Electron Impact and Chemical Ionization High Resolution Time-of-Flight Mass Spectrometry Analyses of Blood Plasma Samples

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

Introduction

Metabolomics has been an important part of medicine and physiology for hundreds of years. It has been used for nutrigenomics, disease diagnosis, drug discovery, and natural product research. This area of science involves detection, quantification, and study of small molecules (Molecular weight <1500) produced and/or transformed in cells of living organisms. While no single instrument is capable of fully profiling the metabolome, the unique capabilities of high resolution time-of-flight mass spectrometry (HRT) make it an essential tool for metabolomic profiling. The HRT provides additional benefits:

- High resolution EI and CI-HRT data
- Reduced analysis times
- Effective peak deconvolution
- Ability to interrogate data sets repeatedly
- Production of high quality, accurate mass spectral data (library database and formula searches)

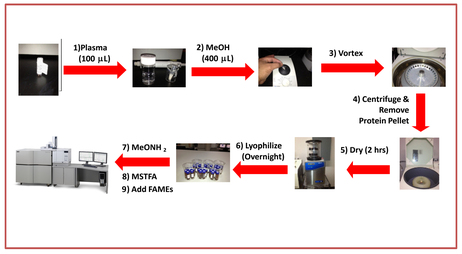
This study included both EI-HRT and CI-HRT data acquisition to obtain comprehensive profiles of derivatized blood plasma samples from twelve lean, fatty, and obese Zucker rats. Mass spectral data was collected in high resolution mode and chromatography was adjusted to maximize throughput without minimizing the number of metabolites identified.

The rats were 7 to 9 weeks old, were fed *ad libitum* chow, which is 18% protein and 6% fat. Plasma was obtained from a terminal bleed and was kept at -80°C and preserved with EDTA.

Pegasus® GC-HRT

Experimental

Sample Preparation



Instrument Parameters

GC Parameters

GC:	Agilent 7890 and 7693 Auto Sampler
Column Type:	Restek Rxi-5Sil MS (30 m, 0.25 mm ID, 0.25 mm df)
Injection:	1 µL, Splitless; Inlet Temp. 250°C
Oven:	70°C (4 min) to 300°C at 20°C/min (6 min)
Carrier Gas:	He, Constant Flow (1.00 mL/min)

MS Parameters

Spectrometer:	LECO Pegasus GC-HRT
Ion Sources:	LECO EI, CI
Source Temp:	250°C (CI 200°C)
Transfer Line Temp:	300°C
Spectral Acquisition:	6 spectra/second
Mass Range (m/z):	60 to 520 (CI 180 to 1400)
Calibration:	PFTBA (Internal)
CI Reagent Gas:	5% Ammonia in Methane
Folded Flight Path:	High Resolution (R = 25,000 FWHM)

Analysis Workflow

Results and Discussion (EI-HRT)

Analytical Ion Chromatograms (AICs) for lean, fatty, and obese Zucker rat plasma are displayed below (Figure 1). The plasma contained chemically diverse compounds including amino acids, acids, diacids, sugars, fatty acids, and sterols (Figure 2). Table 1 lists the retention times, areas, library match values (LM), observed ions, and mass accuracy values (Ave. = 0.90 ppm) for a representative set of the greater than 700 compounds in rat plasma (obese). LECO's ChromaTOF-HRT software facilitated high sample throughput by providing excellent deconvolution of coeluting peaks as shown in Figure 3. High quality spectral data can be searched against commercial libraries such as NIST and Wiley 9 (Figure 4). Accurate mass data resulted in robust formula determinations of molecular and fragment ions (Figure 5).

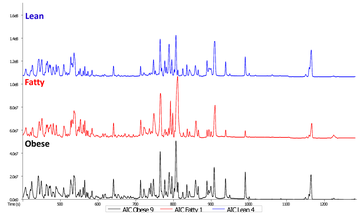


Figure 1: EI-HRT AICs- Lean, Fatty, and Obese Zucker Rat Plasma.

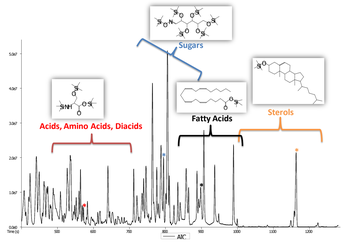


Figure 2: EI-HRT AIC Displaying Classes of Compounds in Zucker Rat Plasma (Obese).

Table 1: Representative Compounds in Zucker Rat Plasma (Obese).
CI-HRT data for compounds in red are shown in Figures 6 through 8.

Name	Formula	R.T. (s)	Area	LM (1000)	Ion	Observed Ion m/z	Mass Accuracy (ppm)
Alanine (3TMS)	$C_9H_{23}NO_2Si_3$	448.6	12732975	829	$[M\text{-}CH_3]^+$	218.10235	-3.62
Oxalic Acid (2TMS)	$C_8H_{20}O_4Si_2$	464.5	27487671	889	$[M\text{-}CH_3]^+$	219.05012	-1.00
Valine (2TMS)	$C_{11}H_{25}NO_2Si_2$	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)	$C_{18}H_{44}O_7Si_4$	739.9	39155972	861	$[M\text{-}CH_3]^+$	465.16066	-0.92
D-(+)-Galactose Oxime (hexaTMS)	$C_{24}H_{63}NO_6Si_6$	795.5	38182656	792	$[M\text{-}C_4H_9O_2Si_2]^+$	364.17887	-0.41
D-(+)-Glucose Oxime (hexaTMS)	$C_{24}H_{63}NO_6Si_6$	801.5	12382092	821	$[M\text{-}C_4H_9O_2Si_2]^+$	332.15293	0.39
Inositol (hexaTMS)	$C_{24}H_{66}O_6Si_6$	824.4	13117024	850	$[M\text{-}C_4H_9O_2Si_2]^+$	432.19929	-1.23
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	$C_{27}H_{46}$	1061.1	384812	782	$M^{+\bullet}$	368.34413	1.01
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	$M\text{-}\bullet$	472.40950	0.01
							Ave = 0.90 ppm

