Application Note

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EMPOWERING RESULTS

Instrument: Pegasus® BT 4D

GC, GC×GC, and TOFMS for Characterization and Roast Level Differentiation of Coffee

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Key Words: GC-MS, GC×GC-MS, GC×GC-TOFMS, Pegasus BT 4D, HS-SPME, Coffee, Sample Differentiation

Introduction

Coffee is one of the most consumed beverages in the world and the industry around it is an important part of the global economy. As expected with commodities, there is a large amount of taste and flavor variation in coffee that can relate to differences in the variety and geographical origin of the beans, storage and processing conditions, roasting conditions, and brewing methods. An understanding of these differences can be helpful for quality control, process optimization, and also for providing information on flavors and characteristics that direct consumers to their preferred styles. In addition to the expected variation, the aroma profile for coffee is quite complex and comprised of a large number of individual analytes, creating an analytical challenge. Non-targeted chemical analysis techniques, like gas chromatography with mass spectrometry (GC-MS) and headspace solid phase micro-extraction (HS-SPME), are well-suited to address these challenges. With these methods, volatile and semi-volatile analytes were collected from the coffee samples, separated, and detected, resulting in identification and relative quantification information for hundreds of analytes. Analytes of interest do not need to be determined prior to acquisition, so the data were generally characterized to investigate the samples and their differences. Comprehensive two-dimensional gas chromatography (GC×GC) increases peak capacity and enhances S/N compared to GC, and also creates structured chromatograms. These additional analytical capabilities were explored and led to the detection of more analytes and an improved understanding of these complex samples. In this work, coffee brewed from a variety of beans was compared to investigate variations related to roast level.

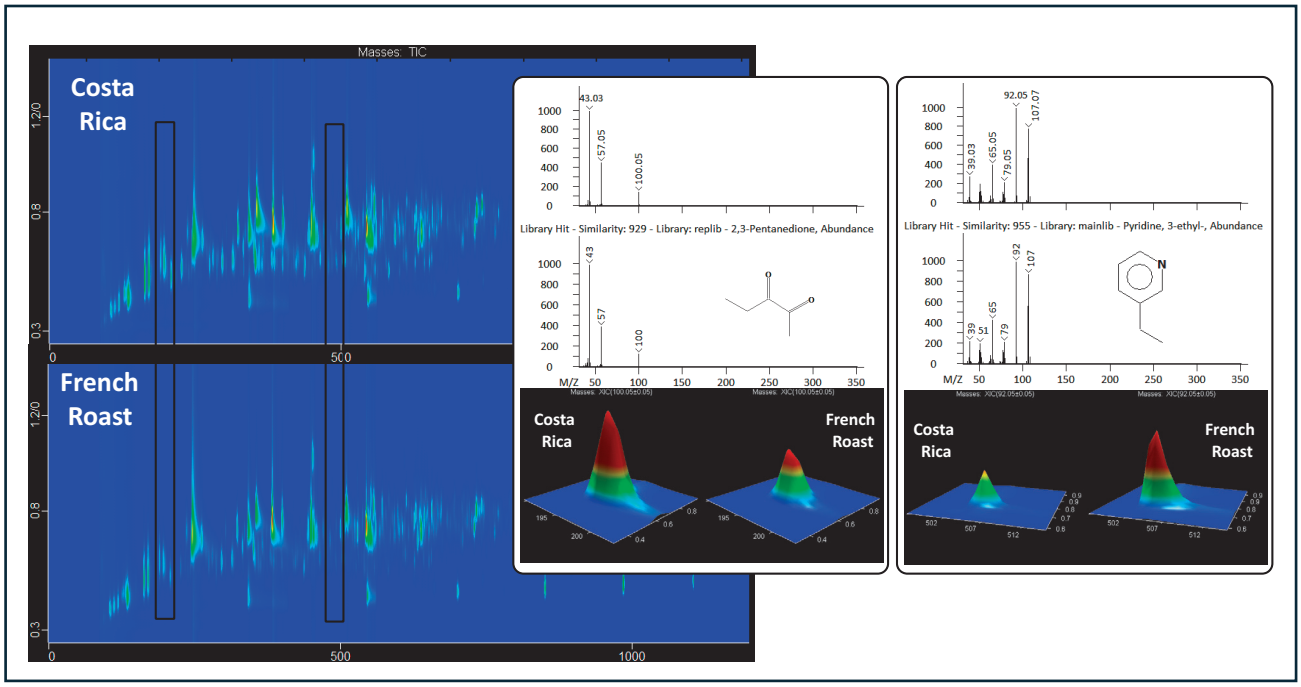


Figure 1. GC×GC chromatograms for coffee from medium and dark roasted coffee beans from Costa Rica are shown. Representative analytes that are present at different levels are shown along with their library matched spectra.

