The Development of Untargeted Metabolite Profiling Methodology for the Analysis of Type-2 Diabetes Patient Plasma

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EMPOWERING RESULTS
Elemental Analysis | GC Mass Spectrometry | Metallography

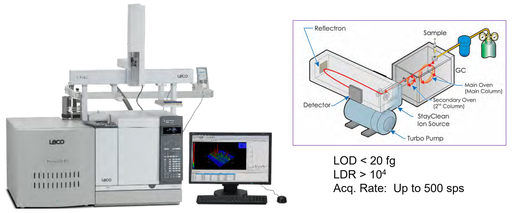
Introduction

- Greater than 400 million adults are living with diabetes worldwide
- Diabetes is a major cause of blindness, kidney failure, heart attacks, strokes, and lower limb amputations
- Type 2 diabetes (T2D) accounts for about 90% of cases
- Current T2D monitoring: 1) Blood glucose, 2) periodic glycated hemoglobin (HbA1c), and 3) self-reporting
- There is a critical need for T2D biomarkers for early disease detection

Objectives

1) To use automated sample preparation and GC- and GCxGC-TOFMS analysis to collect plasma sample data
2) To use statistical processing software for the identification of potential T2D biomarkers

Analytical Platform



LECO Pegasus® BT 4D

LOD < 20 fg
LDR > 10^4
Acq. Rate: Up to 500 sps

Figure 1. GC- and GCxGC-TOFMS Instrument.

Sample Extraction & Automated Derivatization

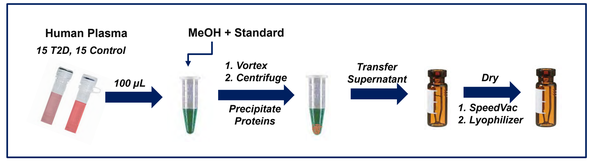


Figure 2. Plasma general extraction procedure.

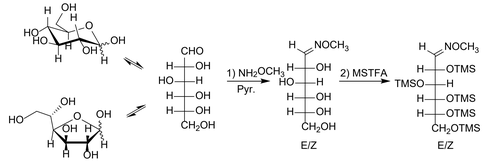
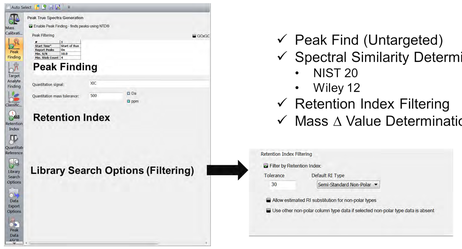


Figure 3. Two-step derivatization of glucose.

Instrument Acquisition Parameters & Data Processing

Table 1. BT 4D instrument acquisition parameters.

Gas Chromatograph	Agilent 7890 and L-PAL 3 Autosampler
Injection	1.0 µL (Split 30:1; 250 °C)
Carrier Gas	He @ 1.4 mL/min, Constant Flow
Columns (1st Dimension) (2nd Dimension)	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm (Restek) Rxi-17 sil ms 0.6 m x 0.25 mm i.d. x 0.25 µm (Restek)
Temperature Program	50 °C (1 min), ramped 5 °C/min to 150 °C (2 min), ramped 10 °C/min to 300 °C (12 min) Secondary oven maintained +5 °C relative to primary oven
Modulation	3 s with temperature maintained +15 °C relative to secondary oven
Mass Spectrometer	**LECO *Pegasus* BT 4D**
Ion Source Temperature	250 °C
Ionization Mode	EI
Mass Range (m/z)	45-650
Acquisition Rate	1D: 12 spectra/s; 2D: 200 spectra/s



- ✓ Peak Find (Untargeted)
- ✓ Spectral Similarity Determinations
  - NIST 20
  - Wiley 12
- ✓ Retention Index Filtering
- ✓ Mass Δ Value Determinations

Figure 4. Untargeted processing of comprehensive data.

Results and Discussion

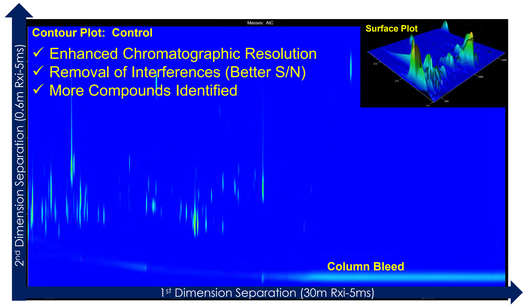


Figure 5. EI contour plot for a control sample.

GC → GCxGC-TOFMS Data: More Metabolites Identified

Table 2. Database search results for GC- and GCxGC-TOFMS of a control plasma sample.

