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Metabolomics Study - Human Breast Cancer Tissue Analysis by Comprehensive 2D-GC Coupled with High Resolution and High Speed Time-of-Flight Mass Spectrometry

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Introduction

Human breast cancer tissue may have more than 2,000 metabolites. In order to distinguish the cancer grades, both qualitative and quantitative analysis of those metabolites are critical. Comprehensive 2D GC (GC x GC) is the only technology so far that can provide the separation power needed for this analysis. GCxGC is an advanced technology for chemical separations that provides significant improvements over traditional one-dimensional gas chromatography (GC), including an order-of-magnitude increase in chemical separation capacity, multidimensional ordering by chemical properties, and a significant increase in signal-to-noise ratio [1, 2]. Coupling the GC×GC to a high speed time-of-flight mass spectrometer (TOFMS), yields an increase in information over a GC×GC–FID instrument, thus making the GC×GC–TOFMS more ideal to study complex samples. GC×GC x TOFMS instrumentation provides a third dimension of data that allows the analyst to more readily distinguish compounds [3]. Here, we report a method using Zoex novel high resolution and high speed *FasTOF* ™ GC x GC x TOFMS system for both qualitative and quantitative analysis of human breast cancer tissue samples.

Experimental

GC x GC x TOFMS system consists of an Agilent 7890A GC, a Zoex ZX-2 closed cycle refrigerated loop modulation system, and a Zoex *FasTOF* ™ high resolution time-of-flight mass spectrometer. The primary GC column is an HP-1MS (10m x 0.25mm, 1 um film thickness). The secondary column is a BPX-50 (1m x 0.1mm, 0.1 um film thickness). The oven temperature was ramped from 40 °C to 310 °C with 3.1 °C/min rate. Helium was served as carrier gas. The injection port was held at 300 °C with a pressure ramp of 0.35 psi/min from 45 psi to 75 psi. The modulation period and the modulation duration are 6 s and 260 ms, respectively. The TOF MS was tuned to achieve 6,000 mass resolution (FWHM) with standard EI source. The data acquisition rate was set at 100 Hz. All data was processed by Zoex GC Image™ software.

The breast cancer tissue sample extracts were courtesy of Dr. Oliver Fiehn at the University of California at Davis. All sample extracts were fresh derivatized by MSTFA and 1 µL was injected with splitless mode.

Results and Discussion

Figure 1 shows the two-dimensional total ion chromatogram for one human breast cancer tissue sample obtained by Zoex *FasTOF* ™ GC x GC x TOFMS system. Over 2,000 components were separated and detected. The marked peaks represent identified compounds listed on table 1. Figure 1b shows the details in the rectangular area in Figure 1a. Combining NIST library search and elemental composition estimation by HR-TOFMS data, nearly 35 different compounds listed in Table 1 were identified.

Total 12 samples in 3 different breast cancer stages were analyzed. External standard quantitation results for identified 35 components are listed in Table 1. Figure 2 shows the external standard calibration curve for one of the components, lysine. Excellent linearity was obtained with $R^2 = 0.9957$.

Ongoing Work

The accurate mass measurement capability in *FasTOF*™ allows us to estimate the elemental composition for all ions detected. We are trying to identify more components in the samples which mass spectra may not present in NIST library.

By comparing all current 12 TIC images in GC Image™ software, we have found that several metabolites successively disappear as the cancer grade advances (images are not showed). We are developing more sophisticated software to do image comparison statistically.

Conclusion

GC x GC x TOFMS provides three dimensions for sample analysis. The accurate mass measurement and elemental composition estimation for high resolution time-of-flight mass spectrometry offers additional dimension for analyzing complex samples, such as human breast cancer tissue. The combination of GC x GC and high resolution and high speed time-of-flight mass spectrometry makes it a powerful tool for metabolomics study.

Acknowledgement

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References

[1]Wolfgang Bertsch. Two-dimensional gas chromatography. Concepts, instrumentation, and applications — Part 2: Comprehensive two-dimensional gas chromatography. Journal of High Resolution Chromatography, 23(3):167–181, 2000.
[2] Lourdes Ramos. Comprehensive Two Dimensional Gas Chromatography. Elsevier, Oxford UK, 2009.

