Identification of Biomarkers for Alcoholic Liver Disease from Mice Fed with Unsaturated Fat Diets by Gas Chromatography-High Resolution Time-of-Flight Mass Spectrometry

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Introduction

Chronic liver diseases such as Hepatitis B and C affect more than 800 million people worldwide causing at least 1.5 million deaths yearly. Alcohol is one of the major causes for liver-related diseases. Alcoholic liver disease (ALD) continues to be a major cause of morbidity and mortality in the US. Polychlorinated biphenyls (PCBs) are persistent environmental pollutants which have been associated with non-alcoholic fatty liver disease. The mouse provides a good model for research and possible treatment studies of these life-threatening diseases. To study the role of alcohol and PCBs on mice fed with a fatty diet, it is important to identify metabolites that have significant abundance changes in mice liver induced by these agents. In this study, we employ a high resolution time of flight mass spectrometer (LECO Pegasus® GC-HRT) to identify major metabolites and their changes from two different conditions from mouse liver samples.[1-2]

Methods & Instrumentation

Mouse liver extracts (methanolic) were reacted with ethoxyamine and MTBSTFA (1% TBS). A Pegasus GC-HRT equipped with an Agilent 7890 gas chromatograph and a Restek Rxi 5Sil MS 60 m × 0.25 mm i.d. × 0.25 µm column was used in this investigation. The MS was set at high resolution mode with mass range of m/z 45−1000. Acquisition rate was at 4 spectra/second. The ion source chamber was set at 230°C, transfer line temperature set to 280°C, and the detector voltage was 1600 V. EI ionization was achieved at 70 eV.

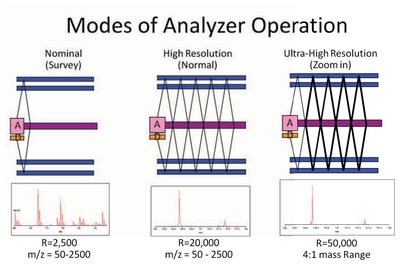

Figure 1. A LECO Pegasus GC-HRT TOFMS (above) with a novel high performance platform employing folded flight path (FFP™) technology (right).

Results

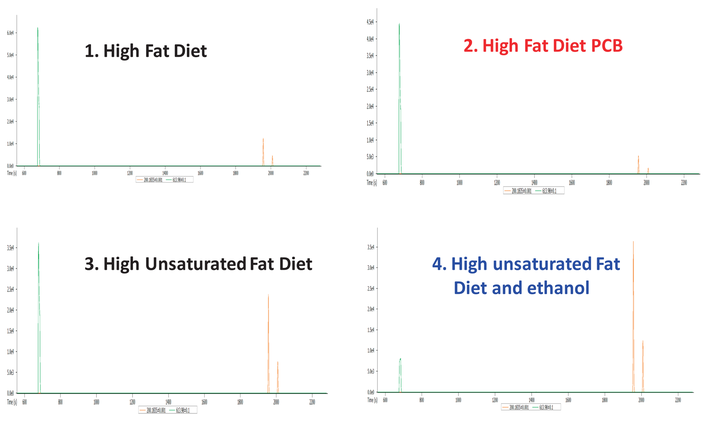

Figure 2. Extracted ion chromatograms (0.001 Da) of ion 200.183±0.001 (leucine/isoleucine) and 613.981±0.001 (PFTBA).

Results

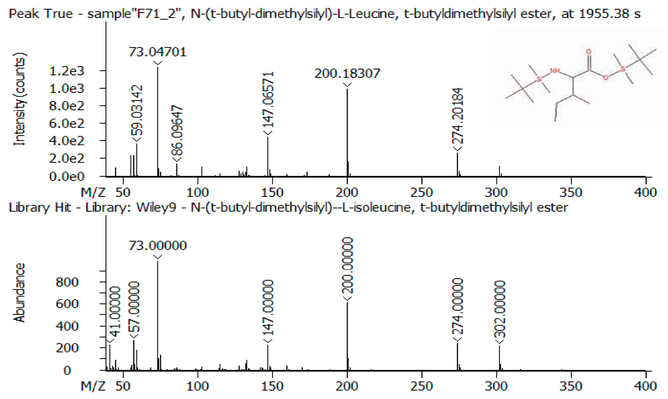

Figure 3. Library match of the peak at 1956s with Isoleucine bis-TBDMS ($C_{18}H_{41}NO_2Si_2$).

