GCxGC-TOFMS Analysis of Mouse Plasma Extracts to Determine Metabolite Profiles From a Traumatic Brain Injury Study

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INTRODUCTION

Comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry has gained recognition as a principal technology for profiling semi-volatile and volatile small molecule metabolites for specific disease states. GCxGC-TOFMS provides an analysis platform that can resolve and identify metabolites even in complex sample matrices. Traditionally, analysis of volatile and semi-volatile compounds found in metabolomic samples has been performed with gas chromatography and mass spectrometry (GC-MS). This study utilizes GCxGC-TOFMS for the analysis of mouse plasma extracts from an experimental and control group of a traumatic brain injury model. Four experimental and four normal control samples were prepared by addition of a four-component internal standard mixture, followed by extraction with methanol. Subsequently, the extracted samples were dried to completion, then derivatized with 50 µL BSTFA plus 1% TMCS at 60°C for 1 hour prior to triplicate analyses by GCxGC-TOFMS. GCxGC provided enhanced chromatographic resolution and peak capacity to successfully resolve complex metabolite profiles. TOFMS provided relative quantitation between the experimental and control groups, as well as library searchable mass spectral data needed for biomarker identification. TOFMS provides the acquisition rates (up to 500 Hz) necessary for thermally modulated GCxGC in addition to non-skewed mass spectra, allowing optimal performance of deconvolution algorithms. The results from this comparative study between normal and brain injured mice present the benefits of GCxGC-TOFMS to characterize and discover potential metabolite variation between normal and diseased states.

EXPERIMENTAL APPROACH

A comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry analysis was conducted using a LECO Pegasus® 4D GCxGC-TOFMS instrument. A total of 8 mouse brain sample extracts—4 controls and 4 traumatic brain injured—were received. The GCxGC-TOFMS analysis was performed in triplicate for each sample, following derivatization per the protocol provided.

SAMPLE PREPARATION

Eight samples were derivatized by injecting 50 µL of BSTFA +1% TMCS into each sample vial. The vials were first vortexed and then heated at 80°C for 60 minutes. The vials were placed in an autosampler tray for 24 hours prior to analysis by GCxGC-TOFMS.

METHODS

GCxGC–TOFMS Analysis Parameters

- Gas Chromatograph: Agilent 7890 equipped with a LECO dual stage, quad jet thermal modulator, secondary oven, and a GERSTEL MPS2 autosampler
- GC Primary Column: 30 m x 0.25 mm id. x 0.25 µm film thickness Rxi-5SilMS (Restek Corp.)
- GC Secondary Column: 1.25 m x 0.25 mm id. x 0.25 µm film thickness Rxi-17Sil-MS (Restek Corp.)
- Carrier Gas: Helium set @ 1.5 mL/min
- Injection Mode: Splitless
- Injection Volume: 1 µL
- Inlet Temperature: 275°C
- Primary Column Temperature Program: Initial temperature 50°C for 0.5 min ramped @ 5.0°C/min to 300°C, held for 6 min
- Secondary Column Temperature Program: Initial temperature 55°C for 0.5 min ramped @ 5.0°C/min to 305°C, held for 6 min
- GCxGC Modulator Temperature Offset: 30°C
- Modulation Period: 4.0 s
- Hot Pulse: 0.5 s
- Cool Time Between Stages: 1.5 s
- Transfer Line Temperature: 280°C
- Total Run Time: 56.50 min

Mass Spectrometer: Pegasus® 4D TOFMS Analysis Parameters

- Acquisition Delay: 450 s
- Mass Range : 45–700 m/z
- Acquisition Rate: 200 spectra/s
- Ion Source Temperature: 230°C

True Signal Deconvolution®

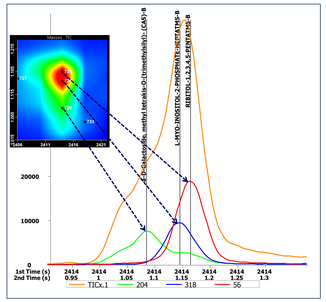

Figure 1. A truly unskewed mass spectral signal is produced when the entire mass range is sampled from the ion source at the same instant in time as conducted with simultaneous sampling obtained with time-of-flight mass spectrometry. The contour plot above shows peaks labeled 729, 730, and 731. The linear 1st dimension chromatogram illustrates the total ion chromatogram and the deconvolution of unique masses for three identified analytes within a time window of approximately 80 milliseconds.