[3] Rachel E. Mohler, et al. Identification and evaluation of cycling yeast metabolites in two-dimensional comprehensive gas chromatography–time-of-flight-mass spectrometry data Journal of Chromatography A, 1186 (2008) 401–411

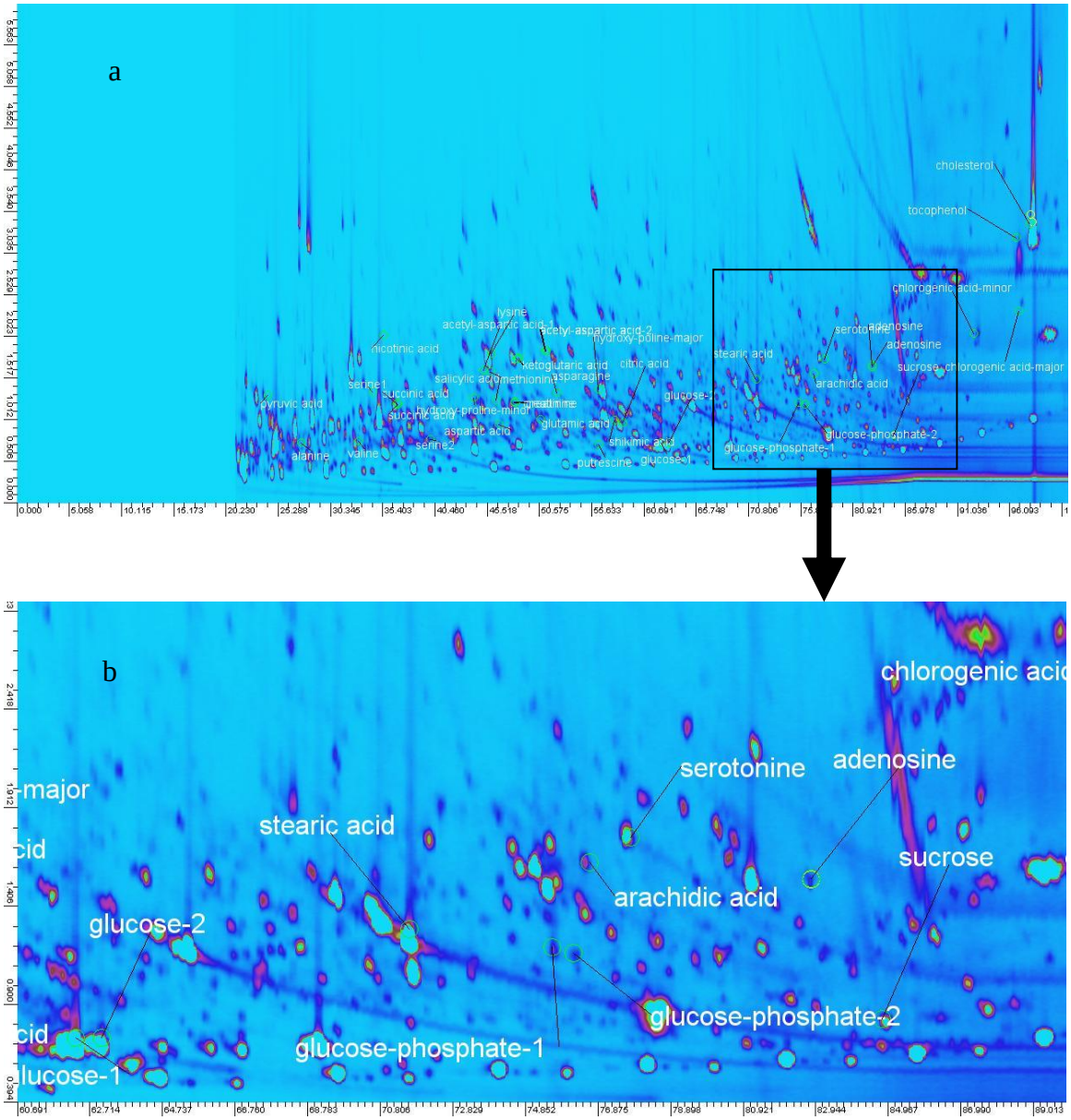

Fig 1a. GC x GC total ion chromatogram for a breast cancer tissue sample
Fig 1b. The details in the rectangular area in Figure 1a.

