Improved Methodology for Metabolomics Data Acquisition Workflow: Utilization of Electron Impact and Chemical Ionization High Resolution Time-of-Flight Mass Spectrometry

David E. Alonso*, Joe Binkley, John Heim and Jeff Patrick | LECO Corporation, St. Joseph, Michigan USA

Introduction

Liver disease is a worldwide dilemma that is responsible for the death of over 1.5 million individuals annually. The utilization of modern instrumental techniques for systemic determination of metabolite profiles is critical for understanding diseases both in individual organs and at the organism level[1]. Metabolomics entails instrumental detection, characterization and quantification of small molecules (Molecular weight <1500) produced, and/or transformed in the cells of living organisms. The high sensitivity, peak resolution and reproducibility of gas chromatography mass spectrometry (GCMS) have made it one of the most utilized techniques for plant and animal metabolite profiling. High resolution time-of-flight mass spectrometry (HRT) provides additional benefits.

- Robust and comprehensive data acquisition
- Effective peak deconvolution for high-throughput methods
- High resolving power minimizes background interferences
- Excellent mass accuracy values for confident elemental formula determination
- Complete and high quality spectral data for database matching
- The ability to interrogate rich data sets repeatedly for novel materials

In this study, a combination of EI-HRT and CI-HRT data was collected to obtain comprehensive profiles of mouse liver extracts. Formula and spectral similarity searches using commercially available libraries and online databases facilitated confident identification of analytes in complex samples (Figure 1). Mass spectral data was collected in high-resolution mode and mass accuracies at 1 ppm allowed for robust formula determinations for molecular and fragment ions.

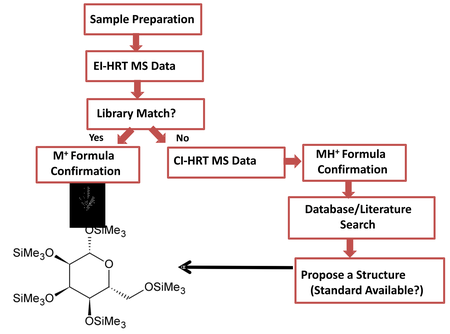

Figure 1: EI and CI HRT Analysis – Confident Identification.

Experimental

Sample Preparation

Samples were dried, derivatized using a two-step procedure, and transferred to GCMS vials for analysis (Figure 2).[2]

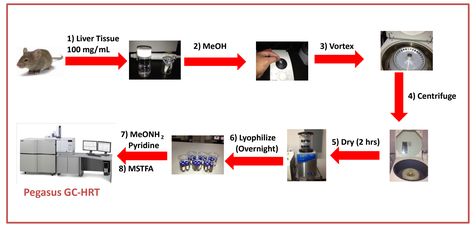

Figure 2. Sample Preparation Procedures.

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:	50°C (2 min) to 140°C at 10°C/min, to 240°C at 4°C/min, to 300°C at 10°C/min (10 min)
Carrier Gas:	He, Constant Flow (1.0 mL/min)

MS Parameters

Spectrometer:	LECO Pegasus® GC-HRT
Ion Sources:	LECO EI, CI
Source Temperature:	EI 250°C (CI 200°C)
Folded Flight Path:	HR (R = 25,000 FWHM)
Spectral Acquisition:	6 spectra/second
Mass Range (m/z):	60 to 520 (CI 50 to 1200)
Calibration:	PFTBA (Internal)
CI Reagent Gas:	5% Ammonia in Methane

Project Workflow

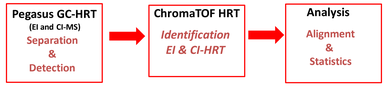

Figure 3: General Project Workflow.

Results (EI-HRT)

An Analytical Ion Chromatogram (AIC) for a mouse liver extract sample is shown in Figure 4. The sample contained a wide variety of metabolites including acids, diacids, amino acids, sugars, fatty acids, and sterols. The formulas, retention times, peak areas, spectral similarity values (Ave. = 819/1000), and mass accuracies (Ave. = 0.69 ppm) for a representative set of compounds in this sample are listed in Table 1.