RESULTS

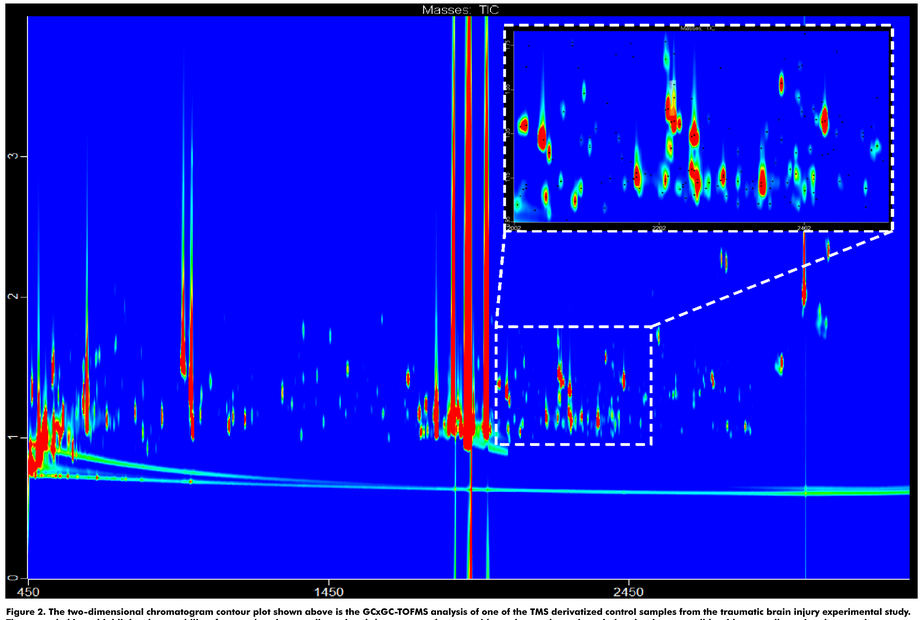

Figure 2. The two-dimensional chromatogram contour plot shown above is the GCxGC-TOFMS analysis of one of the TMS derivatized control samples from the traumatic brain injury experimental study. The expanded inset highlights the capability of comprehensive two-dimensional chromatography to provide peak capacity and resolution that is not possible with a one-dimensional separation.

Three Analytes Identified in an 80 Millisecond Retention Window

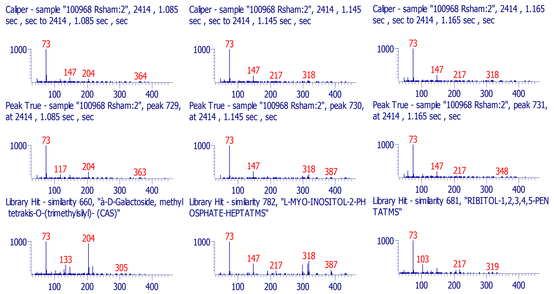

Figure 3. The mass spectral series above displays the identifications from the deconvolution example illustrated in Figure 1. The Caliper (raw) mass spectrum for each of the three peaks are displayed at the top of each series. The middle spectra for each peak are the Peak True (deconvoluted) spectra. The bottom mass spectra in the series are the Library Hits. Notice that the Caliper mass spectra for each peak are very similar. The three peaks were identified with confidence values of greater than 66% in approximately 80 milliseconds between peak apices.

GCxGC Expanded Peak Capacity

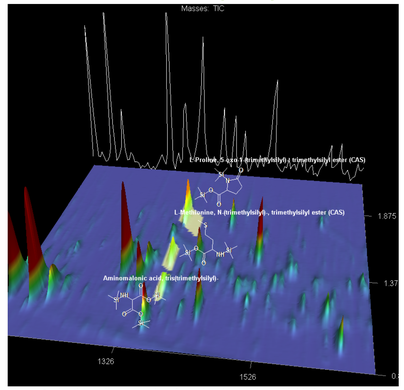

Figure 4. The three-dimensional surface plot shown above highlights aminomalonic acid-3TMS, L-Methionine-2TMS, and oxo-L-Proline-2TMS, which are baseline resolved in the second dimension separation. Notice that the white linear first dimension trace in the background illustrates that these three distinct analytes would be totally coeluted by one-dimensional chromatography.

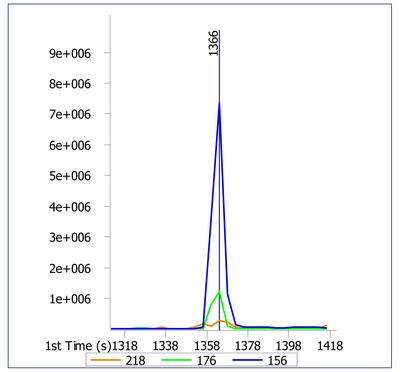

Figure 5. Shown above is a depiction of the reconstructed first-dimension chromatogram for aminomalonic acid-3TMS, L-Methionine-2TMS, and oxo-L-Proline-2TMS. In the first dimension separation, all three metabolites are eluting at the same retention time, 1366s. This example illustrates how comprehensive two-dimensional chromatography separates analytes that would otherwise be buried and masked by higher concentration components.

