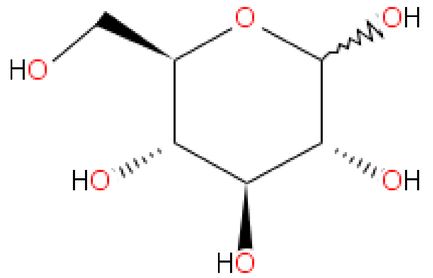


FIA-MRMS high-throughput solution

Access compounds not readily detectable by LC-MS analysis:



- *Like* $C_6H_{12}O_6Na$ = *hexose sugars* in plasma extract
- Hexose sugars are not well retained on revered phase LC-MS and therefore typically not detected!
- Glucose a hexose sugar

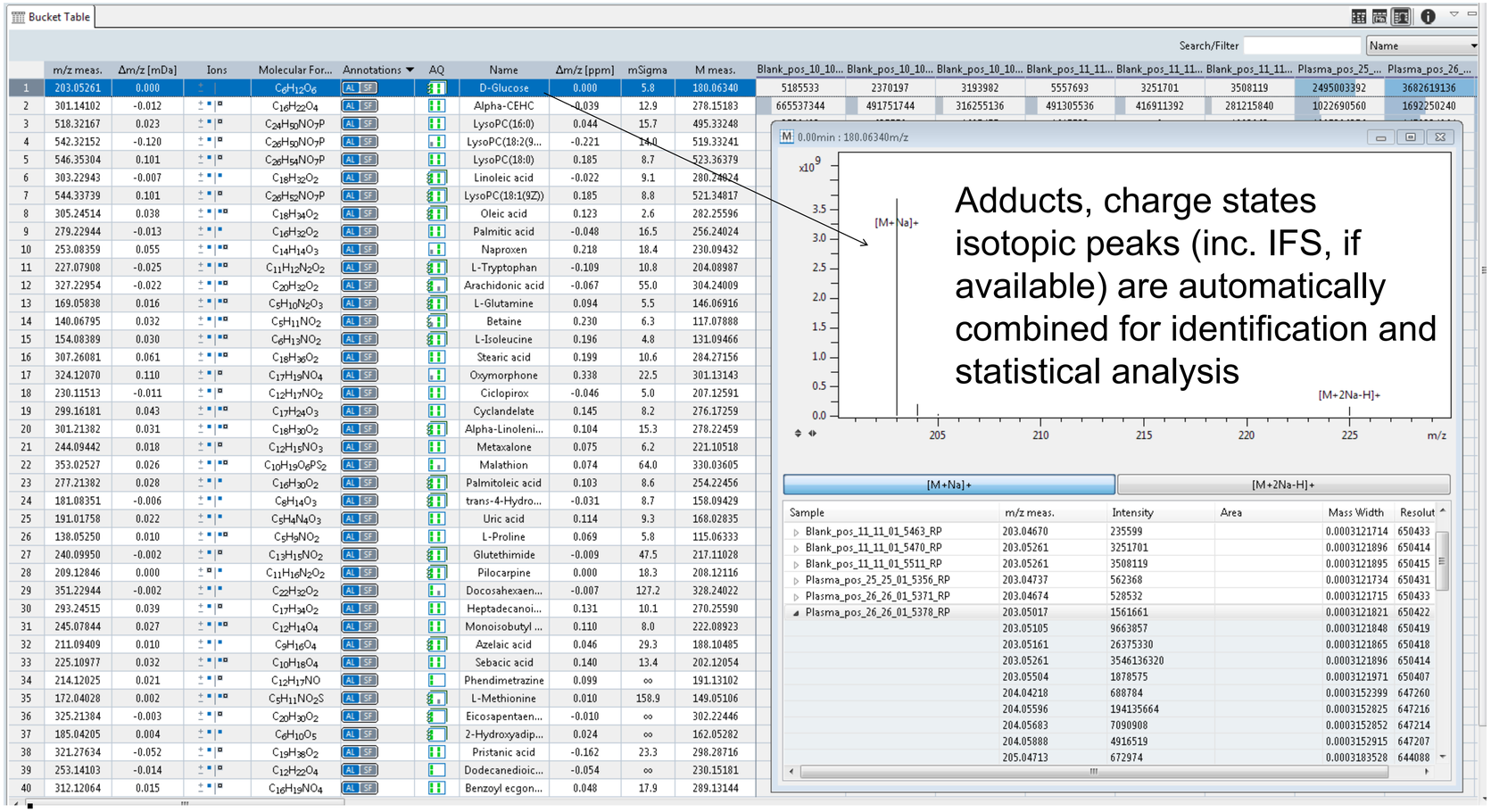


- **Glucose is the typical biomarker in diabetes**

MetaboScape

Powerful data extraction by **T-ReX 2D**

Automatic declustering & deisotoping *Simplifies* the data for statistical analysis

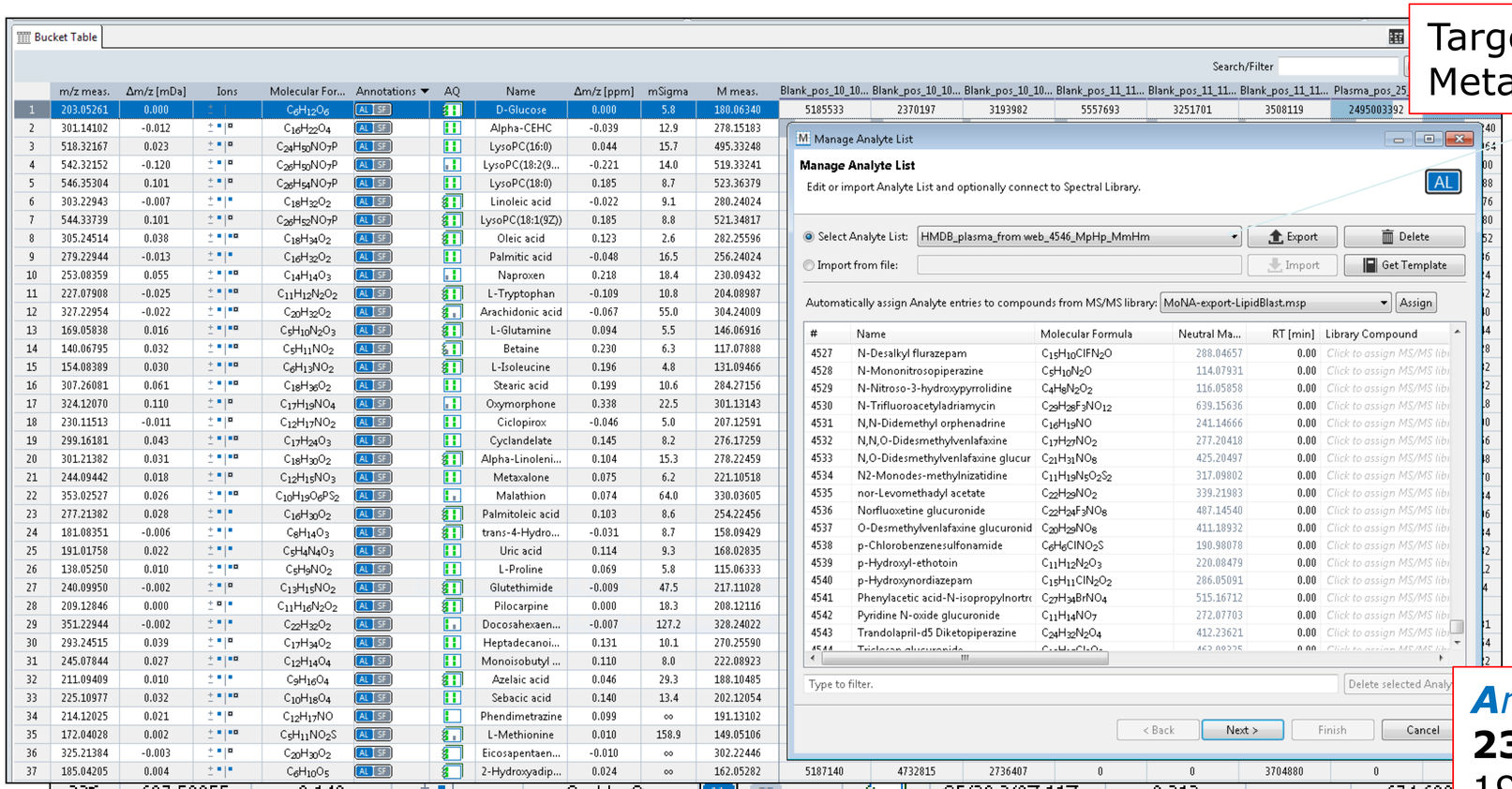


Here, 3963 reproducibly extracted buckets were deisotoped and declustered from the FIA-MRMS data of the plasma extract by T-ReX 2D.

MetaboScape - Automatic Annotation

Analyte List: >4,500 Human plasma metabolites from HMDB (*www.hmdb.ca/*)

Target list derived from Human Metabolome Data Base (HMDB)



Annotation Quality Scoring

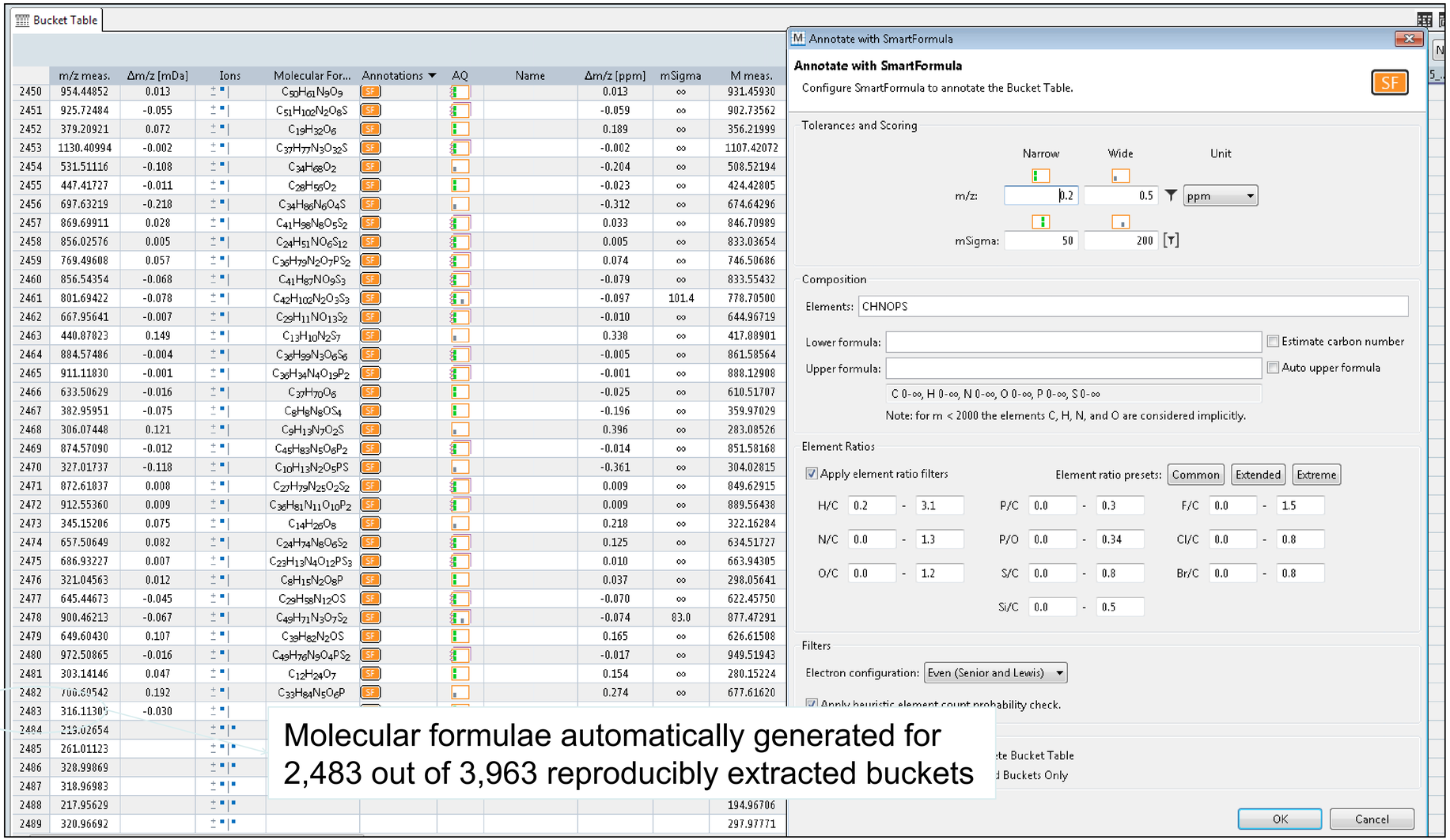
231 automatic annotations

196 within 0.2 ppm mass accuracy

Mass accuracy
Isotopic pattern quality

MetaboScape - Automatic Annotation

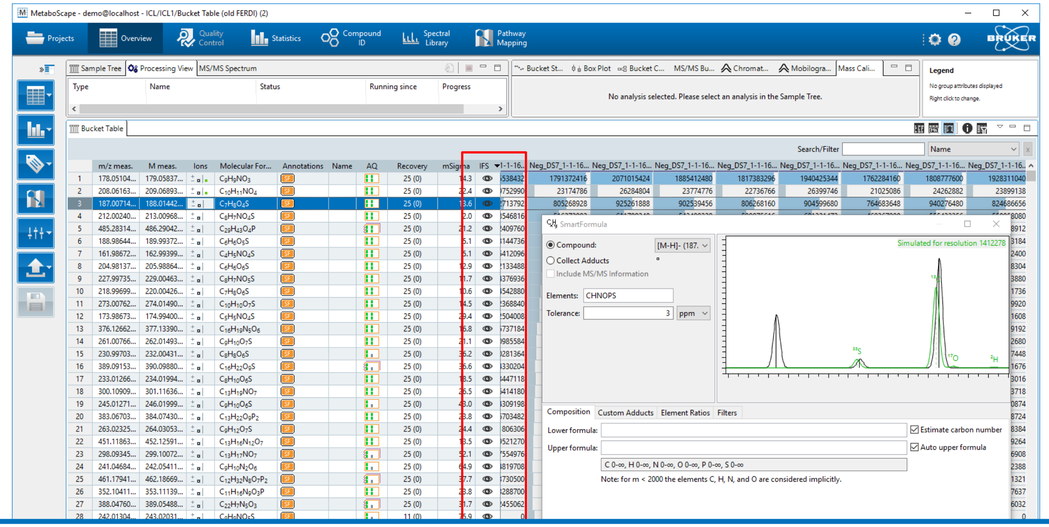
SmartFormula: Mass Accuracy, True Isotopic Pattern & Isotopic Fine Structure