Name	GC-TOFMS	GCxGC-TOFMS
Aspartic acid, 3TMS	662	897
Methionine, 2TMS		902
Creatinine enol, 3TMS		786
Phenylalanine, 2TMS	747	901
Galactopyranose, 5TMS		791
Ornithine, 4TMS	762	922
1,5-Anhydroglucitol, 4TMS	935	932
2'-Hydroxy-5'-methylacetophenone, TMS		767

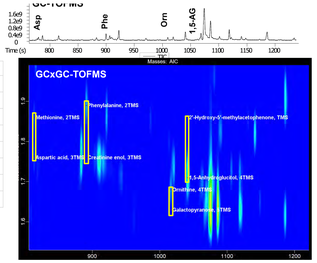


Figure 6. A comparison of a GC-TOFMS TIC and GCxGC-TOFMS contour plot for a control Plasma sample.

Table 3. Representative compounds in a control plasma sample.

Name	Formula	R.T. (s)	Similarity
Lactic Acid, 2TMS	$C_9H_{22}O_3Si_2$	430, 1.743	916
Glycolic acid, 2TMS	$C_8H_{20}O_3Si_2$	442.5, 1.742	910
Benzoic acid, methyl ester	$C_8H_8O_2$	462.5, 2.250	939
L-Alanine, 2TMS	$C_9H_{23}NO_2Si_2$	467.5, 1.704	927
2-Hydroxybutyric acid, 2TMS	$C_{10}H_{24}O_3Si_2$	492.5, 1.707	917
Oxalic acid, 2TMS	$C_8H_{18}O_4Si_2$	495, 1.853	898
Sarcosine, 2TMS	$C_9H_{23}NO_2Si_2$	500, 1.726	882
2-Ethylhexanoic acid, TMS	$C_{11}H_{24}O_2Si$	522.5, 1.739	869
α-Hydroxyisovaleric acid, 2TMS	$C_{11}H_{26}O_3Si_2$	525, 1.699	938
2-Aminobutanoic acid, 2TMS	$C_{10}H_{25}NO_2Si_2$	532.5, 1.702	918
2-Ketoisocaproic acid, TMS	$C_{10}H_{20}O_3Si$	567.5, 1.894	908
L-Valine, 2TMS	$C_{11}H_{25}NO_2Si_2$	570, 1.703	922
Benzoic Acid, TMS	$C_{10}H_{14}O_2Si$	595, 2.023	898
Niacin, TMS	$C_9H_{13}NO_2Si$	615, 2.054	845
L-Leucine, 2TMS	$C_{12}H_{27}NO_2Si_2$	617.5, 1.704	935
L-Isoleucine, 2TMS	$C_{12}H_{27}NO_2Si_2$	635, 1.708	911
Benzeneacetic acid, TMS	$C_{11}H_{16}O_2Si$	637.5, 2.067	830
L-Proline, 2TMS	$C_{11}H_{23}NO_2Si_2$	640, 1.794	894
Glycine, 3TMS	$C_{11}H_{29}NO_2Si_3$	647.5, 1.714	911
Succinic acid, 2TMS	$C_{10}H_{22}O_4Si_2$	650, 1.850	913
Glyceric acid, 3TMS	$C_{12}H_{30}O_4Si_3$	667.5, 1.714	909
Nonanoic acid, TMS	$C_{12}H_{26}O_2Si$	685, 1.801	864
Serine, 3TMS	$C_{12}H_{29}NO_3Si_3$	692.5, 1.698	909
N-Formylglycine, 2TMS	$C_8H_{17}NO_3Si_2$	702.5, 1.989	871
L-Threonine, 3TMS	$C_{13}H_{31}NO_3Si_3$	712.5, 1.688	927
Glutaric acid, 2TMS	$C_{11}H_{24}O_4Si_2$	720, 1.852	871
(R)-5-Methylhydantoin, 2TMS	$C_{10}H_{20}N_2O_2Si_2$	725, 1.978	863
2-Deoxytetronic acid, 3TMS	$C_{13}H_{30}O_4Si_3$	747.5, 1.706	893
Decanoic acid, TMS derivative	$C_{13}H_{28}O_2Si$	760, 1.804	877
2-Aminomalonic acid, 3TMS	$C_{12}H_{29}NO_4Si_3$	777.5, 1.807	910
Niacinamide, TMS	$C_9H_{14}N_2OSi$	780, 2.305	884
Timonacic, 2TMS	$C_{10}H_{21}NO_2SSi_2$	782.5, 1.945	853
Malic acid, 3TMS derivative	$C_{13}H_{30}O_5Si_3$	790, 1.753	921
Pyroglutamic acid, TMS derivative	$C_8H_{15}NO_3Si$	792.5, 2.397	896
Adipic acid, 2TMS	$C_{12}H_{26}O_4Si_2$	797.5, 1.847	842
Erythritol, 4TMS	$C_{16}H_{38}O_4Si_4$	807.5, 1.621	931
Aspartic acid, 3TMS	$C_{13}H_{29}NO_4Si_3$	812.5, 1.759	897
Methionine, 2TMS	$C_{11}H_{25}NO_2SSi_2$	812.5, 1.857	902
cis-4-Hydroxy-L-proline, 3TMS	$C_{14}H_{31}NO_4Si_3$	817.5, 1.727	808
L-Cysteine, 3TMS	$C_{12}H_{29}NO_2SSi_3$	840, 1.785	899
Anthranilic acid, 2TMS derivative	$C_{13}H_{23}NO_2Si_2$	880, 1.966	784
L-Glutamic acid, 3TMS	$C_{14}H_{31}NO_4Si_3$	882.5, 1.763	916
