High Performance Gas Chromatography Mass Spectrometry in Addressing the Challenges of Metabolomic Studies – Separation in Time and Mass

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LECO Separation Science

Outline of Presentation

The Metabolomics Problem – Technologies and Challenges

The Zucker Rat Biological Problem and Other Metabolomic Data

The Outcome of the Study – What is Expressionist?

GC-HRMS – Technology and Data

GCxGC-TOF-HRMS – Rats and Breath

Leco
Delivering the Right Results

Genedata
solutions in silico

Objectives

- **GCMS** provides capabilities to define modulated analytes in populations and phenotypes which complements LCMS
- **HRMS** enables identification of unknowns and confident identification of knowns
  - **Accurate m/z** for fragments
  - **Isotopic Abundance** for knowns and unknown
  - Mass accuracy and Isotopic abundance confirm formulae for m/z
  - **Chemical Ionization** with accurate m/z enables unknown ID
  - Provides linearity and sensitivity needed for metabolomics analysis
- **Deconvolution** enables the ability to:
  - detect and quantify metabolites
  - provide searchable spectra from difficult peak pairs
  - provide interpretable spectra from difficult peak pairs
- **GCxGC TOF MS –**
  - Separation of additional analytes
  - Differential Analysis and enhanced Sensitivity
- **Genedata** enables an HRMS-optimized tool for differential analysis of phenotypes and populations.

The Metabolomic Problem

Analytical Challenges in Metabolomics

Accurate Differential Analysis of Biochemistry

Complex Samples and Analytes
•Diverse samples (plants, phys fluids, insects, tissue, etc)
•Matrix Effects
•Spectral Dynamic Range
•Isomer Differentiation
•Reproducibility

Comprehensive Analysis (vs. Targeted)
> 60% Unknowns!

Peak Capacity (1000s of Analytes/hour)

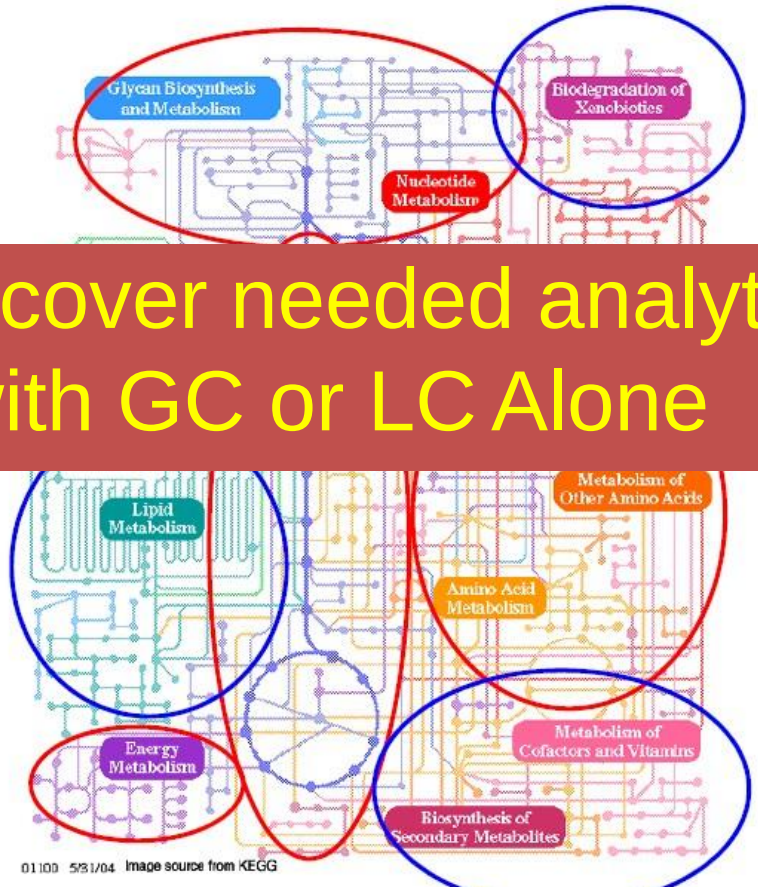
Range of Analyte Concentrations

Data Interpretation
Systems Biology and Contextual Information

GC-MS *vs.* LC-MS

METABOLIC PATHWAYS

Glycolysis
TCA cycle
Pentos
Amino aci
Gluconeogenesis
Urea cycle
Inositol metabolism
Carbohydrate metabolism



METABOLIC PATHWAYS

Lipid and fatty acid metabolism

ary metabolite
is

olism of co-factors and vitamins

Metabolism of Xenobiotics

Can't cover needed analytes with GC or LC Alone

MANCHESTER 1824

PROVIDES GOOD METABOLITE COVERAGE IN COMPLEMENTARY PATHWAYS

Why GCMS?

Fast and easy to adopt

Sensitive, Reliable, Robust and Quantitative

Linear Response and Good Dynamic Range

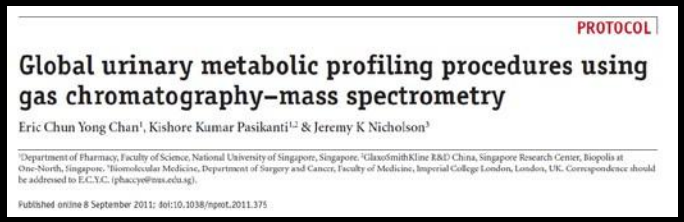
Variety of MS Opportunities (Nominal, HRMS, MSMS)

HUGE Well-established Database (>250k spectra)

Over 50 yrs of Application and Largest Presence of ANY MS System

Universal Application for M < 600 (w/ Deriv)

Highest Peak Capacity Chromatography

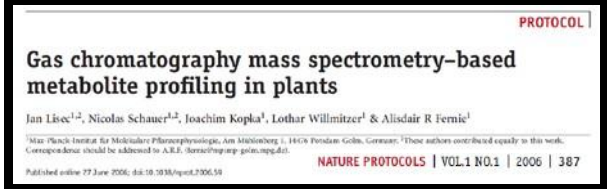

PROTOCOL

Global urinary metabolic profiling procedures using gas chromatography–mass spectrometry

Eric Chun Yong Chan[1], Kishore Kumar Pasikanti[1,2] & Jeremy K Nicholson[3]

[1]Department of Pharmacy, Faculty of Science, National University of Singapore, Singapore. [2]GlaxoSmithKline R&D China, Singapore Research Center, Biopolis at One-North, Singapore. [3]Biomolecular Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London, UK. Correspondence should be addressed to E.C.Y.C. (phaccye@nus.edu.sg).

Published online 8 September 2011; doi:10.1038/nprot.2011.375


PROTOCOL

Gas chromatography mass spectrometry–based metabolite profiling in plants

Jan Lisec[1,2], Nicolas Schauer[1,2], Joachim Kopka[1], Lothar Willmitzer[1] & Alisdair R Fernie[1]

[1]Max Planck Institut für Molekulare Pflanzenphysiologie, Am Mühlenberg 1, 14476 Potsdam Golm, Germany. [2]These authors contributed equally to this work. Correspondence should be addressed to A.R.F. (fernie@mpimp-golm.mpg.de).

Published online 29 June 2006; doi:10.1038/nprot.2006.59

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Metabolic Fingerprinting Using Comprehensive Two-Dimensional Gas Chromatography – Time-of-Flight Mass Spectrometry

Martin F. Almstetter, Peter J. Oefner, and Katja Dettmer

Michael Kaufmann and Claudia Klinger (eds.), *Functional Genomics: Methods and Protocols*, Methods in Molecular Biology, vol. 815, DOI 10.1007/978-1-61779-424-7_29, © Springer Science+Business Media, LLC 2012

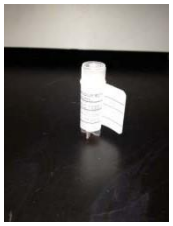
System Under Study

- Zucker Rats
  - 3 Phenotypes/Strains w/ Animals bred to be
    - Lean (n=12), Fatty (n=12), Obese (n=12)
  - 7-9 Weeks old (terminal bleed)
    - Disodium EDTA as anti-coagulant
    - 0.1 µm filtered
- Objectives
  - Identify analytes which are up or down regulated with phenotype using high performance MS
  - Test the capabilities of HRTs

"Fit"

"Husky??"

Zucker Rat Study: Sample Preparation



1)Plasma (100 µL)

2) MeOH (400 µL)

3) Vortex

4) Centrifuge & Remove Protein Pellet

5) Dry (2 hrs)

6) Lyophilize (Overnight)

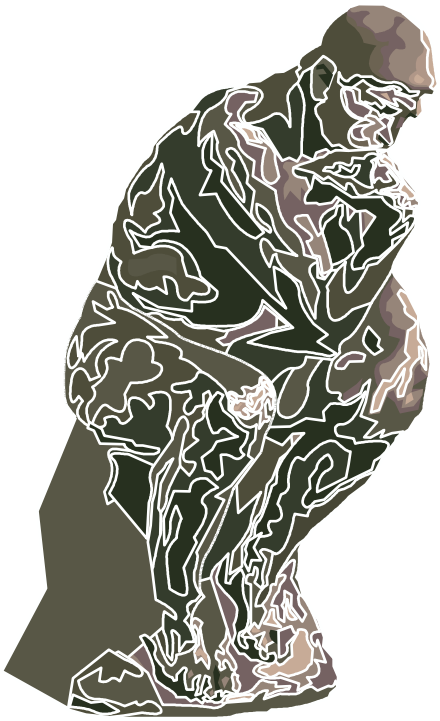
7) $MeONH_2$

8) MSTFA

9) FAMEs

Pegasus GC-HRT

Why High Resolution and Accurate Mass?



What are these values?

Why??

Corrupt or Impure Spectra
Uncertainty in m/z
EI only with no M
Limited MS/MS or accurate mass databases
Limited Libraries to match Derivatives or Analytes
Inadequate Chemical Analysis Tools

What is the real value of Accurate Mass?