Molecular formulae automatically generated for 2,483 out of 3,963 reproducibly extracted buckets

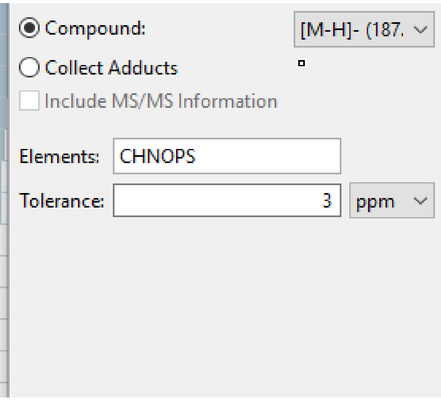
MetaboScape - Automatic Annotation

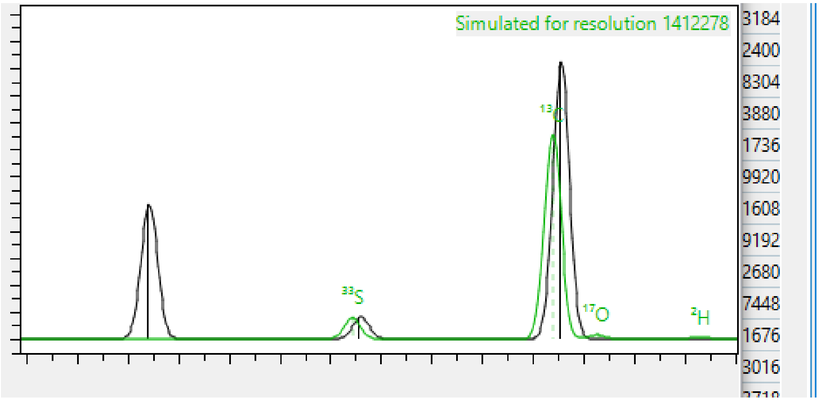
SmartFormula: Mass Accuracy, True Isotopic Pattern & Isotopic Fine Structure



- MetaboScape can use the Isotopic Fine Structure and flag the compounds identified on the basis of IFS (only for high abundant peaks)

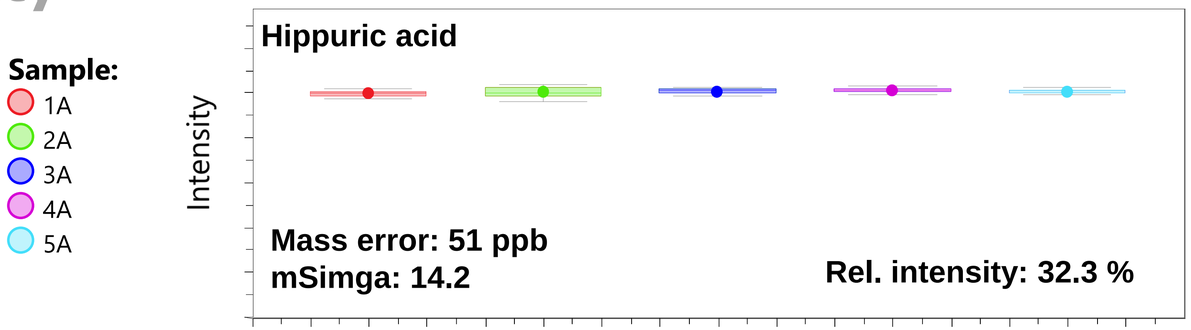
6	188.98644...	189.99372...	$C_6H_6O_5S$	SF			25 (0)	5.1		3144736
7	161.98672...	162.99399...	$C_4H_5NO_4S$	SF			25 (0)	5.1		3412096
8	204.98137...	205.98864...	$C_6H_6O_6S$	SF			25 (0)	12.9		3133488
9	227.99735...	229.00463...	$C_8H_7NO_5S$	SF			25 (0)	11.7		3379936
10	218.99699...	220.00426...	$C_7H_8O_6S$	SF			25 (0)	10.6		3542880
11	273.00762...	274.01490...	$C_{10}H_{10}O_7S$	SF			25 (0)	14.5		3363840
12	173.98673...	174.99400...	$C_5H_5NO_4S$	SF			25 (0)	29.4		3504008
13	376.12662...	377.13390...	$C_{16}H_{19}N_5O_6$	SF			25 (0)	16.8		3737184
14	261.00766...	262.01493...	$C_9H_{10}O_7S$	SF			25 (0)	21.1		3985584
15	230.99703...	232.00431...	$C_8H_8O_6S$	SF			25 (0)	36.2		3028364
16	389.09153...	390.09880...	$C_{16}H_{22}O_9S$	SF			25 (0)	36.6		3330204
17	233.01266...	234.01994...	$C_8H_{10}O_6S$	SF			25 (0)	18.5		3447118



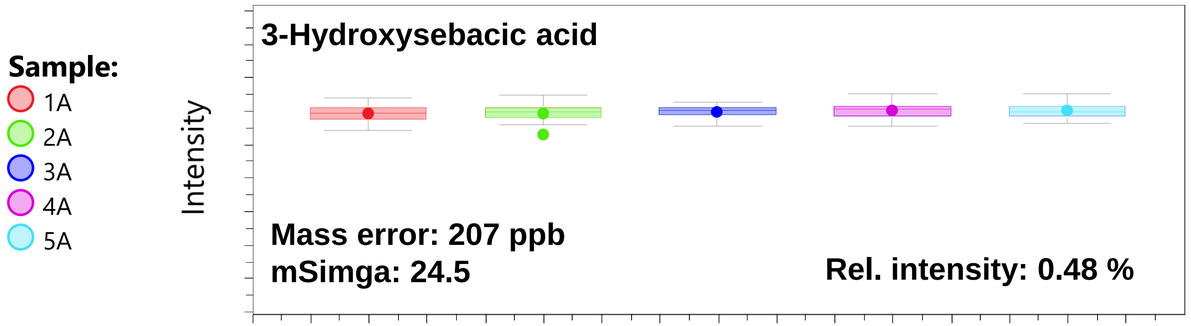


FIA-MRMS of urine samples

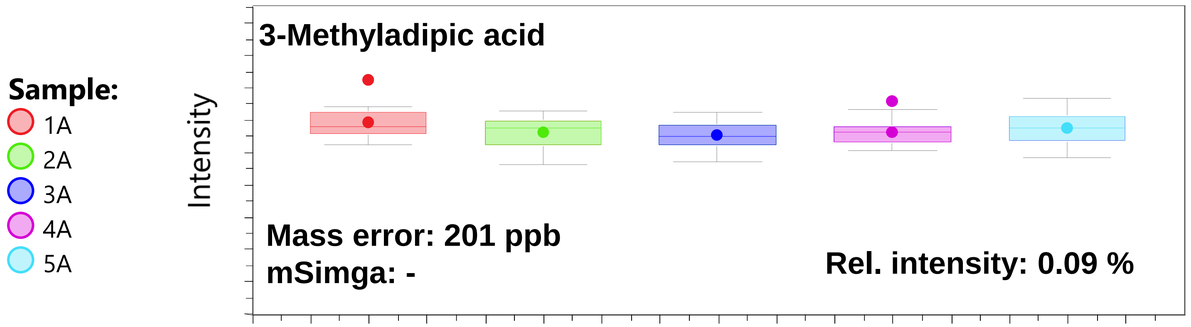
Reproducibility



Standard deviation:
Average each vial 1.2 %
All injections: 1.4 %



Standard deviation:
Average each vial 3.8 %
All injections: 3.8 %



Standard deviation:
Average each vial 7.7 %
All injections: 8.0 %

FIA-MRMS of urine samples

- Desalted urine samples have been measured in 125 replicates for each mode (ESI(+) and ESI(-)) using FIA-MRMS and MetaboScape

Measurement	Features	Analytes with HMDB urine list	Mol. Formula with SF calc.
FIA-MRMS(+)	4502	203	2838
FIA-MRMS(-)	1738	221	1176
FIA-MRMS(+) and FIA-MRMS(-) combined	6055	**363**	3748

Mass tolerance for HMDB search and SF calculation: 0.5 ppm

- Pooled desalted urine samples have been measured in multiple replicates* for

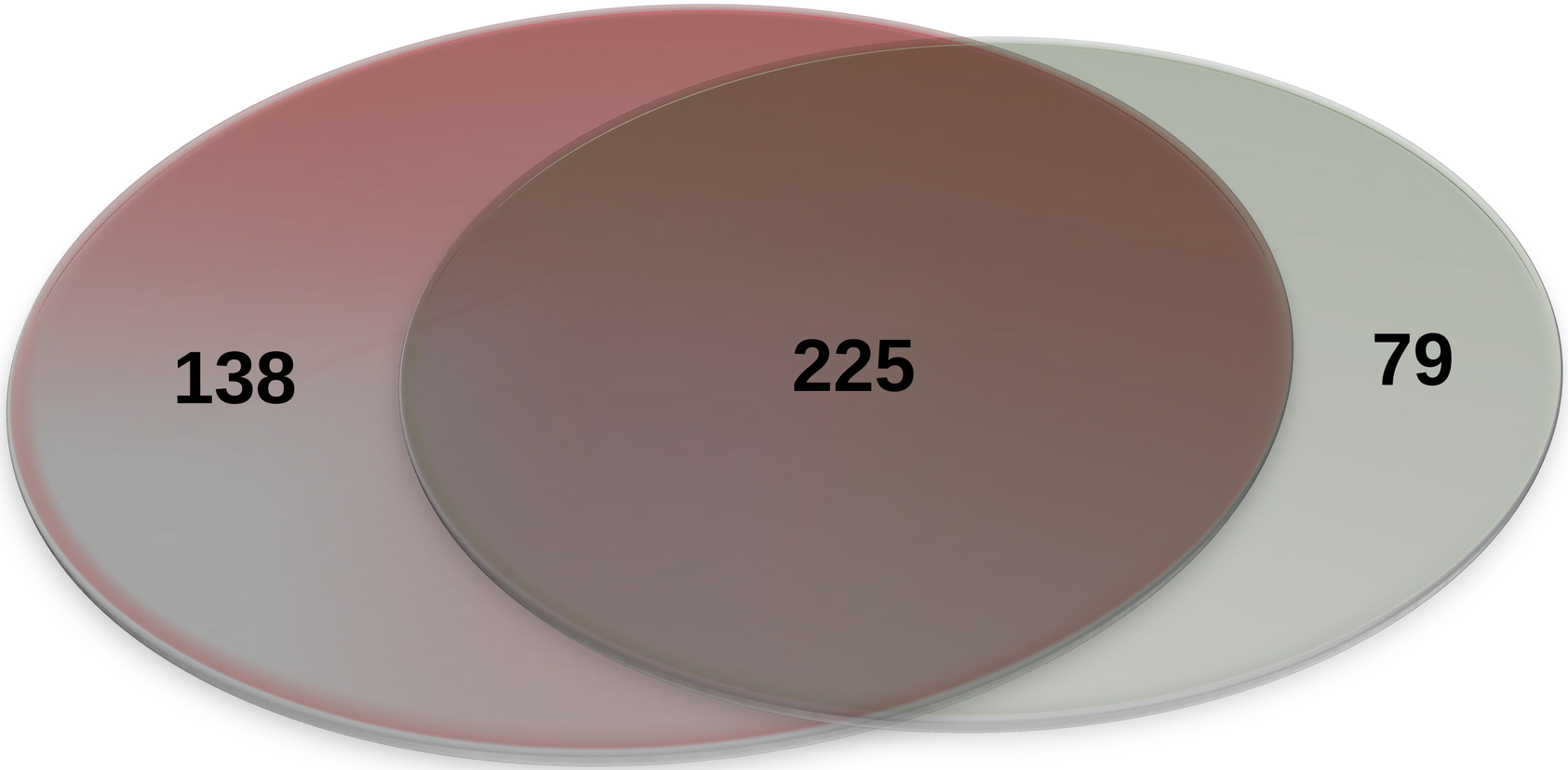
Measurement	Features	Analytes with HMDB urine list	Mol. Formula with SF calc.
LC-MRMS(+)	1374	202	1212
LC-MRMS(-)	1448	203	1126
LC-MRMS(+) and LC-MRMS(-) combined	2585	**304**	2113

