Utilization of GCxGC-TOFMS to Screen for Potential Metabolite Differences in Pooled Plasma Samples from Lean, Fat, and Obese Rats

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1. Introduction

This GCxGC-TOFMS research presents the comparison of pooled derivatized plasma samples from lean, fat, and diabetic obese Zucker rats. The small molecule metabolite profiles from the different sample pools were compared for similarity in mass spectral identification, retention time, and analyte variation by peak area. The enhanced chromatographic resolution and peak capacity available with comprehensive two-dimensional chromatography together with fast acquisition rates provided by TOFMS (up to 500 spectra/second), provided the ability to characterize low levels of metabolites common in complex sample matrices such as blood plasma. These complex and data rich files were processed with LECO's ChromaTOF® software. ChromaTOF utilizes automated peak find algorithms to detect, deconvolve and identify components present by performing mass spectral searches against commercially available libraries.

GCxGC-TOFMS was used to evaluate pooled trimethylsilyl-derivatized rat plasma samples in various states of health. Plasma from each of the strains of rat (Zucker Lean, Zucker Fatty and Zucker Diabetic Obese from Charles River Labs) was derivatized with BSTFA after the proteins were removed. Sample analysis was conducted on the lean, fat, and obese pooled samples utilizing a GCxGC separation followed by TOFMS detection at an acquisition rate of 150 spectra per second. The raw data was processed and mass spectral searches conducted using Max Planck, Fiehn Rtx5, and NIST libraries. The data mining strategy compared the combined peak tables, mass spectral similarities, and overlaid chromatograms to discover significant metabolite variations between the lean, fat, and obese sample pools.

This exploratory research was conducted as a screening tool to characterize and measure semi-quantitative variations in metabolites between the sample pools. The raw data files were first processed then compared by the chromatographic first and second dimension retention times and mass spectral similarity. A total of twelve metabolites between the different sample pools were found to have significant variation measured by their chromatographic peak areas. This exploratory research demonstrates the favorable and practical applicability of GCxGC-TOFMS as a screening tool to discover potential metabolic biomarkers which can be utilized to distinguish between healthy and disease state sample pools.

2. Experimental Conditions

Samples

- Proteins were removed from lean, fat and obese Zucker rat plasma with a 5000 MWCO (molecular weight cut-off) filter
- 100 µL samples were dried in a Savant SpeedVac
- The dried residue was suspended in 100 µL BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) and incubated overnight at 60°C

Experimental

GCxGC-TOFMS Analysis Parameters

Gas Chromatograph:
Agilent 7890 equipped with a LECO dual stage quad jet thermal modulator

Primary Column:
30 m x 0.25 mm id. x 0.25 µm film thickness
Rxi-5Sil MS (Restek Corp., Bellefonte, PA)

Secondary Column:
1.24 m x 0.15 mm id. x 0.15 µm film thickness
Rxi-17Sil MS (Restek Corp., Bellefonte, PA)

Carrier Gas: Helium set @ 1.5 mL/min

Injection: 1 µL splitless @ 260°C

Primary Column Temperature Program:
Initial temperature 70°C for 0.5 min, then ramped 6°C/min to 305°C for 5 min

Secondary Column Temperature Program:
Parallel ramp offset by +5°C

Total Run Time: 44.67 min.

Mass Spectrometer: LECO Pegasus® 4D

Mass Range: 45 to 750 m/z
Acquisition Rate: 150 spectra/s
Ion source Temp: 240°C
Detector Voltage: 1650 V
Electron Energy: -70 eV