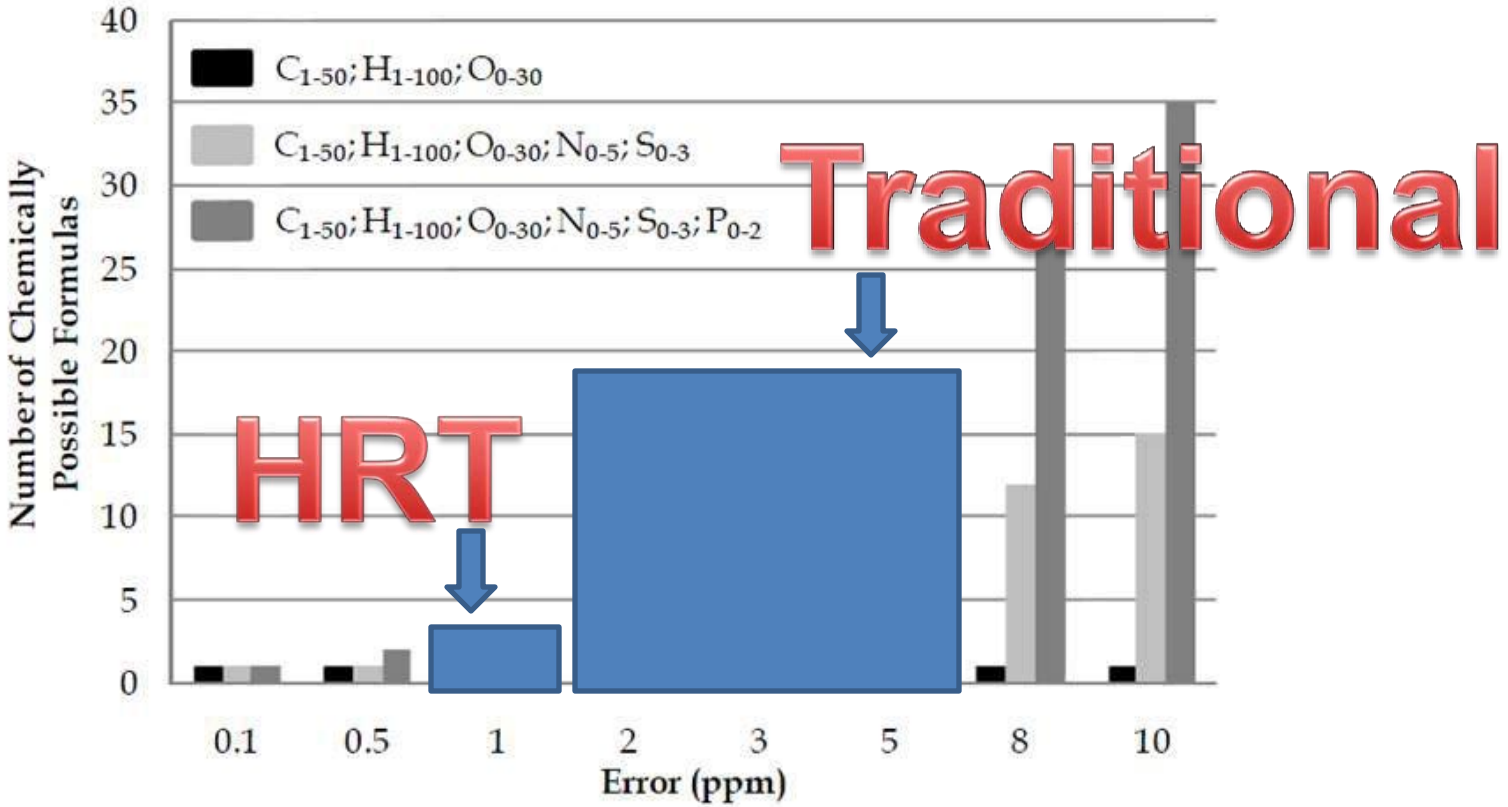
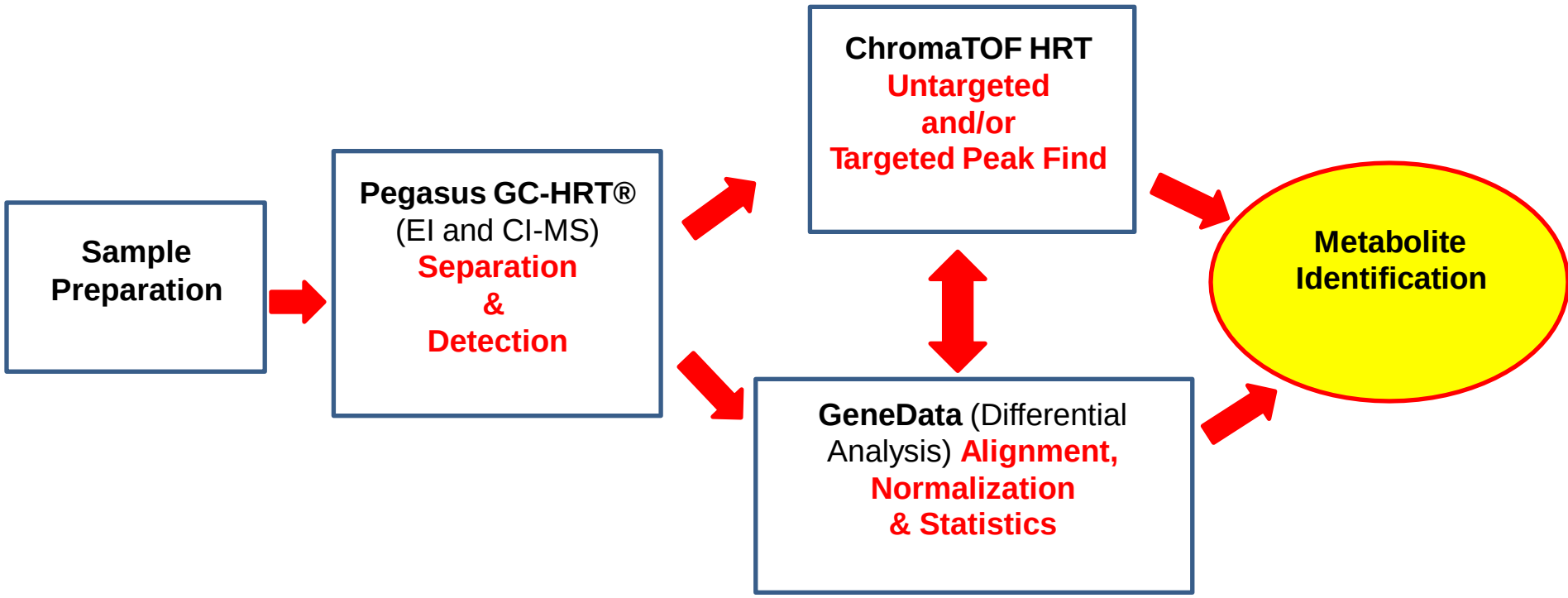


Fig. 8. The number of chemically possible molecular formulas for hypothetical m/z 499.21257 at various error values for the different elemental compositions specified in the legend

Rachel L. Sleighter and Patrick G. Hatcher (2011). Fourier Transform Mass Spectrometry for the Molecular Level Characterization of Natural Organic Matter: Instrument Capabilities, Applications, and Limitations, Fourier Transforms - Approach to Scientific Principles, Prof. Goran Nikolic (Ed.), ISBN: 978-953-307-231-9, InTech, DOI: 10.5772/15959. Available from: http://www.intechopen.com/books/fourier-transforms-approach-to-scientific-principles/fourier-transform-mass-spectrometry-for-the-molecular-level-characterization-of-natural-organic-matt

Zucker Rat Study: Workflow

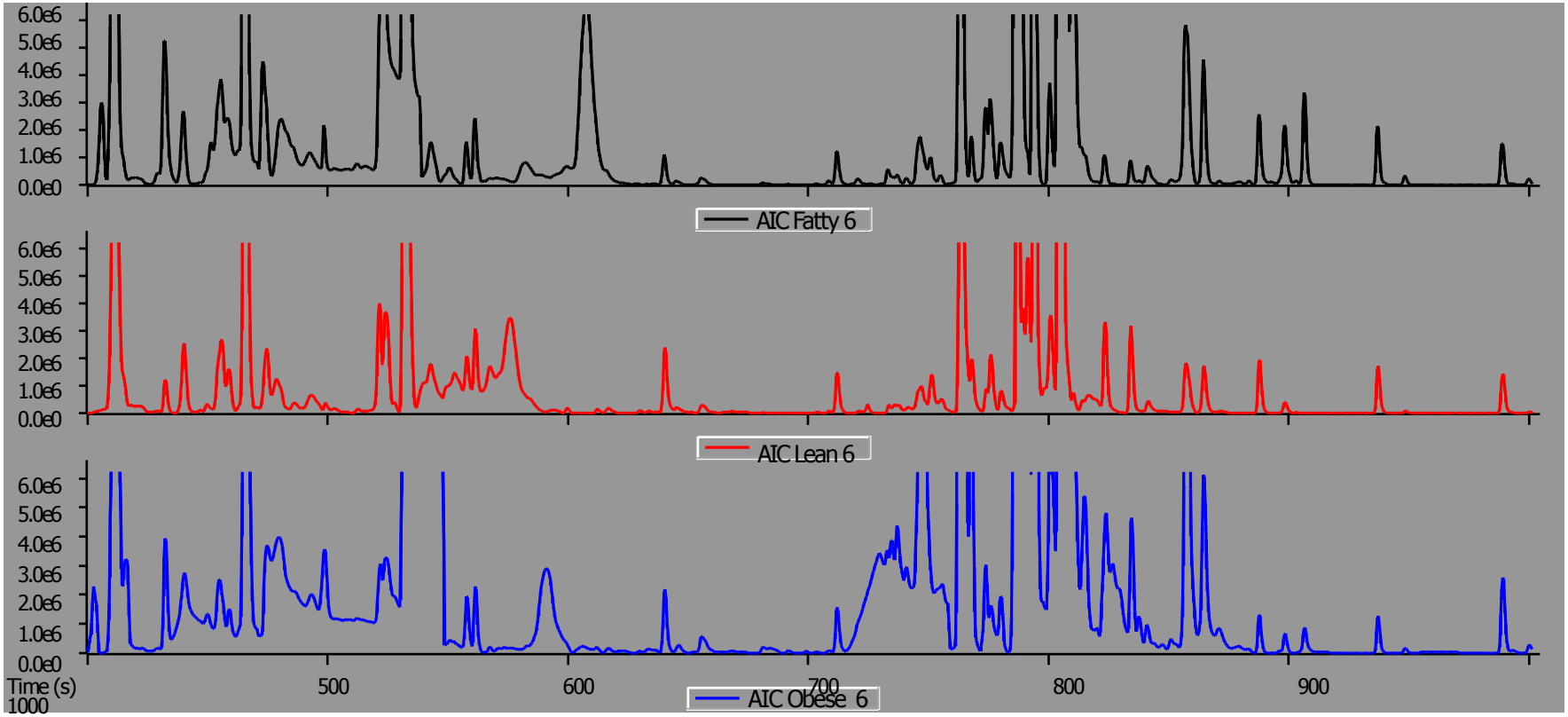


Zucker Rat Study: Instrument Parameters

GC	Agilent 7890 with 7693 Auto Sampler
Column	Restek Rxi-5Sil MS (30m x 0.25mm x 0.25mm) & 5m Guard
Carrier Gas, Flow	He, 1.0 mL/min Constant Flow
Injection/Volume	Splitless, 1 µL (CI 2 µL)
Temp. Program	70 °C (4 min) to 300 °C at 20 °C/min (6 min)

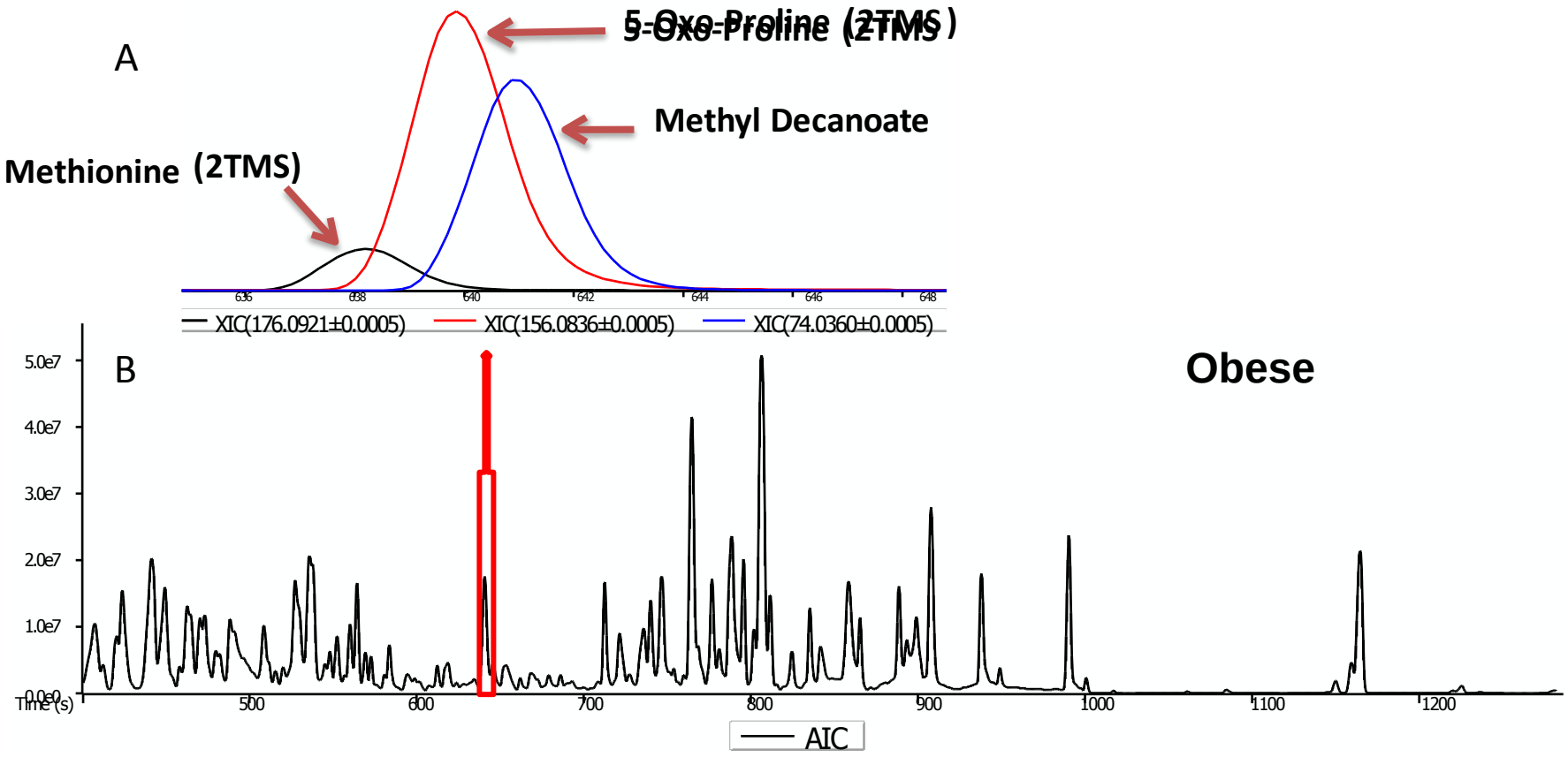
MS	LECO Pegasus® GC-HRT
Transfer Line Temp.	300 °C
Ion Source Temp.	250 °C (CI 200 °C)
Ionization	EI (70 eV); CI (140 eV)
Mass Range	60 – 520 (CI 100 – 1000, Reagent Gas = 5% NH_3 in CH_4)
Acquisition Rate	6 sps
Mass Calibration	PFTBA (Internal)