Mass tolerance for HMDB search and SF calculation: 0.5 ppm

* Pooled urine sample was measure in 12 replicates ** column: Waters Acquity BEH C18 1.7 um (2.1 x 50 mm), flow 0.6 ml/min

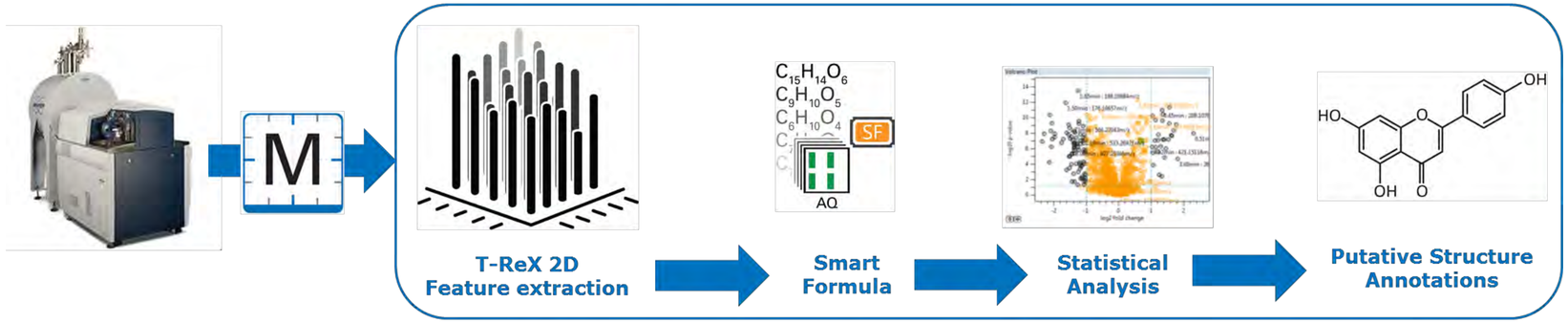
FIA-MRMS of urine samples

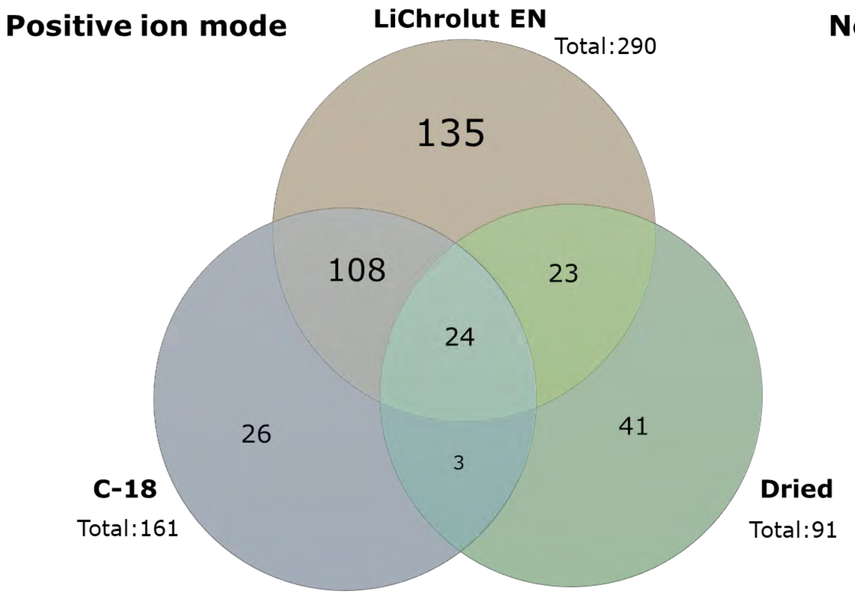
Comparison of FIA and LCMS results (ESI(+) and ESI(-) were merged)

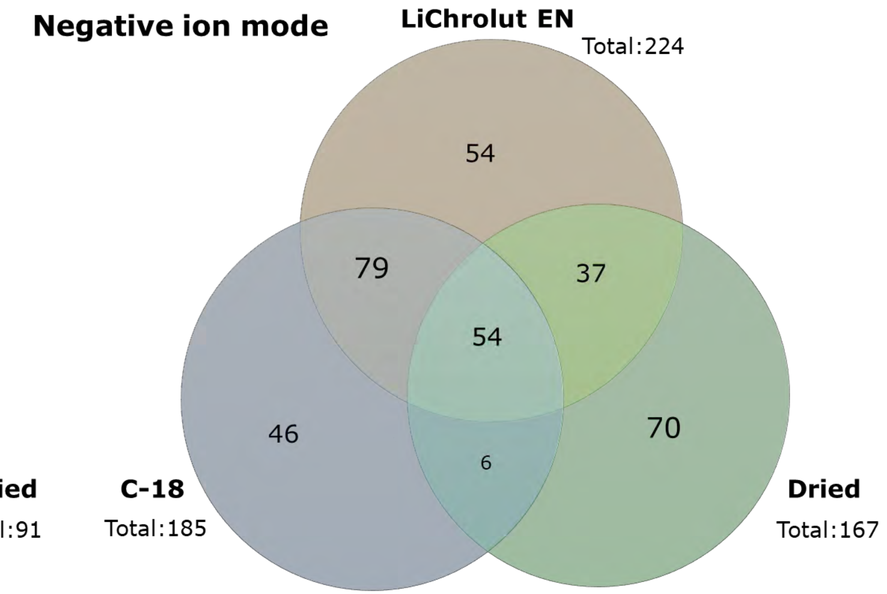


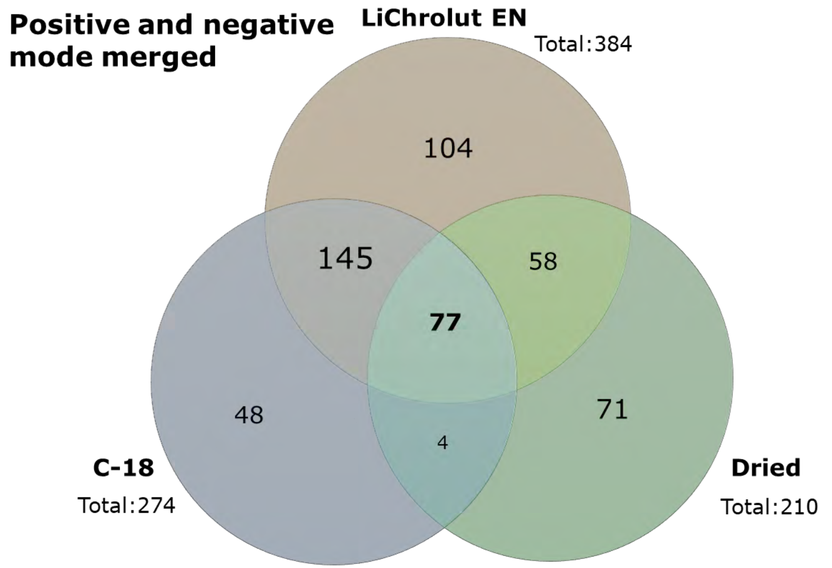
FIA-MRMS of urine samples

Comparison of sample preparation of urine

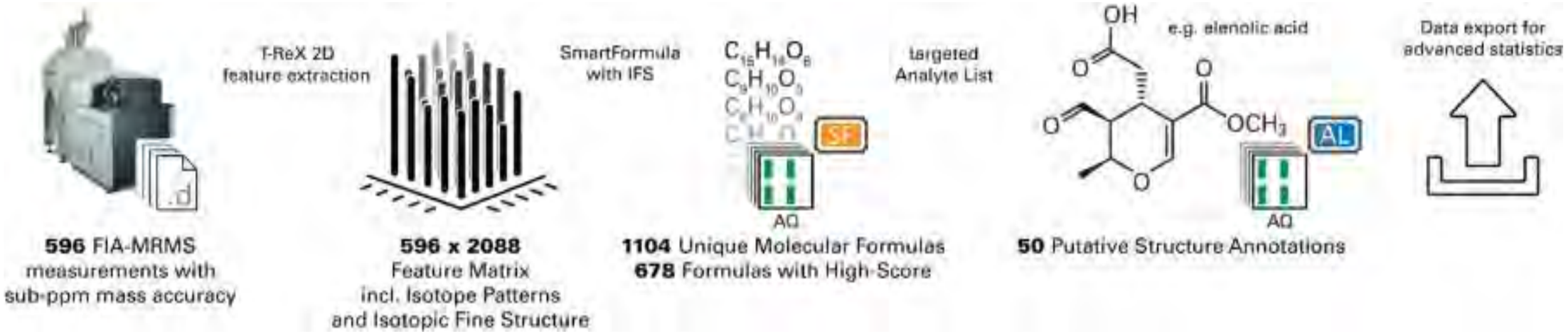








FIA-MRMS of EEVO samples and polyphenols



- **Samples collection:** Samples were collected from the main Greek olive oil producing regions and stored at room temperature, in darkness, under nitrogen.

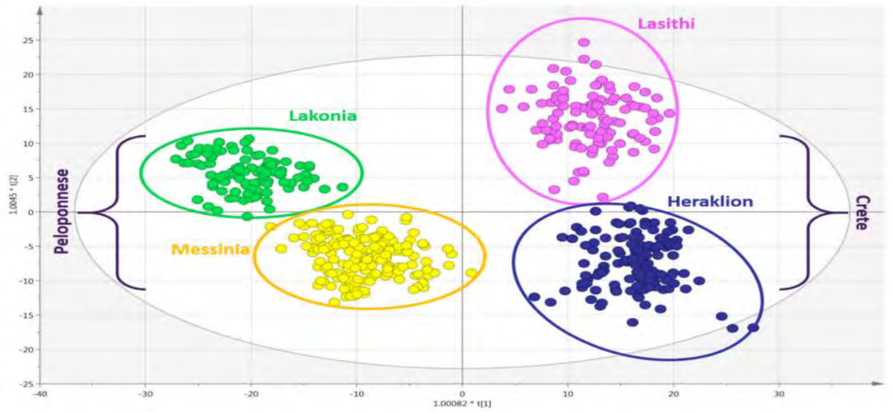
- **Sample Preparation:** Stock solutions were prepared by dissolving 10uL of samples in 500 mL MeOH. The stock solutions were then diluted 1:20 in 50% MeOH + 10 mM Ammonium Acetate.

- **MS analysis:** EVOO (extra virgin olive oil) samples and their biophenolic extracts were analyzed using a Bruker solariX-XR 7T mass spectrometer using ESI (-) mode by FIA.