Calculated Mass	Species	Neutral Formula	Measured Mass	ΔPPM
302.19661	$(M-C_4H_9)^+$	$C_{14}H_{32}NO_2Si_2$	302.1971	-1.62
274.20169	$(M-C_4H_9-CO)^+$	$C_{13}H_{32}NOSi_2$	274.20184	-0.55
200.1829	$(M-C_7H_{15}O_2Si)^+$	$C_{11}H_{26}NSi$	200.18307	-0.85

Table 1. Fragment ions of Isoleucine bis-TBDMS

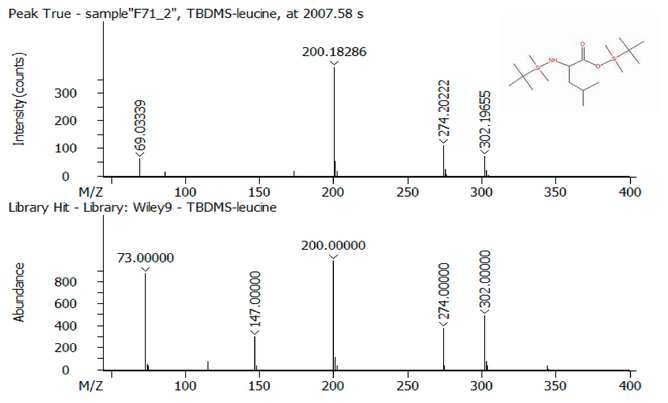

Figure 4. Library match of the peak at 2008s with Leucine bis-TBDMS.

Calculated Mass	Species	Neutral Formula	Measured Mass	ΔPPM
302.19661	$(M-C_4H_9)^+$	$C_{14}H_{32}NO_2Si_2$	302.19655	0.20
274.20169	$(M-C_4H_9-CO)^+$	$C_{13}H_{39}NOSi_2$	274.20222	-1.93
200.1829	$(M-C_7H_{15}O_2Si)^+$	$C_{11}H_{26}NSi$	200.18286	0.20

Table 2. Fragment ions of Leucine bis-TBDMS

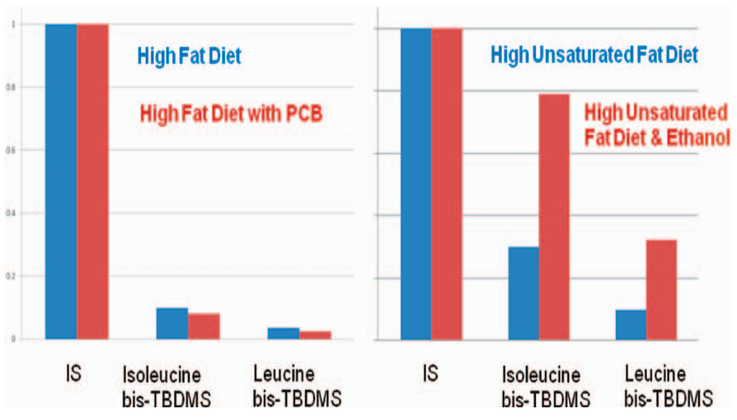

Figure 5. Relative changes of isoleucine and leucine species under high fat diet and additional PCBs (left). Relative changes of isoleucine and leucine species under high unsaturated fat diet and additional ethanol (right).