General Findings

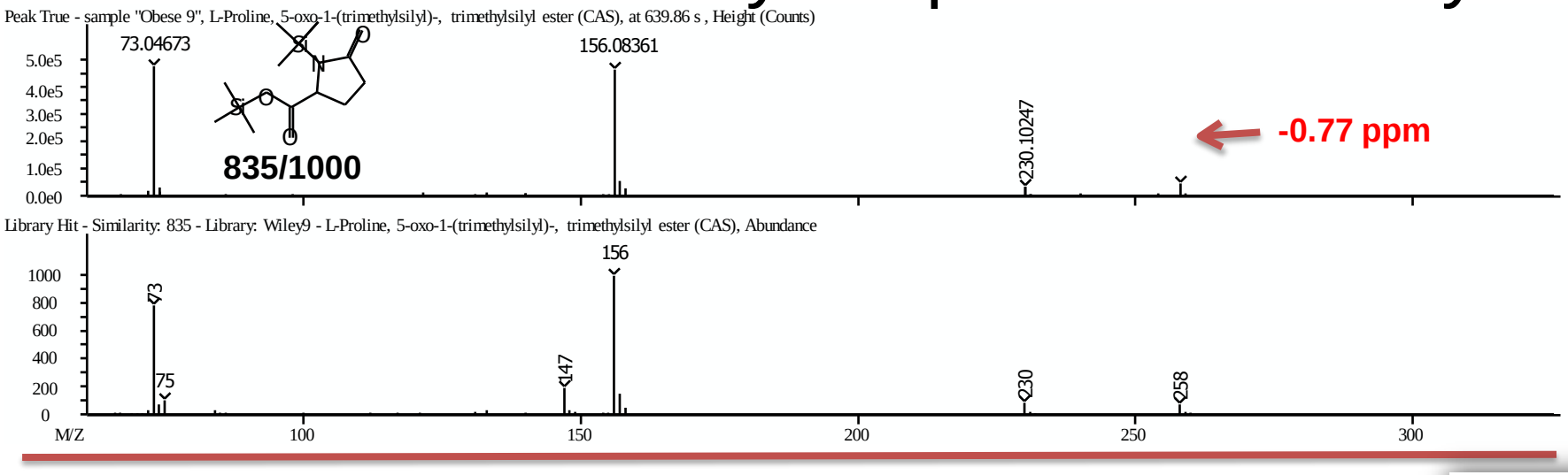


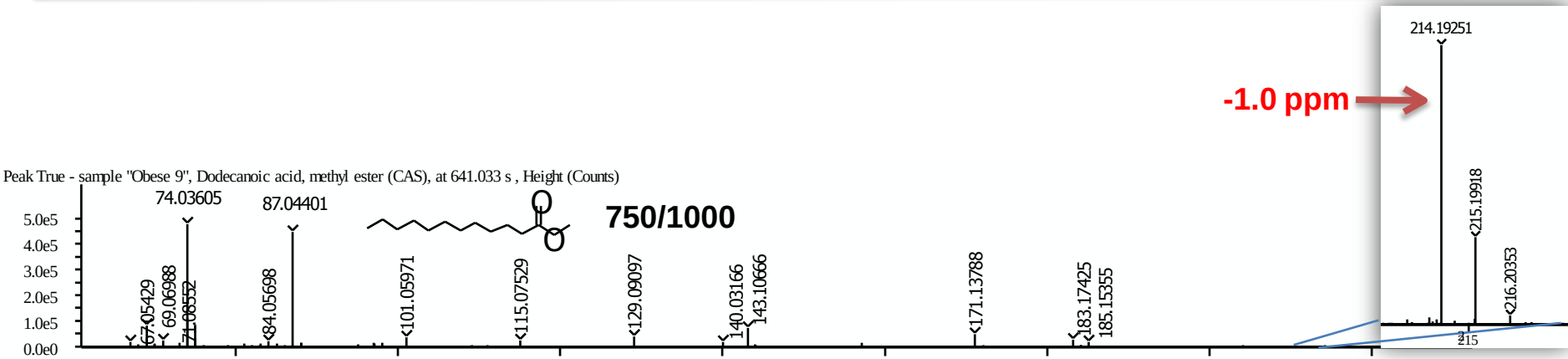
- Total Average Features Found (S/N > 100) – 662 (+/- 57)
- Analytes having ID Match > 800 – 274 (+/- 34)
- Analytes at > 600 and M , 2ppm – 266 (+/- 26) (N = 36)

ChromaTOF HRT: Deconvolution



EI-HRT: Accuracy & Spectral Similarity





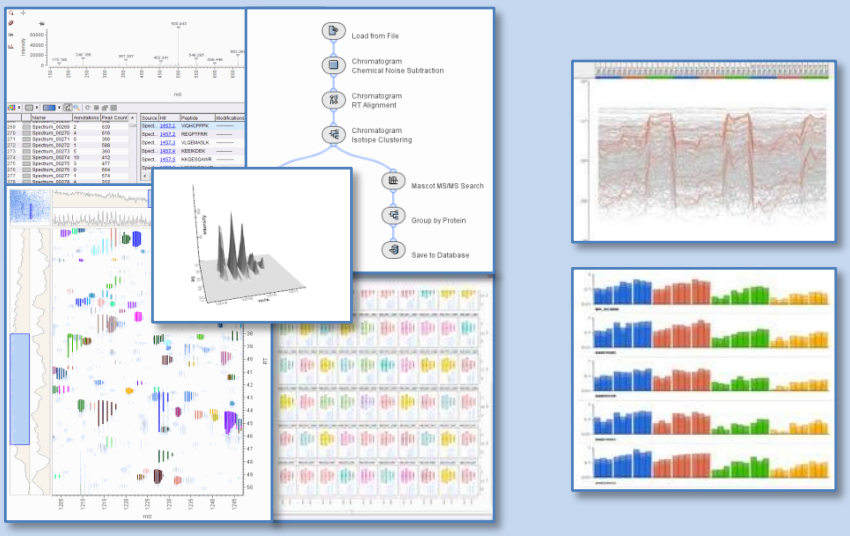
ChromaTOF HRT: Representative Compounds (0.9 ppm mass error)

Name	Formula	R.T. (s)	Area	LM (1000)	Ion	Observed Ion m/z	Mass Accuracy (ppm)
Alanine (3TMS)	$C_9H_{23}NO_2Si_2$	448.6	127332975	829	$[M\text{-}CH_3]^+$	218.10235	-1.62
Oxalic Acid (2TMS)	$C_8H_{18}O_4Si_2$	464.5	27487671	889	$[M\text{-}CH_3]^+$	219.05012	-1.00
Valine (2TMS)	$C_{11}H_{27}NO_2Si_2$	508.2	50953068	820	$[M\text{-}C_3H_7]^+$	218.10250	-0.95
Serine (3TMS)	$C_{12}H_{31}NO_3Si_3$	572.5	16161374	811	$[M\text{-}CH_3]^+$	306.13720	0.16
Threonine (3TMS)	$C_{13}H_{33}NO_3Si_3$	583.6	17103360	807	$[M\text{-}CH_3]^+$	320.15264	-0.51
L-Proline, 5-oxo- (2TMS)	$C_{11}H_{23}NO_3Si_2$	639.9	47799304	835	$M^{+\bullet}$	273.12089	-0.77
					$[M\text{-}CH_3]^+$	258.09738	-0.94
Citric Acid (4TMS)	$C_{18}H_{40}O_7Si_4$	739.9	39155972	861	$[M\text{-}CH_3]^+$	465.16066	-0.92
Galactose (MEOX, 5TMS)	$C_{22}H_{55}NO_6Si_5$	795.467	38182656	792	$[M\text{-}C_{10}H_{27}O_2Si_3]^+$	364.1788657	-0.4088
Glucose (MEOX, 5TMS)	$C_{22}H_{55}NO_6Si_5$	801.498	12382092	821	$[M\text{-}C_{11}H_{31}O_3Si_3]^+$	332.1529305	0.3934
Inositol (6TMS)	$C_{24}H_{60}O_6Si5$	824.445	11117824	850	$[M\text{-}C_6H_{20}O_2Si_2]^+$	432.1992864	-1.2284
Octadecanoic acid (TMS)	$C_{21}H_{44}O_2Si$	865.1	18735936	895	$M^{+\bullet}$	356.30982	-1.94
Arachidonic acid (TMS)	$C_{23}H_{40}O_2Si$	899.0	16205056	889	$M^{+\bullet}$	376.27853	-1.80
Cholestadiene	$C_{27}H_{44}$	1061.05	384812	782	$M^{+\bullet}$	368.3441255	1.0118
Cholesterol TMS	$C_{30}H_{54}OSi$	1164.7	24326300	736	$M^{+\bullet}$	458.39349	-0.76
Campesterol, TMS	$C_{31}H_{56}OSi$	1224.6	1668712	888	$M^{+\bullet}$	472.40950	0.01
							Ave = 0.90 ppm