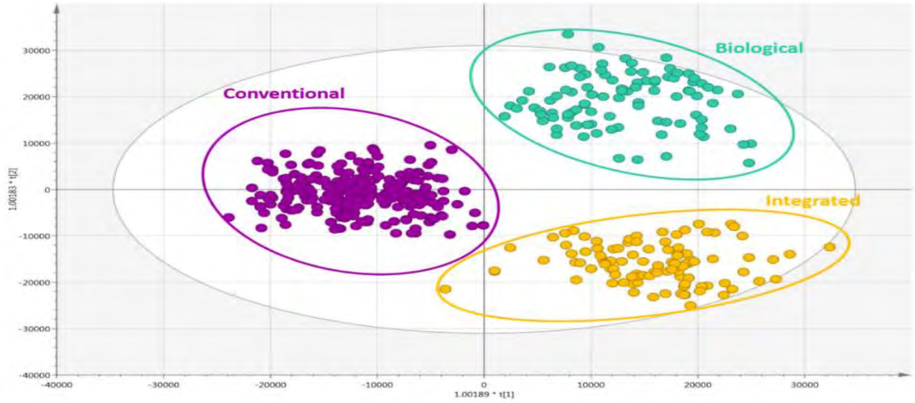
FIA-MRMS of EEVO samples and polyphenols

Geographical origin

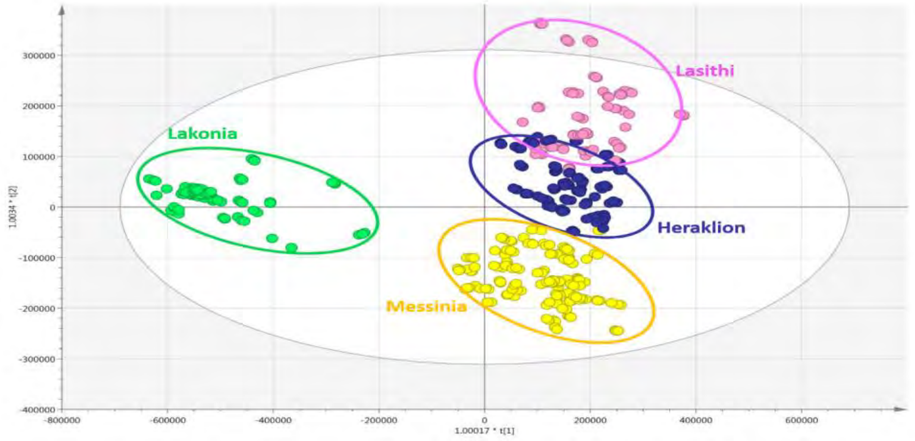
Geographical origin-EVOO



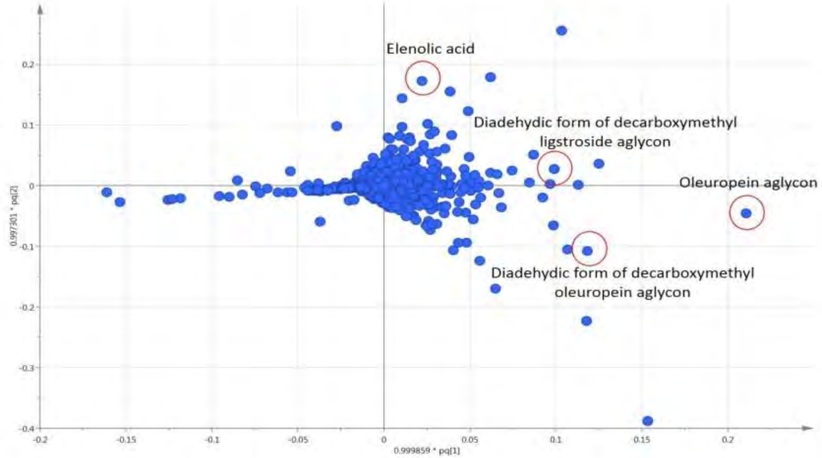
Cultivation practice-EVOO



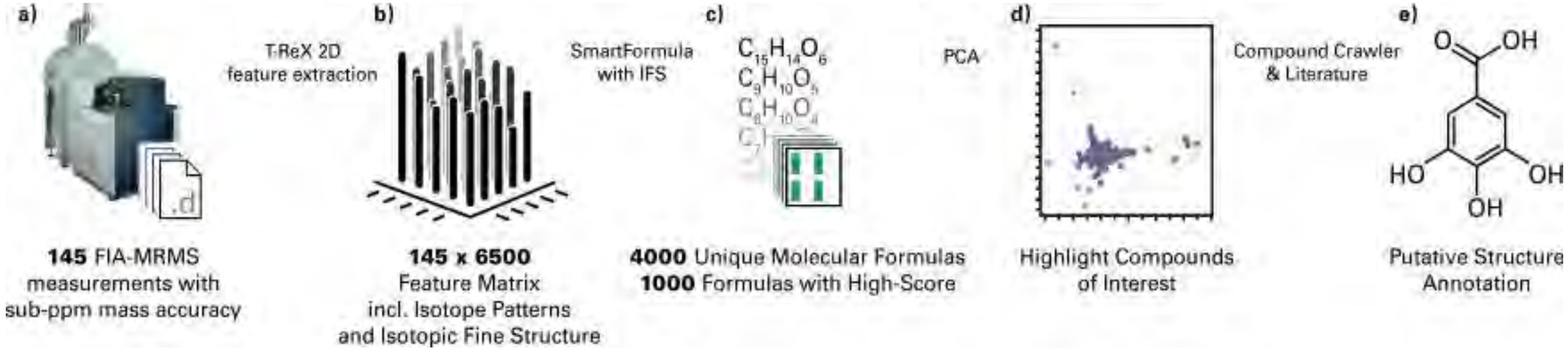
Geographical origin- Biophenols



loading plot



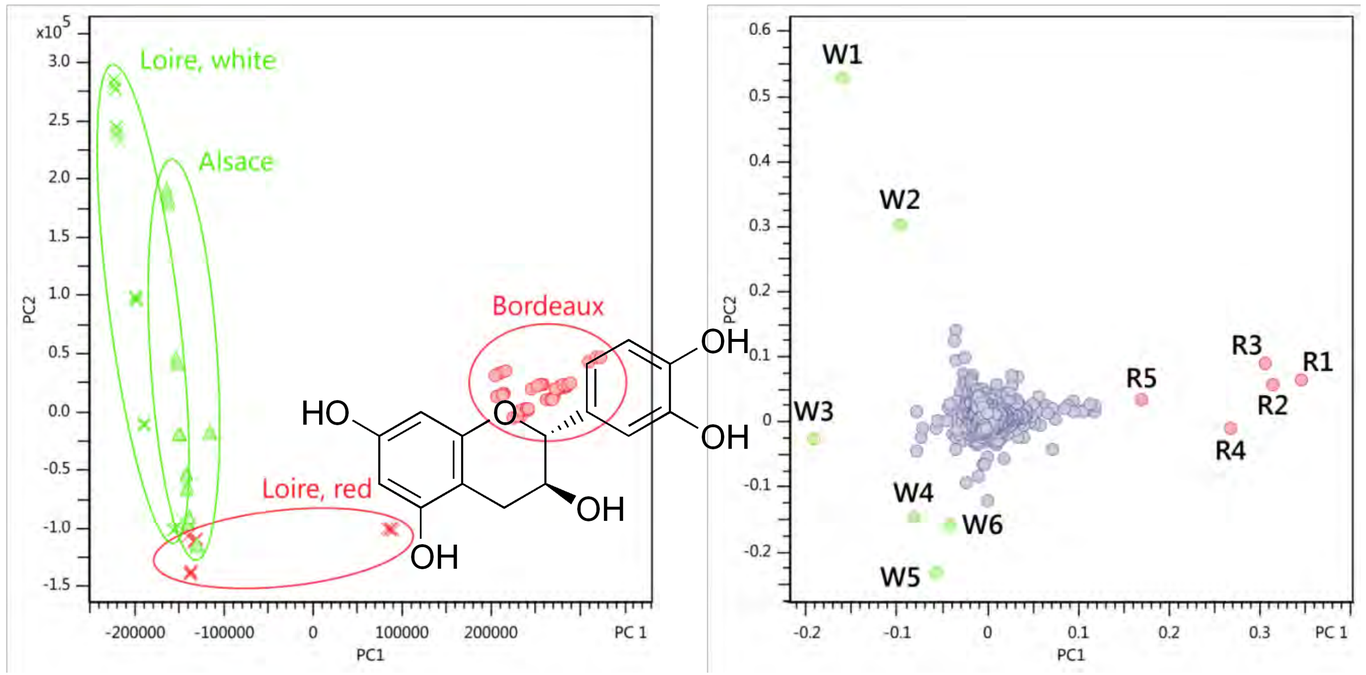
FIA-MRMS of wine samples



- **Sample preparation:** Solid phase extraction (SPE)
- **MS Analysis:** Bruker solariX XR 7T mass spectrometer using ESI (-) with a resolving power of 300,000 at m/z 400.
- **Data Processing:** Deisotoping and adduct collation in MetaboScape.
- **Statistical analysis:** PCA as well as molecular formula calculation based on accurate mass, IFS and filtering based on elemental composition in MetaboScape. Annotated features were investigated using filters for mass defects and DBE.

FIA-MRMS of wine samples

PCA plots



High abundance in white wine

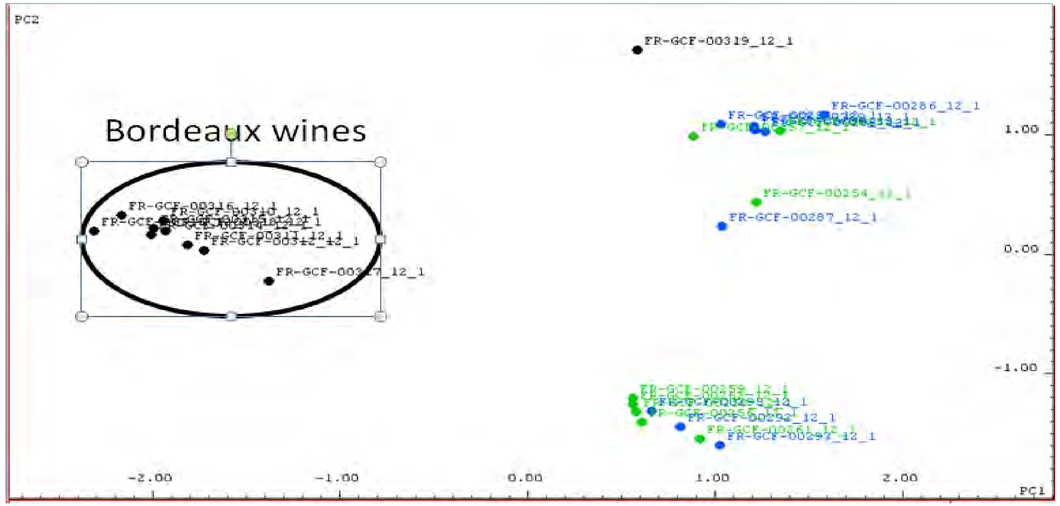
Compound	Molecular formula	Name
W1	$C_{15}H_{18}O_6$	Methoxycoumarin
W2	$C_{14}H_{16}O_3$	-
W3	$C_6H_{10}O_5$	3-Hydroxy-3-methyl-glutaric acid
W4	$C_6H_8O_2$	Sorbic acid
W5	$C_{13}H_{12}O_9$	Caftaric acid
W6	$C_{14}H_{14}O_9$	Fertaric acid

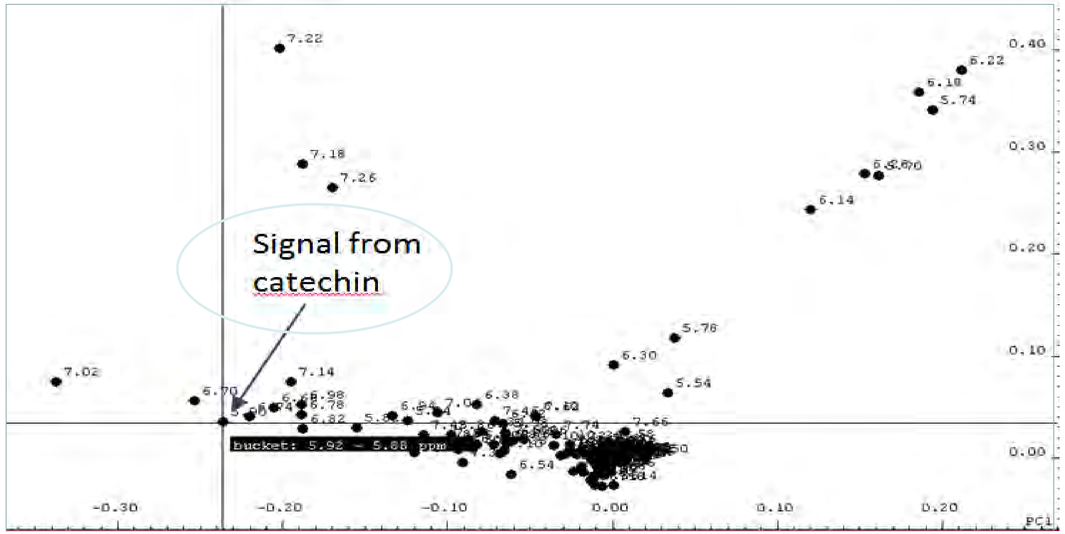
High abundance in red wine

Compound	Molecular formula	Name
R1	$C_{15}H_{14}O_6$	Catechin
R2	$C_9H_{10}O_5$	Syringic acid
R3	$C_6H_{10}O_4$	Adipic acid / 3-Methyl glutamic acid
R4	$C_7H_{12}O_5$	Diacetin
R5	$C_7H_6O_5$	Gallic acid

FIA-MRMS of wine samples

^{1}H-NMR analysis





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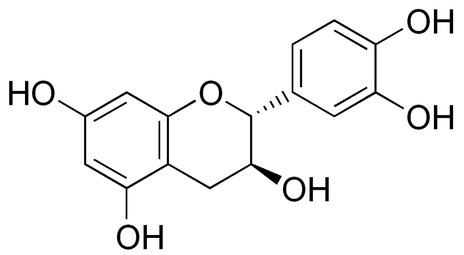
R4	$C_7H_{12}O_5$	Diacetin
R5	$C_7H_6O_5$	Gallic acid

- MRMS and 1H-NMR anaylses results in same grouping of wines.
- Catechin is mainly responsible to separate white wine and red wine in PCA plot.