Creatinine enol, 3TMS	$C_{10}H_{23}N_3O_2Si_3$	890, 1.761	786
Phenylalanine, 2TMS	$C_{15}H_{25}NO_2Si_2$	890, 1.893	901
Paracetamol, 2TMS	$C_{14}H_{23}NO_2Si_2$	892.5, 1.894	899
Taurine, 3TMS	$C_{11}H_{31}NO_3SSi_3$	920, 1.865	842
L-Lysine, 3TMS	$C_{15}H_{34}N_2O_2Si_3$	942.5, 1.714	836
Cotinine	$C_{14}H_{14}N_2O$	947.5, 3.083	922
Adonitol, 5TMS	$C_{19}H_{46}O_5Si_5$	952.5, 1.608	820
Xylitol, 5TMS	$C_{19}H_{46}O_5Si_5$	960, 1.600	925
L-Glutamine, 3TMS	$C_{14}H_{32}N_2O_3Si_3$	985, 1.855	884
9H-Purin-6-ol, 2TMS	$C_{11}H_{20}N_4OSi_2$	1007.5, 2.202	891
L-3-Methylhistidine, 2TMS derivative	$C_{13}H_{23}N_3O_2Si_2$	1010, 2.173	829
Ornithine, 4TMS	$C_{17}H_{46}N_2O_2Si_4$	1015, 1.684	922
Citric acid, 4TMS	$C_{18}H_{44}O_7Si_4$	1020, 1.736	870
Myristic acid, TMS	$C_{17}H_{34}O_2Si$	1025, 1.809	917
Hippuric acid, TMS	$C_{12}H_{17}NO_3Si$	1025, 2.398	803
1,5-Anhydroglucitol, 4TMS	$C_{18}H_{44}O_5Si_4$	1040, 1.713	932
2'-Hydroxy-5'-methylacetophenone, TMS	$C_{14}H_{22}O_3Si$	1040, 1.864	767
Tyrosine, 2TMS	$C_{15}H_{25}NO_3Si_2$	1055, 2.029	798
α-D-Mannopyranose, 5TMS	$C_{21}H_{54}O_6Si_5$	1067.5, 1.657	870
Galactose, MOX 5TMS	$C_{22}H_{55}NO_6Si_5$	1075, 1.630	913
Histidine, 3TMS	$C_{15}H_{31}N_3O_2Si_3$	1080, 1.961	864
Glucose, (1Z)-, MOX 5TMS	$C_{22}H_{55}NO_6Si_5$	1085, 1.634	913
L-Tyrosine, 3TMS	$C_{18}H_{33}NO_3Si_3$	1090, 1.824	924
D-Mannitol, 6TMS	$C_{24}H_{62}O_6Si_6$	1092.5, 1.587	928
Ethyl α-D-glucopyranoside, 4TMS	$C_{20}H_{48}O_6Si_4$	1092.5, 1.682	874
Glucuronic acid, MOX 5TMS	$C_{22}H_{53}NO_7Si_5$	1097.5, 1.668	816
Glucopyranose, 5TMS	$C_{21}H_{54}O_6Si_5$	1117.5, 1.633	927
Palmitelaidic acid, TMS	$C_{19}H_{36}O_2Si$	1130, 1.856	909
Xanthine, 3TMS	$C_{17}H_{36}N_4O_2Si_3$	1137.5, 2.138	792
Palmitic Acid, TMS	$C_{19}H_{40}O_2Si$	1140, 1.821	920
5-Hydroxytryptophan, 4TMS	$C_{23}H_{48}N_2O_3Si_4$	1142.5, 2.051	832
L-Citrulline, 3TMS	$C_{18}H_{41}N_3O_3Si_3$	1180, 1.997	813
Scyllo-Inositol, 6TMS	$C_{24}H_{60}O_6Si_6$	1185, 1.616	898
O-Desmethylnaproxen, 2TMS	$C_{19}H_{26}O_3Si_2$	1212.5, 2.102	853
Indole-3-lactic acid, 3TMS	$C_{20}H_{33}NO_3Si_3$	1225, 1.999	824
9-Octadecenoic acid, (E)-, TMS	$C_{21}H_{42}O_2Si$	1232.5, 1.871	858
Stearic acid, TMS	$C_{21}H_{42}O_2Si$	1245, 1.831	901
L-Tryptophan, 3TMS	$C_{20}H_{36}N_2O_2Si_3$	1247.5, 1.992	921
Methyl linoleiadate	$C_{19}H_{34}O_2$	1255, 1.925	808
L-Cystine, 4TMS	$C_{24}H_{56}N_2O_4S_2Si_4$	1285, 1.825	860
Benzoylecgonine, TMS	$C_{19}H_{27}NO_4Si$	1292.5, 2.452	891
Arachidonic acid, TMS	$C_{23}H_{40}O_2Si$	1312.5, 1.961	903
Myo-Inositol, phosphate, 7TMS	$C_{27}H_{67}O_9PSi_7$	1355, 1.674	853
Uridine, 4TMS	$C_{21}H_{48}N_2O_6Si_4$	1360, 1.929	808
Uridine, 3TMS	$C_{18}H_{36}N_2O_6Si_3$	1362.5, 2.017	798
Docosanexent, TMS	$C_{25}H_{42}O_2Si$	1402.5, 2.035	913
1-Monopalmitin, 2TMS	$C_{25}H_{54}O_4Si_2$	1415, 1.808	838
Sucrose, 8TMS	$C_{24}H_{62}O_{11}Si_8$	1460, 1.614	831
γ-Tocopherol, TMS	$C_{31}H_{54}O_2Si$	1597.5, 2.213	804
Cholest-5-en-3-ol, (3α)-, TMS	$C_{30}H_{54}OSi$	1692.5, 2.658	843