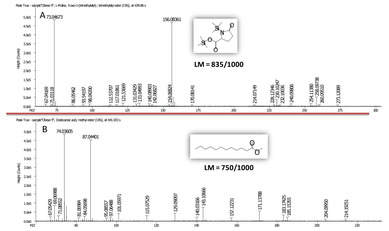


Figure 4: EI-HRT Peak True Mass Spectral Data for coeluting TMS Derivative of 5-Oxo-Proline (A) and Methyl Decanoate (B).

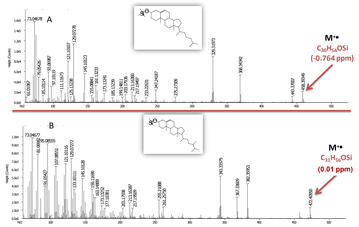


Figure 5: EI-HRT Peak True Mass Spectral Data for TMS Derivatives of Cholesterol (A) and Campesterol (B).

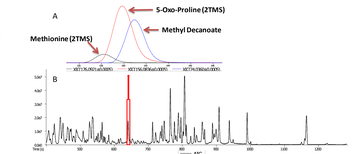


Figure 3: EI-HRT AIC (B) and XIC (A) Showing ChromaTOF-HRT's Deconvolution Capabilities.

Results and Discussion (CI-HRT)

Complementary CI-HRT data provided an extra level of confidence for metabolite characterization. For example, derivatized serine, galactose, glucose, and inositol did not display molecular ions in their EI-HRT mass spectra (Table 1); however, protonated molecular ions were readily observed in their CI-HRT spectra (Figures 6 and 7). Searches using m/z values for their adducts resulted in formulas with accurate mass values ranging from -0.053 to -0.424 ppm.

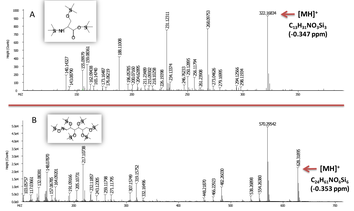


Figure 6: CI-HRT Peak True Mass Spectral Data for TMS Derivatives of Serine (A) and Galactose (B).

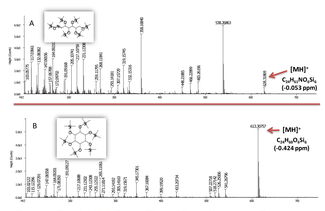


Figure 7: CI-HRT Peak True Mass Spectral Data for TMS Derivatives of Glucose (A) and Inositol (B).

CI-HRT can also be used to eliminate incorrect spectral similarity hits. For example, the number 1 and 2 library hits for a peak with retention time 1061.1 s (Obese Rat) were cholesteryl propionate ($C_{30}H_{50}O_2$). Quick inspection of the CI-HRT data (Figure 8) revealed that the 3rd hit, cholestadiene ($[MH]^+ = C_{27}H_{45}$, -0.208 PPM), was the actual compound.

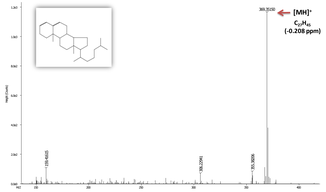
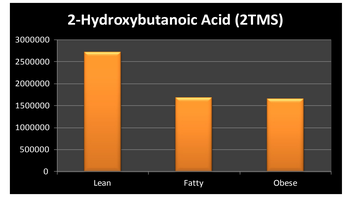


Figure 8: CI-HRT Peak True Mass Spectrum of Cholestadiene.



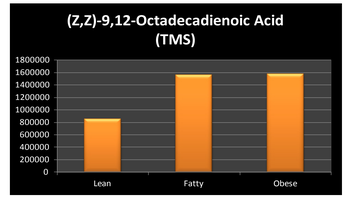


Figure 9: Trends in Zucker Rats (Lean → Fatty → Obese) 2-Hydroxybutanoic acid (top) and (Z,Z)-9,10-Octadecadienoic Acid (bottom).

Statistical Analysis

Three sets of twelve (lean, fatty, obese) were processed using ChromaTOF-HRT's Reference utility. Figure 9 shows the decreasing concentration trend in 2-hydroxybutanoic acid (2TMS) and increasing trend in (Z,Z)-9,10-octadecadienoic acid (TMS) for lean → fatty → obese Zucker rats. The Pegasus HRT data files can be readily exported in various file formats for further statistical analysis using 3rd party software packages such as Genedata. Additional statistical analyses are under way, but are beyond the scope of this poster presentation.

Conclusion

The Pegasus GC-HRT is an ideal instrument for the analysis of plasma samples. The combination of EI and CI-HRT greatly facilitate confident identification of metabolites.