Catechin
antioxidant (polyphenol), flavanol

Detection of intermediates

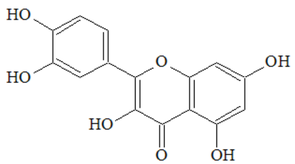
Anti-inflammatory Effects of Hop bitter acids in Dentritic cells

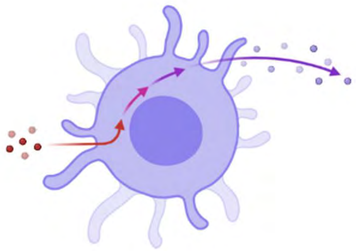
https://DOI: 10.1039/d1fo02361f (E. Sommella)

Stimulation of dendritic cells (DCs) and sample preparation protocol for extraction of metabolites.

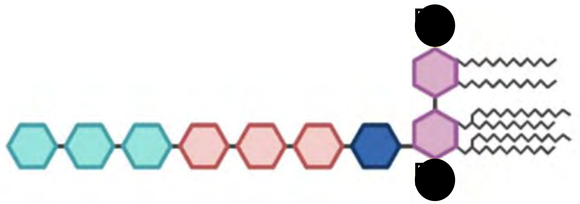
DCs from murine bone marrow (BMDCs) from six- to eight-week-old mice.

or





24 h later **LPS stimulation** (1 µL/mL)



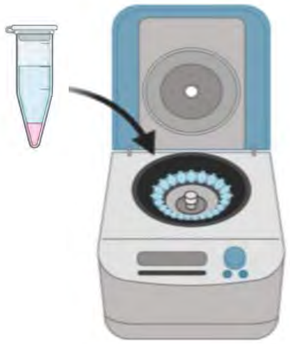
On day 10. **Hop fractions (A, B, C)** 25 µg/mL **or quercetin** 25 µM **administration**

Pelleted cells thawed on ice

100 µL of ice-cold $MeOH/H_2O$ (80:20 *v/v*).

Sonicate 6 min, 25 °C + vortex for 30 sec

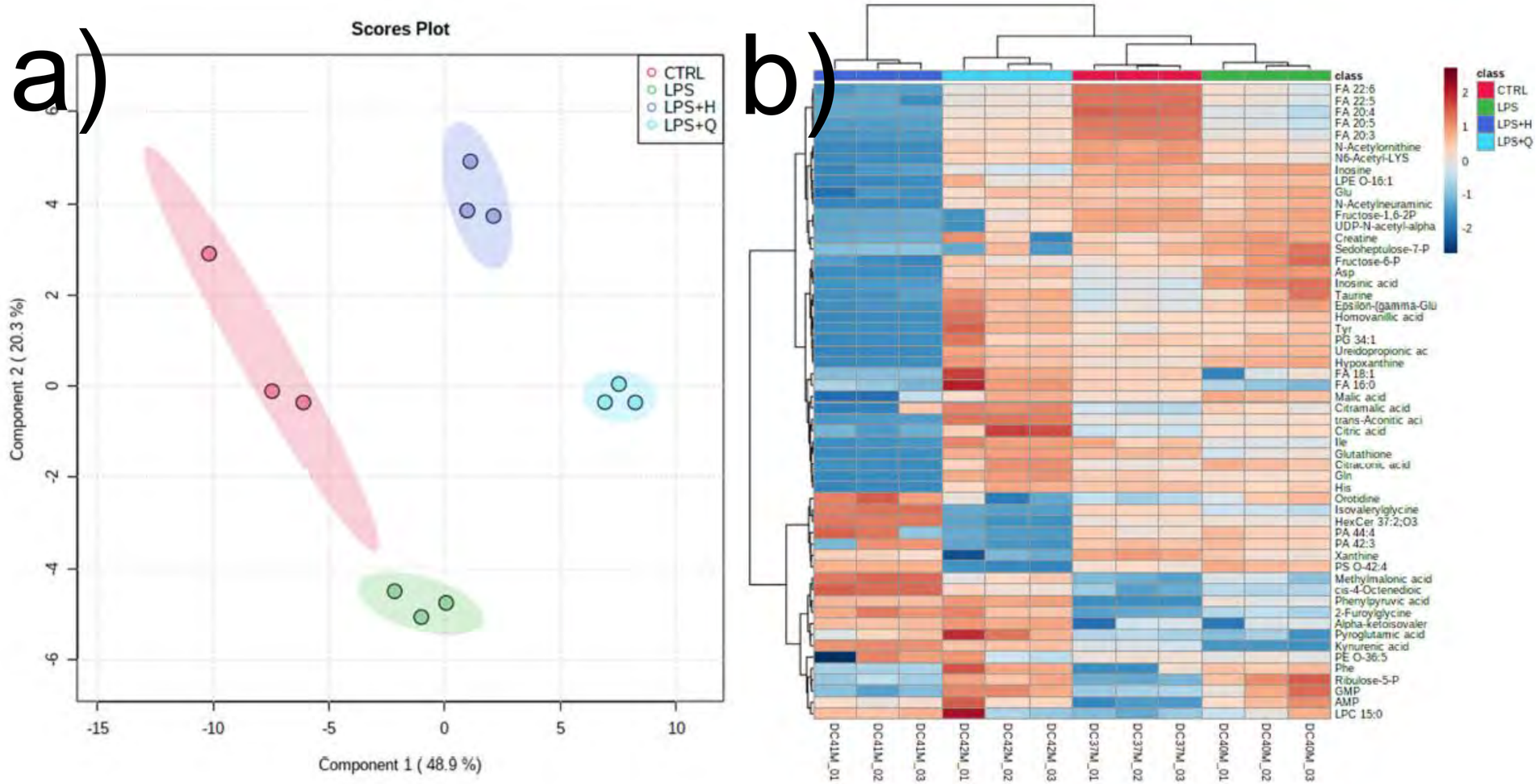
14680 rpm, 10 min, 4°C.



Detection of intermediates

Anti-inflammatory Effects of Hop bitter acids in Dentritic cells

https://DOI: 10.1039/d1fo02361f (E. Sommella)

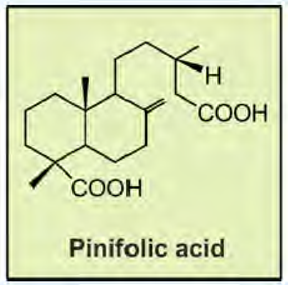


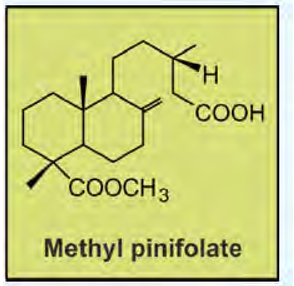
PLS-DA score plot (A) and heat map (B) of statistically relevant (ANOVA, $p < 0.05$) DC metabolites modulated by LPS, HOP C, and quercetin. Color changes reflect the normalized intensity.

Fingerprinting of Plant extracts

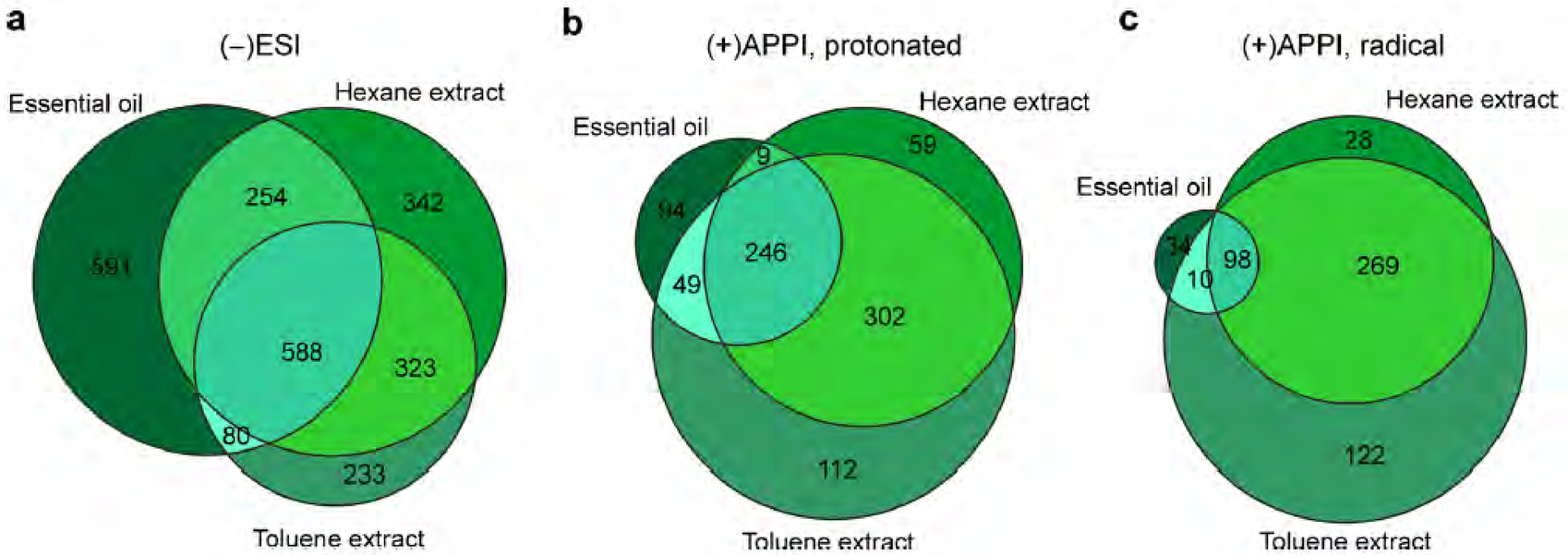
Detection of compounds in Conifer Needle Essential Oils

O. O. Mofikoya et al., ACS Omega 2020, 5, 18, 10543–10552.





Venn diagrams showing distribution of compounds between pine needle essential oil and two solvent extracts: (a) (−)ESI, (b) (+)APPI (protonated molecules), (c) (+)APPI (radical cations)