Genedata Expressionist MSX

Integrated MS data processing and analysis

MS Data → Integrated → Data Processing → Statistical Analysis

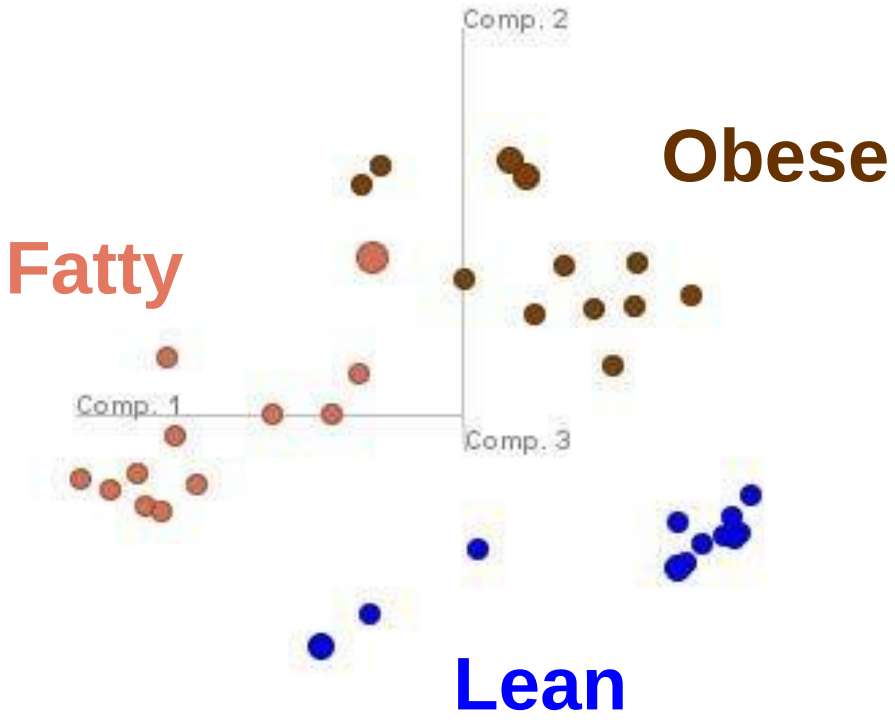


Software truly optimized to handle GC-HRMS data

Leco Delivering The Right Results

Genedata solutions in silico

GeneData Results



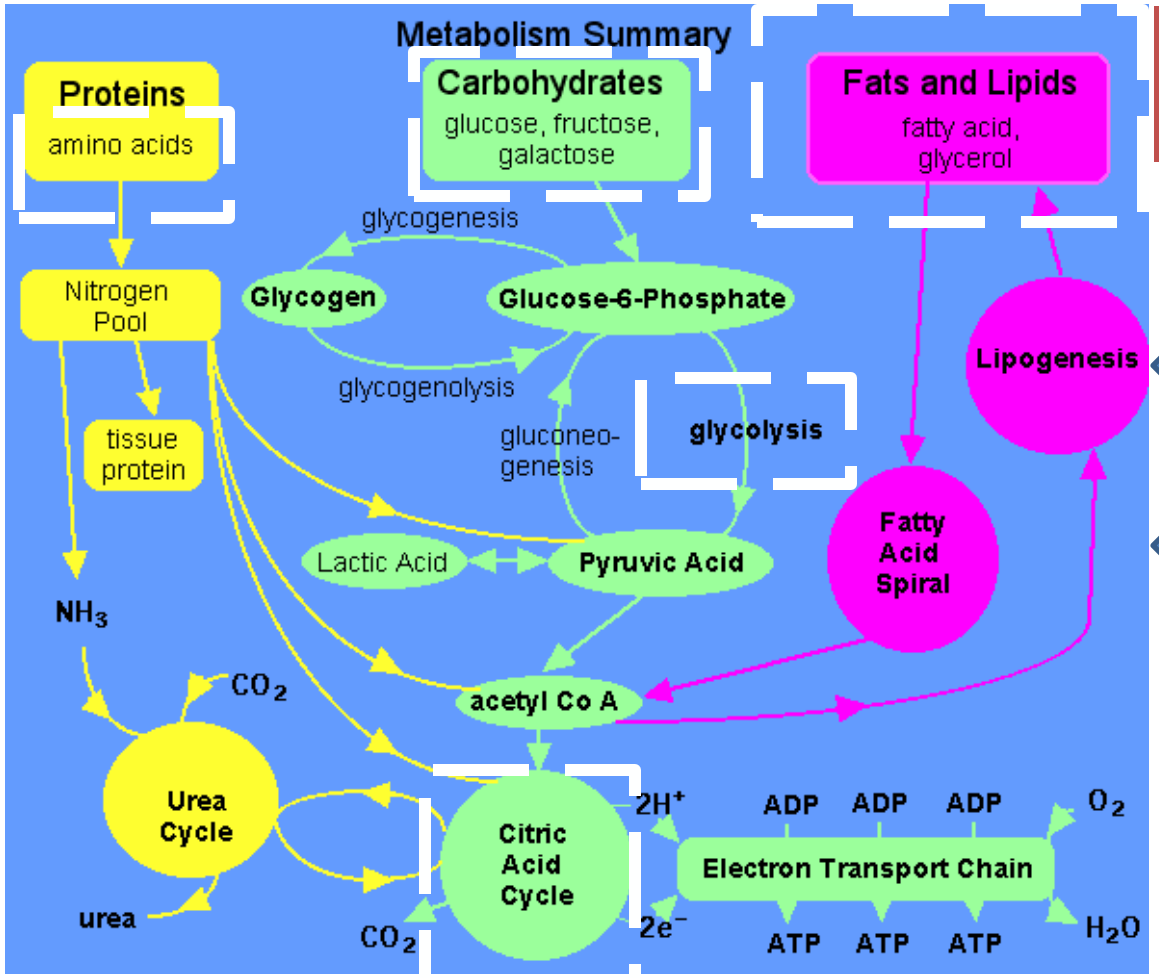
Fold Change > 1.5

P values < 0.05

Leco®
Delivering the Right Results

Genedata
solutions in silico

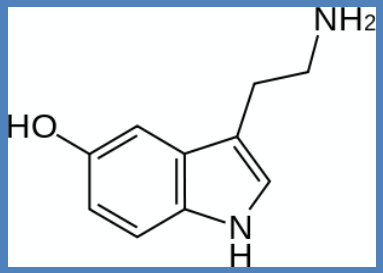
Pathways Implicated in GCMS



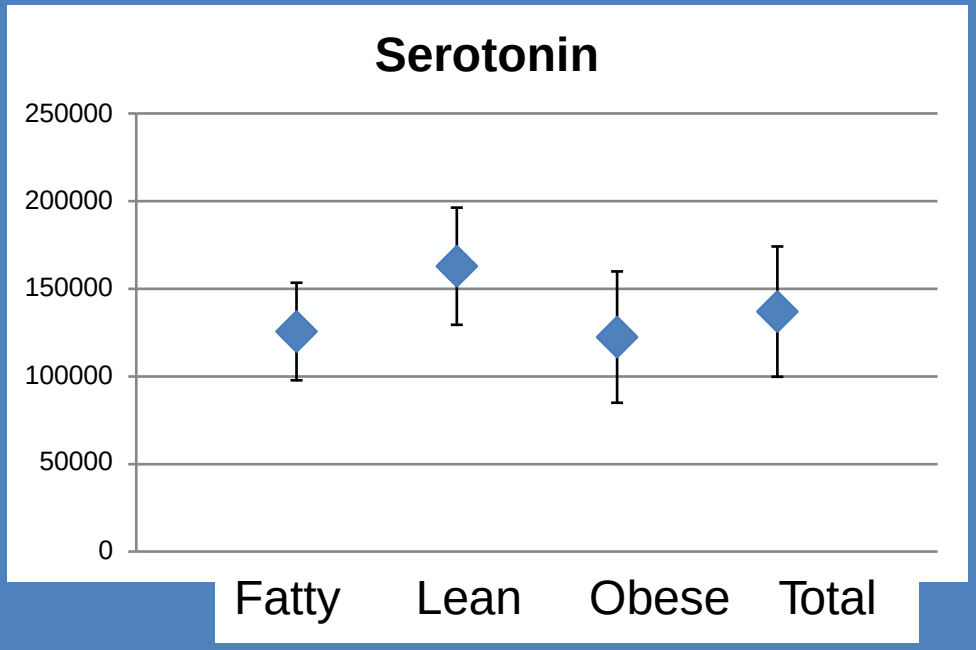
BCAAs which Feed fatty acids and lipids

Statistics and Pathways are important but individual metabolites are important as well

Serotonin – Obesity, Sleep and Appetite

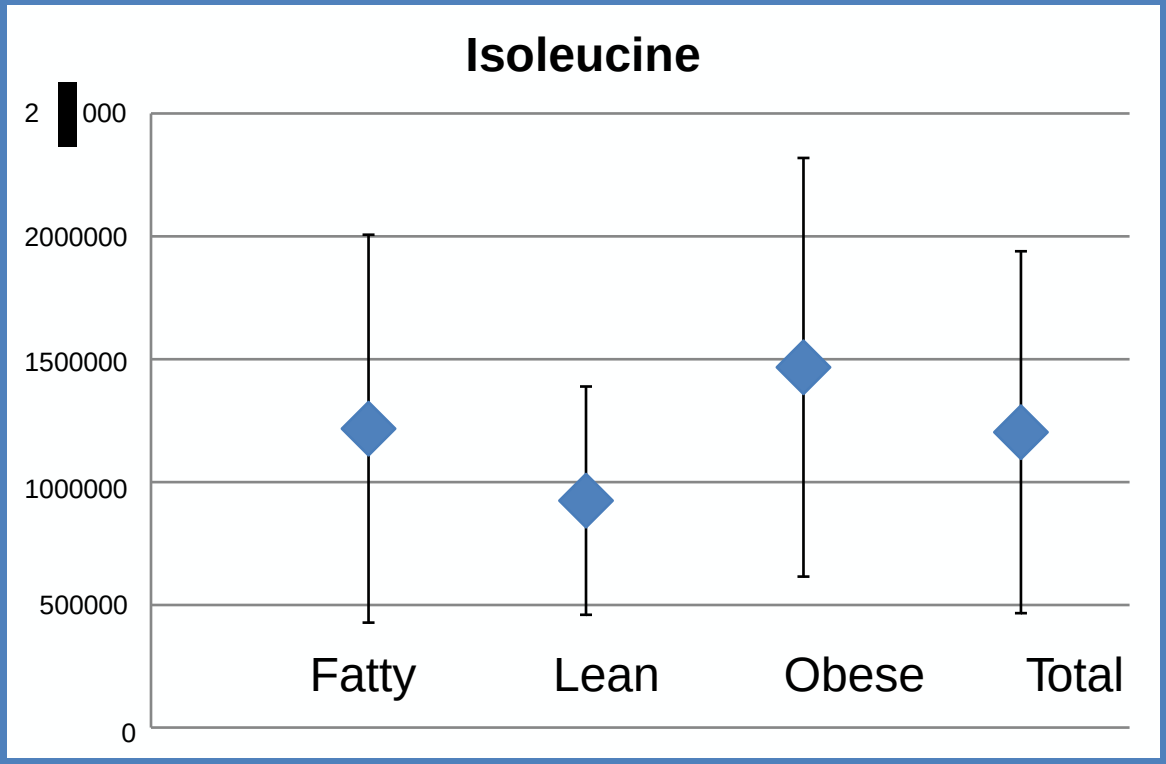


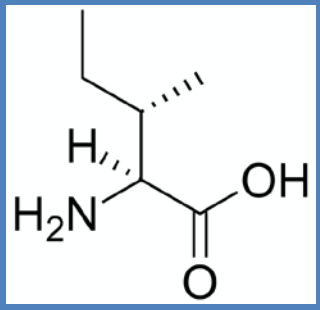
5-Hydroxytryptamine



Implicated Pathways and Physiology
Tryptophan Biosynthesis
Neurotransmission
Appetite control
Depression
Obesity