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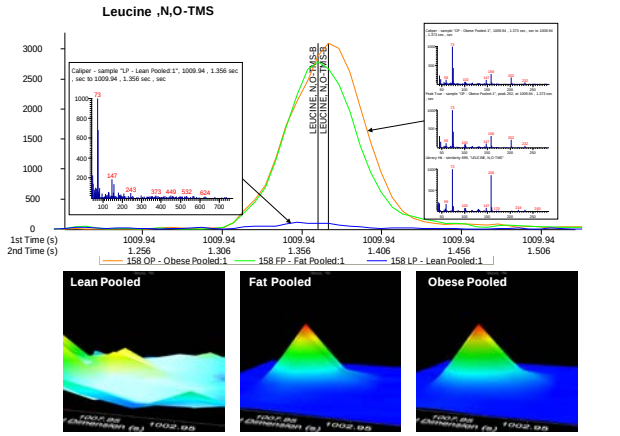
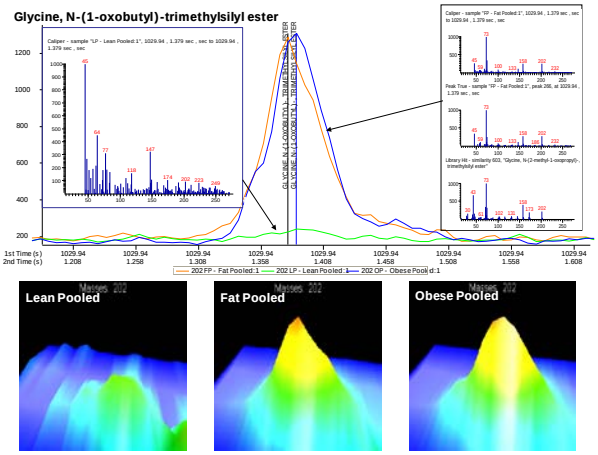
4. Results



Sample Pool Name	Peak #	Name	R.T. (s)	Area	Height	UniqueMass	Similarity	S/N	Library
Lean pooled	0	NOT FOUND	0	0	0	0	0	0	0
Fat pooled	260	LEUCINE,N,O-TMS	1009.94 , 1.366	28250	3317.2	158	715	278.61	Max Planck1
Obese pooled	254	LEUCINE,N,O-TMS	1009.94 , 1.373	33093	3885.1	158	699	312.62	Max Planck1

Figure 1. The figure above shows the overlaid linear unique mass ion chromatograms and 3D surface plots for Leucine, N,O-TMS in the lean, fat, and diabetic obese pooled samples. The mass spectra show that leucine was not found in the lean pooled sample while the mass spectra for the fat and obese pooled sample are identified as leucine. The 3D surface plot chromatograms for lean, fat, and obese pooled samples indicate that leucine is not found in the lean pooled sample and is upregulated in the fat and diabetic obese sample pools.



Sample Pool Name	Peak #	Name	R.T. (s)	Area	Height	UniqueMass	Similarity	S/N	Library
Lean pooled	0	NOT FOUND	0	0	0	0	0	0	0
Fat pooled	265	Glycine, N-(2-methyl-1-oxopropyl)-, trimethylsilyl ester	1029.94 , 1.379	9346.2	1094.9	202	603	118.16	mainlib
Obese pooled	260	GLYCINE, N-(1-OXOBUTYL)-, TRIMETHYLSILYL ESTER	1029.94 , 1.386	10781	1307.9	158	599	131.89	Wiley7

Figure 2. Figure 2 above shows the overlaid linear unique mass ion chromatograms and 3D surface plots for TMS-ester-oxobutyl-glycine in the lean, fat and diabetic obese pooled samples. The mass spectra show that TMS-ester-oxobutyl-glycine was not found in the lean pooled sample. However, it is identified in both the fat and obese pools. The 3D surface plots of the lean, fat, and obese pooled samples indicate that TMS-oxobutyl-glycine is upregulated in the fat and diabetic obese sample pools and not present in the lean sample pool.

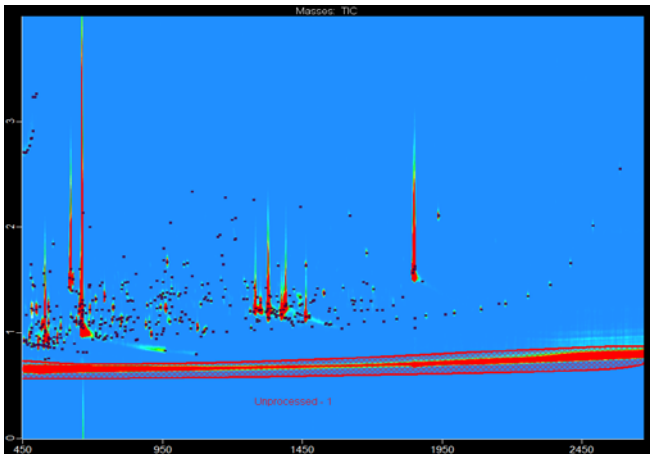
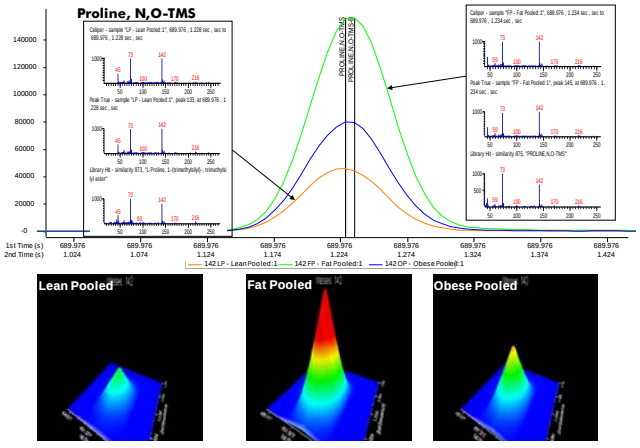


