Myth Busters: The Truth About Metabolomics and High Resolution Gas Chromatography Time-of-Flight Mass Spectrometry

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Introduction

Background

- The best approach for comprehensive profiling of metabolites is through a combination of instrumental techniques (e.g., GC-MS, LC-MS, FT-NMR, etc.).
- GC-MS offers analytical advantages that complement other analytical techniques.
- The intent of this poster is to dispel the myths about the shortcomings of GC-MS as a technique for metabolomic studies.

The Myths

1) GC-MS is only applicable for the analysis of non-polar compounds.
2) Derivatization is required, is a waste of time, and provides inconclusive results.
3) Only compounds with m/z values <400 can be analyzed by GC-MS.
4) GC-EI MS fragmentation is problematic and hinders identification of metabolites.
5) MS/MS experiments are necessary for confident characterization of metabolites (See 4 above).
6) High resolution mass spectrometry is not required for metabolomic studies.

Metabolomics: General Analytical Challenges

- Chemical Diversity of Compounds
- Complexity of Biological Matrices
- Confident Compound Identification and Quantification (bottleneck)

The Solution: High Resolution GC-TOFMS

- High Quality Spectral Data
  - Comprehensive
  - Search Against Well-Established Databases (NIST, Wiley)
- Excellent Mass Accuracy Values (<1 ppm) = Robust Formulas for Fragment, Molecular, and Adduct Ions
- High Resolution Deconvolution™ (HRD™)
- High Resolving Power (up to 50,000) = Increased Selectivity for:
  - Discovery
  - Confirmation
  - Comprehensive Profiling of Complex Samples

Experimental

GC-MS Analysis Capabilities

Alcohols	Ionic Compounds	Organophosphates
Alkaloids	Lipids	Phenolics
Amino Acids	Nucleosides	Polar Organics
Carotenoids	Nucleotides	Prostaglandins
Catecholamines	Organic Acids/Diacids	Sterols
Eicosanoids	Organic Amines	Terpenes
Essential Oils	Organic Bases	Waxes

General Sample Preparation Methods

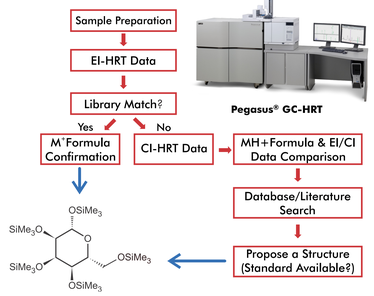
Samples (100 µL) were dried for 2 hours using a Speed Vac and then for 4 hours in a lyophilizer (3 mbar, -50°C). The samples were derivatized with MEOX (25 µL, 60°C, 1 hr) and MSTFA (75 µL, 60°C, 1hr) and transferred to autosampler vials for analysis.

Instrument Parameters

GC	Agilent 7890 with Gerstel MPS Auto Sampler
Column	Restek *Rxi*-5 MS (30 m x 0.25 mm x 0.25 µm)
Carrier Gas, Flow	He, 1.0 mL/min Constant Flow
Injection Volume & Split	1 µL, Split 10:1 (CI, Splitless)
Inlet Temperature	270°C
Temp. Program	70°C (2 min) to 280°C at 20°C/min (3 min)

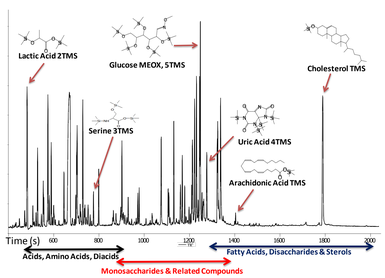
MS	LECO Pegasus® GC-HRT
Transfer Line Temp.	300°C
Ion Source Temp.	EI 250°C; CI 200°C
Ionization	EI (70 eV); CI (140 eV)
Mass Range	EI 35–510; CI 60–1000
Acquisition Rate	12 spectra/second
Calibration (Internal)	PFTBA
Reagent Gas	5% Ammonia in Methane

Workflow



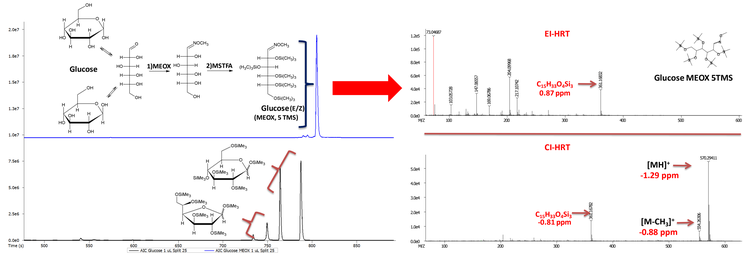
Debunking The Myths (1–3)

1) Analysis of a wide variety of metabolites (Human Plasma).

