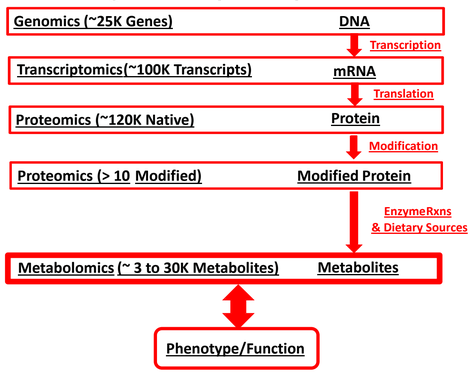
Strategic Utilization of Gas Chromatography with Both Nominal and High Resolution Time-of-Flight Mass Spectrometers for Metabolomic Studies

David E. Alonso, Jeffrey S. Patrick, and Joe Binkley | LECO Corporation, St. Joseph, MI USA

Introduction

Metabolomics is a critical tool for biological research[1]

- Close proximity to phenotype
- Quick insight into system perturbations



Metabolomics – The Challenges[2,3]

- Overwhelming number of metabolites
- Chemical diversity of metabolites
- Large concentration range
- Variability – Diet, metabolism
- Bottleneck – Confident compound identification

GC-TOFMS – Pegasus® HT/4D (Throughput & Discovery)

Pegasus HT/4D

- Speed and robustness
- Enhanced sensitivity – Cryogenic Focusing
- Unprecedented chromatographic resolution
- Peak True Deconvolution
- Large, well established databases (e.g., NIST)
- **One Injection → Rich Data**

~~Sim Mode~~ ~~MS/MS~~

GC-HR TOFMS – Pegasus HRT (Discovery & Confirmation)

Pegasus HRT

- Speed and robustness
- Chromatographic resolution
- High Resolution Deconvolution™
- High resolution accurate mass:
  1. Spectral similarity searches
  2. Formulas for fragment, molecular and adduct ions

Experimental

Sample Preparation

GC-TOF Metabolomics

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

Derivatization:

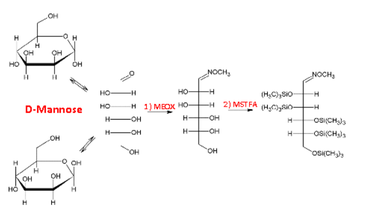


Fig. 1: A Wide Variety of Compounds Amenable to GC-TOFMS Analysis (Top); After Derivatization (Bottom).

Blood plasma samples were treated with 25 µL of MEOX (20 mg/mL in pyridine) and heated at 60°C for 1 hour. Samples were then treated with 50 µL of MSTFA and heated at 60°C for 1 hour. The MEOX reagent was spiked with OFN to monitor instrument performance and fatty acid methyl esters for calculation of retention index values.

Instrument Parameters

Pegasus HT	
Carrier Gas	He @ 1.0 ml/min
Column One	Rxi-5 MS, 30 m x 0.25 mm x 0.25 µm (Restek)
Temp Program	4 min at 70°C, ramped 20°C/min to 300°C, held 6 min
Mass Range	50-510 m/z
Acquisition Rate	10 spectra/s
Source Temp	250°C

Pegasus 4D (Additions and Changes to HT Parameters)	
Column Two	Rtx-200, 1.5 m x 0.25 mm x 0.25 µm (Restek)
Temp Program	4 min at 70°C, ramped 5°C/min to 300°C, held 10 min; Secondary oven maintained +5°C relative to primary
Modulation	5 s with temperature maintained +15 relative to 2nd oven
Acquisition Rate	200 spectra/s

Pegasus HRT	
Carrier Gas	He @ 1.0 ml/min
Column	Rxi-5 MS, 30 m x 0.25 mm x 0.25 µm (Restek)
Temp Program	4 min at 70°C, ramped 10°C/min to 300°C and held 7 min
Mass Range	30-600 m/z (CI 65-1000 m/z; reagent gas 5% NH_3 in CH_4)
Acquisition Rate	12 spectra/s
Source Temp	250°C