Experimental

Coffee brewed from six different types of beans was compared with HS-SPME and GC or GC×GC coupled to TOFMS. The beans were from four geographical origins (Peru, Costa Rica, Kona, and Colombia) with a medium roast style from all four, and a dark roasted style from Costa Rica and Kona. The coffee was prepared by coarsely grinding each bean and brewing by French Press. Four ounces of water (100 °C) were added per 1 Tbs. whole beans and the coffee was pressed after 4 min of steeping. For HS-SPME analysis, 4 mL of coffee were transferred to a 20 mL vial which was incubated for 5 min at 60 °C, and then extracted with a DVB/CAR/PDMS fiber (Supelco) for 5 min at the same temperature. The samples were subsequently analyzed by GC-TOFMS and GC×GC-TOFMS with instrument conditions listed in Table 1. Single dimension GC data were acquired with the GC×GC column configuration by simply turning the modulator off, which allowed for rapidly switching between acquisition modes. Data for an alkane standard was also acquired for retention index calculations.

Table 1. GC-TOFMS and GC×GC-TOFMS (*Pegasus* BT 4D) Conditions

Gas Chromatograph	**LECO GCxGC Quad Jet Thermal Modulator & L-PAL 3 Autosampler**
Injection	SPME, 3 min desorption, split 5:1 in 250 °C inlet
Carrier Gas	He @ 1.4 mL/min, Corrected Constant Flow
Column One	Rxi-5Sil MS, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Column Two	Rxi-17SilMS, 0.3 m x 0.25 mm x 0.25 µm coating (Restek)
Temperature Program	3 min at 40 °C, ramped 10 °C/min to 250 °C, hold 5 min Secondary oven maintained +10 °C relative to primary oven
Modulation	1.2 s with temperature maintained +15 °C relative to 2nd oven
Transfer Line	250 °C with uncoated guard column
Mass Spectrometer	**LECO *Pegasus* BT**
Ion Source Temperature	250 °C
Mass Range	33-510 m/z
Acquisition Rate	10 spectra/s (GC) and 100 spectra/s (GC×GC)

Results and Discussion

Representative GC×GC chromatograms for two of the coffee samples, brewed from medium and dark roasted beans from Costa Rica, are shown in Figure 1. The complexity of this type of sample is apparent with hundreds of peaks visible in the TIC. One of the benefits of coupling these separation techniques with full mass range TOFMS detection is that comprehensive data are acquired for each sample. This non-targeted data provides the spectra for library matching and can be reviewed for the discovery of unknowns. Two specific analytes are highlighted in Figure 1. 2,3-pentanedione is observed at higher levels in the medium roast coffee, and 3-ethyl pyridine is observed at elevated levels in coffee brewed from the darker roasted beans. 2,3-pentanedione has known odor characteristics of caramel, nutty, sweet, and creamy while the pyridine has odor descriptors like roasted, tobacco, and leather. Automated data processing results in peak information for the entire sample and many other analytes from a wide range of compound types including alkanes, terpenes, aldehydes, ketones, furans, nitrogen-containing rings, aromatic compounds, and thiophenes were found. All identifications were tentatively determined with retention index and spectral similarity to library databases.

The use of GC×GC for this experiment provided several key benefits for these complex samples by adding a complementary 2nd separation dimension. GC×GC is typically expected to increase the peak capacity, enhance S/N, and create structured chromatograms, and all of these benefits were observed in these data. This led to more analytes detected, better library similarity scores, and more information on these complex samples compared to a GC separation. Examples are shown in Figures 2-4.