Table I: Compound Amounts in µg/mL

Compounds	Grade 3	Grade 3	Grade 3	Grade 3	Grade 2	Grade 2	Grade 2	Grade 2	Grade 1	Grade 1	Grade 1	Grade 1
acetyl-aspartic acid-1	1.199	1.211	1.386	3.513	1.157	1.153	1.247	1.627	0.33	1.283	1.137	1.326
acetyl-aspartic acid-2	0.502	0.490	0.697	0.507	0.496	0.489	0.504	1.263	1.55	0.495	0.513	0.631
adenosine	1.188	1.182	1.182	1.166	1.296	1.180	1.177	1.166		1.166	1.166	1.166
alanine	1.371	1.359	1.283			1.008	0.864	2.702	1.82	2.883		2.385
arachidic acid	0.040	0.080	0.055	1.540	0.024	0.071	0.077	0.099	0.31	0.089	0.038	0.163
asparagine	3.358	7.871	1.750	1.750	1.750	1.750	1.750	1.750	0.00	1.750	1.750	1.750
aspartic acid	0.782	0.800	0.799	0.695	0.936	0.762	0.768	1.164	0.52	0.825	0.717	0.746
chlorogenic acid-major												
chlorogenic acid-minor												
cholesterol	10.394	8.543	6.726	30.582	7.895	9.252	9.786	13.749	0.43	12.871	2.086	10.656
citric acid	0.243					0.041	0.081	0.002		0.223		0.093
creatinine				1.121	0.925			2.950				0.890
glucose-1		0.406			0.251		0.723	0.136		1.448		
glucose-2	0.762	1.371	0.735	0.762	1.259	0.352	1.642	1.167		2.078	0.824	0.975
glucose-phosphate-1	0.858	0.843	0.857	0.849	0.861	0.844	0.833	0.833	0.00	0.876	0.848	0.881
glucose-phosphate-2	0.946	1.365	0.856	0.856	0.856	0.976	1.303	1.252		0.856	0.856	0.856
glutamic acid	6.897	2.937	2.588	0.666	4.022	1.679	4.102	6.562	1.46	6.927	1.070	3.625
hydroxy-poline-major	1.481	1.481	1.481	1.481	1.481	1.481	1.481	1.481	0.00	1.481	1.481	1.481
hydroxy-proline-minor												
ketoglutaric acid	0.802	0.802	0.802	0.802	0.802	0.817	0.836	0.802		0.802	0.802	0.802
lysine	5.517	3.318	3.256	8.477	3.335	1.967	3.201	5.352	1.09	8.170	1.468	3.597
methionine	0.859	0.867	0.859	0.843	0.872	0.855	0.866	0.914	0.06	0.873	0.849	0.870
nicotinic acid	1.051	1.051	1.055	1.083	1.064	1.062	1.051	1.051	0.00	1.083	1.058	1.082
putrescine												
pyruvic acid	2.409	2.516	2.365	2.702	2.394	2.260	2.366	2.701	0.15	2.547	2.268	2.492
salicylic acid	2.036	2.036	2.036	2.036	2.055	2.041	2.036	2.036	0.00	2.036	2.042	2.041
serine1	1.386	1.386	1.386	1.386	1.386	1.386	1.386	1.386	0.00	1.386	1.386	1.386
serine2	0.484	0.598	0.467	0.354	0.500	0.414	0.482	1.119	1.54	0.732	0.378	0.517
serotonine	1.934	1.934	1.934	1.934	1.934	1.934	1.934	1.934	0.00	1.934	1.934	1.934
shikimic acid												
stearic acid	1.091	4.037		132.343			3.340					4.956
succinic acid	0.894	0.811	1.081	5.099	0.695	0.437	0.587	1.332	1.71	2.603	0.388	1.190
sucrose												
tocophenol	2.390	1.474	5.564	7.068	2.528	1.275	16.802	1.984		1.862	1.340	2.679
valine	0.113							0.519		0.464		0.070

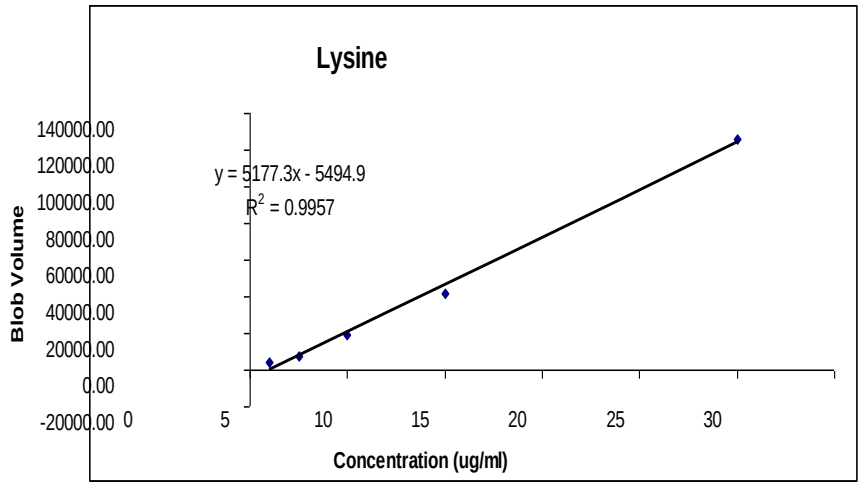

Figure 2. The external standard calibration curve for lysine