Results and Discussion – Instrumental Platforms

Throughput

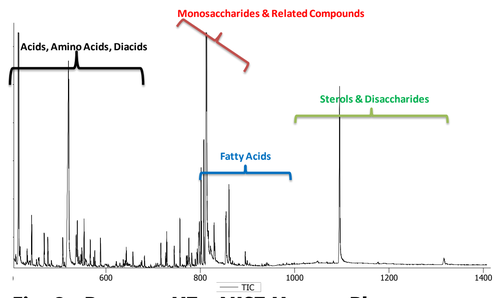
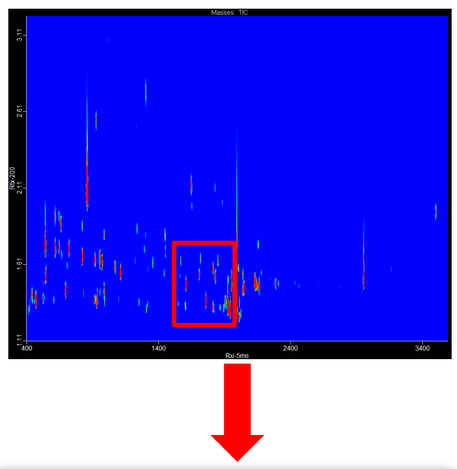


Fig. 2: Pegasus HT – NIST Human Plasma.

Table 1: Pegasus HT – Representative Compounds

Name	Formula	R.T. (s)	Retention Index	Area	Similarity
Lactic Acid 2TMS	$C_9H_{22}O_3Si_2$	414	220344	78412	926
Alanine 2TMS	$C_9H_{23}NO_2Si_2$	442	250961	28186763	853
Pyruvic Acid MEOX 2TMS	$C_8H_{21}NO_3Si_2$	471	282442	96993	809
Valine 2TMS	$C_{11}H_{27}NO_2Si_2$	508	322255	15086154	895
Leucine 2TMS	$C_{12}H_{29}NO_2Si_2$	536	355568	7506780	873
Phosphate 3TMS	$C_9H_{27}O_4PSi_3$	539	359187	14704044	901
Isoleucine 2TMS	$C_{12}H_{29}NO_2Si_2$	547	368354	4513200	878
Proline 2TMS	$C_{11}H_{25}NO_2Si_2$	549	371129	12961273	902
Glycine 3TMS	$C_{11}H_{29}NO_2Si_3$	553	376557	16536857	892
Glyceric Acid 3TMS	$C_{12}H_{30}O_4Si_3$	563	388940	281511	842
Serine 3TMS	$C_{12}H_{31}NO_3Si_3$	576	405947	3262088	900
Threonine 3TMS	$C_{13}H_{33}NO_3Si_3$	588	421527	2432941	897
5-oxo-Proline 2TMS	$C_{11}H_{23}NO_3Si_2$	643	492895	10753295	873
Glutamic Acid 3TMS	$C_{14}H_{33}NO_4Si_3$	676	537217	1057351	827
Phenylalanine 2TMS	$C_{15}H_{27}NO_2Si_2$	682	544919	2205667	868
Asparagine 3TMS	$C_{13}H_{32}N_2O_3Si_3$	695	563027	499990	898
Glutamine 4TMS	$C_{17}H_{42}N_2O_5Si_4$	716	591899	1907951	847
Glutamine 3TMS	$C_{14}H_{34}N_2O_4Si_3$	729	609091	12998376	870
Ornithine 4TMS	$C_{17}H_{44}N_2O_5Si_4$	745	630514	4933987	882
Citric Acid 4TMS	$C_{18}H_{40}O_7Si_4$	745	630923	943974	803
1,5-Anhydro-D-sorbitol 4TMS	$C_{18}H_{44}O_5Si_4$	757	647024	4551170	901
Caffeine	$C_8H_{10}N_4O_2$	759	650163	239658	817
Lysine 4TMS	$C_{18}H_{46}N_2O_2Si_4$	776	672963	4824013	739
Tyrosine 3TMS	$C_{18}H_{35}NO_3Si_3$	782	681175	5195386	860
Glucose MEOX 5TMS	$C_{22}H_{55}NO_6Si_5$	813	723878	70168404	791
9Z,12Z-Octadecadienoic acid TMS	$C_{21}H_{40}O_2Si$	823	737702	2409582	916
Uric acid 4TMS	$C_{17}H_{36}N_4O_3Si_4$	829	746051	2464071	819
Oleic acid TMS	$C_{21}H_{42}O_2Si$	855	781656	1174025	926
Octadecanoic acid TMS	$C_{21}H_{44}O_2Si$	861	789663	25117086	902
Tryptophan 3TMS	$C_{20}H_{36}N_2O_2Si_3$	863	793114	3414435	921
Arachidonic Acid TMS	$C_{23}H_{40}O_2Si$	895	837441	1703921	933
Vitamine E TMS	$C_{32}H_{58}O_2Si$	1082	1076776	450887	787
Cholesterol TMS	$C_{30}H_{54}OSi$	1095	1091081	46976466	870