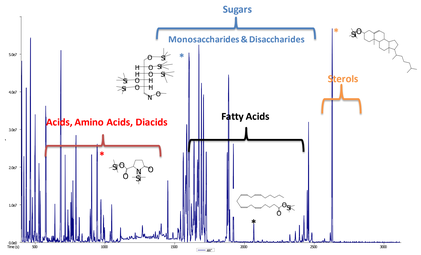

Figure 4: AIC – Mouse Liver Extract Data.

Table 1: Representative Compounds in Liver Extract

Name	Formula	R.T. (s)	Area	Similarity	Species	Observed m/z	Mass Accuracy (ppm)
Lactic Acid (2 TMS)	$C_9H_{22}O_3Si_2$	499	24946624	854	$[M-CH_3]^+$	219.08667	-0.26
Glycolic Acid, (2 TMS)	$C_8H_{20}O_3Si_2$	512	4397888	724	$[M-CH_3]^+$	205.07112	0.20
Glycine (3 TMS)	$C_{11}H_{29}NO_2Si_3$	714	30923198	864	$M^{+\bullet}$	291.15018	0.42
Glyceric Acid (3 TMS)	$C_{12}H_{30}O_4Si_3$	734	21222912	793	$M^{+\bullet}$	322.14501	1.16
2,5-Dihydroxypyrazine (2 TMS)	$C_{10}H_{20}N_2O_2Si_2$	764	21408124	815	$M^{+\bullet}$	256.10546	-1.25
Aminomalonic Acid (3 TMS)	$C_{12}H_{31}NO_4Si_3$	882	1005968	834	$[M-CH_3]^+$	320.11621	-0.65
Malic acid (3TMS)	$C_{13}H_{30}O_5Si_3$	905	71845120	867	$M^{+\bullet}$	350.13944	-0.34
5-Oxo-Proline (2 TMS)	$C_{11}H_{23}NO_3Si_2$	944	88032864	699	$M^{+\bullet}$	273.12081	-1.07
Threonic Acid (4 TMS)	$C_{16}H_{40}O_5Si4$	971	45927360	870	$[M-C_5H_{11}O_2Si]^+$	292.13396	-0.40
Mannose (MEOX, 5 TMS)	$C_{22}H_{53}NO_6Si_5$	1604	149664757	602	$[M-C_4H_{10}O_2Si_2]^+$	364.17883	-0.51
Inositol (6 TMS)	$C_{24}H_{60}O_6Si_6$	1721	18598229	900	$[M-C_{11}H_{29}O_2Si_2]^+$	318.14970	-0.09
Linoleic Acid (TMS)	$C_{21}H_{40}O_2Si$	1876	3987501	889	$M^{+\bullet}$	352.27928	0.19
Oleic Acid (TMS)	$C_{21}H_{42}O_2Si$	1886	66299648	728	$M^{+\bullet}$	354.29444	-1.18
cis-13-Octadecenoic Acid (TMS)	$C_{21}H_{42}O_2Si$	1894	17079936	841	$M^{+\bullet}$	354.29510	0.69
Octadecanoic Acid (TMS)	$C_{21}H_{44}O_2Si$	1921	65739823	879	$M^{+\bullet}$	356.31025	-0.73
Arachidonic Acid (TMS)	$C_{23}H_{40}O_2Si$	2067	8086857	855	$M^{+\bullet}$	376.27890	-0.82
Maltose (MEOX, 8 TMS)	$C_{35}H_{81}NO_{11}Si_8$	2459	48139520	852	$[M-C_4H_{10}O_2Si_2]^+$	480.24412	-1.32
Cholesterol (TMS)	$C_{30}H_{54}OSi$	2629	64955952	886	$M^{+\bullet}$	458.39333	-1.11
						Ave. =	0.69

Peak True (deconvoluted) and library mass spectral data for derivatives of 5-oxo-proline, mannose, arachidonic acid, and cholesterol are displayed in Figures 5 through 8.

