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Optimized Column Selectivity for Orthogonal Separation of Fatty Acid Methyl Esters (FAMEs) Using GCxGC

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Introduction

Gas chromatography coupled to mass spectrometry is a powerful tool often used to resolve volatile and semi-volatile analytes. An advanced version of GC is GCxGC, which employs two GC columns of complementary selectivity. As shown in **Figure 1**, the columns are placed in two separate GC ovens and are connected in series to enhance GC separation power. A cryo-focusing modulator serves as the heart of GCxGC separations; it slices the peak produced by the first column and focuses it on the head of the second column, which provides a comprehensive separation of analytes in both dimensions. Generally, a relatively long column (30 meter) is chosen for the first dimension and for the second dimension, a 1 to 2 meter column with complementary selectivity is considered. Based on the goal of analysis, either a polar primary column and a non-polar secondary column, or a non-polar primary column and polar secondary column can be used. The best separation power is realized if the columns offer complementary selectivity. Presented here is an analysis of FAMEs, where a Zebron™ ZB-FAME column, 30 meter x 0.25 mm x 0.20 µm, is used as a first dimension and a Zebron ZB- 5MS*PLUS*™ column, 1.5 meter x 0.25 mm x 0.25 µm, is used as the second dimension.

Materials and Methods

Sample Preparation

The analytical 37 FAME standard mix was purchased from Supelco®. The mix was dissolved/present in dichloromethane, with analytes in varied concentrations from 200 to 400 µg/mL.

GC Conditions

	Primary Column	Secondary Column
Columns:	Zebron ZB-FAME	Zebron 5MS*PLUS*
Dimensions:	30 meter x 0.25 mm x 0.20 µm	1.5 meter x 0.25 mm x 0.25 µm
Part No.:	7HG-G033-10	7XG-G030-11
Injection:	Split 1:50 @ 250 °C, 2 µL	Same as Primary Column
Recommended Liner:	Zebron PLUS Single Taper Z-Liner™ (for Agilent® systems)	Same as Primary Column
Liner Part No.:	AG2-0A13-05	Same as Primary Column
Carrier Gas:	Helium @ 1 mL/min (constant flow)	Same as Primary Column
Oven Program:	40 °C for 2 min to 160 °C @ 30 °C/min to 250 °C @ 2°C/min hold for 1 min	60 °C for 2 min to 180 °C @ 30 °C/min to 270 °C @ 2 °C/min hold for 1 min
Detector:	Leco® Pegasus® 4D GCxGC-TOFMS	Same as Primary Column
MS Parameters:	Ion Source Temperature: 220°C Transfer Line Temperature: 240 °C m/z Range: 45 – 650 Acquisition Rate: 100 spectra/sec Solvent Cut Time: 3 min	Same as Primary Column
Modular Parameters:	Modulator Temperature Offset (above primary oven): 45 °C Modulation Time: 10 sec Hot Pulse Time: 1 sec Total Run Time: 56 min	Same as Primary Column

Figure 1.
GCxGC Schematic

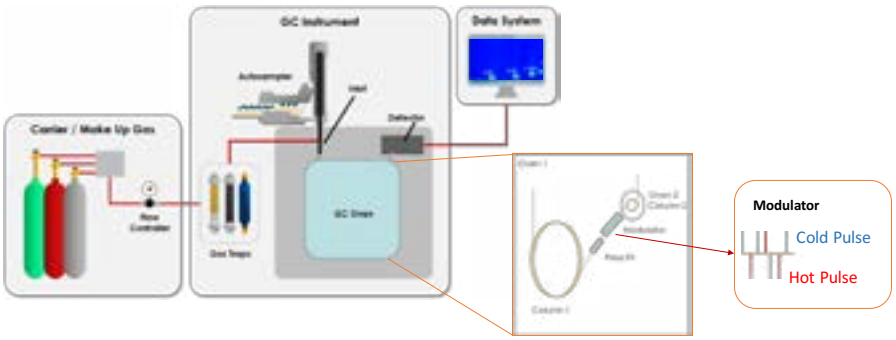

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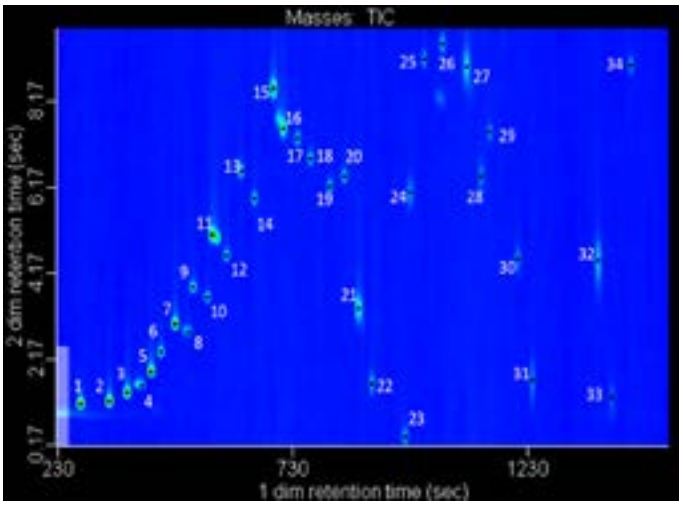
Figure 2.
Contour Pot (2D Chromatogram) for FAMEs Mix



