Flow Modulated GCxGC Coupled to TOFMS for Non-Target Profiling of Food, Flavor, and Fragrance Samples

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INTRODUCTION

Two-dimensional gas chromatography (GCxGC) is a well-established analytical technique that offers better characterization of complex samples compared to GC. GCxGC pairs two columns with complementary stationary phases in series with a modulating device between. The role of the modulator is to collect effluent from the first column and reinject into the second column at set intervals throughout the separation, such that each sample is chromatographically separated by both mechanisms. This additional dimension of separation provides increased chromatographic resolution and the possibility for first dimension coelutions to be separated in the second dimension. In this work, a GCxGC system with a new and easy-to-use flow-based modulator was coupled to a benchtop time-of-flight mass spectrometer to investigate a wide variety of samples. In each case, the samples were prepared for analysis with solid-phase micro extraction (SPME) prior to the separation and detection. Deconvolution offered an additional level of information based on the mathematical separation of the full m/z range TOFMS data, which allowed for identifying and quantifying individual analytes even when chromatographically coeluting with other analytes in the matrix. In some cases, these coelutions were important sample-distinguishing features that would be hidden without this capability. This analytical approach is readily applied to many different types of samples including foods, beverages, and personal care products. Individual analytes were compared and contrasted in these types of samples, and several characterization and differentiation examples are demonstrated.

METHOD

A variety of food and fragrance samples (honey, liquors, perfumes, etc.) were analyzed with HS-SPME and GCxGC-TOFMS (LECO, Pegasus® BT 4D FLUX™). For each sample type, a specific amount (see details in each section) was transferred to a 20 mL headspace vial and sealed with a septum cap. Instrument conditions are listed in Table 1.

Table 1. Instrument Conditions

AS	**LECO L-PAL3 Autosampler**
SPME	5 min incubation, 10 min extraction at 40 °C
SPME fiber	DVB/Car/PDMS
Fiber Conditioning	5 min pre-injection and 5 min post-injection at 250 °C
GC	**LECO FLUX GCxGC**
Injection	Desorb fiber 3 min at 250 °C, splitless
Columns	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating Rxi-17SilMS, 0.91* m x 0.10 mm i.d. x 0.10 µm coating (*0.31 m in transfer line and 0.60 m in secondary oven)
Carrier Gas	He @ 0.80 mL/min
Oven Program	3 min 40 °C, ramp 4.2 °C/min to 250 °C, hold 5 min Secondary oven +20 °C
2nd Dimension Separation Time	1 s, injection duration 0.05 s
MS	**LECO Pegasus BT**
Ion Source Temp	250 °C
Mass range	33-550 m/z
Acquisition Rate	200 spectra/s

FLOW MODULATION

The modulator is a crucial part of the GCxGC system, as it collects the primary column effluent and reinjects into the second column for the second dimension separation. This work was done with a robust and easy-to-use flow-based system. The modulator cycles between inject mode and divert mode at set time periods. Inject mode transfers a portion of the first column effluent to the secondary column. Divert mode directs first column effluent to waste between injections, as shown in Figure 1.

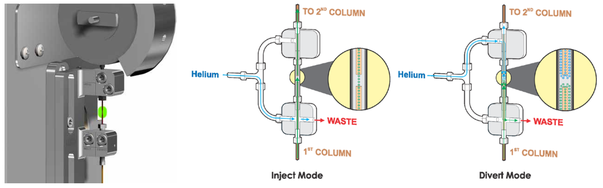


Figure 1. Diagrams showing LECO's Pegasus BT FLUX modulator and its modes of operation

GCxGC BENEFITS – INCREASED CHROMATOGRAPHIC RESOLUTION

One of the important benefits of GCxGC is an increase in chromatographic resolution, or peak capacity. Analytes that coelute in the first dimension can often be separated on the complementary stationary phase in the second dimension. An example of this benefit from a chromatogram of Jägermeister liquor is shown in Figure 2 (Sample preparation: 2 mL in 20 mL vial). What appeared to be a single peak in the GC separation was revealed to be two coeluting analytes. Both analytes have interesting odor characteristics.

