# Non-Targeted Screening of Nutritional Supplements with GC-TOFMS and GC×GC-TOFMS

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## Background

Understanding the composition of nutritional supplements that are commonly taken by many consumers and athletes is important. Nutritional supplements are more loosely regulated by the FDA, and are not subjected to the same processes and requirements as pharmaceutical drugs, so questions of efficacy and safety can occur. Typically, manufacturers and distributors are responsible for ensuring that all claims and information on their packaging material are truthful and not misleading, and the consumer relies on their compliance. Athletes are often concerned about unintentional consumption of unreported substances in supplements that are performance enhancing or otherwise banned within their sport. Thus, it is not uncommon for athletes to pay to have their supplements screened for banned substances. The work reported here aims to explore what else is present in supplements through complementary analytical technologies. We performed a general non-targeted extraction of a variety of nutritional powders and pills, followed by non-targeted analyses with gas chromatography (GC), two-dimensional GC (GC×GC), and time-of-flight mass spectrometry (TOFMS). GC×GC allowed for the chromatographic separation of analytes that coelute in a one-dimensional separation and TOFMS detection provided identifications through library matching. Further insight to these complex supplements was gained and is reported.

## Methods

*Samples and Sample Preparation*

A variety of commercially available nutritional supplements were analyzed, including two protein powders, three vitamin tablets/capsules, and two sports drink powders. The samples were prepared for analysis with QuEChERS. For each sample, 1 g of sample (crushed with mortar and pestle, if needed) was soaked in 10 mL of water for 30 minutes. 10 mL of acetonitrile was added and the sample was placed on an automatic shaker for 20 minutes. The EN QuEChERS salts (each packet contains: 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogencitrate sesquihydrate) were added, followed by another minute of shaking. The samples were centrifuged for 10 minutes and the supernatant was analyzed.

*Instrument conditions*

GC and GC×GC analyses were performed with LECO's Pegasus® 4D, consisting of an Agilent 7890 GC modified with LECO's dual-stage quad jet thermal modulator and secondary oven, and paired with LECO's Pegasus TOFMS. Method parameters are listed in Table 1.

**Table 1. Instrument Conditions for GC and GC×GC**

| | |
|---|---|
| Injection | 1 µL splitless to 250°C inlet via Gerstel MPS2 Autosampler |
| Carrier gas | He at 1.4 mL/min (pressure corrected constant flow for GC×GC) |
| Columns | Rxi-5Sil MS, 30 m x 0.25 mm x 0.25 µm (Restek) paired with Rxi-17Sil MS, 0.7 m x 0.25 mm x 0.25 µm (Restek) |
| Temperature Program | 2 min at 90°C, ramped 10°C/min to 325°C, held 5 min; Secondary oven maintained +10°C relative to primary |
| Modulation | 2 s with temperature maintained +15°C relative to 2nd oven |
| **Pegasus Conditions** | |
| Mass Range | 33-550 m/z |
| Acquisition Rate | 10 spectra/s for GC and 100 spectra/s for GC×GC |
| Source Temp | 250°C |

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## Benefits of GC×GC

GC×GC, shown in Figure 1, subjects a complex sample to two complementary separations simultaneously. Relative to GC, GC×GC often separates more individual analytes and provides better characterization for complex samples. The primary benefits (improved peak capacity, enhanced S/N, and structured chromatograms) are demonstrated in Figures 2-4.

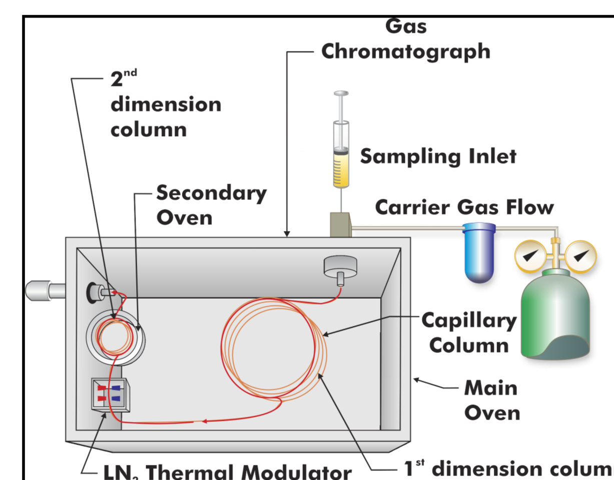



**Figure 1: GC×GC couples two columns with complementary stationary phases in series. A thermal modulator connects the two columns, and effluent from the first is cryogenically focused and injected to the second at set intervals (modulation periods). The effluent from the second column is introduced to TOFMS which provides full mass range data for identification and quantification.**