Branched Amino Acids





Figures of merit

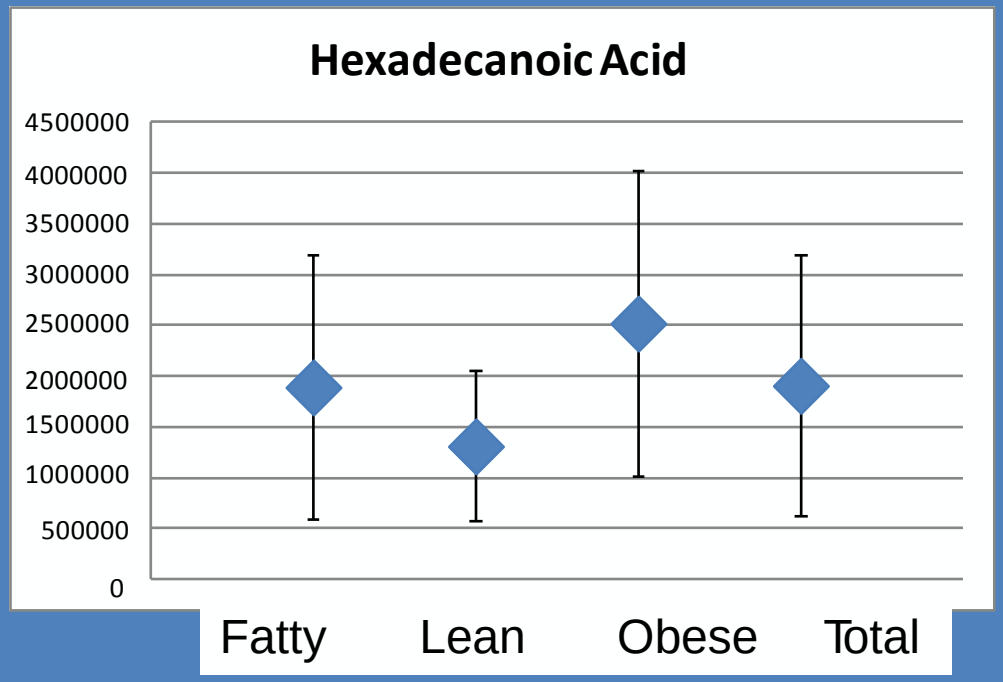
- Average m/z = 86.096368
- Target m/z 86.096430
- Avg. Mass Error = 0.72 ppm (Abs)

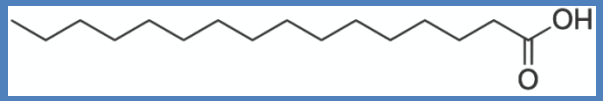
(N = 36 over 2 days)

Leucine and Valine show similar trends

Branched Chain AA**, Fatty Acid** and other Metabolism

Modulated Fatty Acid



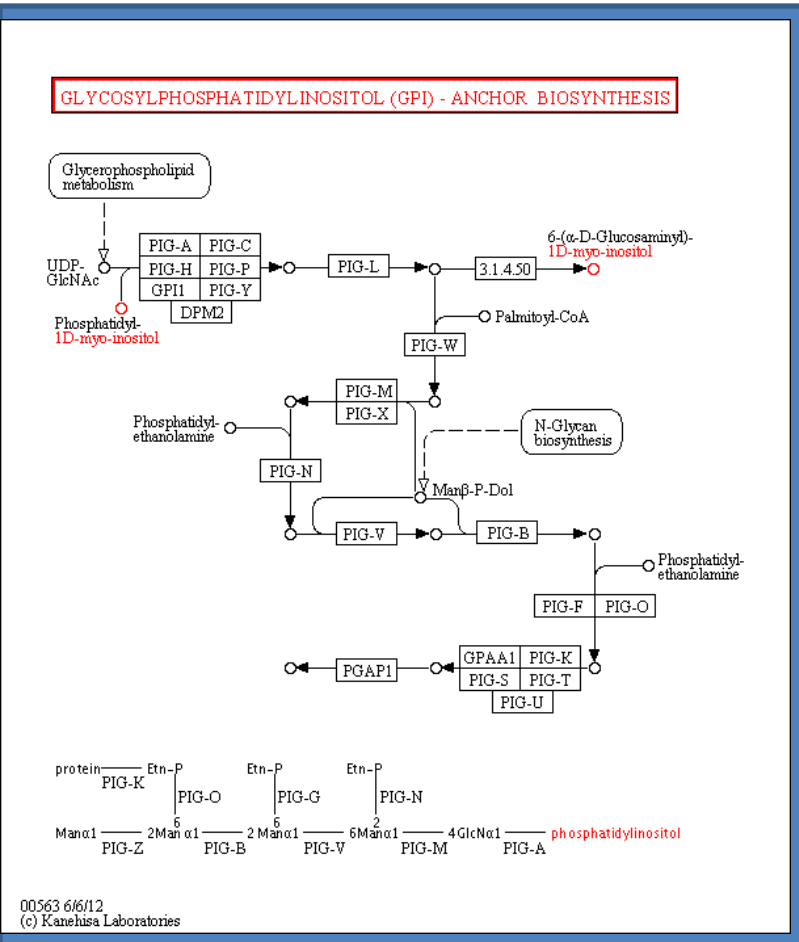


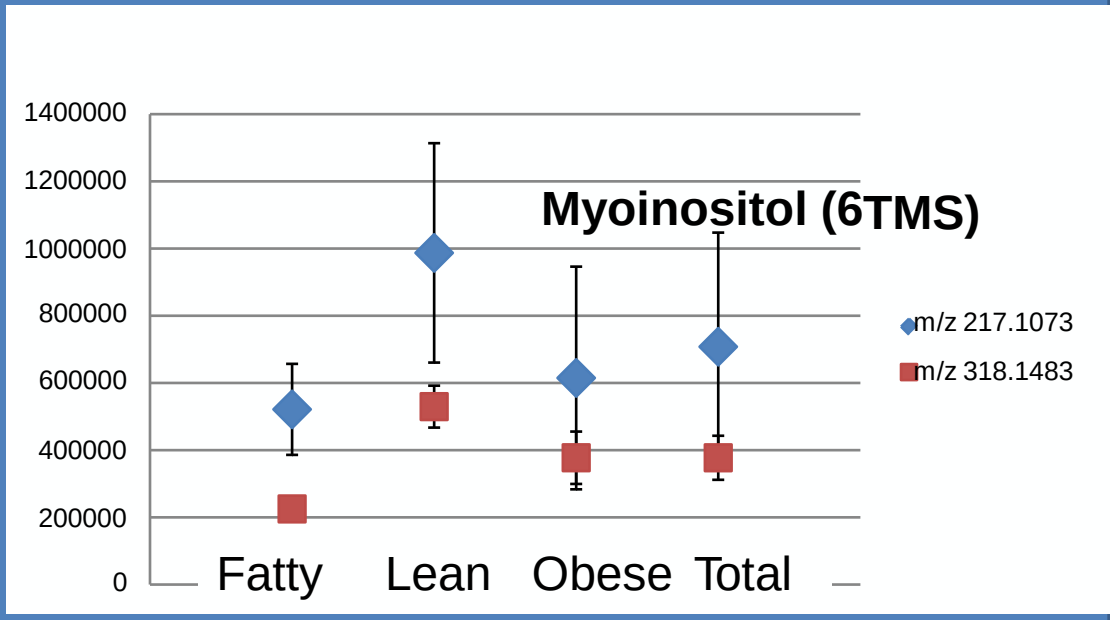
Figures of merit

- Average m/z = 328.278977
- Target m/z 328.27889
- Avg. Mass Error = 0.97 ppm (Abs)

(N = 36 over 2 days)

Other Modulated Metabolites



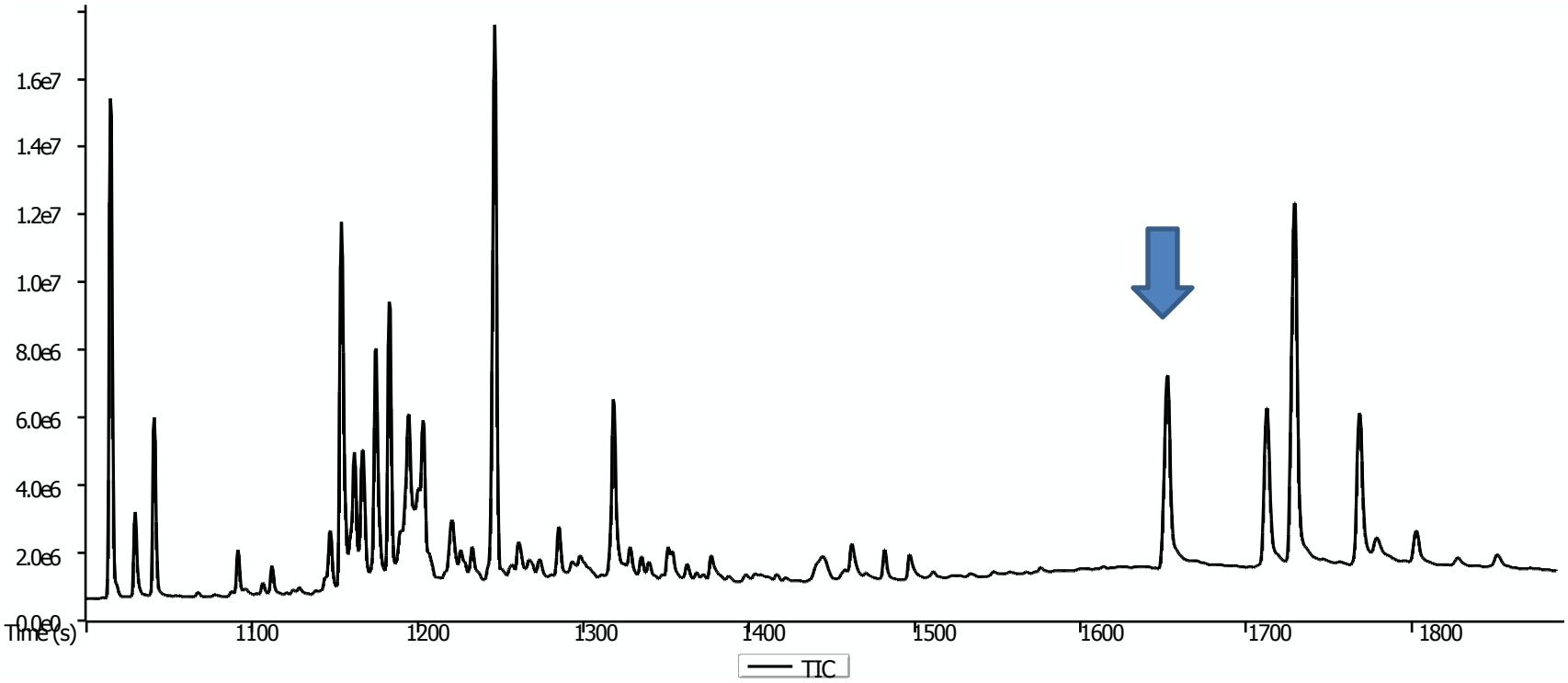


Linked to selected lipid metabolism

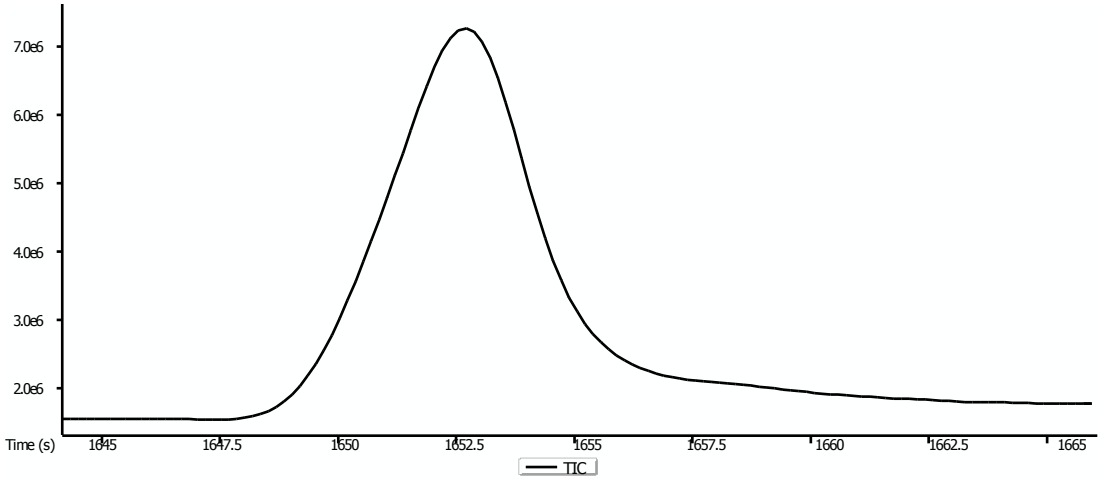
Monitored by each of 2 accurate m/z values one is more consistent/selective than the other