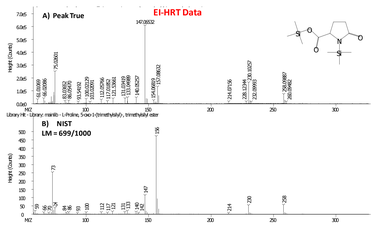

Figure 5: Peak True EI-HRT (A) and Library Data (B) for 5-Oxo-Proline (2 TMS).

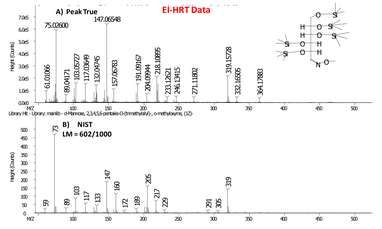

Figure 6: Peak True EI-HRT (A) and Library Data (B) for Mannose (MEOX 5 TMS).

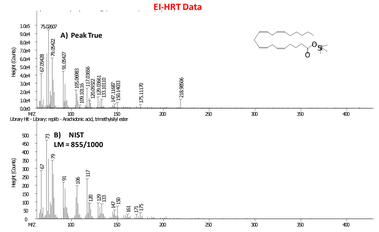

Figure 7: Peak True EI-HRT (A) and Library Data (B) for Arachidonic Acid (TMS).

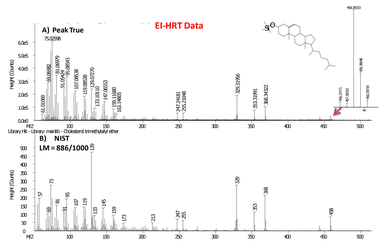

Figure 8: Peak True EI-HRT (A) and Library Data (B) for Cholesterol (TMS).

Results (CI-HRT)

Characterization of labile molecules present in low concentrations can be challenging due to poor quality data and/or lack of molecular ions in their mass spectra. Indeed, a significant number of derivatized compounds in mouse liver extracts did not exhibit molecular ions in their mass spectra (i.e., inositol, threonic acid, mannose, and maltose). The utilization of accurate mass EI-HRT fragment formula determination, coupled with spectral similarity searches resulted in acceptable structural assignments for compounds; however, parallel analyses using CI-HRT provided an extra level of confidence for full characterization of metabolites. For example, a spectral similarity search using EI-HRT data for mannose (MEOX, 5 TMS) in liver sample resulted in a low match value (602/1000). In addition, there was no observable molecular ion in its EI-mass spectrum (Figure 9). A strong signal for the protonated molecular ion at m/z = 570.29431 was present in the corresponding CI-HRT mass spectrum. A formula search of this ion resulted in $C_{22}H_{56}NO_6Si_5$ as the number one hit with a mass accuracy of -0.94 ppm.

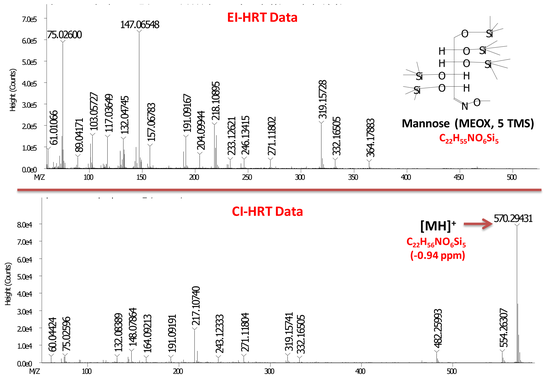

Figure 9: EI (Top) and CI-HRT Data (Bottom) for Mannose (MEOX, 5 TMS).

Summary

The Pegasus GC-HRT is ideal for metabolomic studies. This high resolution instrument provides quality spectral data and excellent mass accuracy values which can be used to characterize metabolites in complex biological matrices. The addition of CI-HRT data facilitates confident identification of low-level, labile compounds in samples.

References

[1] Xie B. Waters M.J and Schirra H.J. "Investigating Potential Mechanisms of Obesity by Metabolomics" J. Biomed Biotechnol. 2012; 2012: 805683. Published online 2012 May 16.

[2] Mouse liver extracts were provided by Dr. Xiang Zhang (Department of Chemistry, University of Louisville, KY 40292, USA).