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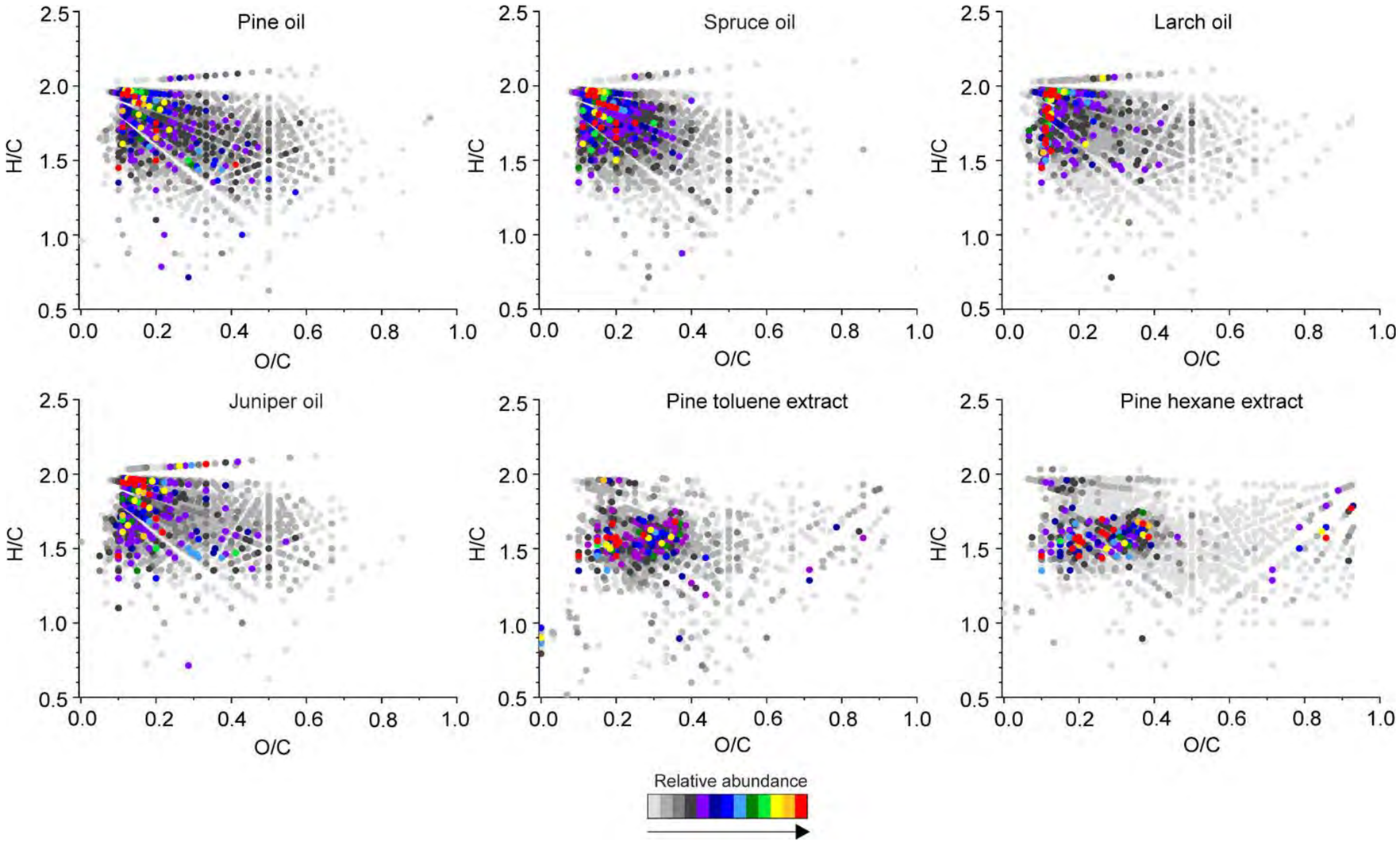
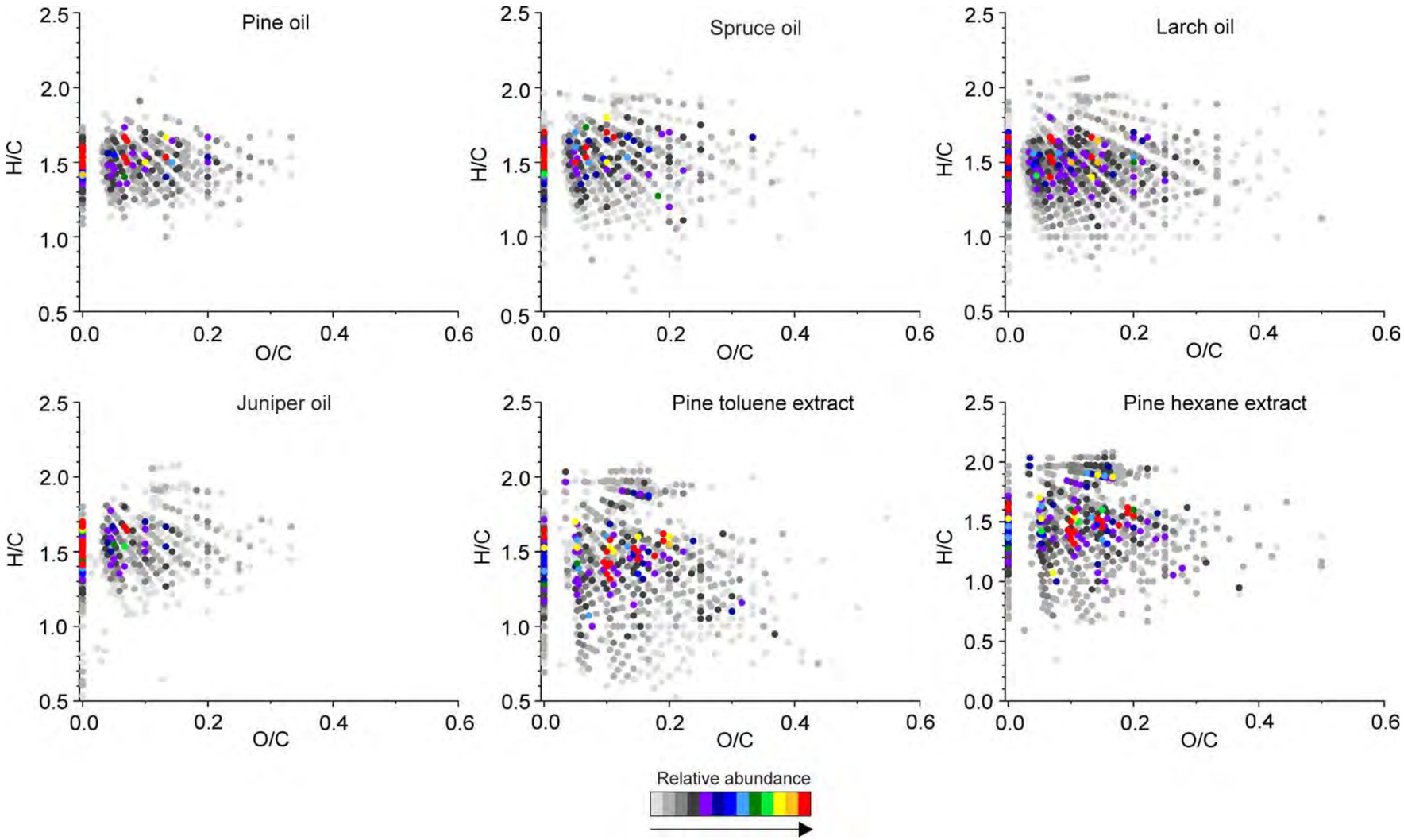


Van Krevelen diagrams of the compounds detected in the conifer needle essential oils and solvent extracts by (−)ESI FT-ICR MS.

Fingerprinting of Plant extracts

Detection of compounds in Conifer Needle Essential Oils

O. O. Mofikoya et al., ACS Omega 2020, 5, 18, 10543–10552.

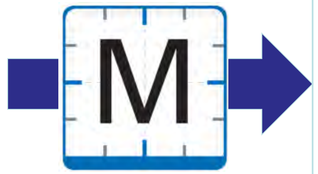


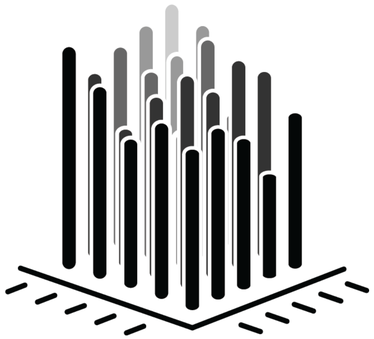
Van Krevelen diagrams of the compounds detected in the conifer needle essential oils and solvent extracts by (+)APPI FT-ICR MS.

MRMS aXelerate

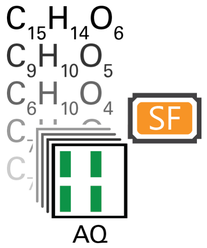
Now everyone can achieve high sample throughput on Phenomics research…

scimaX MRMS

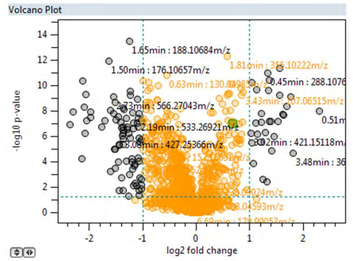




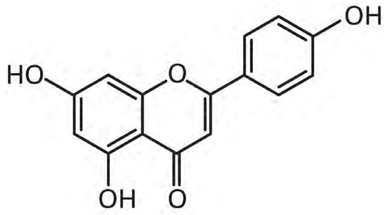
T-ReX 2D Feature extraction



Smart Formula



Statistical Analysis

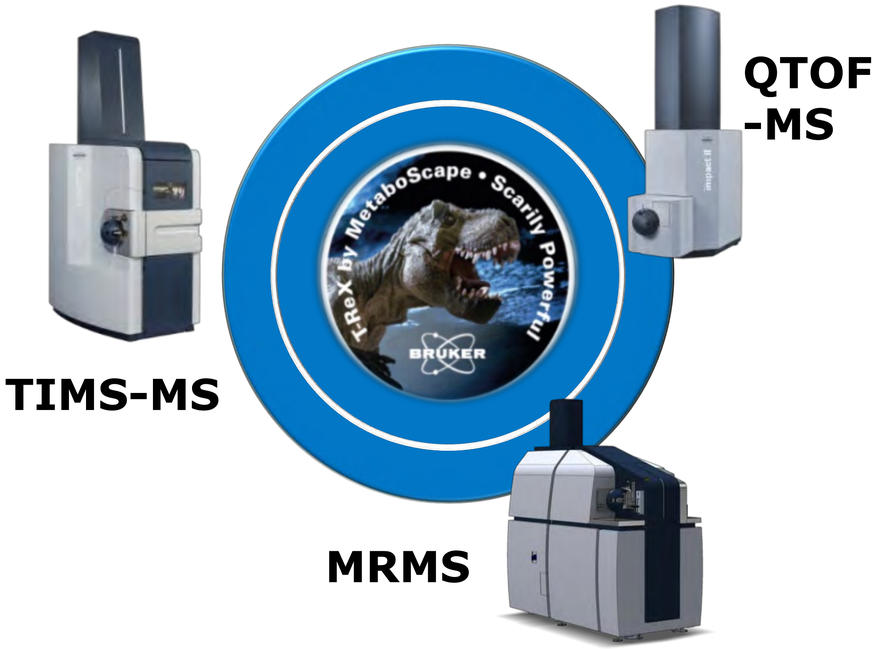


Putative Structure Annotations

- Extreme resolving power of **scimaX MRMS** system enables chromatography-free FIA or MALDI data acquisition for >200 samples / day
- Powerful data extraction in **MetaboScape: T-ReX 2D** for evaluating > 1000 samples
- Automatic ID with 3 tier confidence:
  - *Routinely <0.2ppm mass accuracy*
  - *True Isotopic Pattern*
  - *Mass resolution >1 million, boosts Isotopic Fine Structure fidelity*

MetaboScape

Key advantages



T-ReX (**T**ime aligned **R**egion complete **eX**traction) feature extraction technology for processing data from **complementary systems**:

T-ReX 2D for FIA-MRMS

High-throughput phenomics by MRMS

~5 min/sample, 3-tier confidence in formula, complementary to LC-MS

T-ReX 3D for LC-QTOF-MS/MS

Deep metabolome profiling by different LC methods and integrated processing of positive / negative mode data

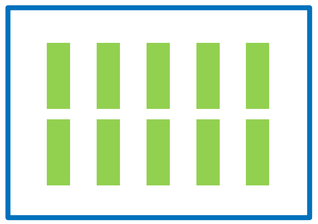
>1000 LC-MS runs from Phenomics Workhorse can be evaluated

T-ReX 4D for LC-TIMS-QTOF-MS/MS

For metabolomics methods development with 4D separations, robust CCS raised to next level with TIMS

Accurate Collisional Cross Sections (CCS) from **TIMS**

AQ score



A Precursor mass accuracy
B Retention time
C Isotopic pattern
D MS/MS fragment spectra
E CCS values

Intuitive Annotation Quality scoring for high confidence ID

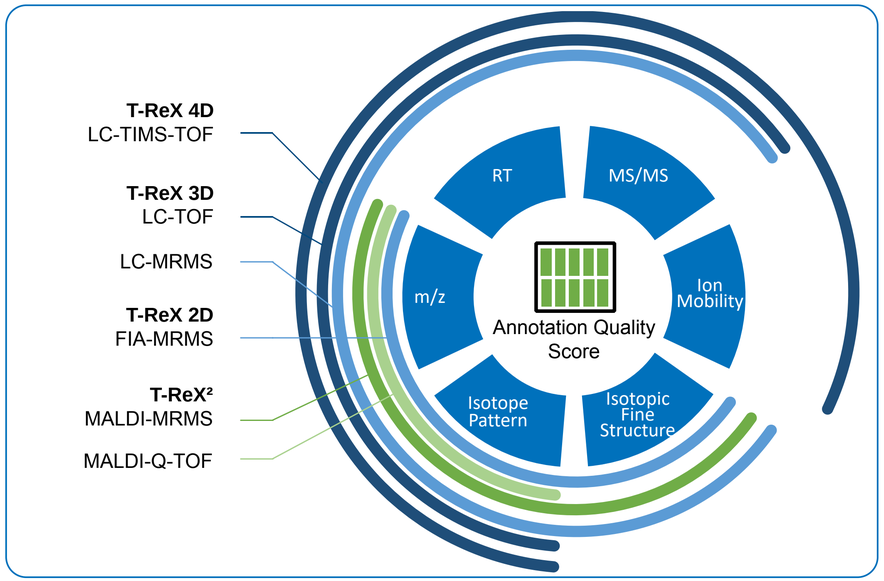
Rates all annotations with up to five quality factors

New: CCS matching for timsTOF and timsTOF Pro

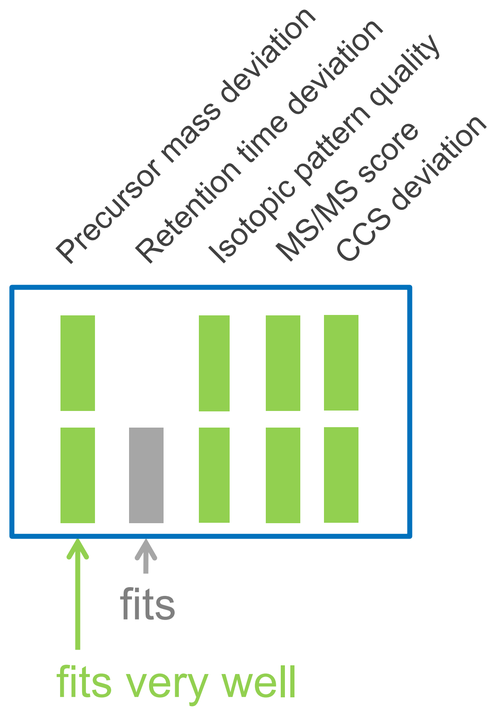
MetaboScape

Identification / Dereplication

The Goodness of annotation is indicated by the **annotation quality** symbol



MetaboScape and T-ReX support different instrument types, exploiting the respective strengths of each: For the sake of separation, annotation quality, or throughput.