Discovery



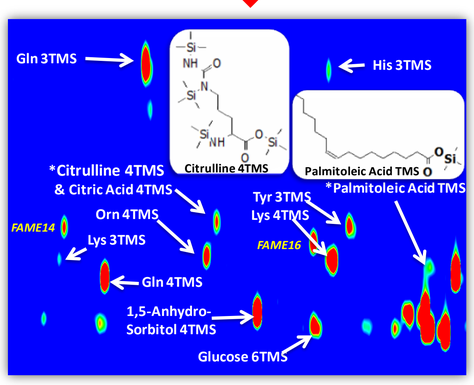


Fig. 3: Pegasus 4D Contour Plot of NIST Human Plasma (Top). Plot Expansion Highlighting Identified Compounds In NIST Human Plasma (Bottom).

Table 2: Citrulline and Palmitoleic Acid TMS Derivatives in NIST Human Plasma[4]

Name	Formula	R.T. (s)	Area	Similarity
Palmitoleic Acid TMS	$C_{19}H_{38}O_2Si$	1925 , 1.515	75121	892
Citrulline 4TMS	$C_{18}H_{45}N_3O_3Si_4$	1710 , 1.690	22895	700

Confirmation

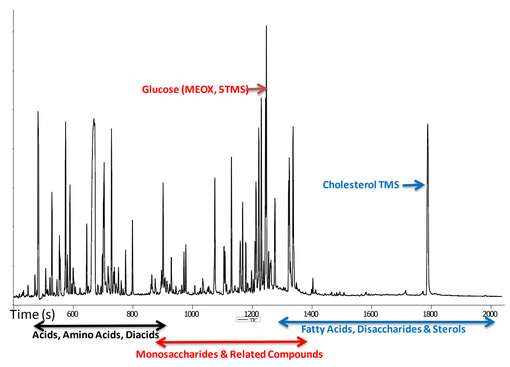


Fig. 4: Pegasus HRT – NIST Human Plasma.

