Application of GC High Resolution Time-of-Flight Mass Spectrometry to Mouse Liver Metabolomic Analyses

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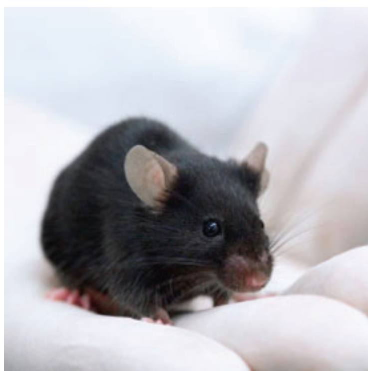
Key Words: GC-HRT MS, High Resolution Time-of-Flight MS, Mass Accuracy, Metabolites, Liver Disease, Library Match

1. Introduction

Liver disease affects more than 800 million people worldwide causing at least 1.5 million deaths annually. Alcohol consumption and alcoholic liver disease (ALD) continue to be a major cause of morbidity and mortality in the US. Polychlorinated biphenyls (PCBs) are persistent environmental pollutants. Exposures to PCBs have been associated with non-alcoholic fatty liver disease. Monitoring these and other diseases of the liver can be achieved by evaluating metabolites in serum or plasma but a key, basal understanding can come from evaluation of the diseased tissues themselves. Changes detected here will help understand the biology and physiology and may translate to the circulation. Mice provide a good model for this type of research.[1]



A key tool in these studies is gas chromatography coupled to mass spectrometry owing to its sensitivity and identification capabilities. Recent advances have allowed for the use of high resolution Time-of-Flight MS which further enhances the selectivity and comprehensive nature of the analysis. Here LECO's Pegasus® GC High Resolution Time-of-Flight Mass Spectrometer is applied to the identification and differential analysis of metabolites extracted for diseased livers from mice exposed to alcohol with and without fatty diets. To study the role of alcohol and PCBs on mice fed with an unsaturated fat diet, it is important to identify metabolites that have significant abundance changes in mice liver induced by alcohol.[2]

2. Results

Liver extracts from treated (fatty diet plus PCB or ethanol) and untreated (fatty diet only) were analyzed after derivatization to the silyl forms. Figure 1 shows the response of a pair of isomeric analytes, isoleucine and leucine (201.183) from 4 samples. These two amino acids are integrally linked to fatty acid metabolism. Note the differences in relative intensity between the pair and between samples.

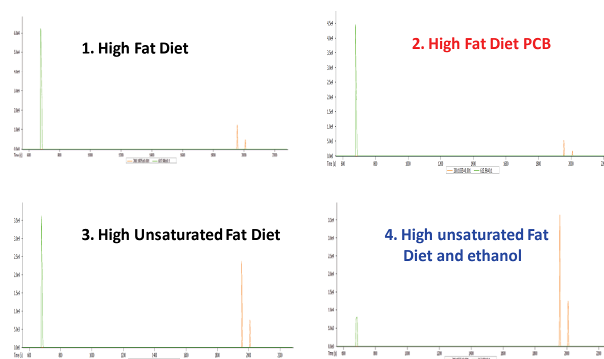


Figure 1. Extracted ion chromatograms of m/z 200.183 (±0.001) (Gold) and 613.981 (±0.001) (Green) in representative liver extract samples.

The identification of isoleucine and leucine are supported by library searches with spectral similarity scores of 830 and 740, respectively as shown in Figures 2 and 3. Additional confirmation comes with the determination of accurate fragment ions with mass accuracies of less than 2 ppm shown in Tables 1 and 2.

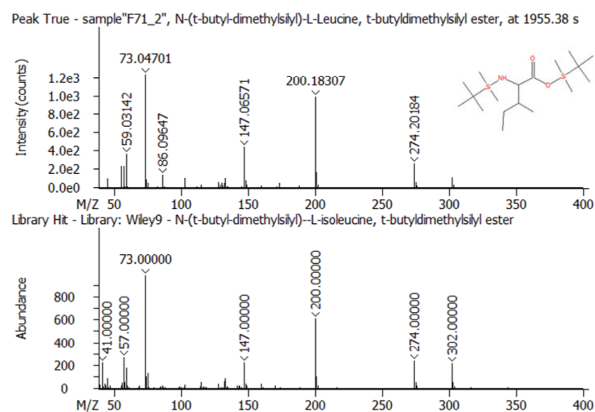


Figure 2. Peak True and library match of the Isoleucine bis-TBDMS peak.