Results and Discussion

Comprehensive GCxGC requires the use of 2 columns of complementary selectivity to assist in orthogonal separation of analytes. Presented in **Figure 1** is a schematic of GCxGC. As outlined in the Materials and Methods section, two Zebron™ GC columns of differing selectivity were used for analysis. **Figure 2** is a contour plot, also referred to as a 2D plot, from a comprehensive GCxGC analysis. The x-axis of this plot is the retention time of the analyte in the first dimension and the y-axis is the retention time of the analyte in the second dimension. The chromatogram shows that the peaks are distributed very well between the two axes. This signifies that the two columns selected, ZB-FAME (30 meter) and ZB-5MS*PLUS*™ (1.5 meter), provide adequate selectivity for the orthogonal separation required for FAME analysis. **Figure 3** offers a zoomed in view of individual isomers of the FAMEs mix. Excellent separation of C18 isomers between the two GC columns can be seen from the zoomed in contour plot. To visualize the separation power of a comprehensive GCxGC analysis, a 3D plot can be explored. This plot has first and second dimension retention on the x- and y-axis, and on the z-axis, the peak intensity (sometimes referred to as relative abundance) is plotted as shown in **Figure 4**.

Figure 3.
Zoomed Contour Plot of C6 to C18 FAMEs

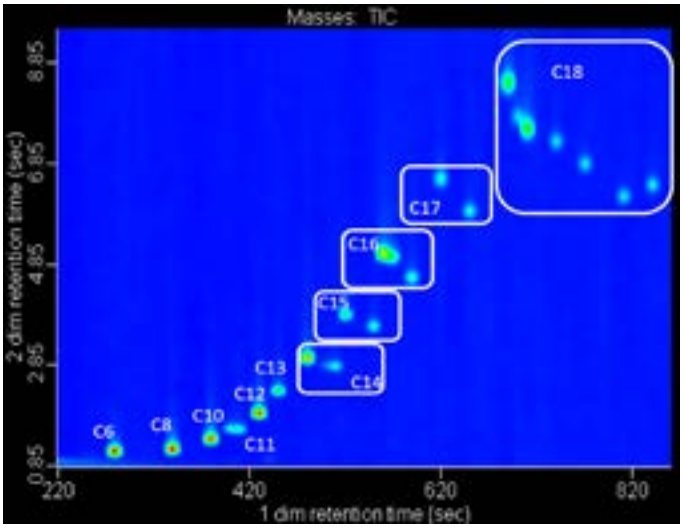
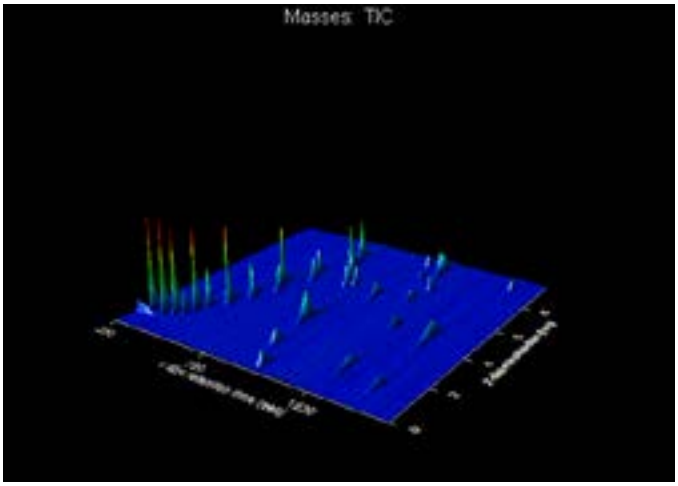


Figure 4.
3D Chromatogram for FAMEs Mix



Conclusion

In order to get very symmetric peaks in GCxGC as displayed in the plots shown here, it is important that the GC stationary phase selected has inert performance, so as to reduce any peak tailing. Based on the circular shape of target analytes in the chromatogram, it is clear that the combination of ZB-FAME and ZB-5MS*PLUS* not only offered complementary selectivity, but also produced highly symmetric peaks compared to traditional cyano phases. ZB-FAME has a higher maximum temperture (280 °C) which extends its use in GCXGC as either a primary or secondary column. This method serves as a proof of concept to demonstrate the successful use of complementary selectivities and GCxGC for FAMEs analysis; the method can be further optimized for complete separation of more complex FAMEs isomers. This study also suggests that the complementary column pair used to generate the data is successful, and warrants additional study for use with other analytes such as hydrocarbons, aromatics, PAHs, PCBs, PDBEs – which are closely related in terms of their structure and could benefit from the enhanced separation power of GCxGC.