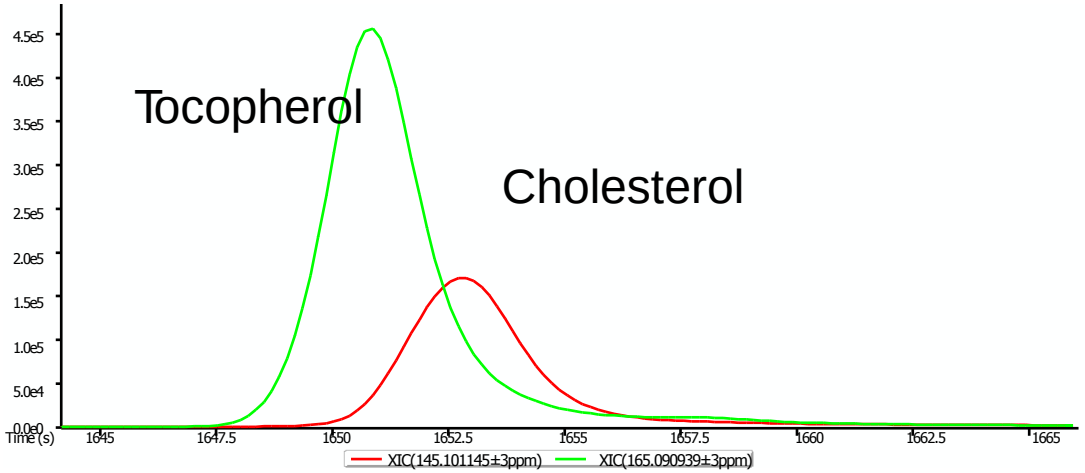
Metabolite ID in Tobacco Leaf

Cholesterol in Green Tobacco Leaf



Two components?

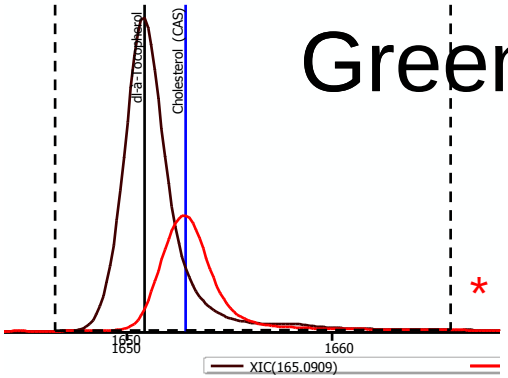


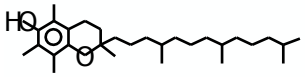


What else are you missing?

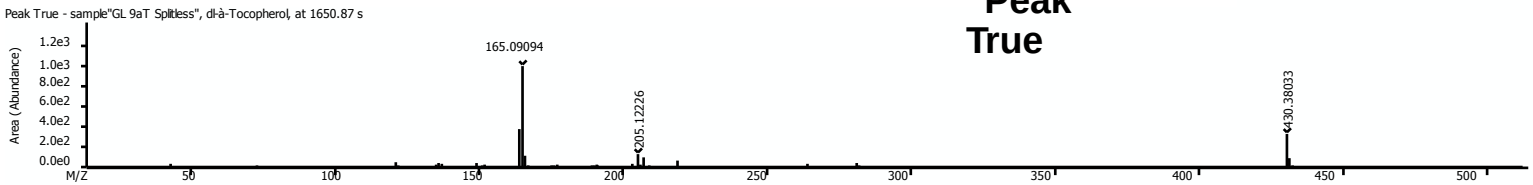
The same proven deconvolution applied high resolution data

Green Leaf : Co-eluting Compounds

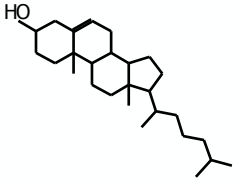




Peak True

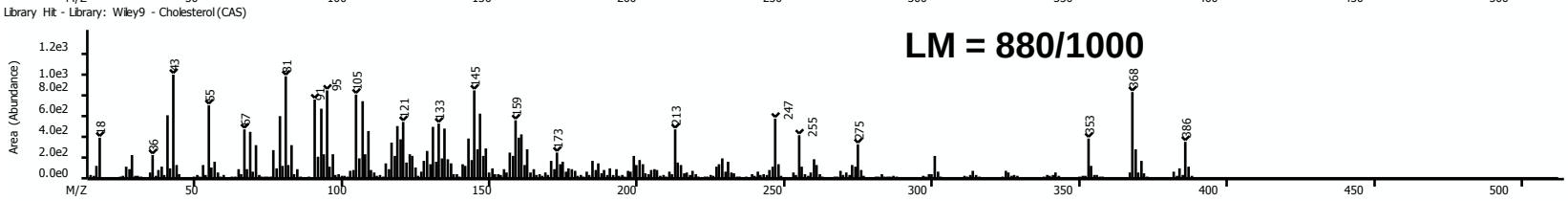


LM = 833/100

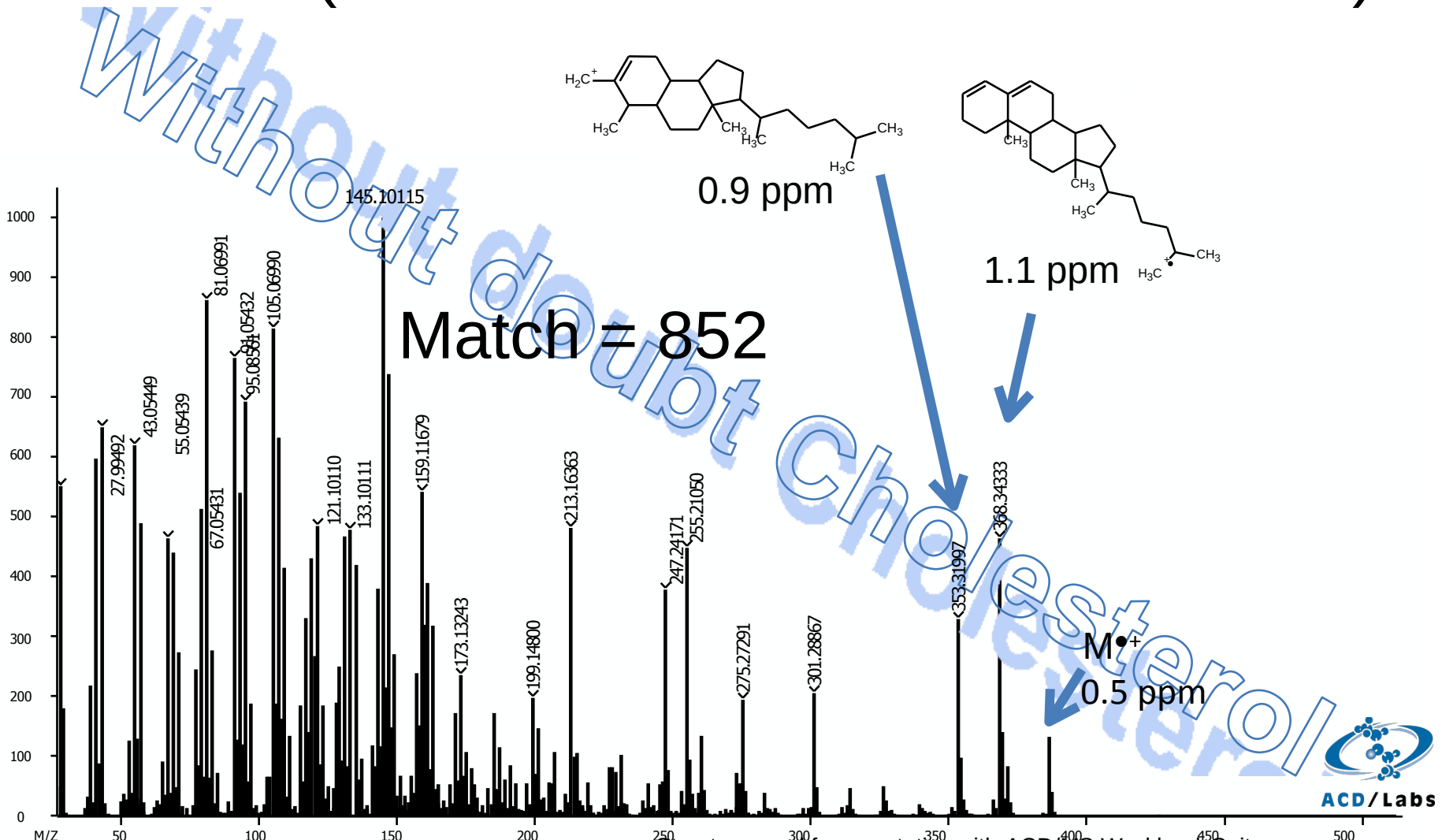


Peak True

LM = 880/1000



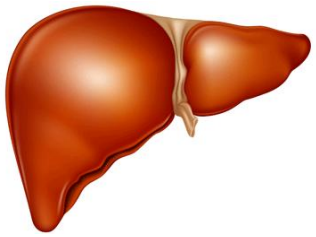
Interpretative Power of Accurate Mass – Cholesterol (in Tobacco Leaf Extract NOT TMS)