Table 3: Pegasus HRT – Representative Compounds

Metabolite	Formula	Ion	Observed m/z	Expected m/z	Mass Accuracy (ppm)
Lactic Acid 2TMS	$C_9H_{22}O_3Si_2$	$[M-C_4H_9O_2Si]^+$	117.07302	117.07318	1.37
Alanine 2TMS	$C_9H_{23}NO_2Si_2$	$[M-C_4H_9O_2Si]^+$	116.089	116.08908	0.70
Pyruvic Acid MEOX 2TMS	$C_8H_{21}NO_3Si_2$	$M^{+\cdot}$	247.10526	247.10545	-0.75
Valine 2TMS	$C_{11}H_{27}NO_2Si_2$	$[M-C_4H_9O_2Si]^+$	144.1203	144.12038	0.53
Leucine 2TMS	$C_{12}H_{29}NO_2Si_2$	$[M-C_4H_9O_2Si]^+$	158.13595	158.13601	0.36
Phosphate 3TMS	$C_9H_{27}O_4PSi_3$	$M^{+\cdot}$	314.09492	314.09493	-0.02
Isoleucine 2TMS	$C_{12}H_{29}NO_2Si_2$	$[M-C_4H_9O_2Si]^+$	158.13595	158.13609	0.90
Proline 2TMS	$C_{11}H_{25}NO_2Si_2$	$[M-C_4H_9O_2Si]^+$	142.10465	142.10468	0.17
Glycine 3TMS	$C_{11}H_{29}NO_2Si_3$	$[M-C_4H_9O_2Si]^+$	174.11288	174.1131	1.26
Glyceric Acid 2TMS	$C_{12}H_{30}O_4Si_3$	$[M-C_5H_{13}O_2Si]^+$	189.07616	189.07614	-0.09
Serine 3TMS	$C_{12}H_{31}NO_3Si_3$	$[M-C_4H_9O_2]^+$	204.12344	204.1235	0.28
Threonine 3TMS	$C_{13}H_{33}NO_3Si_3$	$[M-C_5H_{13}OSi]^+$	218.10271	218.10269	-0.06
5-oxo-Proline 2TMS	$C_{11}H_{23}NO_3Si_2$	$M^{+\cdot}$	273.12073	273.1211	-1.35
Glutamic Acid 3TMS	$C_{14}H_{33}NO_4Si_3$	$M^{+\cdot}$	363.17109	363.17119	-0.28
Phenylalanine 2TMS	$C_{15}H_{27}NO_2Si_2$	$[M-C_7H_7]^+$	218.10271	218.10274	0.15
Asparagine 3TMS	$C_{13}H_{32}N_2O_3Si_3$	$M^{+\cdot}$	348.17193	348.17152	1.16
Glutamine 4TMS	$C_{17}H_{42}N_2O_5Si_4$	$[M-C_4H_9O_2Si]^+$	317.18952	317.18906	-1.45
Glutamine 3TMS	$C_{14}H_{34}N_2O_4Si_3$	$M^{+\cdot}$	362.1873	362.18717	0.35
Ornithine 4TMS	$C_{17}H_{44}N_2O_5Si_4$	$M^{+\cdot}$	420.24755	420.24744	0.26
Citric Acid 4TMS	$C_{18}H_{40}O_7Si_4$	$[M-C_7H_{19}O_3Si_2]^+$	273.09729	273.0974	0.40
Caffeine	$C_8H_{10}N_4O_2$	$M^{+\cdot}$	194.07969	194.07983	-0.69
1,5-Anhydro-D-sorbitol 4TMS	$C_{18}H_{44}O_5Si_4$	$M^{+\cdot}$	452.22579	452.22603	-0.54
Lysine 4TMS	$C_{18}H_{46}N_2O_2Si_4$	$M^{+\cdot}$	434.26306	434.26309	-0.06
Tyrosine 3TMS	$C_{18}H_{35}NO_3Si_3$	$[M-C_{10}H_{15}OSi]^+$	218.10271	218.10292	0.95
Glucose MEOX 5TMS	$C_{22}H_{55}NO_6Si_5$	$[M-C_7H_{22}NO_2Si_2]^+$	361.16812	361.16857	1.26
Uric Acid 4TMS	$C_{17}H_{36}N_4O_3Si_4$	$M^{+\cdot}$	456.18649	456.1859	1.29
9Z,12Z-Octadecadienoic Acid TMS	$C_{21}H_{40}O_2Si$	$M^{+\cdot}$	352.27892	352.27921	-0.83
Oleic acid TMS	$C_{21}H_{42}O_2Si$	$M^{+\cdot}$	354.2944	354.29486	-1.28
Octadecanoic acid TMS	$C_{21}H_{44}O_2Si$	$M^{+\cdot}$	356.31019	356.31051	-0.90
Tryptophan 3TMS	$C_{20}H_{36}N_2O_2Si_3$	$[M-C_8H_{20}NO_2Si_2]^+$	202.10465	202.10471	0.29
Arachidonic acid TMS	$C_{23}H_{40}O_2Si$	$[M-C_{19}H_{31}]^+$	117.03663	117.03661	-0.19
Vitamin E TMS	$C_{32}H_{58}O_2Si$	$M^{+\cdot}$	502.42006	502.42006	0.00
Cholesterol TMS	$C_{30}H_{54}OSi$	$M^{+\cdot}$	458.39421	458.39384	0.79