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Table 1.
Analyte List

Peak #	Name	R.T. (s)
1	Hexanoic acid methyl ester	280 , 1.170
2	Octanoic acid methyl ester	340 , 1.230
3	Decanoic acid methyl ester	380 , 1.420
4	Undecanoic acid methyl ester	410 , 1.600
5	Dodecanoic acid methyl ester	430 , 1.920
6	Tridecanoic acid methyl ester	450 , 2.370
7	Methyl tetradecanoate	480 , 3.020
8	10-Undecenoic acid methyl ester	510 , 2.850
9	Pentadecanoic acid methyl ester	520 , 3.870
10	7-Hexadecenoic acid methyl ester, (Z)-	550 , 3.640
11	Pentadecanoic acid, 14-methyl-, methyl ester	560 , 5.080
12	9-Hexadecenoic acid, methyl ester, (Z)-	590 , 4.610
13	Hexadecanoic acid, 15-methyl-, methyl ester	620 , 6.560
14	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	650 , 5.920
15	Octadecanoic acid, methyl ester	690 , 8.470
16	9-Octadecenoic acid (Z)-, methyl ester	710 , 7.550
17	9,12-Octadecadienoic acid, methyl ester, (E,E)-	740 , 7.300
18	11,14-Eicosadienoic acid, methyl ester	770 , 6.850
19	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	810 , 6.210
20	11,14,17-Eicosatrienoic acid, methyl ester	840 , 6.440
21	Eicosanoic acid, methyl ester	870 , 3.390
22	15-Tetracosenoic acid, methyl ester, (Z)-	900 , 1.640
23	11-Octadecynoic acid methyl ester	970 , 0.410
24	Heneicosanoic acid methyl ester	980 , 6.060
25	11,14,17-Eicosatrienoic acid methyl ester	1010 , 9.150
26	9,12,15-Octadecatrienoic acid methyl ester, (Z,Z,Z)-	1050 , 9.500
27	Docosanoic acid methyl ester	1100 , 8.980
28	15-Tetracosenoic acid methyl ester, (Z)-	1130 , 6.460
29	4,7,10,13,16,19-Docosahexaenoic acid methyl ester, (all-Z)-	1150 , 7.450
30	11,14-Eicosadienoic acid methyl ester	1210 , 4.510
31	Heneicosanoic acid methyl ester	1240 , 1.720
32	Hexacosanoic acid methyl ester	1380 , 4.490
33	15-Tetracosenoic acid methyl ester, (Z)-	1410 , 1.320
34	4,7,10,13,16,19-Docosahexaenoic acid methyl ester, (all-Z)-	1450 , 9.020

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Ordering Information

Zebron™ ZB-FAME GC Columns

Length (m)	ID (mm)	Film (µm)	Temp. Limits (°C)	Standard	5 m Guardian™
				Part No.	Part No.
20	0.18	0.15	- 60 to 280	7FD-G033-05	–
30	0.25	0.20	- 60 to 280	7HG-G033-10	7HG-G033-10-GGA
60	0.25	0.20	- 60 to 280	7KG-G033-10	–

Zebron ZB-5MS*PLUS*™ GC Columns

ID(mm)	df(µm)	Temp. Limits °C	Part No.
15-Meter			
0.25	0.25	-60 to 325/350	7EG-G030-11
20-Meter			
0.18	0.18	-60 to 325/350	7FD-G030-08
0.18	0.36	-60 to 325/350	7FD-G030-53
30-Meter			
0.25	0.25	-60 to 325/350	7HG-G030-11
0.25	0.50	-60 to 325/350	7HG-G030-17
0.25	1.00	-60 to 325/350	7HG-G030-22
0.32	0.25	-60 to 325/350	7HM-G030-11
0.32	1.00	-60 to 325/350	7HM-G030-22
30-Meter with 5-Meter Guardian™ Integrated Guard			
0.25	0.25	-60 to 325/350	7HG-G030-11-GGA
30-Meter with 10-Meter Guardian Integrated Guard			
0.25	0.25	-60 to 325/350	7HG-G030-11-GGC
0.25	0.50	-60 to 325/350	7HG-G030-17-GGC
60-Meter			
0.25	0.25	-60 to 325/350	7KG-G030-11
1.5-Meter			
0.25	0.25	-60 to 325/350	7XG-G030-11

Zebron PLUS GC Inlet Liners

Description	Dimensions	Part No.	Unit
For Agilent® and Thermo Scientific® GC Systems			
Single Taper Z-Liner™	4 x 78.5	AG2-0A13-05	5/pk
		AG2-0A11-25	25/pk

guarantee

If Zebron columns do not provide you with equivalent or better separations as compared to any other GC column of the same phase and comparable dimensions, return the column with comparative data within 45 days for a FULL REFUND.

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