The increased peak capacity provides better separations for complex samples as coelutions in the first dimension can often be separated in the second dimension. Figure 2 shows an example where a single peak marker was determined in the GC data that was revealed to be two analytes in the GC×GC data. These analytes coelute in the first dimension and are chromatographically separated in the second dimension. Plotting unique m/z for each analyte shows near perfect coelution in the GC data and distinct chromatographically separated peaks in the GC×GC data. The mass spectrum derived from the single dimension GC separation is the combination of the spectra of the two analytes and has a poor library similarity score, while the chromatographically separated peaks in the GC×GC data have library matches with similarity scores into the 800s. What was once unknown with GC was determined to be two knowns with GC×GC: 2,3-dimethyl pyridine with coffee and caramel odor notes and the furan, 5-methyl-2(5H) furanone.

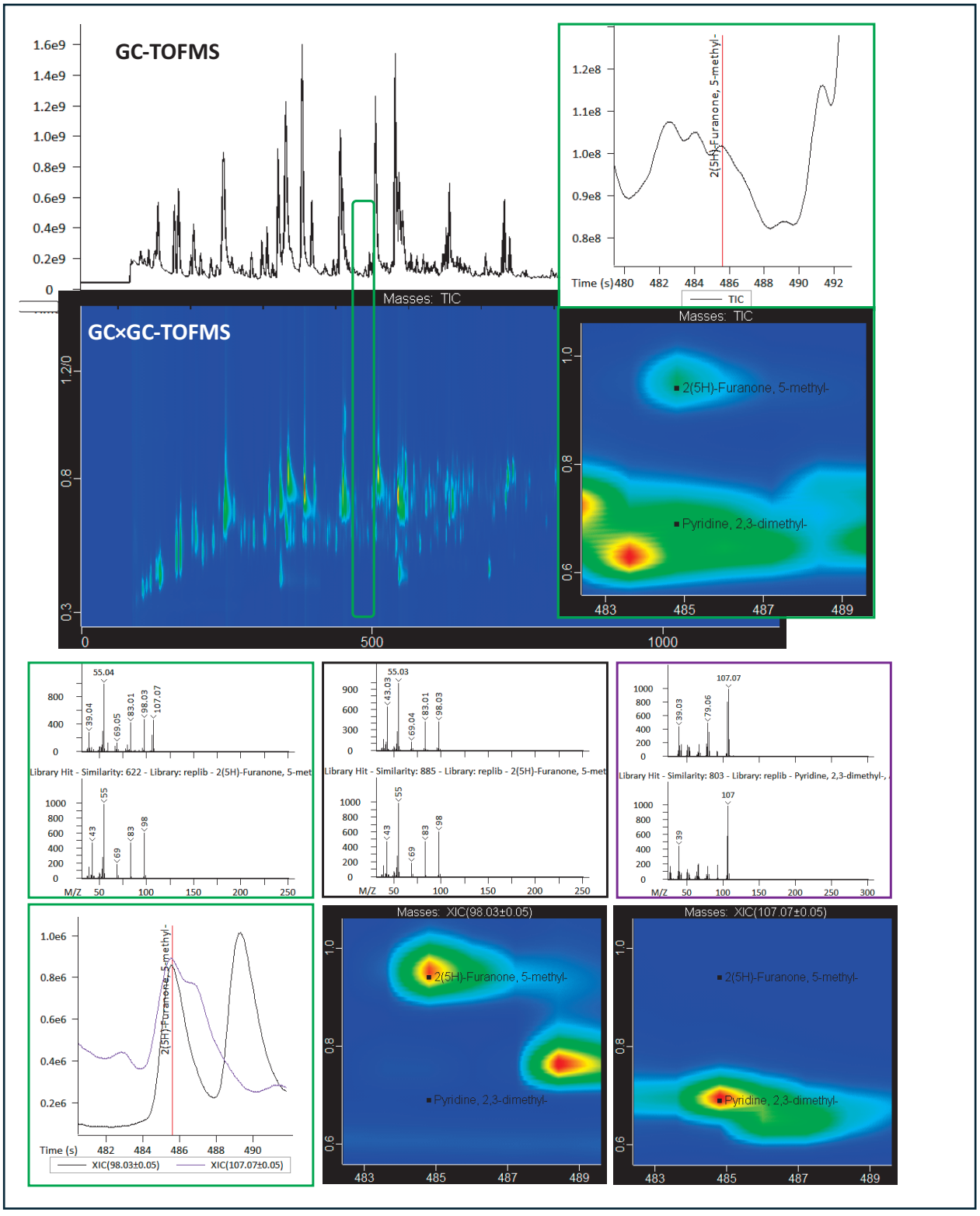


Figure 2. GC×GC provides an increased peak capacity compared to GC. Coelutions that exceed deconvolution in the first dimension can often be separated in the second dimension, as shown here.