Figure 3. A two dimensional contour plot of the diabetic obese pooled sample is shown above in Figure 3. A total of 438 peaks were found with a S/N ratio of greater than or equal to 100. The GCxGC contour plot visually illustrates the enhanced peak capacity and resolution that is not possible by a one dimension separation.



Sample Pool Name	Peak #	Name	R.T. (s)	Area	Height	UniqueMass	Similarity	S/N	Library
Obese pooled	132	PROLINE,N,O-TMS	689.976 , 1.228	946687	82531	142	865	7498.5	Max Planck1
Lean pooled	132	L-Proline, 1-(trimethylsilyl)-, trimethylsilyl ester	689.976 , 1.228	531417	46136	142	873	4231.6	replib
Fat pooled	142	PROLINE,N,O-TMS	689.976 , 1.234	1828229	166557	142	879	14233	Max Planck1

Figure 4. Figure 4 above shows the overlaid linear unique mass ion chromatograms and 3D surface plots for N,O-TMS-Proline in the lean, fat and diabetic obese pooled samples. The mass spectra show that N,O-TMS-Proline was found in all 3 sample pools. The surface plots for lean, fat, and obese pooled samples indicate that N,O-TMS-Proline is upregulated from the lean to the fat sample pool and then downregulated in the diabetic obese sample pool.

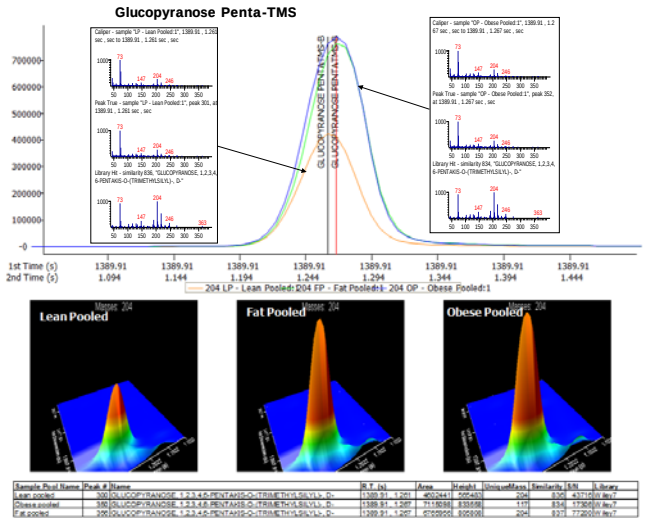
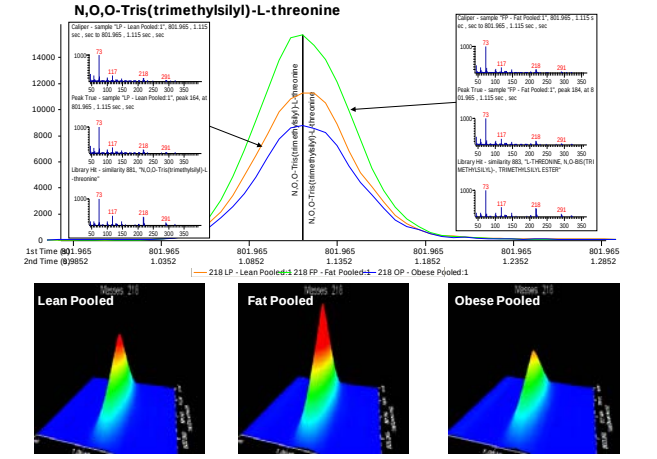


Figure 5. Figure 5 shows the overlaid linear unique mass ion chromatograms and 3D surface plots for Penta-TMS-Glucopyranose in the lean, fat, and diabetic obese pooled samples. The mass spectra show that Penta-TMS-Glucopyranose was found in all 3 sample pools. The surface plots for lean, fat, and obese pooled samples indicate the expected trend that Penta-TMS-Glucopyranose is upregulated from the lean to the fat and the obese diabetic sample pools.