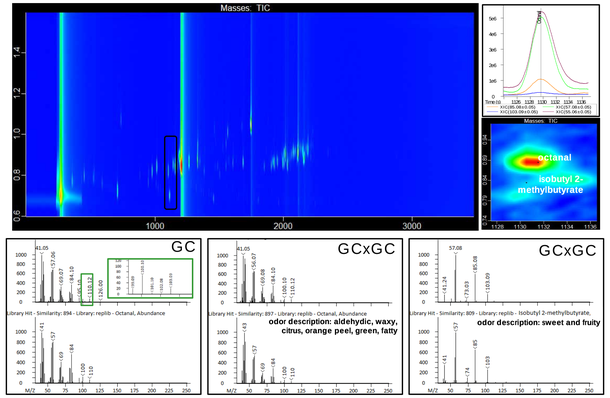
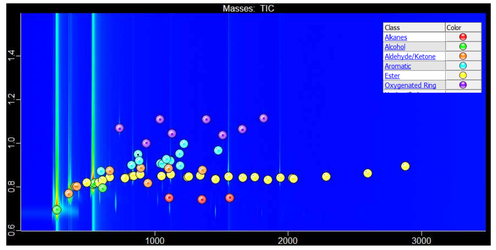


Figure 2. The increased chromatographic resolution with GCxGC is highlighted with data from a sample of Jägermeister. A single peak that combined both analytes was identified as octanal in the GC data. With GCxGC, an ester (isobutyl 2-methylbutyrate), that was completely coeluting in the first dimension is separated in the second.

GCxGC BENEFITS – STRUCTURED CHROMATOGRAMS

Another important benefit of GCxGC is the generation of structured chromatograms. Due to the complementary nature of the stationary phases, functional group classes tend to elute in structured bands across the separation space. An example of this from a bourbon sample is shown in Figure 3 (Sample preparation: 1.5 mL in a 20 mL vial).



Class	Name	Formula	Sim	R.T. (s,s)	RI	Lib. RI	CAS	Odor Description
	dodecane	$C_{12}H_{26}$	915	1557.9, 0.753	1200.5	1200	112-40-3	
	isoamyl alcohol	$C_5H_{12}O$	918	539.965, 0.814	751.3	736	123-51-3	fusel, oil, alcoholic, whiskey, fruity, banana
	nonanal	$C_9H_{18}O$	818	1355.91, 0.880	1105.6	1104	124-19-6	waxy aldehydic rose fresh orris orange peel fatty peely
	styrene	C_8H_8	827	873.944, 0.952	895.2	893	100-42-5	sweet balsam floral plastic
	ethyl heptanoate	$C_9H_{18}O_2$	911	1339.91, 0.854	1098.2	1097	106-30-9	fruity pineapple cognac rum wine
	benzaldehyde	C_7H_6O	878	1036.93, 1.111	964.9	962	100-52-7	strong sharp sweet bitter almond cherry

Figure 3. The structured nature of the chromatograms is highlighted with data from a bourbon sample. Representative analytes from various compound classes are shown with different colored peak markers. Specific examples of each class are shown in the associated table.

HONEY DIFFERENTIATION

This method is readily transferrable to a wide range of sample types. In addition to liquor (shown in Figures 2 and 3), the same method was used to analyze honey samples. (Sample preparation: 3.5 g were added to 20 mL vial.) Aroma profiles of three flavors of honey (clover, blueberry, and orange blossom) were compared. The clover honey was described as light, mild, and floral. The blueberry honey was described as dark, spicy, fruity, and tangy. The orange blossom honey was described as mild, fruity, and citrusy. The benefits of GCxGC can also be noted in these samples and helped uncover differences that were difficult to detect with GC alone.