GC×GC with thermal modulation is also expected to provide an enhancement in the S/N, demonstrated in Figure 3. This enhancement comes from cryogenic focusing at the modulator, which sharpens and focuses peaks just prior to detection. In the GC data, a single peak was identified as 3-phenyl furan. This furan is known to occur in coffee and had a S/N above the threshold with both GC and GC×GC. The GC×GC data revealed that a second analyte was also present that was below the S/N threshold in the GC separation, but above the threshold after cryogenic focusing. The analyte, 5-hydroxymethyl furfural, has caramel and buttery odor properties and was only detected with the GC×GC separation.

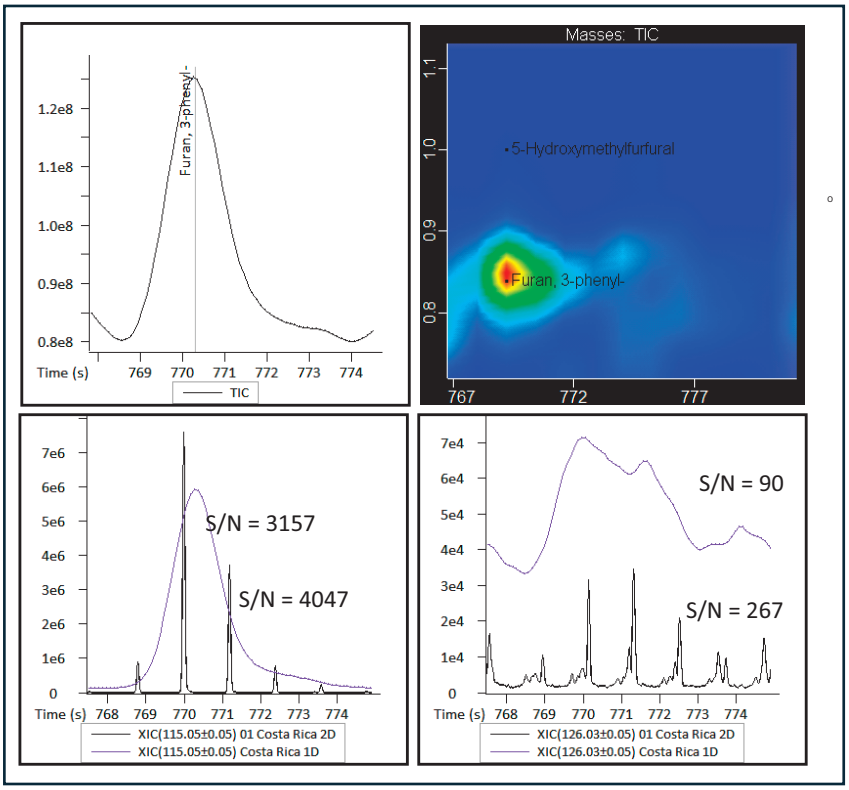


Figure 3. GC×GC with thermal modulation leads to an enhanced S/N because of cryogenic focusing at the modulator.

The structured nature of the chromatograms is demonstrated in Figure 4. The complementary nature of the stationary phases leads to chromatograms where compound classes tend to elute in bands across the GC×GC separation space. In this figure, peak markers are color coded by compound class for a collection of representative analytes to help visualize these bands. This aspect of GC×GC allows for rapid characterization of the samples and visual determination of the types of analytes present.