Delivering the Right Results

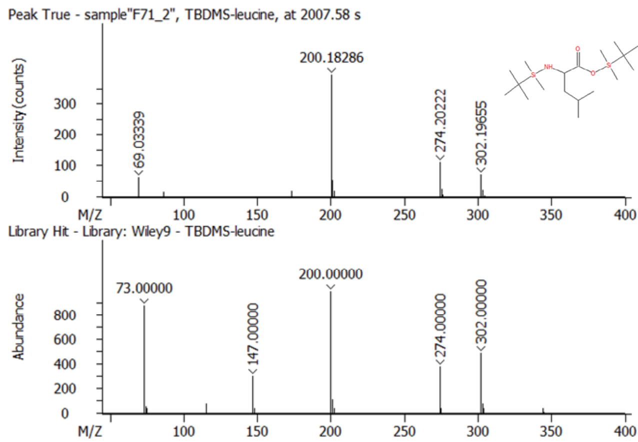


Figure 3. Peak True and Library match of Leucine bis-TBDMS ($C_{18}H_{41}NO_2Si_2$).

Table 1. Fragment ions of Isoleucine bis-TBDMS.

Ions	Species	Neutral Formula	Mass	ΔPPM
302.1966	$(M-C_4H_9)^+$	$C_{14}H_{32}NO_2Si_2$	302.1971	-1.62146
274.2017	$(M-C_4H_9-CO)^+$	$C_{13}H_{32}NOSi_2$	274.2018	-0.54704
200.1829	$(M-C_7H_{15}O_2Si)^+$	$C_{11}H_{26}NSi$	200.1831	-0.84922

Table 2. Fragment ions of Leucine bis-TBDMS.

Ions	Species	Neutral Formula	Mass	ΔPPM
302.1966	$(M-C_4H_9)^+$	$C_{14}H_{32}NO_2Si_2$	302.19655	0.1985462
274.2017	$(M-C_4H_9-CO)^+$	$C_{13}H_{32}NOSi_2$	274.20222	-1.932884
200.1829	$(M-C_7H_{15}O_2Si)^+$	$C_{11}H_{26}NSi$	200.18286	0.1998173

Figure 4 shows the relative levels of leucine and isoleucine in liver extracts under the various treatment conditions. Treatment with PCBs decreases both isoleucine and leucine by approximately 20% compared to a fatty diet only as shown in Figure 4. In contrast, ethanol consumption caused a 2-3 fold increase in leucine and isoleucine compared to a fatty diet alone.

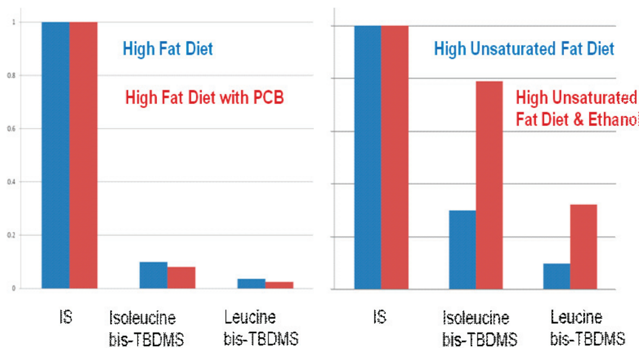


Figure 4. Relative changes of isoleucine and leucine upon ingestion of PCB (left) or ethanol (right).

Liver as the target tissue and a diet rich in fats suggest this might also be an opportunity to monitor fatty acid changes in a diseased state. As an example, trans-13-Octadecenoic acid, t-butylmethylsilyl ester is confirmed to be present in four samples (Figure 5) by library search with a spectral similarity score of better than 770 (Figure 6). Further structural confirmation comes from accurate fragment ion mass determination by GC-HRT with mass accuracies of less than 2 ppm. Exposure to PCBs with a high unsaturated fat diet increases the relative amount of Octadecenoic acid more than three-fold compared to the untreated animals with the same diet (Figure 7), while consumption of ethanol instead of PCBs while on a diet high in unsaturated fats increases it nearly eight-fold as shown in Figure 7. These are three of numerous metabolites observed to change in the study.