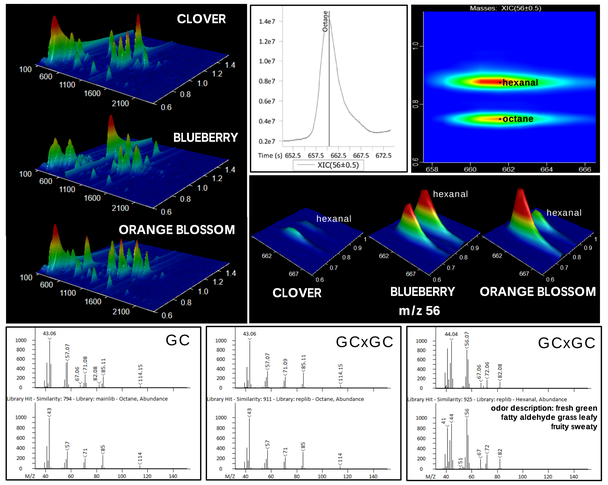


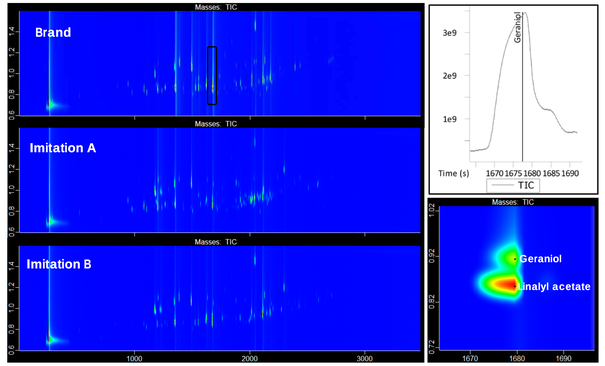
Figure 4. Representative chromatograms for three honey varieties are shown. A GC coelution that exceeds deconvolution is also shown. What appeared as a single peak in the GC data was actual two coeluting analytes. Hexanal that was obscured without GCxGC was an analyte that differentiated the samples.

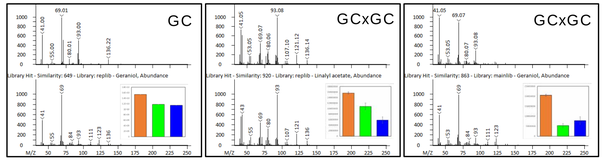
Table 2. Representative analytes that differ between the honey varieties.