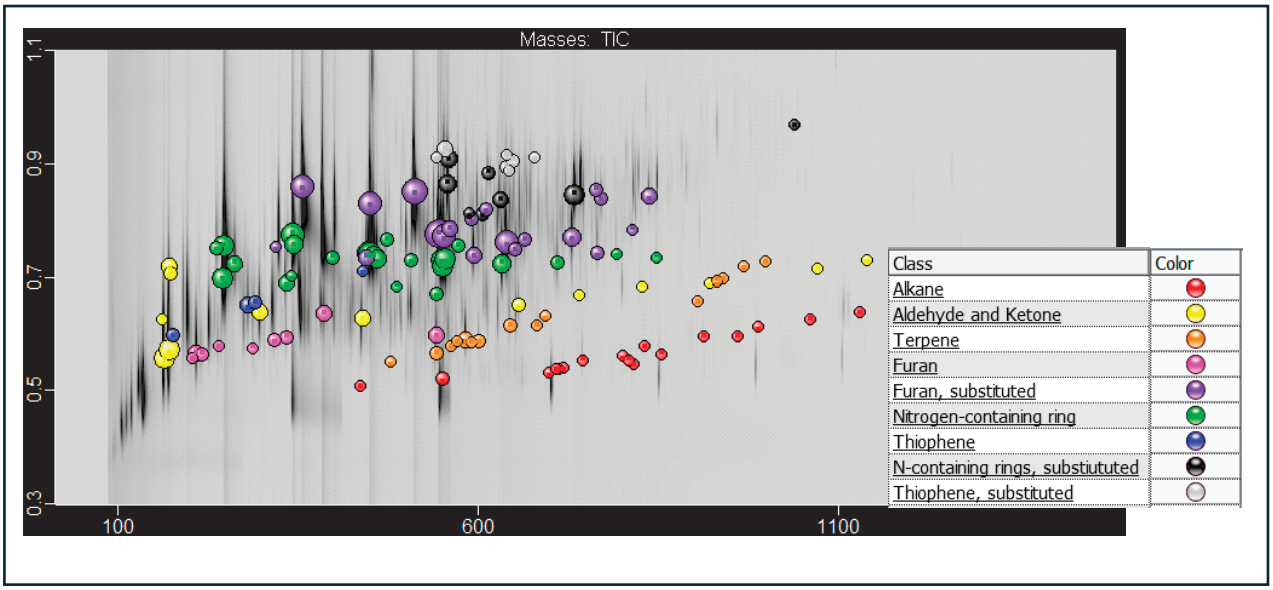


Figure 4. The complementary nature of the stationary phases leads to compound classes eluting in structured bands in the GC×GC separation space. This helps with visual characterization and highlights many of the types of analytes that were observed in these data.

The benefits of GC×GC provided information about these complex samples and uncovered some analytes that were difficult to measure with GC alone. Having information on hundreds of individual analytes allowed for making comparisons of the coffee prepared from the different beans. We looked at beans from four geographical origins (Peru, Costa Rica, Colombia, and Kona) with a medium roast from each and a dark roast from Costa Rica (French Roast) and Kona (Dark Kona). A representative TIC chromatogram of each coffee sample is shown in Figure 5. These samples have many similarities, but some differences can be noted. The structured nature of the plot gives insight to these differences even before peak finding has been performed. For example, the medium roast Peru sample has some unique peaks in the alkane band that are not present in the other samples. The dark roast Dark Kona sample has more intensity in the nitrogen-containing ring band compared to the other samples. Automated data processing provided identification information on these individual analytes based on RI and MS matching to library databases, and peak areas were compared for each analyte across the sample set to observe general trends and differences. A collection of information for some of the sample-distinguishing analytes observed here, including those analytes highlighted in Figure 1, is shown in Table 2. These differences appear to relate to roast level with peak area trends distinguishing the medium and dark roasted beans for these samples. Of note, several specific alkanes were observed elevated in the Peru samples and several specific pyridines were observed elevated in the Dark Kona samples. The aroma properties were also compiled for these sample-distinguishing analytes. Several analytes that were observed at higher levels in the coffee from darker roasted beans had odor descriptors like roasted, coffee, smoke, and burnt; and, several analytes at higher levels in the coffee from the medium roasted beans had odor descriptors like caramel, buttery, baked bread, and nutty. Many other analytes were observed that would also contribute to the overall aroma profile.