Ave.= 878

Compound Identification

1) Database Comparisons + Mass Delta Calculations

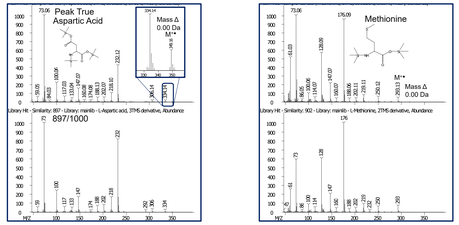


Figure 7. Peak True (Deconvoluted) and library mass spectra for aspartic acid (left) and methionine (right). The spectral similarity values were 897 and 902/1000 respectively. The mass delta values for the molecular ions was zero.

Compound Identification

2) Database Comparisons + Mass Delta Calculations + RI Filtering

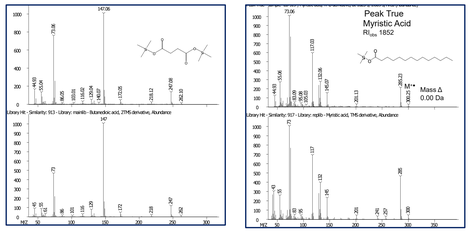


Figure 8. Peak True (Deconvoluted) and library mass spectra for succinic acid (left) and myristic acid (right). The spectral similarity values were 913 and 917/1000 respectively. The mass delta values for the molecular ions was zero. The observed RI values were 1319 (Library RI = 1321) and 1852 (Library RI = 1850) respectively.

Statistical Processing

analytical chemistry
Article
Tile Based Fisher Ratio Analysis of Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (GC×GC-TOFMS) Data using a Null Distribution Approach
Brendon A. Parsons, Luke C. Marney, W. Christopher Siegler, Jamin C. Hoggard, Bob W. Wright, and Robert E. Synovec
Anal. Chem. **2015**, *87*, 7, 3812-3819.

$$Fisher\ Ratio = \frac{\sigma^2_{cl}}{\sigma^2_{err}} = \frac{class\ to\ class\ variation}{within\ class\ variation}$$

Figure 9. ChromaTOF® Tile statistical processing software.

Statistical Results

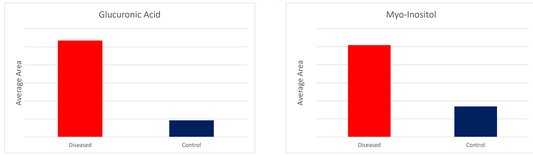


Figure 10. ChromaTOF Tile results showing elevated levels of glucuronic acid and myo-inositol in diseased samples.

Summary

- ✓ We developed analytical methodology for the analysis of T2D plasma samples.
- ✓ The method included automated sample derivatization, data collection, and processing, as well as statistical analysis with *ChromaTOF* Tile software.