Results and Discussion – The EI & CI-HRT Advantage

EI-HRT

Rich Data = Excellent Spectral Similarity Scores

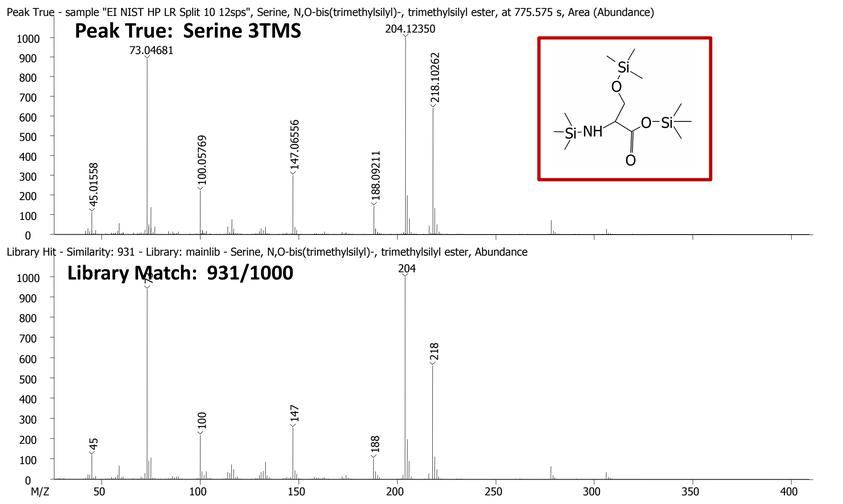


Fig. 5: Pegasus HRT – Peak True (Deconvoluted) and Library Mass Spectra for Serine 3TMS.

EI & CI-HRT

Formulae for Molecular Adducts: Confident Identifications

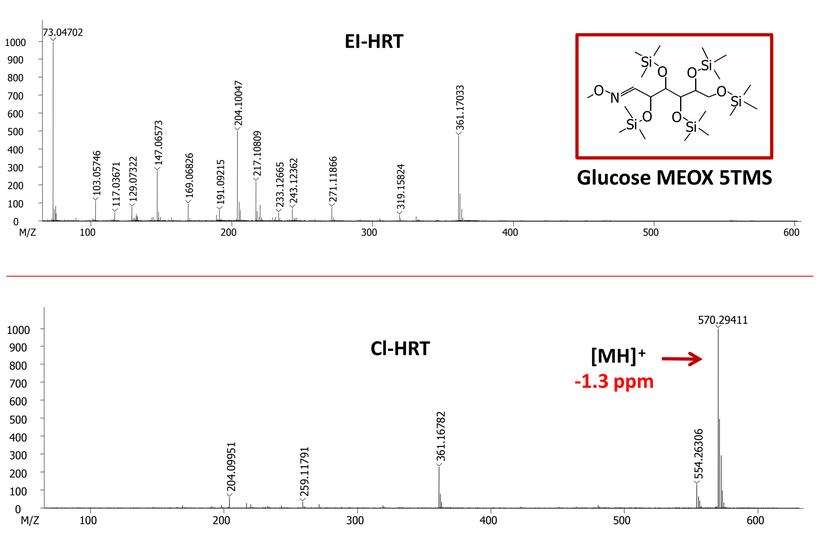
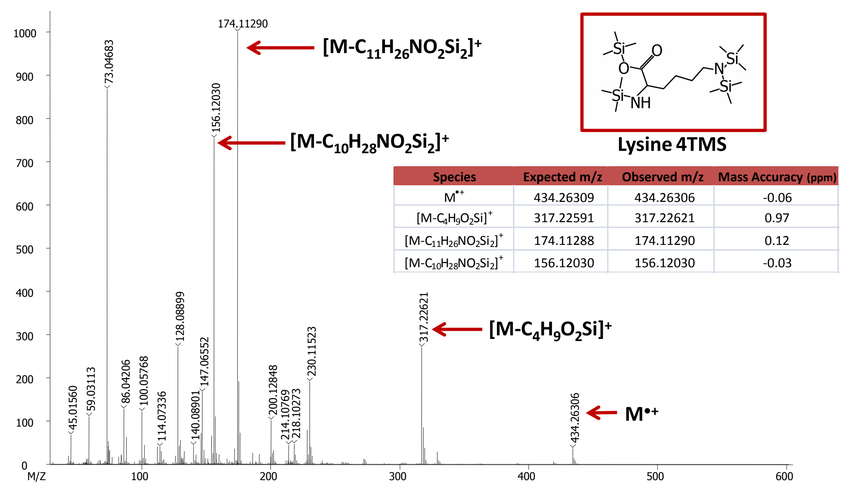