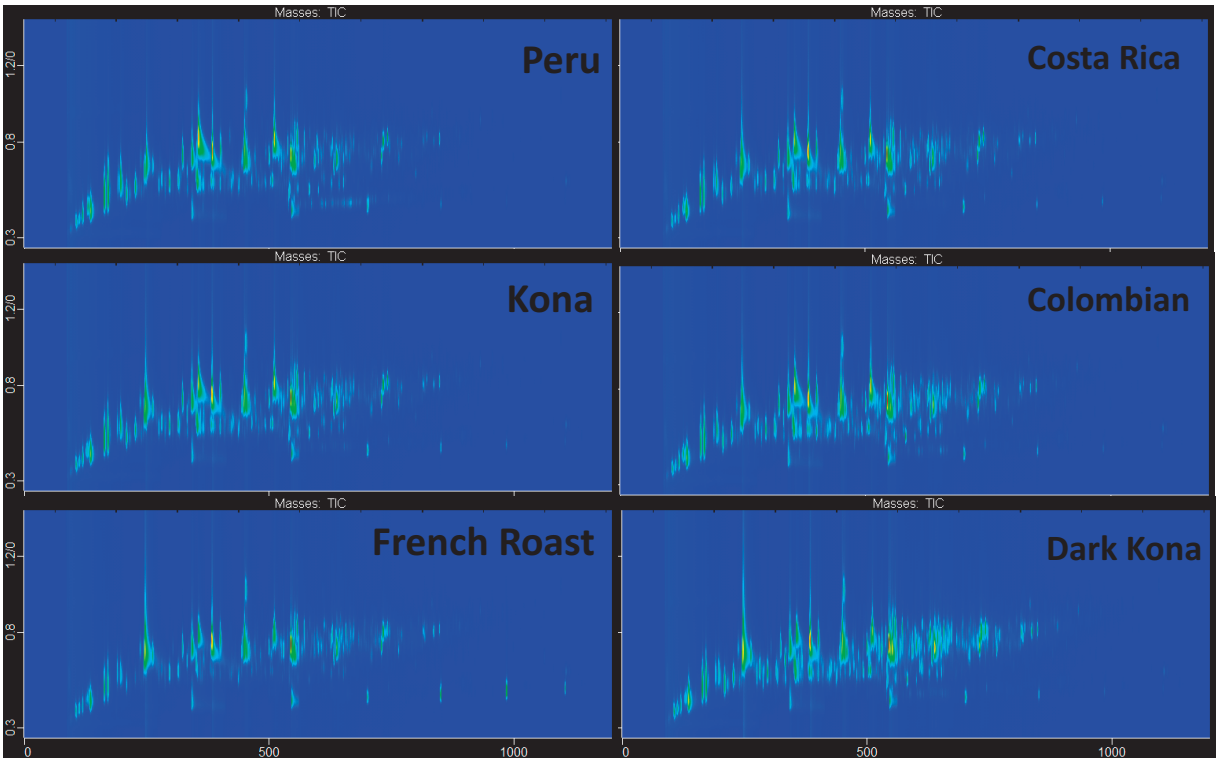
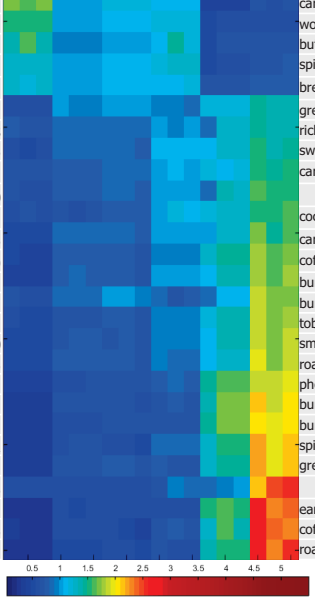


Figure 5. Representative TIC chromatograms of each coffee sample

Table 2. Some analyte differences that appear to relate to roast level.