Structures and fragmentation with ACD/MS Workbook Suite

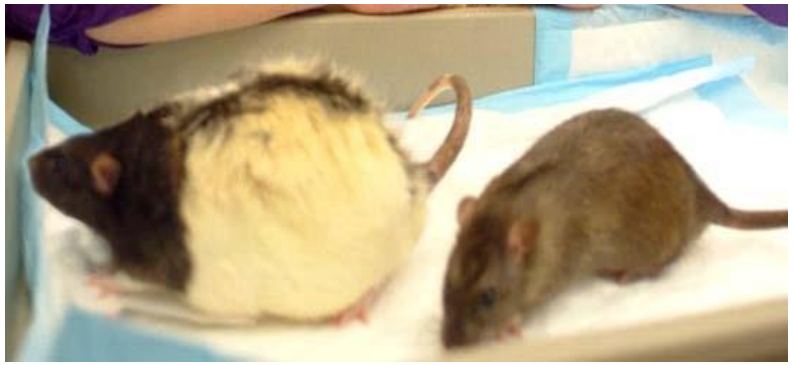
GCxGC TOF MS

Sample Preparation



Freeze; Pulverize;
Extract with MeOH

Centrifuge

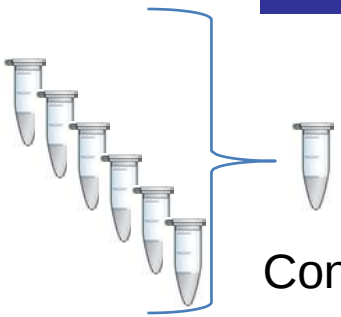


Pool by Condition

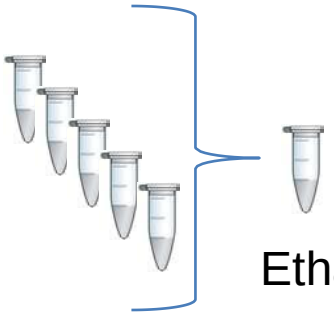
SpeedVac

BSTFA

GCxGC
TOF-MS

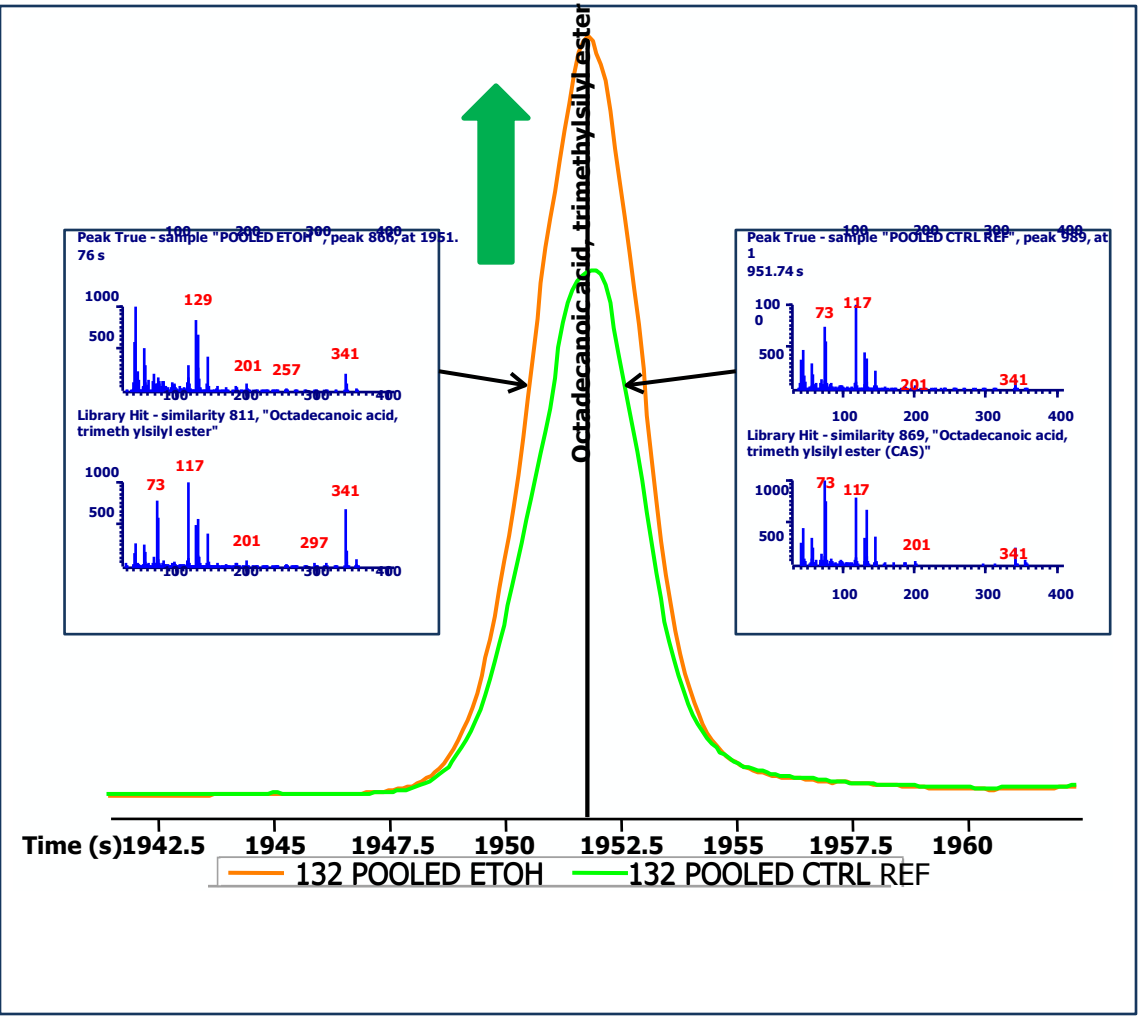


Control

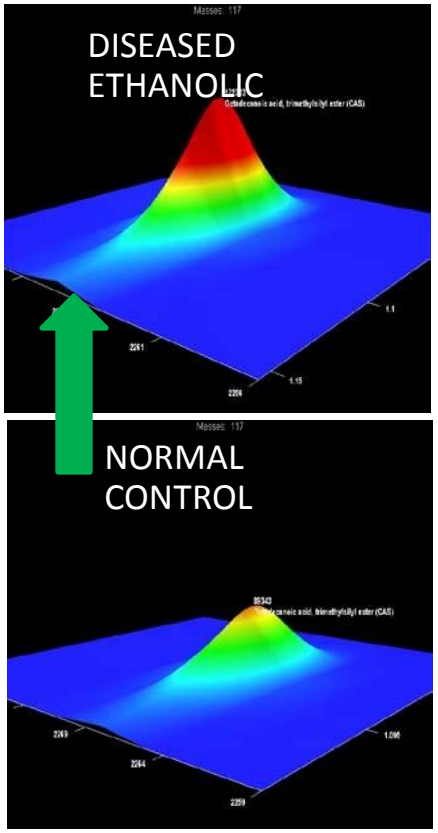


Ethanol

Robust Differential Analysis



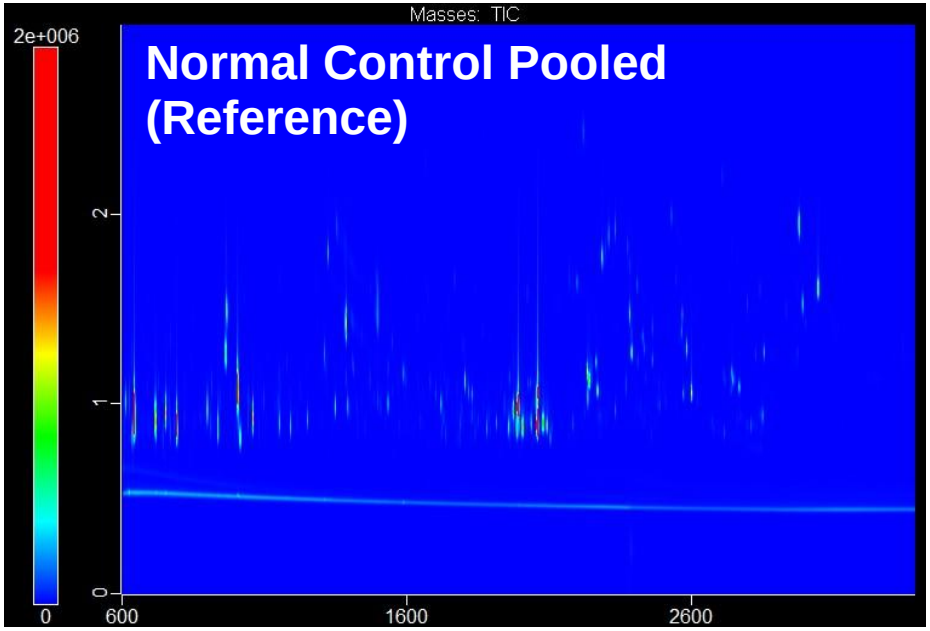
Octadecanoic Acid in Mouse Liver extracts – arrow indicates up regulation

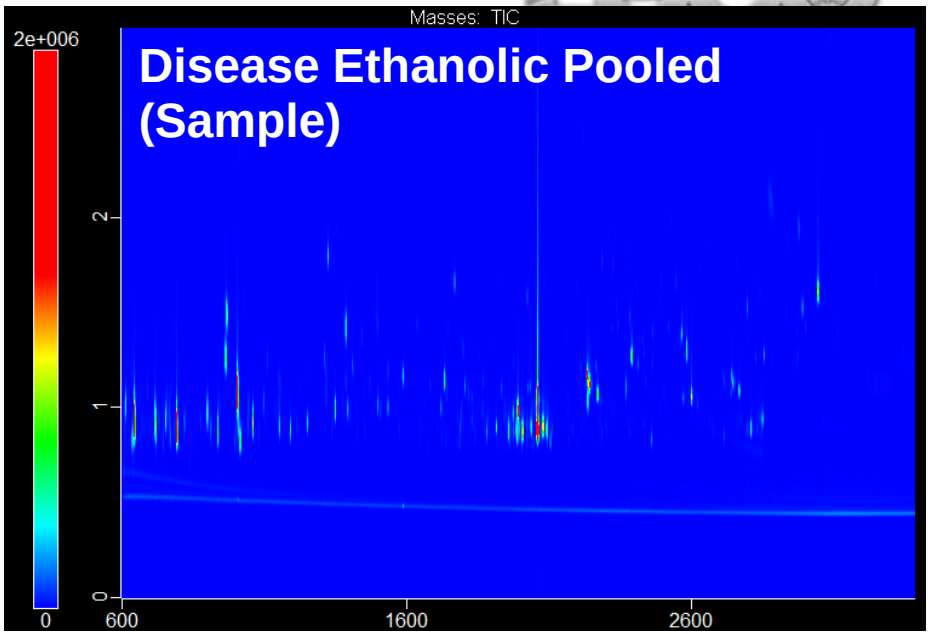


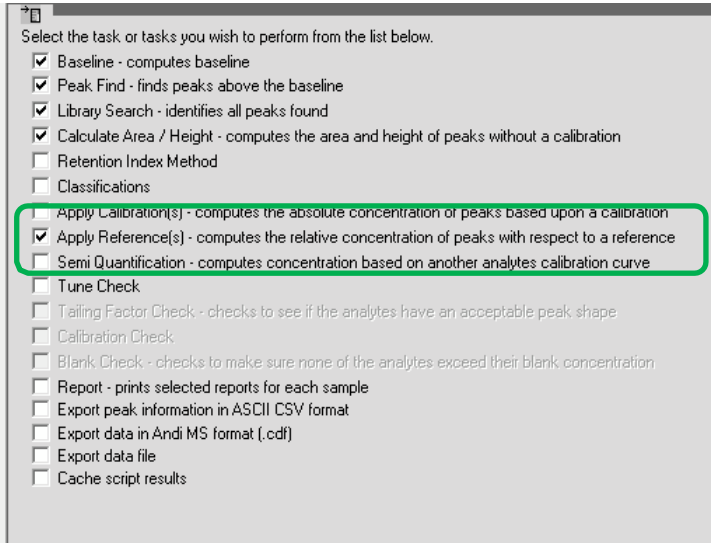
Research has demonstrated significantly higher fatty acid levels in alcoholic liver studies UP Regulation of Biological significance

Similarity > 800

How can we identify differences between similar samples?







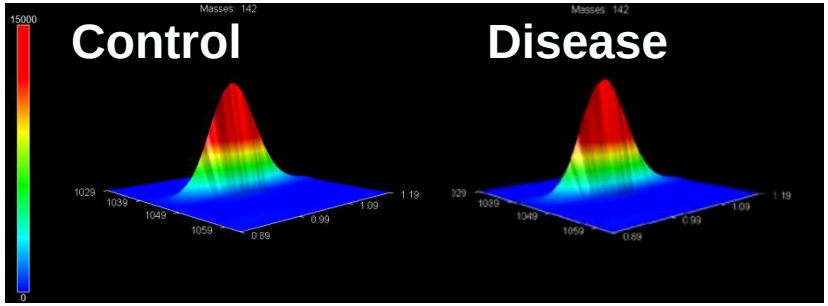
ChromaTOF's Reference Feature computes the relative concentration of peaks in a sample with respect to a user specified reference. Type tags are assigned to each analyte:

Match	Present in reference and sample within user-specified concentration tolerance
Out of Tolerance	Present in reference and sample, but not within user-specified concentration tolerance
Not found	Present in reference, but not in sample
Unknown	Present in sample, but not in reference

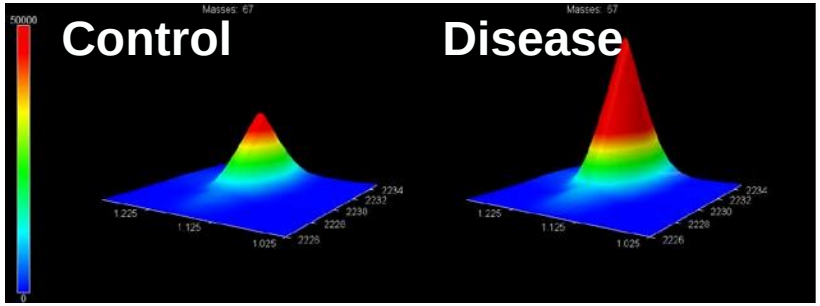
Representative Examples

Type	Concentration	Name	Similarity	R.T. (s)	Quant S/N	Area	Height	Quant Masses
Match	105.68	Proline, di-TMS	888	1044 , 1.025	5419.4	665035	28371	142
Out of Tolerance	199	Linoleic Acid, TMS	901	2232 , 1.175	12237	1858318	141038	67
Not Found		Ala-Gly, N-TMS-, TMS ester	850	1548 , 1.325				116
Unknown		Tryptophan, tri-TMS	844	2229 , 1.360	664.14	48291	3073.3	202