Fig. 7: EI and CI-HRT Data for Glucose MEOX 5TMS.

Accurate Mass = Great Selectivity



Species	Expected m/z	Observed m/z	Mass Accuracy (ppm)
$M^{+\cdot}$	434.26309	434.26306	-0.06
$[M-C_4H_9O_2Si]^+$	317.22591	317.22621	0.97
$[M-C_{11}H_{26}NO_2Si_2]^+$	174.11288	174.11290	0.12
$[M-C_{10}H_{28}NO_2Si_2]^+$	156.12030	156.12030	-0.03

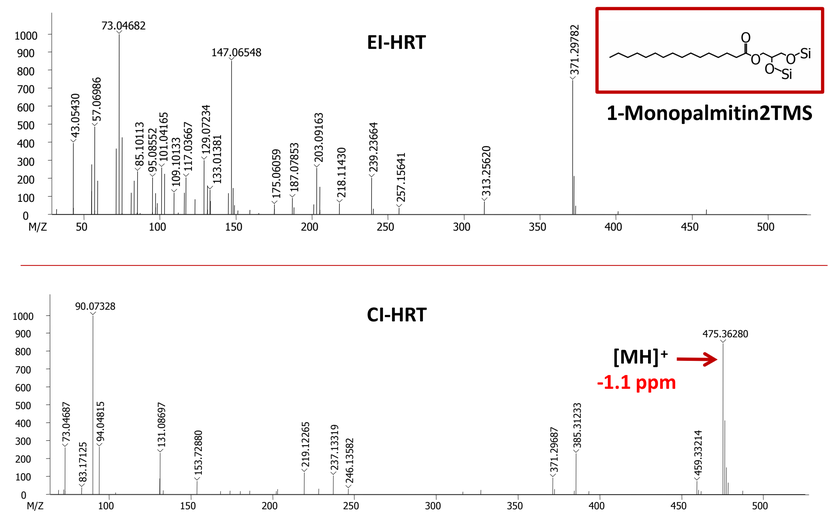


Fig. 8: EI and CI-HRT Data for 1-Monopalmitin 2TMS.

Fig. 6: Pegasus HRT – Peak True Mass Spectrum for Lysine 4TMS.

Summary

The Pegasus series of mass spectrometers are indispensable tools for metabolomics research:

- Throughput
- Discovery
- Confirmation

GC-HRT analyses resulted in confident metabolite identifications:

- Spectral similarity searches
- Formulae for fragment, molecular, and adduct ions

References

1) F. Hadacek, et al., *Metabolomics*, **2013**, *9*. 564-574.
2) P. Britz-McKibbin, et al., *Chemical Reviews*, **2013**, *113*, 2437-2468.
3) W.B. Dunn, et al., *Metabolomics*, **2013**, *9*, S44-S66.
4) Potential candidates to add to the GC-MS compound list in "Metabolite Profiling of a NIST Standard Reference Material for Human Plasma (SRM 1950): GC-MS, LC-MS, NMR and Clinical Laboratory Analysis, Libraries, and Web-Based Resources," *Anal. Chem.*, **2013**, *85*, 11725-11731