	tR	Similarity	Formula	CAS	RI (Obs)	RI (Lib)	Peru	Costa Rica	Kona	Colombian	French Roast	Dark Kona	Odor and flavor notes
decane, 3,7-dimethyl-	673.2	867	$C_{12}H_{26}$	17312-54-8	1124	1125							
undecane, 2-methyl-	710.4	908	$C_{12}H_{26}$	7045-71-8	1163.8	1164							
undecane, 3-methyl-	716.4	949	$C_{12}H_{26}$	1002-43-3	1170	1170							
2,3-pentanedione	195.0	929	$C_5H_8O_2$	600-14-6	707.3	698							caramel, nutty, sweet, butter, creamy, cheese, pungent
furfural	356.0	966	$C_5H_4O_2$	98-01-1	835.9	833							woody, almond, fragrant, baked bread, sweet
2(5H)-furanone	454.6	896	$C_4H_4O_2$	497-23-4	916.6	918							buttery
2-furancarboxaldehyde, 5-methyl-	510.8	950	$C_6H_6O_2$	620-02-0	966.2	965							spice, caramel, maple
3(2H)-furanone, dihydro-2-methyl-	323.5	938	$C_5H_8O_2$	3188-00-9	809.8	809							bread, buttery, nutty, sweet, solvent
thiazole, 2-methyl-	323.5	839	C_4H_5NS	3581-87-1	809.8	815							green, vegetable
furan, 2,2'-methylenebis-	637.9	935	$C_9H_8O_2$	1197-40-6	1086.7	1088							rich, roasted
acetophenone	621.2	894	C_8H_8O	98-86-2	1070.3	1065							sweet, almond, pungent, hawthorn, mimosa, acacia, chemical
pentanoic acid, 4-oxo-, methyl ester	537.2	912	$C_6H_{10}O_3$	624-45-3	989.4	982							caramellic
furan, 2-(2-furanylmethyl)-5-methyl-	728.4	926	$C_{10}H_{10}O_2$	13678-51-8	1183	1190							
2(3H)-furanone, dihydro-5-methyl-	500.1	899	$C_5H_8O_2$	108-29-2	956.7	958							cocoa, woody, sweet, herbal, warm, tobacco
1-(2-thienyl)-1-propanone	736.4	854	C_7H_8OS	13679-75-9	1190.7	1185							caramel, creamy
furan, 2,2'-[oxybis(methylene)]bis-	836.0	915	$C_{10}H_{10}O_3$	4437-22-3	1305.5	1299							coffee, nutty, earthy
benzoxazole, 2-methyl-	670.4	839	C_8H_7NO	95-21-6	1120.1								burnt, tobacco, phenolic, meaty, powdery, capers
1H-pyrrole, 1-ethyl-	331.2	923	C_6H_9N	617-92-5	816.6	821							burnt flavors
methyl 2-furoate	524.0	888	$C_6H_6O_3$	611-13-2	977.8	980							tobacco, fruity, mushroom, fungus, sweet
phenol, 2-methoxy-	644.0	941	$C_7H_8O_2$	90-05-1	1092.6	1090							smoke, spice, vanilla, woody, phenolic
5,6,7,8-tetrahydroquinoxaline	759.2	845	$C_8H_{10}N_2$	34413-35-9	1216.4	1223							roasted, nut, musty, bean, cereal, corn, chip, cheese
phenol	529.9	949	C_6H_6O	108-95-2	983.1	980							phenolic plastic rubber
methyl-2-thiophenecarboxylate	665.6	852	$C_6H_6O_2S$	5380-42-7	1115								burnt flavors
dihydro-2(3H)-thiophenone	554.1	922	C_4H_6OS	1003-10-7	1004.7								burnt, garlic
phenol, 4-ethyl-2-methoxy-	818.0	900	$C_9H_{12}O_2$	2785-89-9	1284.3	1282							spicy, smoky, bacon, phenolic, clove
pyridine, 2-ethyl-	444.6	853	C_7H_9N	100-71-0	908.2	906							green, grassy
pyridine, 3-methoxy-	551.6	831	C_6H_7NO	7295-76-3	1002.3	1005							
pyridine, 3-methyl-	391.9	843	C_6H_7N	108-99-6	864.9	863							earthy, hazelnut, green flavors
furfuryl sulfide	969.3	888	$C_{10}H_{10}O_2S$	13678-67-6	1474.6	1463							coffee, mushroom, earthy, powerful, meaty, sulfury
pyridine, 3-ethyl-	503.5	912	C_7H_9N	536-78-7	959.9	959							roasted flavors; tobacco, oakmoss, leather odors



Conclusion

The *Pegasus* BT 4D is a powerful analytical tool that allows for non-target GC or GC×GC-TOFMS analyses to gain insight and learn more about your complex samples. In this work, variations in coffee samples relating to the roast level of the beans were investigated. HS-SPME collected the volatile and semi-volatile analytes from the coffee samples, GC or GC×GC separated the analytes from each other, and TOFMS detection provided identification and relative quantitation information for hundreds of analytes. A comparison of six different coffee samples prepared from medium and dark roasted beans found specific analytes that appear to relate to the roasting styles, independent of geographical origin. Key GC×GC benefits were demonstrated that uncovered analytes not detected with GC.

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Form No. 203-821-556 9/19-REV1