User-definable **confidence levels** can be set for the **annotation quality**.

	Narrow	Wide	Unit
m/z:	1.0	3.0	ppm
Retention time:	0.15	0.4	minutes
mSigma:	15	50	
MS/MS score:	900	800	
CCS:	1	2	%

Acknowledgements

Australian National Phenome Center, Perth, Australia
- Jeremy Nicholson

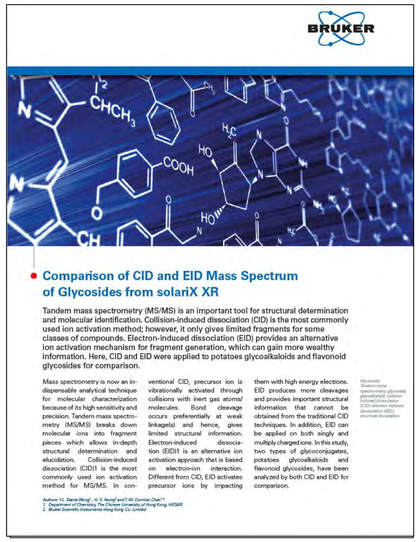
Imperial College London, UK
- Matt Lewis

University of Athens, Greece
- Theodora Nikou
- Maria Halabalaki

Bruker Daltonics GmbH & Co. KG Bremen
- Aiko Barsch
- Sven Meyer
- Ulrike Schweiger-Hufnagel

Bruker MRMS Metabolomics Application Notes

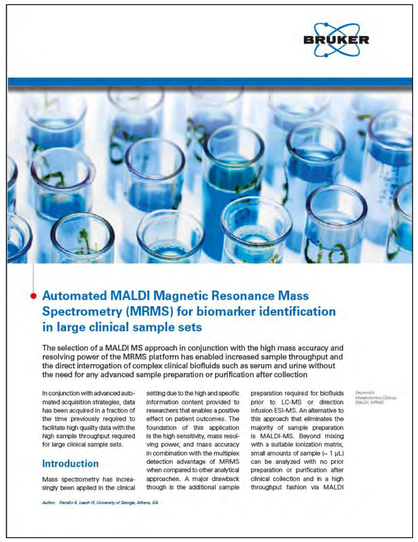
Unambiguous Natural product ID



Comparison of CID and EID Mass Spectrum of Glycosides from solariX XR

MRMS-60

Biomarker Identification in sample cohorts



Automated MALDI Magnetic Resonance Mass Spectrometry (MRMS) for biomarker identification in large clinical sample sets

MRMS-61

Micropollutants and plant response metabolites

MRMS aXelerate – rapidly detected micropollutants and plant response metabolites in poplar leaves

MRMS-62

Metabolic changes in murine hair follicles

Analysis of metabolic changes in murine hair follicles treated with Procyanidin-B2 rich nutraceuticals by DI-MRMS

MRMS-63

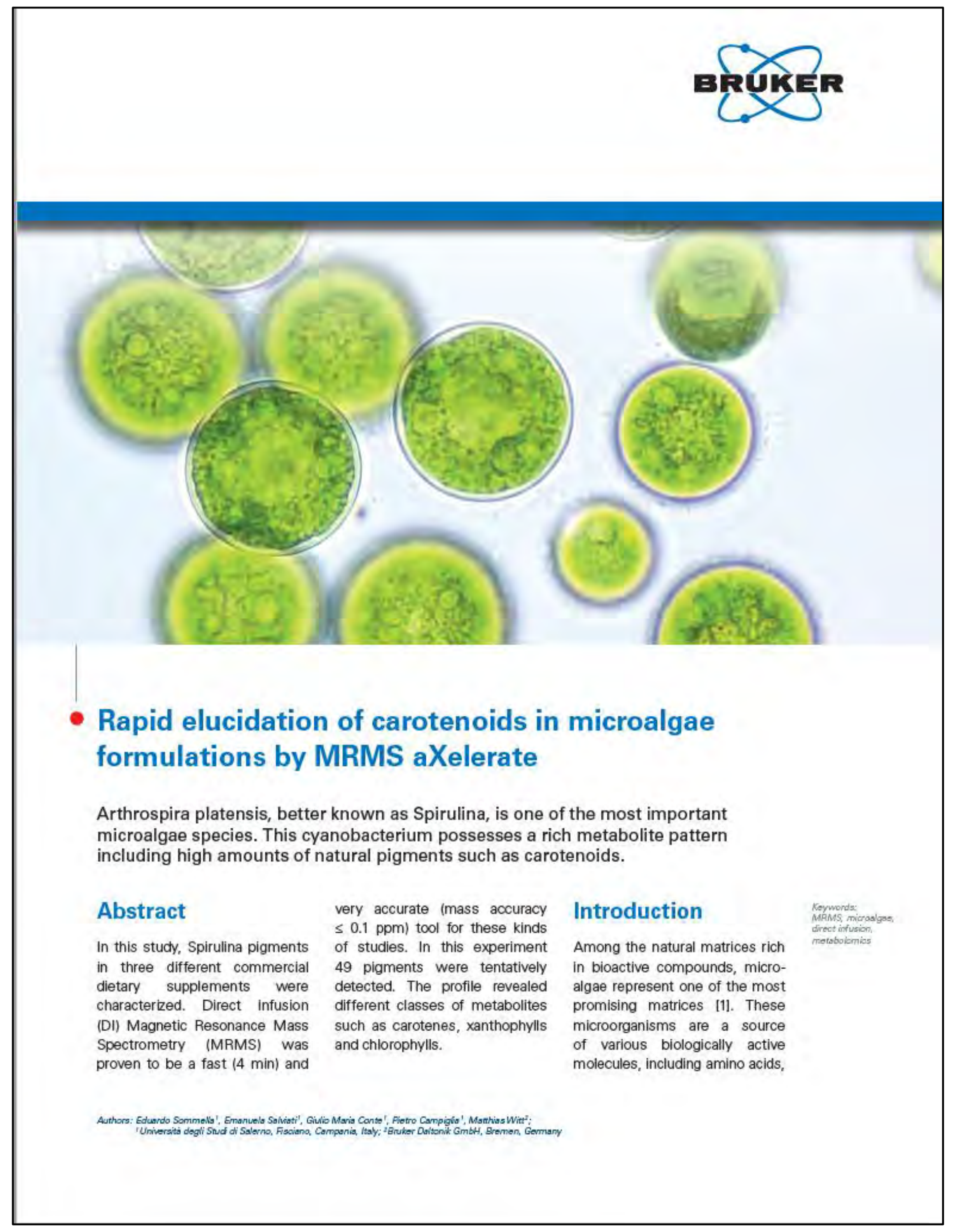
Isotopic fine structure to reveal potential biomarkers

A strategy using isotopic fine structure to reveal potential biomarkers showing the effects of traditional Chinese medicines on Alzheimer disease in rats

MRMS-65

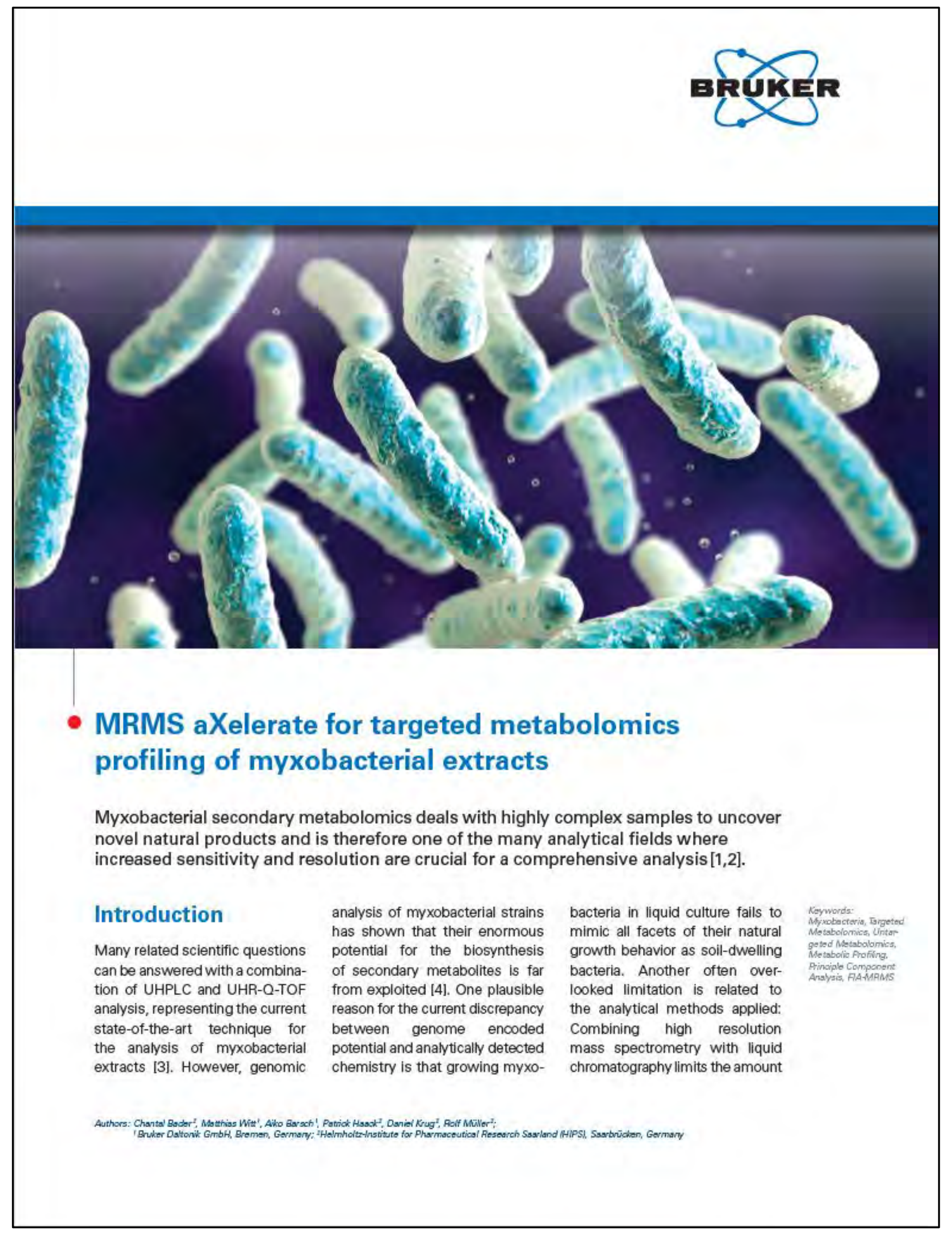
Bruker MRMS Metabolomics Application Notes