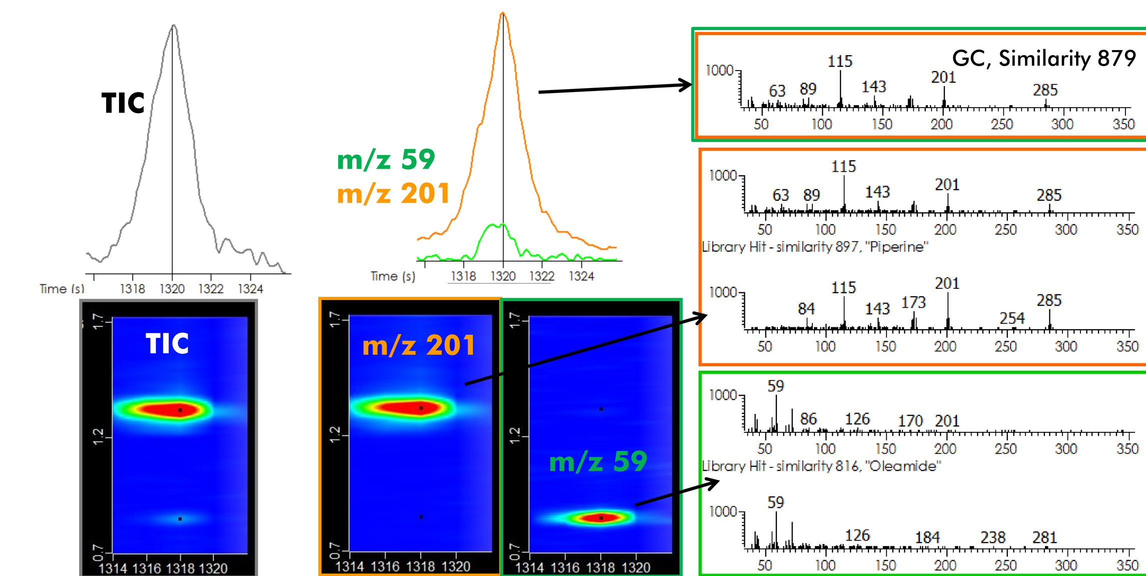



**Figure 2: Increased Peak Capacity - each sample is simultaneously and comprehensively separated on two complementary GC columns. Analytes that coelute in the first separation dimension are often separated in the second dimension. Here, piperine and oleamide in the multivitamin were combined to one peak marker, identified as piperine by GC, and were chromatographically separated with GC×GC.**

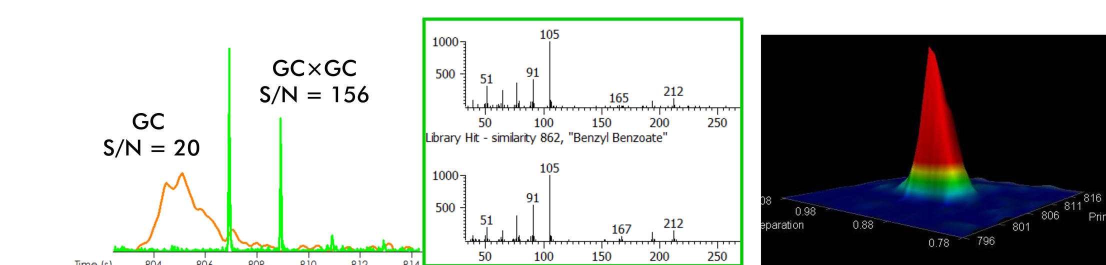



**Figure 3: Lower Limit of Detection - thermal modulation collects and refocuses effluent between the first and second column, sharpening analyte peaks just prior to detection, increasing the S/N, and leading to more detectable analytes. Benzyl benzoate, a preservative detected in the chocolate protein powder, had a S/N of 20 in the GC data and 156 in the GC×GC data.**