Results

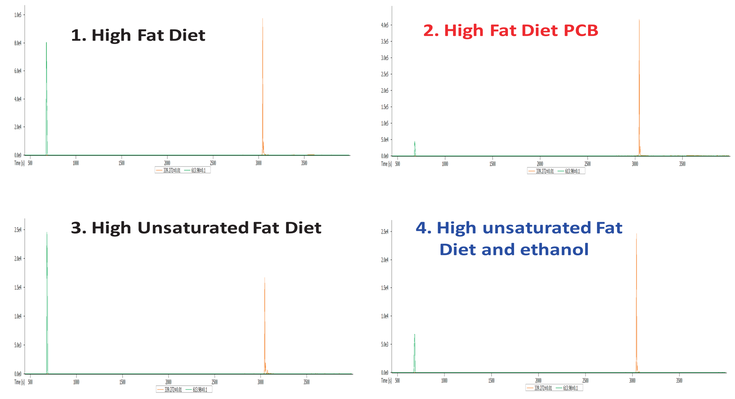

Figure 6. Extracted ion chromatograms (0.001 mDa) of ion 339.272, with ion 613.981 a fragment of PFTBA as the internal standard ion for relative quantitative estimates for metabolites.

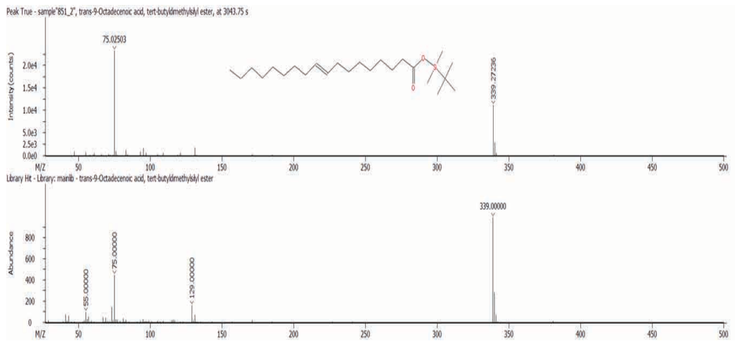

Figure 7. Library match of the peak at 3043s with trans-13-Octadecenoic acid, t-butylmethylsilyl ester.

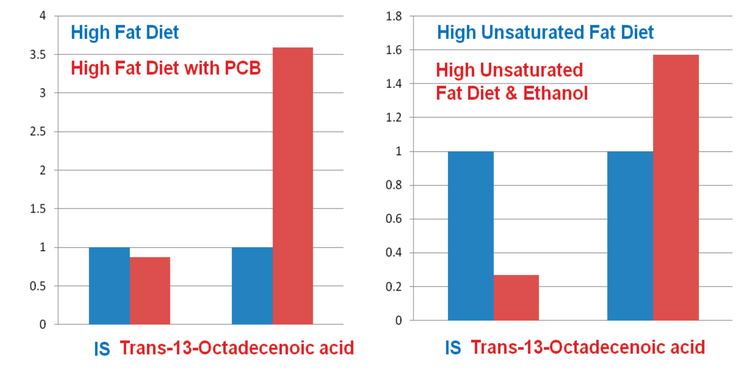

Figure 8. Relative changes of trans-13-Octadecenoic acid under high fat diet (left) and under high unsaturated fat diet plus additional ethanol (right).

CONCLUSIONS

We demonstrate the utility of high performance MS in metabolite identification. Specifically, the unmatched capabilities of the Pegasus GC-HRT have been successfully applied to identify metabolites which are differentially expressed in liver from treated mice. Isoleucine, leucine, and trans-13-octadecenoic acid were observed at different relative concentrations and were identified in samples through database searching and confirmed using accurate mass information (<2 ppm). The mass accuracy and resolving power also permitted the selective detection and comparison of levels in the various tissues. Substantial changes in the relative amounts of these three compounds were detected in the liver extracts and correlated with ingestion of PCBs or alcohol in combination with a high fat diet.

REFERENCES

1) Jessica Wapner. Scientific American. October 21, 2010

2) Neil Loftus, Alan Barnes, Simon Ashton, Filippos Michopoulos, Georgios Theodoridis, Ian Wilson, Cheng Ji, and Neil Kaplowitz. J Proteome Res. 2011 February 4; 10(2): 705–713.