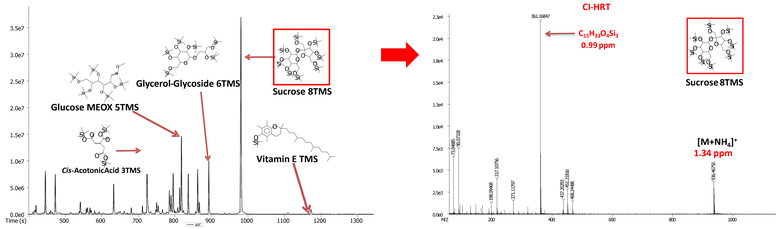


Metabolite	Formula	R.T. (s)	Similarity	Ion	Observed m/z	Expected m/z	Mass Accuracy (ppm)
Lactic Acid 2TMS	$C_9H_{22}O_3Si_2$	484	835	$[M-CH_3O_2]^+$	117.07302	117.07318	1.37
Alanine 2TMS	$C_9H_{23}NO_2Si_2$	529	836	$[M-CH_3O_2]^+$	116.0891	116.08908	0.70
Pyruvic Acid MEOX 2TMS	$C_9H_{21}NO_3Si_2$	581	915	M^+	247.10526	247.10545	-0.75
Valine 2TMS	$C_{11}H_{25}NO_2Si_2$	645	841	$[M-CH_3O_2]^+$	144.10338	144.10358	0.53
Leucine 2TMS	$C_{12}H_{27}NO_2Si_2$	697	890	$[M-CH_3O_2]^+$	158.13935	158.13901	0.36
Phosphate 3TMS	$C_9H_{27}O_4PSi_3$	703	909	M^+	314.09492	314.09493	-0.02
Isoleucine 2TMS	$C_{12}H_{27}NO_2Si_2$	717	871	$[M-CH_3O_2]^+$	158.13935	158.13909	0.90
Proline 2TMS	$C_{11}H_{23}NO_2Si_2$	719	895	$[M-CH_3O_2]^+$	142.10445	142.10468	0.17
Glycine 3TMS	$C_{11}H_{29}NO_2Si_3$	728	929	$[M-CH_3O_2Si]^+$	174.11288	174.1131	1.26
Glycerol-Acid 2TMS	$C_{12}H_{29}O_3Si_2$	751	825	$[M-CH_3O_2Si]^+$	189.07616	189.07614	-0.09
Serine 3TMS	$C_{12}H_{29}NO_3Si_3$	776	931	$[M-CH_3O_2]^+$	204.12344	204.1235	0.26
Threonine 3TMS	$C_{13}H_{31}NO_3Si_3$	799	932	$[M-CH_3O_2Si]^+$	218.10271	218.10269	-0.06
5-oxo-Proline 2TMS	$C_{11}H_{23}NO_3Si_2$	901	907	M^+	273.12073	273.1211	-1.35
Glutamic Acid 3TMS	$C_{14}H_{31}NO_4Si_3$	971	907	M^+	363.17109	363.17119	-0.28
Phenylalanine 2TMS	$C_{15}H_{25}NO_2Si_2$	977	919	$[M-CH_3]^+$	218.10271	218.10274	0.15
Asparagine 3TMS	$C_{15}H_{34}N_2O_3Si_3$	1008	825	M^+	348.17193	348.17152	1.16
Glutamine 4TMS	$C_{18}H_{42}N_2O_4Si_4$	1052	768	$[M-CH_3O_2Si]^+$	317.18952	317.18906	-1.45
Glutamine 3TMS	$C_{17}H_{40}N_2O_4Si_3$	1075	889	M^+	362.1873	362.18717	0.35
Ornithine 4TMS	$C_{17}H_{42}N_2O_3Si_4$	1105	904	M^+	420.24755	420.24744	0.26
Citric Acid 4TMS	$C_{18}H_{36}O_7Si_4$	1109	877	$[M-CH_3O_2Si]^+$	273.09729	273.0974	0.40
Caffeine	$C_8H_{10}N_4O_2$	1122	568	M^+	194.07969	194.07983	-0.69
1,5-Anhydro-D-sorbitol 4TMS	$C_{21}H_{48}O_5Si_4$	1130	933	M^+	452.25579	452.25603	-0.54
Lysine 4TMS	$C_{18}H_{42}N_2O_4Si_4$	1168	799	M^+	434.26306	434.26309	-0.06
Tyrosine 3TMS	$C_{21}H_{37}NO_3Si_3$	1178	935	$[M-CH_3O_2Si]^+$	218.10271	218.10292	-0.95
Glucose MEOX 5TMS	$C_{21}H_{49}NO_6Si_5$	1247	841	$[M-CH_3NO_2Si]^+$	361.16852	361.16857	1.26
Uric Acid 4TMS	$C_{23}H_{48}N_4O_6Si_4$	1276	818	M^+	456.18649	456.1859	1.29
SC-122-Octadecadienoic Acid TMS	$C_{21}H_{42}O_2Si$	1322	775	M^+	352.27892	352.27921	-0.83
Oleic acid TMS	$C_{21}H_{42}O_2Si$	1324	806	M^+	354.2944	354.29486	-1.28
Octadecanoic acid TMS	$C_{21}H_{44}O_2Si$	1337	875	M^+	356.31039	356.31051	-0.90
Tryptophan 3TMS	$C_{20}H_{36}N_2O_2Si_3$	1338	852	$[M-CH_3NO_2Si]^+$	202.10465	202.10471	0.29
Arachidonic acid TMS	$C_{23}H_{42}O_2Si$	1403	888	$[M-C_4H_9]^+$	117.03661	117.03661	-0.19
Vitamin E TMS	$C_{32}H_{58}O_3Si$	1772	856	M^+	502.42006	502.42006	0.00
Cholesterol TMS	$C_{30}H_{54}OSi$	1787	740	M^+	458.39421	458.39384	0.79