Sample Pool Name	Peak #	Name	R.T. (s)	Area	Height	UniqueMass	Similarity	S/N	Library
Fat pooled	183	N,O,O-Tris(trimethylsilyl)-L-threonine	801.965 , 1.115	159764	15771	218	882	1627.2	replib
Obese pooled	177	N,O,O-Tris(trimethylsilyl)-L-threonine	801.965 , 1.115	91111	8767.4	218	882	907.12	replib
Lean pooled	164	N,O,O-Tris(trimethylsilyl)-L-threonine	801.965 , 1.115	115429	11304	218	881	1178.6	replib

Figure 6. Figure 6 shows the overlaid linear unique mass ion chromatograms and 3D surface plots for Tris-TMS-L-Threonine in the lean, fat and diabetic obese pooled samples. The mass spectra show that Tris-TMS-L-Threonine was found in all 3 sample pools. The surface plots for the lean, fat, and obese pooled samples indicate an interesting trend that Tris-TMS-L-Threonine is upregulated from the lean to the fat sample pool and downregulated to the least concentration in the diabetic obese sample pool.

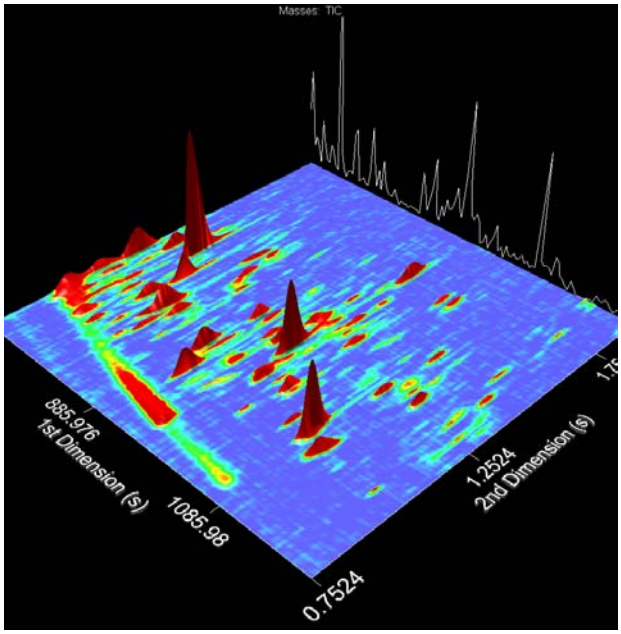


Figure 7. The three dimensional surface plot chromatogram shown above illustrates the increased peak capacity and resolution of comprehensive two-dimensional chromatography. Notice the separation of trace level peaks that are found in the second dimension plane. The white total ion chromatogram of the 1st dimension separation is shown in the top right-hand section of figure 7. This example clearly illustrates that GCxGC resolves and separates components that would otherwise be coeluted and buried under high concentration analytes by a one dimensional separation.

5. Conclusions

This study highlights the use of GCxGC-TOFMS analysis of pooled Zucker rat plasma samples from different states of health. The chromatographic and time-of-flight mass spectral comparison between sample pools establishes this analytical approach as a valuable screening tool for discovery of metabolite variation across different classes. The analysis of derivatized complex blood plasma demonstrates the enhanced detection and increased peak capacity available with GCxGC-TOFMS using the LECO Pegasus 4D instrument. The GCxGC-TOFMS results for the lean, fat, and obese sample pools demonstrates how GCxGC separates and resolves components that would otherwise totally coelute and be buried under high concentration analytes in one-dimensional chromatography. TOFMS provides non-skewed mass spectral data and the fast acquisition necessary to facilitate deconvolution algorithms that yield high quality library searchable mass spectra. This proof of concept research shows a fast, efficient, and simple comparative analysis method for screening large sample pools to distinguish metabolite variations as potential biomarkers for healthy and disease state populations. GCxGC-TOFMS is a valuable time and resource saving instrumental option for the comparative analysis of pooled samples from varying states of health to quickly and efficiently determine significant metabolic differences.

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6. References

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