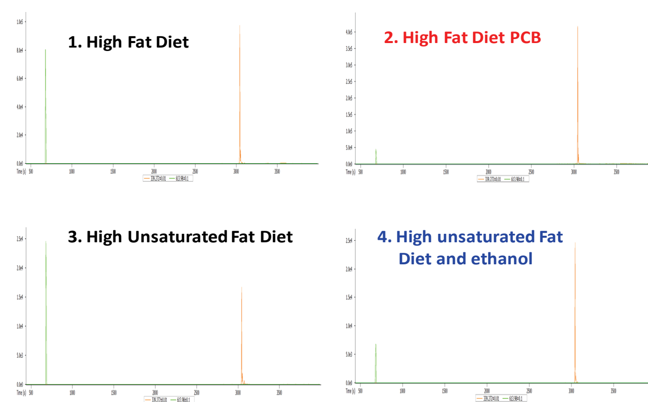


Figure 5. Extracted ion chromatograms of m/z 339.272 (±0.001; octadecenoic acid) in extracts.

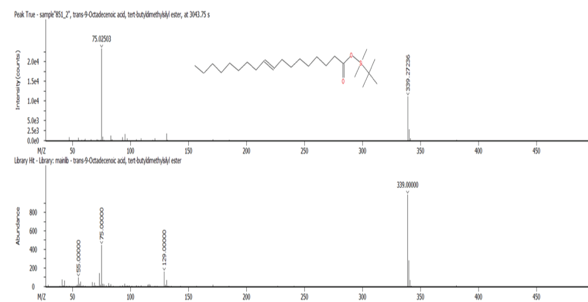


Figure 6. Library match of trans-13-Octadecenoic acid, t-butylmethylsilyl ester.

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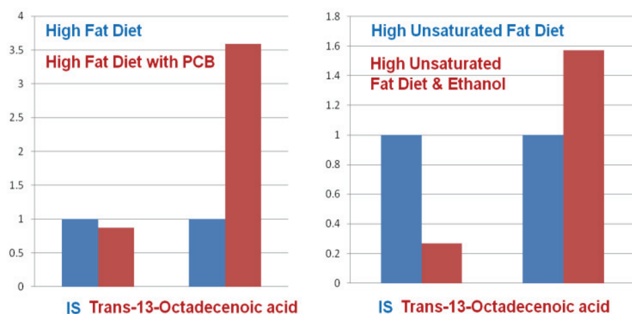

Figure 7. Relative changes of trans-13-Octadecenoic acid under high fat diet (left). Relative changes of trans-13-Octadecenoic acid under high unsaturated fat diet and additional ethanol (right). (Note – the exact isomeric form is the best hit of several octadecenoic acid forms.)

3. Conclusions

We demonstrate here that the Pegasus GC-HRT provides a tool for the analysis of metabolic changes in extracts of normal and diseased liver. Three primary metabolites—isoleucine, leucine, and octadecenoic acid—are identified based on library matches to acquired spectra and accurate mass matches of molecular and fragment ions. It proves a powerful tool in the analysis of metabolic changes due, in this instance, to PCBs and ethanol.

4. Experimental

All samples were stored at -80°C until they were derivatized for GC-HRT analysis. A 20 µl aliquot of each metabolite extract solution was dried with a stream of nitrogen. The dried residue was dissolved in 20 mg/ml freshly prepared ethoxyamine solution in pyridine. The mixture was vortexed and the reaction took placed at 60°C for 30 min. A 40 µl aliquot of MTBSTFA (1% TBS) solution was added to the vial and it was vortexed well before putting in a 60°C oven for 30 min. A 10 µl aliquot of internal standard mix solution (200 ng/µl) was added. A 65 µl aliquot of the final mixture was transferred to vials for GC-HRT analysis. The GC-HRT instrument equipped with an Agilent 7890 gas chromatograph and a Restek Rxi 5Sil MS (5% diphenyl/95% dimethyl polysilox) 60 m × 0.25 mm i.d. × 0.25 µm film thickness column was used in this investigation.

5. 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.

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