Name	Formula	Sim	R.T. (s)	Obs. RI	Lib. RI	CAS	CLOVER	BLUE	ORANGE	ODOR and FLAVOR Descriptions
cocoa butenal	C_4H_6O	886	1717.89,1.167	1280.9	1279	4411-89-6				sweet narcissus cortex beany honey cocoa nutty radish / green vegetative floral cocoa nutty
Benzeneacetaldehyde	C_8H_8O	949	1229.92,1.119	1049.3	1045	122-78-1				green sweet floral hyacinth clover honey cocoa / honey sweet floral chocolate and cocoa with a spicy nuance
benzyl alcohol	C_7H_8O	881	1202.92,1.101	1037.3	1036	100-51-6				floral rose phenolic balsamic / chemical fruity cherry almond balsamic bitter
phenylethyl alcohol	$C_8H_{10}O$	920	1382.91,1.109	1118.3	1116	60-12-8				floral rose dried rose flower rose water / floral sweet rosey and bready
2-linalool oxide	$C_{10}H_{18}O_2$	926	1293.92,0.888	1077.8	1074	5989-33-3				earthy floral sweet woody
limonene	$C_{10}H_{16}$	904	1196.92,0.853	1034.7	1030	138-86-3				citrus herbal terpene camphor
gamma-valerolactone	$C_5H_8O_2$	914	1014.94,1.242	955.5	958	108-29-2				herbal sweet warm tobacco cocoa woody / sweet tonka coumarinic tobacco cocoa dark chocolate coconut
linalool oxide	$C_{10}H_{18}O_2$	826	1512.9,0.955	1179.3	1178	14049-11-7				floral honey
lilac aldehyde A	$C_{10}H_{16}O_2$	926	1445.91,0.959	1147.9	1145	53447-46-4				flowery fresh
lilac aldehyde B	$C_{10}H_{16}O_2$	925	1464.91,0.964	1156.8	1154	53447-45-3				fresh flowery
lilac aldehyde D	$C_{10}H_{16}O_2$	925	1496.9,0.972	1171.8	1169	53447-47-5				sweet flowery
hexanal	$C_6H_{12}O$	934	659.958,0.876	803	800	66-25-1				fresh green fatty aldehyde grass leafy fruity sweaty / green woody vegetative apple grassy citrus and orange with fresh/lingering aftertaste
benzaldehyde	C_7H_6O	959	1036.93,1.106	964.9	962	100-52-7				strong sharp sweet bitter almond cherry / sweet oily almond cherry nutty and woody
ethyl butyrate	$C_6H_{12}O_2$	879	662.958,0.845	804.3	802	105-54-4				fruity juicy fruit pineapple cognac / fruity sweet tutti frutti apple fresh and lifting ethereal
2-heptanone	$C_7H_{14}O$	932	866.945,0.889	892.2	891	110-43-0				fruity spicy sweet herbal coconut woody / cheese fruity coconut waxy green
butanoic acid	$C_4H_8O_2$	951	687.956,0.791	815.1	805	107-92-6				sharp acetic cheese butter fruit / acidic sour cheesy dairy creamy with a fruity nuance
furfural	$C_5H_4O_2$	958	737.953,1.064	836.6	833	98-01-1				sweet woody almond fragrant baked bread / brown sweet woody bready nutty caramellic with burnt astringent nuance
isophorone	$C_9H_{14}O$	882	1402.91,1.047	1127.7	1124	78-59-1				cooling woody sweet green camphor fruity musty cedarwood tobacco leather / sweet green waxy woody cooling pulpy mouthfeel and citrus
cyclohexanol	$C_6H_{12}O$	895	855.945,0.949	887.5	880	108-93-0				camphor menthol phenol
cyclohexanone	$C_6H_{10}O$	881	878.944,1.060	897.4	894	108-94-1				minty acetone
linalool	$C_{10}H_{18}O$	894	1347.91,0.870	1101.9	1099	78-70-6				citrus floral sweet bois de rose woody green blueberry / citrus orange lemon floral waxy aldehydic and woody
methyl heptenone	$C_8H_{14}O$	877	1091.93,0.920	988.5	986	110-93-0				citrus green musty lemongrass apple / green vegetative musty apple banana and green bean-like
perilla alcohol	$C_{10}H_{16}O$	851	1750.89,1.011	1297.5	1296	536-59-4				green linalool terpineol fatty / sweet woody aromatic spicy cardamom green cumin like with dried orange peel and green waxy floral nuances

PERFUME DIFFERENTIATION

The same GCxGC method was used to analyze perfume samples. A brand perfume and two drugstore imitations were compared. (Sample preparation: 10 µL were added to 20 mL vial.) The GCxGC benefit of increased chromatographic resolution can be noted in these samples as well. Differences that were difficult to detect with a GC separation were separated and distinguished in the second dimension.





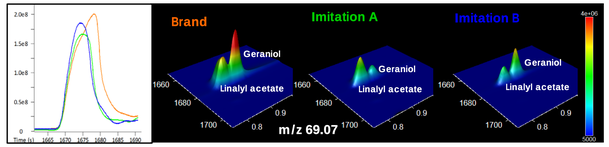


Figure 5. Representative chromatograms for three perfume samples are shown. A complete coelution that exceeded deconvolution in the GC data is separated in the second dimension with GCxGC. In this case, most masses were shared. The differences in expression between samples was obscured with only GC, but revealed with GCxGC.

CONCLUSIONS

A single GCxGC method was applied to several food, flavor, and fragrance samples. In this work, Jägermeister, bourbon, honey, and perfumes are used to demonstrate the capabilities of this instrument configuration. GCxGC (with flow modulation) provides both improved chromatographic resolution and structured chromatograms as the primary benefits. The structured nature of the chromatogram was highlighted with the bourbon sample, providing rapid characterization. Several coelutions that exceeded deconvolution capabilities in a GC separation were shown separated with GCxGC. In many cases, these analytes that were obscured without GCxGC were important for distinguishing the samples, and/or had aroma properties of interest. The use of a flow-based modulator with this method simplified the GCxGC data acquisition. This is a robust and easy-to-use option for performing GCxGC.