Carotenoids in microalgae formulations



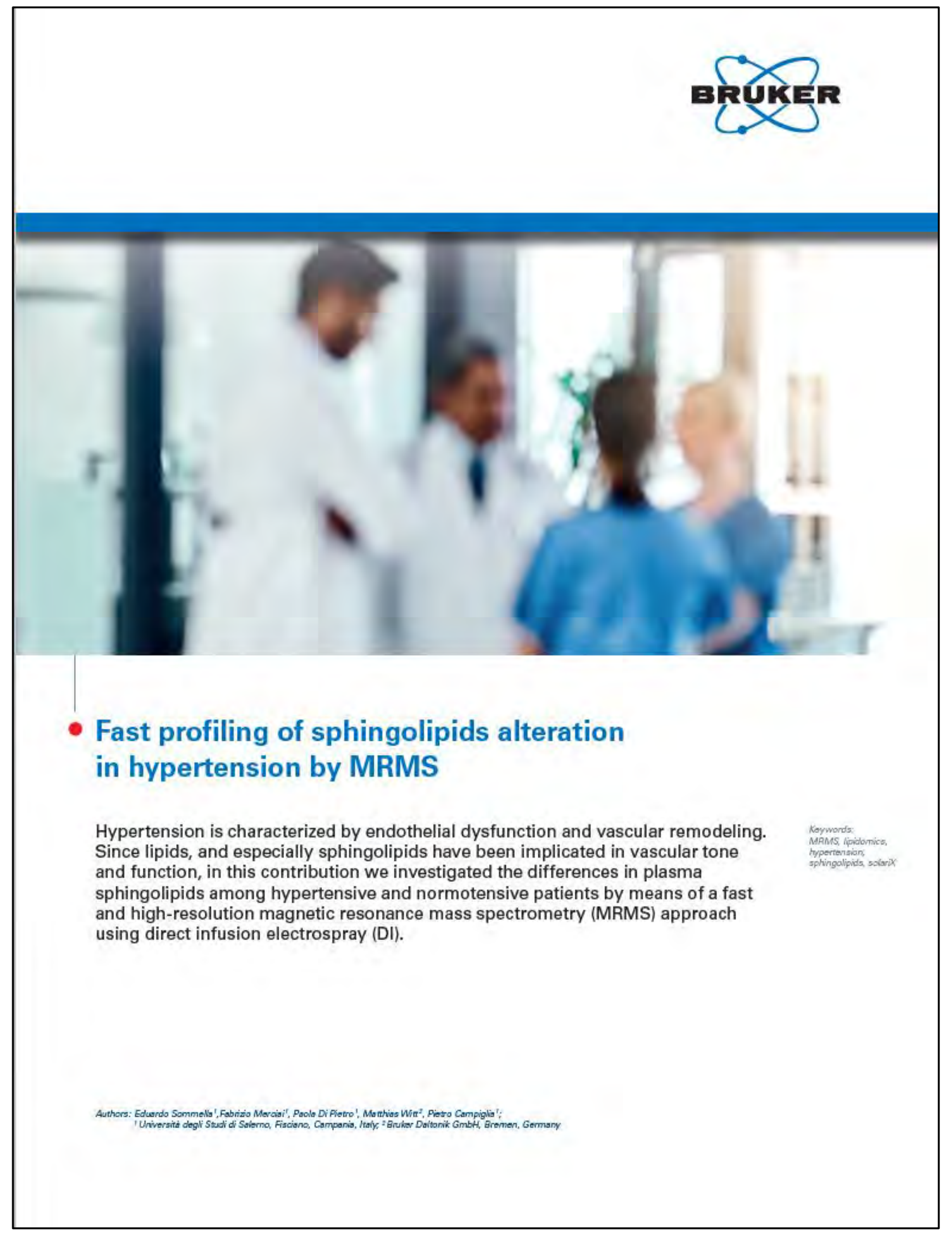
MRMS-66

Profiling of myxobacterial extracts



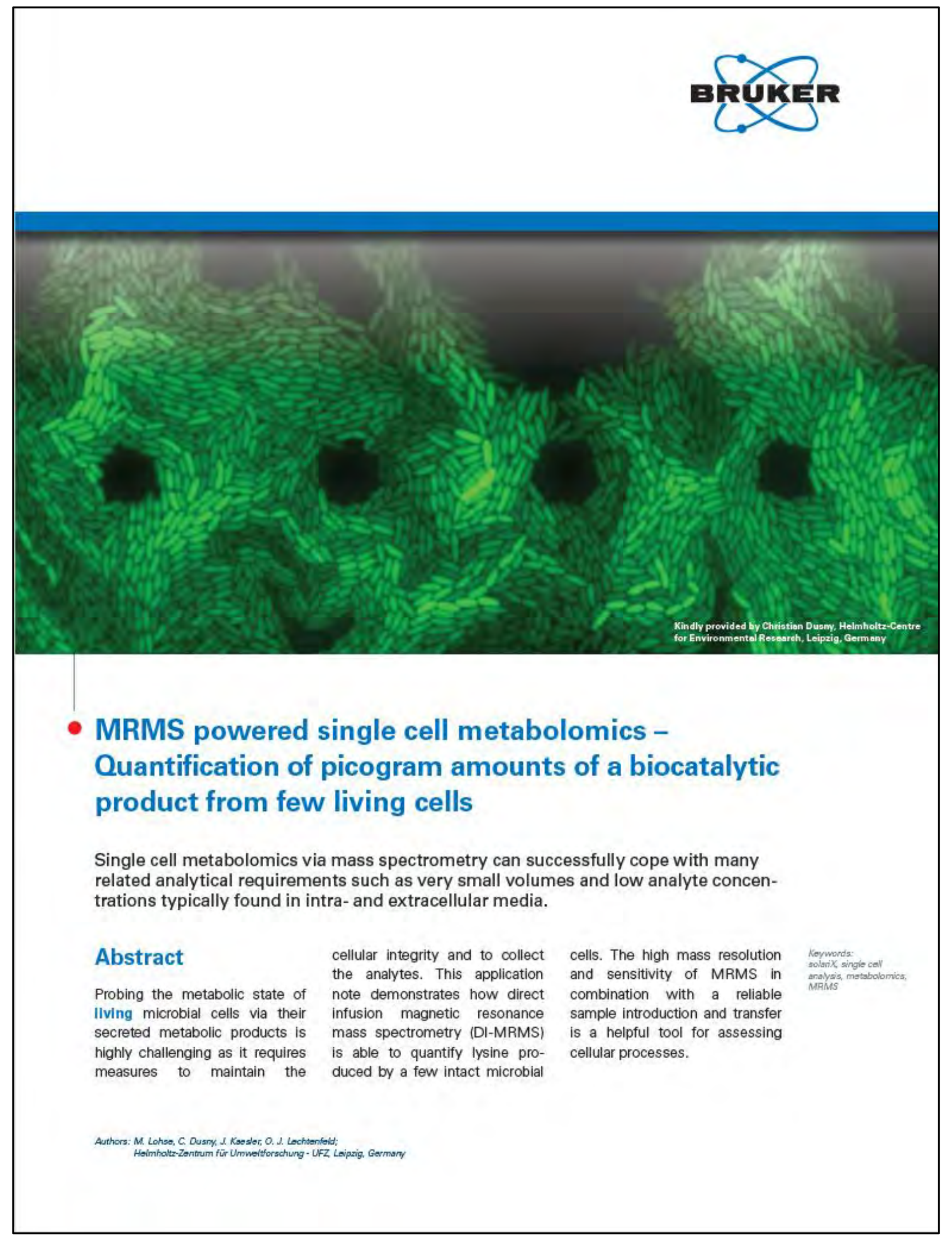
MRMS-67

Fast profiling of sphingolipids



MRMS-68

Single cell metabolomics



MRMS-70

Fast profiling of sphingolipids

MRMS-71

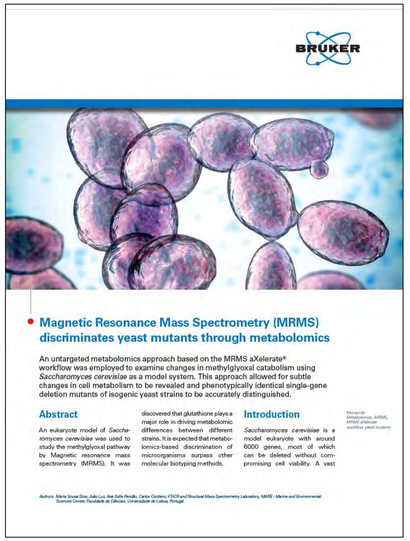
Bruker MRMS Metabolomics Application Notes

Elucidation of metabolic changes

Elucidation of metabolic changes in HFD-ApoE$^{-/-}$ model by SP6 peptide: A flow injection analysis magnetic resonance mass spectrometry (FIA-MRMS) study

MRMS-73

Discrimination of yeast mutants by metabolomics



Magnetic Resonance Mass Spectrometry (MRMS) discriminates yeast mutants through metabolomics

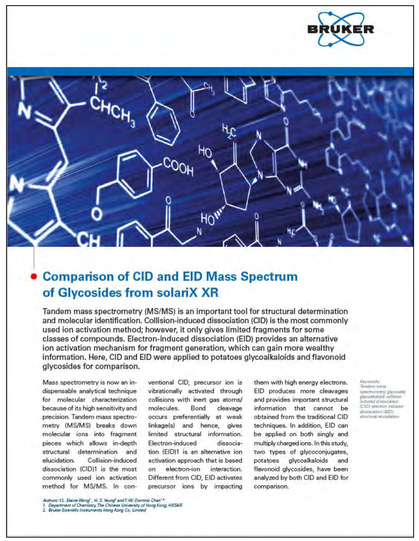
MRMS-75

Structural characterization of pesticide products

Electron-Induced Dissociation (EID) as a complementary tool to collision induced dissociation (CID) for structural characterization of pesticides photo-oxidation products

MRMS-76

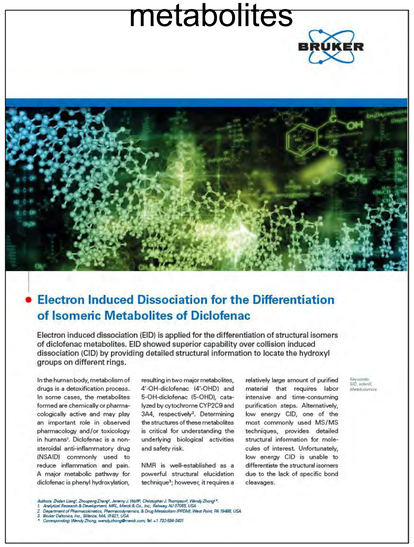
CID and EID of Glycosides



Comparison of CID and EID Mass Spectrum of Glycosides from solariX XR

FTMS-57

EID of isomeric metabolites



Electron Induced Dissociation for the Differentiation of Isomeric Metabolites of Diclofenac

FTMS-58

Bruker MRMS Metabolomics Application Notes

Mapping of olive oil

Mapping of Greek olive oil using magnetic resonance mass spectrometry flow injection analysis and multivariate data analysis

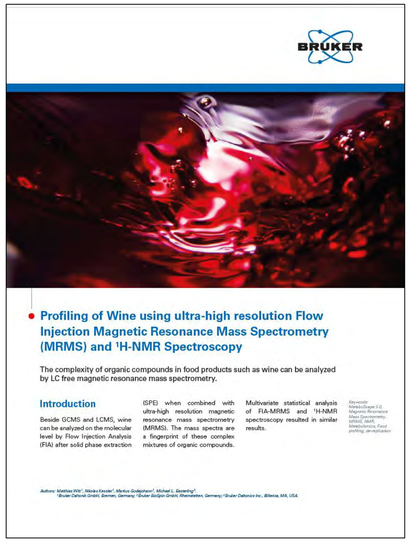
In this study, LC free magnetic resonance mass spectrometry (MRMS) analysis was employed for mapping and quality control assessment of Greek extra virgin olive oil (EVOO) using a new and intuitive software workflow.

Introduction

The increasing popularity of EVOO over the last decade has provided the need for quality and authenticity control[1]. Its chemical complexity impedes the transaction of the typical analytical methodologies[2]. In this study, a holistic approach to map Greek EVOO is presented. The workflow takes advantage of the rapid, LC free, flow injection analysis (FIA) based data acquisition by ultra-high resolution MRMS. The obtained mass spectra were evaluated using the new MetaboScape® 3.0 software for deisotoping, as well as for identifying the most significant metabolites.

PN-45

Profiling of wine



Profiling of Wine using ultra-high resolution Flow Injection Magnetic Resonance Mass Spectrometry (MRMS) and ^{1}H-NMR Spectroscopy

The complexity of organic compounds in food products such as wine can be analyzed by LC free magnetic resonance mass spectrometry.

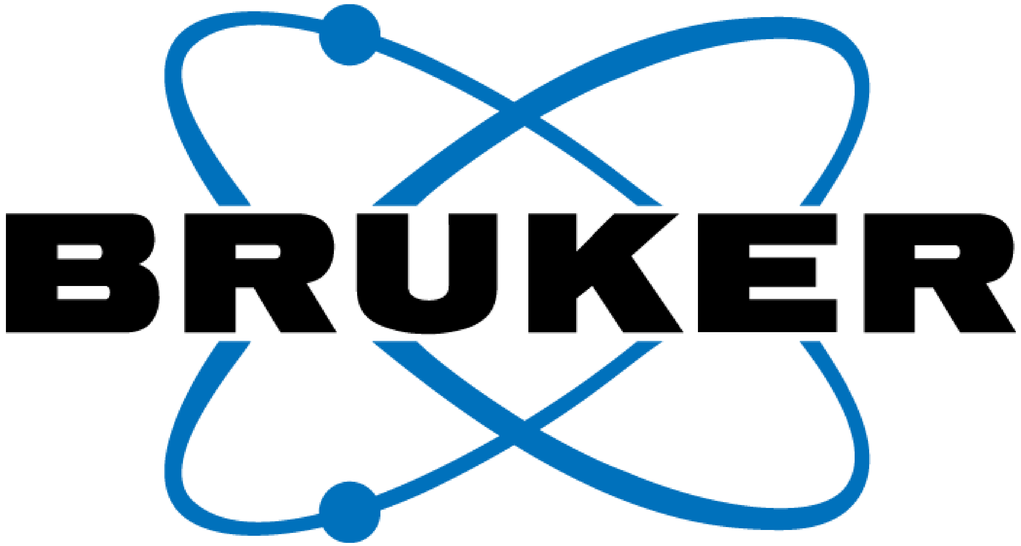
Introduction

Beside GCMS and LCMS, wine can be analyzed on the molecular level by Flow Injection Analysis (FIA) after solid phase extraction (SPE) when combined with ultra-high resolution magnetic resonance mass spectrometry (MRMS). The mass spectra are a fingerprint of these complex mixtures of organic compounds. Multivariate statistical analysis of FIA-MRMS and ^{1}H-NMR spectroscopy resulted in similar results.

PN-48

http://eftms2022.campus.ciencias.ulisboa.pt/


Thank you for your attention



www.bruker.com