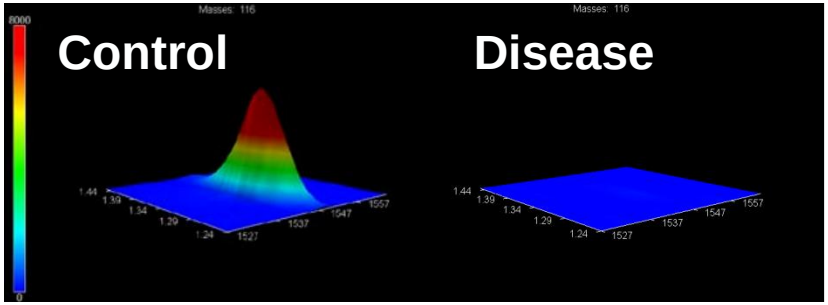
Match: Proline, di-TMS



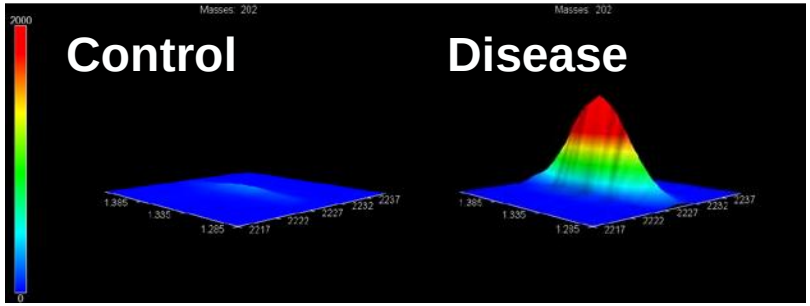
Out of Tolerance: Linoleic Acid, TMS



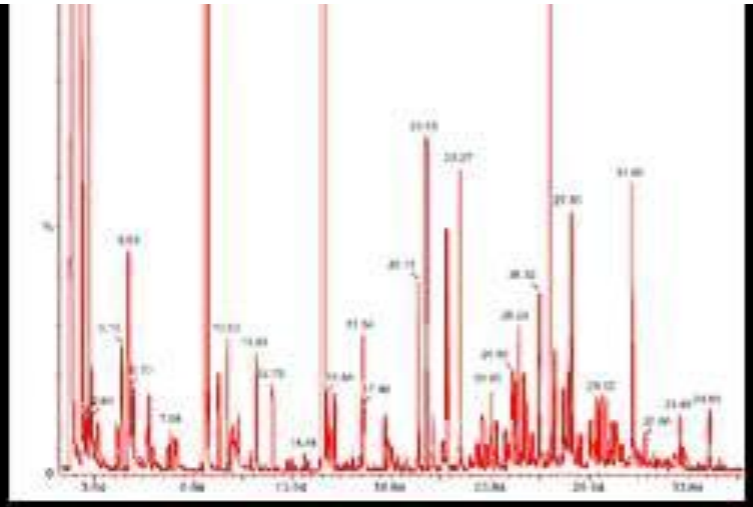
Not Found: Ala-Gly, N-TMS-, TMS ester



Unknown: Tryptophan, tri-TMS



Breath Analysis



MENSSANA RESEARCH, INC.

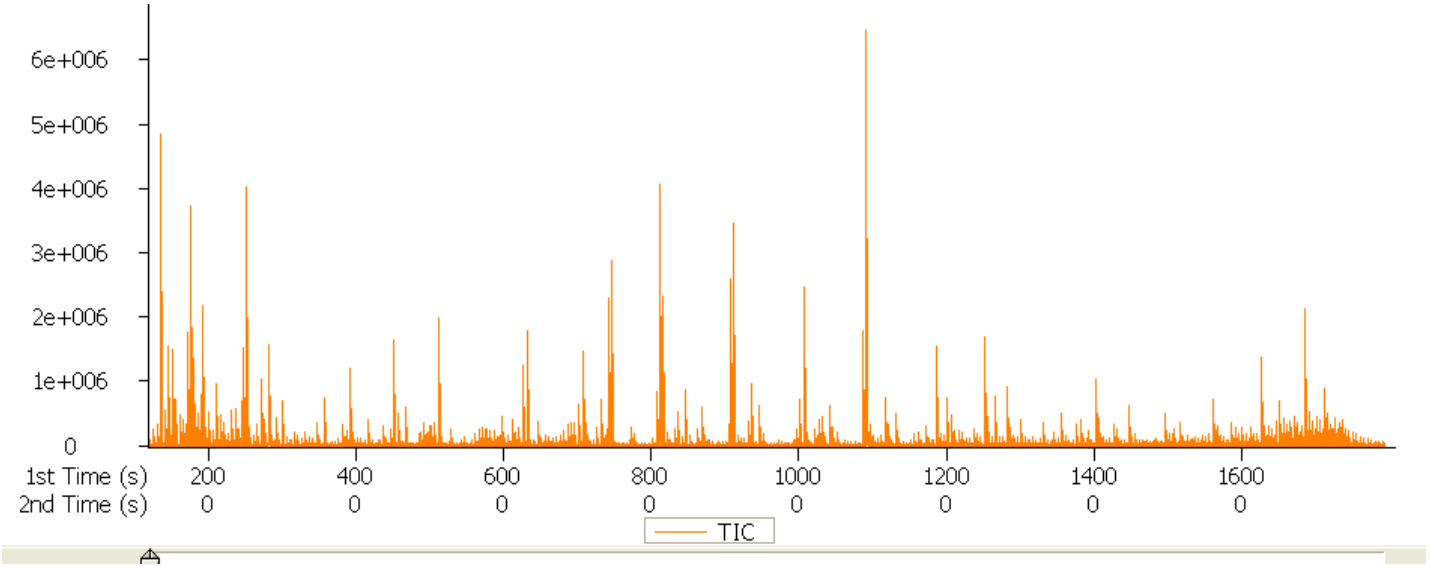
Breath tests using 1D GC MS

Demonstrated proof of principle in:

- *Lung cancer*
- *Breast cancer*
- *Heart transplant rejection*
- *Pulmonary tuberculosis*
- *Environmental toxicology*
- *Influenza*

MENSSANA RESEARCH, INC.

All of these studies were performed with 1D GC MS…



…and then 2D GC MS came along

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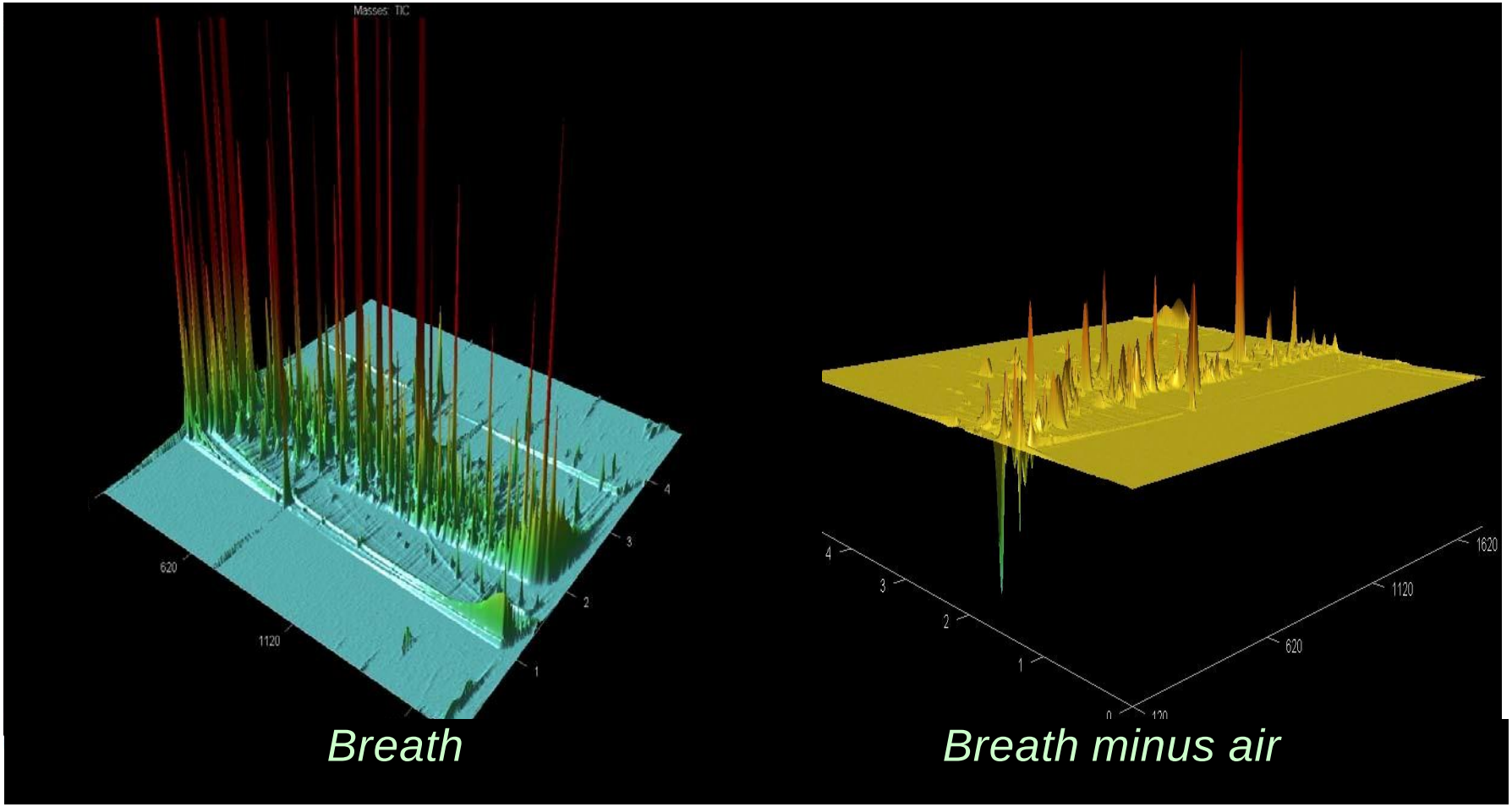
Human breath VOCs

Two-dimensional gas chromatography and time-of-flight mass spectrometry



Breath

Breath minus air

MENSSANA RESEARCH, INC.

The limitations of 1D GC MS	*The advantages of GCxGC TOF MS*
Poor selectivity ~ 200 VOCs in breath	*Excellent selectivity ~ 2,000 VOCs in breath*
Co-elution +++	*Co-elution – minimal*
Biomarker ID not consistent	*Biomarker ID highly consistent*

MENSSANA RESEARCH, INC.

Conclusions

- Metabolomics Studies using **GCMS** provide capabilities to define modulated analytes in populations and phenotypes which complements LCMS
- **HRMS** provides an ability to identify unknowns and to have confident identification of knowns
  - **Accurate m/z** for fragments
  - **Isotopic Abundance** for knowns and unknown
  - Mass accuracy and isotopic Abundance confirm formulae for m/z
  - **CI** enables molecular ion m/z
  - Provides linearity and sensitivity needed for metabolomics analysis
- **Deconvolution** enables the ability to:
  - detect and quantify metabolites
  - provide searchable spectra from difficult peak pairs
  - provide interpretable spectra from difficult peak pairs
- **GCxGC TOF MS –**
  - Separation of additional analytes
  - Differential Analysis and enhanced Sensitivity
- **Genedata** enables an HRMS-optimized tool for differential analysis of phenotypes and populations.

People Doing the Work

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