Table 1. Peak Table for the amino acid metabolites illustrated in Figures 4 and 5. The peak area for 5-oxo-Proline-2TMS is 26 times greater than the peak area for Aminomalonic acid - 3TMS highlighting the value of the two-dimensional separation

Peak #	Name	R.T. (s)	Similarity	Area	Height	UniqueMass	S/N	Library
260	Aminomalonic acid, tris(trimethylsilyl)-	1366 , 1.220	681	455186	28135	218	1738.8	mainlib
261	L-Methionine, N-(trimethylsilyl)-, trimethylsilyl ester (CAS)	1366 , 1.420	904	2064635	119370	176	10686	Wiley9
262	L-Proline, 5-oxo-1-(trimethylsilyl)-, trimethylsilyl ester (CAS)	1366 , 1.760	893	11985408	486265	156	32623	Wiley9

Metabolites Of Change (Control Vs. TBI)

Table 2 shown below is a peak list of sixteen identified metabolites that were determined to show variance between the control and traumatic brain injured sample classes. The list is comprised mainly of trimethylsilyl derivatized amino acids, as well as several sugars. Amino acid levels such as glutamate, taurine, and aspartate are known to show increased levels in TBI individuals. The metabolites listed illustrate the capability of comprehensive GCxGC-TOFMS analysis to detect and differentiate potentially important biomarkers of a disease process.

Table 2. Metabolites displaying variance between Control and TBI models

Name	R.T. (s)	Similarity
L-Alanine, N-(trimethylsilyl)-, trimethylsilyl ester (CAS)	722 , 1,115	859
L-VALINE, N-(TRIMETHYLSILYL)-, TRIMETHYLSILYL ESTER	902 , 1,150	907
L-Isoleucine, N-(trimethylsilyl)-, trimethylsilyl ester (CAS)	1026 , 1,160	910
Serine trims	1130 , 1,155	924
N,O,O-Tris(trimethylsilyl)-L-threonine	1170 , 1,135	926
L-Methionine, N-(trimethylsilyl)-, trimethylsilyl ester (CAS)	1366 , 1,415	915
L-Proline, 1-(trimethylsilyl)-4-[(trimethylsilyl)oxy]-, trimethylsilyl ester, trans- (CAS)	1378 , 1,190	822
L-Aspartic acid, N-(trimethylsilyl)-, bis(trimethylsilyl) ester (CAS)	1386 , 1,260	729
L-Glutamic acid, N-(trimethylsilyl)-, bis(trimethylsilyl) ester (CAS)	1514 , 1,255	790
N,O-Bis-(trimethylsilyl)phenylalanine	1514 , 1,490	872
L-(-)-Arabitol, pentakis(trimethylsilyl) ether	1650 , 0,995	917
l-Glutamine, tris(trimethylsilyl) deriv. (CAS)	1710 , 1,420	878
Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	2086 , 1,045	922
TRYPTOPHAN 2TMS	2198 , 2,145	832
Uridine, 2',3',5'-tris-O-(trimethylsilyl)-	2446 , 1,735	709
D-Mannitol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	2486 , 1,020	856

CONCLUSIONS

This study between normal control and traumatic brain injury mouse extract samples demonstrates that comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry (GCxGC-TOFMS) is an ideal instrumental platform for identification of the small molecule metabolite profile in complex biological samples. GCxGC-TOFMS analysis was conducted in triplicate for 8 samples from 4 normal control mouse brain extracts and 4 diseased traumatic brain injured mouse brain extracts. True Signal Deconvolution is illustrated in Figure 1 by the contour plot and linear unique mass ion traces identifying 3 distinct analytes in approximately 80 milliseconds. Figure 3 shows the time-of-flight mass spectral data of the Figure 1 deconvolution example with library search matches of greater than 66%. The increased peak capacity, enhanced resolution, and improved peak detectability that comprehensive GCxGC offers is demonstrated with greater than 1000 peaks found per sample with a S/N of ≥300. The benefit of GCxGC to separate analytes that would otherwise be buried and undetected by conventional one-dimensional methods is illustrated by examples in Figures 4 and 5. The results in Table 2 list the metabolites found to have the highest variation between the normal and injured sample classes, validating the capability of the comprehensive GCxGC-TOFMS platform to detect and differentiate potentially important biomarkers of a diseased state.

For further information regarding the results obtained in this study, please contact the authors at john_heim@leco.com.

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