2) Derivatization is well-understood, routine, and can increase sensitivity while decreasing the complexity of samples (Human Serum).

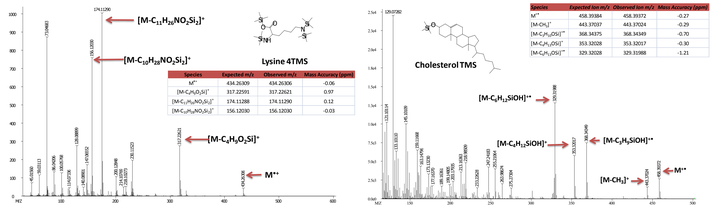


3) A workflow that includes CI-HRT data acquisition can provide valuable information for compounds with molecular weights >900 Da (Corn Extract).



Debunking The Myths (4-6)

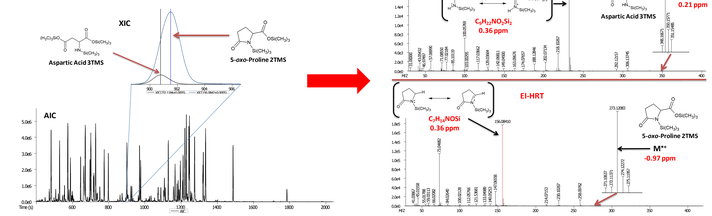
4) Fragmentation = structural characterization (Human Plasma).



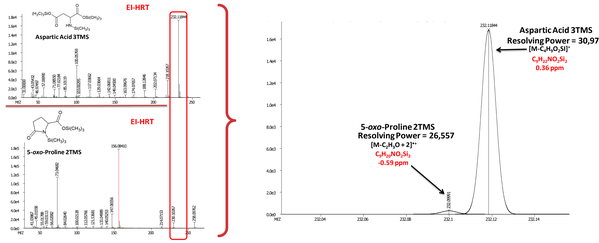
Species	Expected m/z	Observed m/z	Mass Accuracy (ppm)
M^+	434.26309	434.26306	-0.06
$[M-C_4H_9OSi]^+$	317.22591	317.22621	0.97
$[M-C_{10}H_{26}NO_2Si_2]^+$	174.11288	174.11290	0.12
$[M-C_{11}H_{26}NO_2Si_2]^+$	156.12030	156.12030	-0.03

Species	Expected m/z	Observed m/z	Mass Accuracy (ppm)
M^+	458.39384	458.39372	-0.27
$[M-CH_3]^+$	443.37037	443.37024	-0.29
$[M-C_3H_7OSi]^+$	368.34375	368.34349	-0.70
$[M-C_4H_9OSi]^+$	353.32028	353.32017	-0.30
$[M-C_7H_{15}OSi]^+$	329.32028	329.31988	-1.21

5) Accurate mass ions provide confident structural characterization of metabolites (Human Serum). This enhanced selectivity, combined with HRD, facilitates the analysis of analytes in complex biological matrices.



6) High resolution minimizes isobaric interferences (Human Serum).



Summary

- The data summarized in this presentation dispels myths associated with the utilization of GC-MS for analytes which are critical for metabolomic studies.
- GC-HRT provides high quality, accurate mass data for:
  - Spectral similarity searches (NIST, Wiley, etc.)
  - Formula determination (fragment, molecular & adduct ions)
- GC-HRT is an indispensible tool for the analysis of complex biological samples.