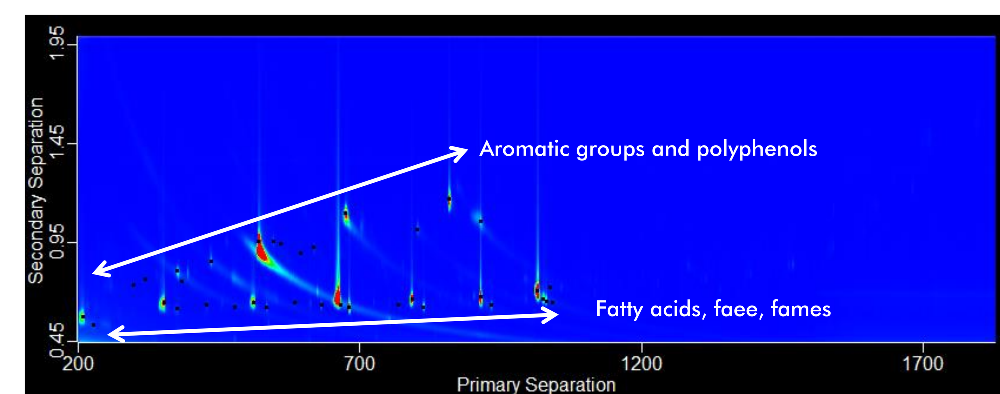
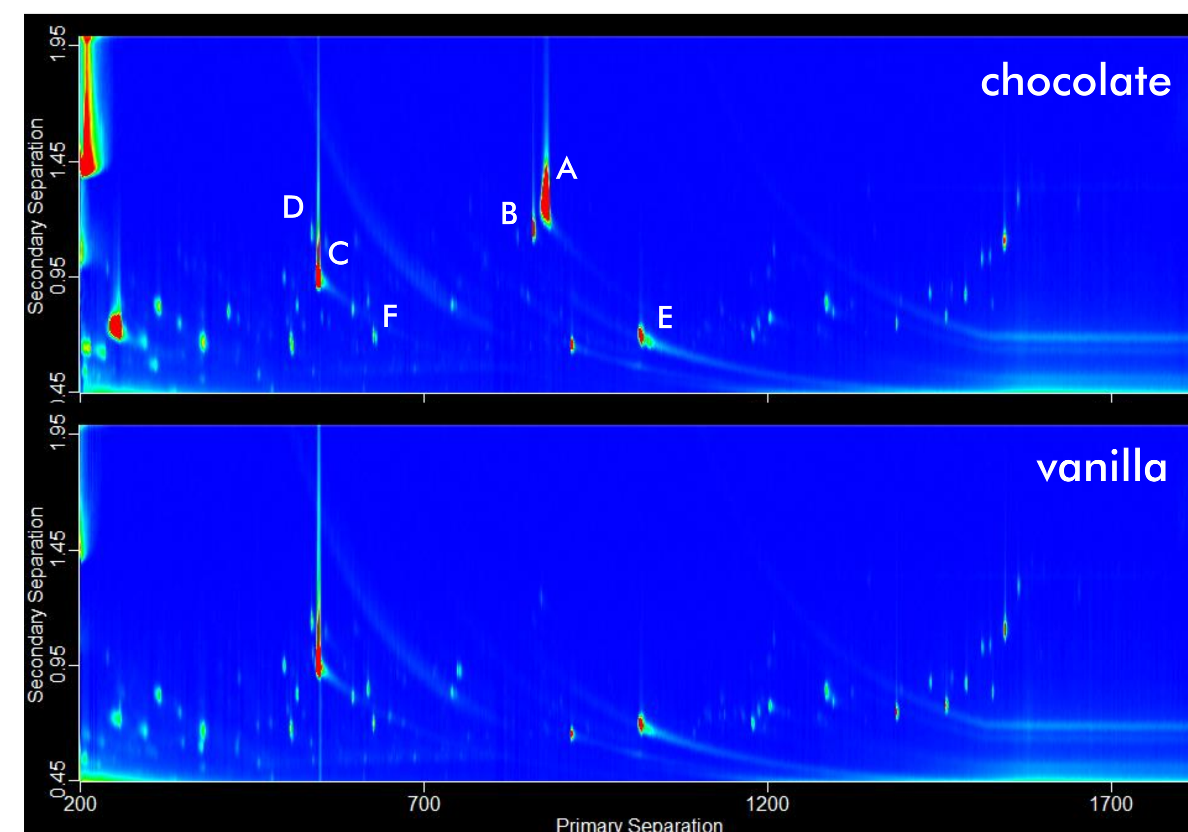



**Figure 4: Structured Chromatograms - analytes with similar functional groups elute in structured bands through the 2D space providing characterization information. Here, the fatty acids and fatty acid methyl and ethyl esters elute at earlier 2nd dimension retention times, while the aromatic and polyphenols in the antioxidant tablet are later eluting.**

## Protein Powder Characterization

A chocolate and vanilla protein powder were analyzed. These samples contained a variety of flavor compounds, vitamins, fatty acids, fatty acid ethyl and methyl esters, preservatives, and additives. Some representative examples are shown below in Figure 5.




| | Name and (CAS) | Similarity | R.T. (s) | Notes | Observed in |
|---|---|---|---|---|---|
| A | Theobromine (83-67-0) | 902 | 878 , 1.26 | Naturally occurring in chocolate | Chocolate |
| B | Caffeine (58-08-2) | 951 | 858, 1.16 | Stimulant | Chocolate |
| C | Vanillin (121-33-5) | 963 | 546, 0.98 | Flavor analyte | Both |
| D | Niacinamide (98-92-0) | 950 | 536, 1.14 | Vitamin | Both |
| E | Linoleic acid (60-33-3) | 915 | 1014, 0.71 | Fatty Acid | Both |
| F | BHT (128-37-0) | 872 | 630, 0.69 | Controversial additive | Chocolate |

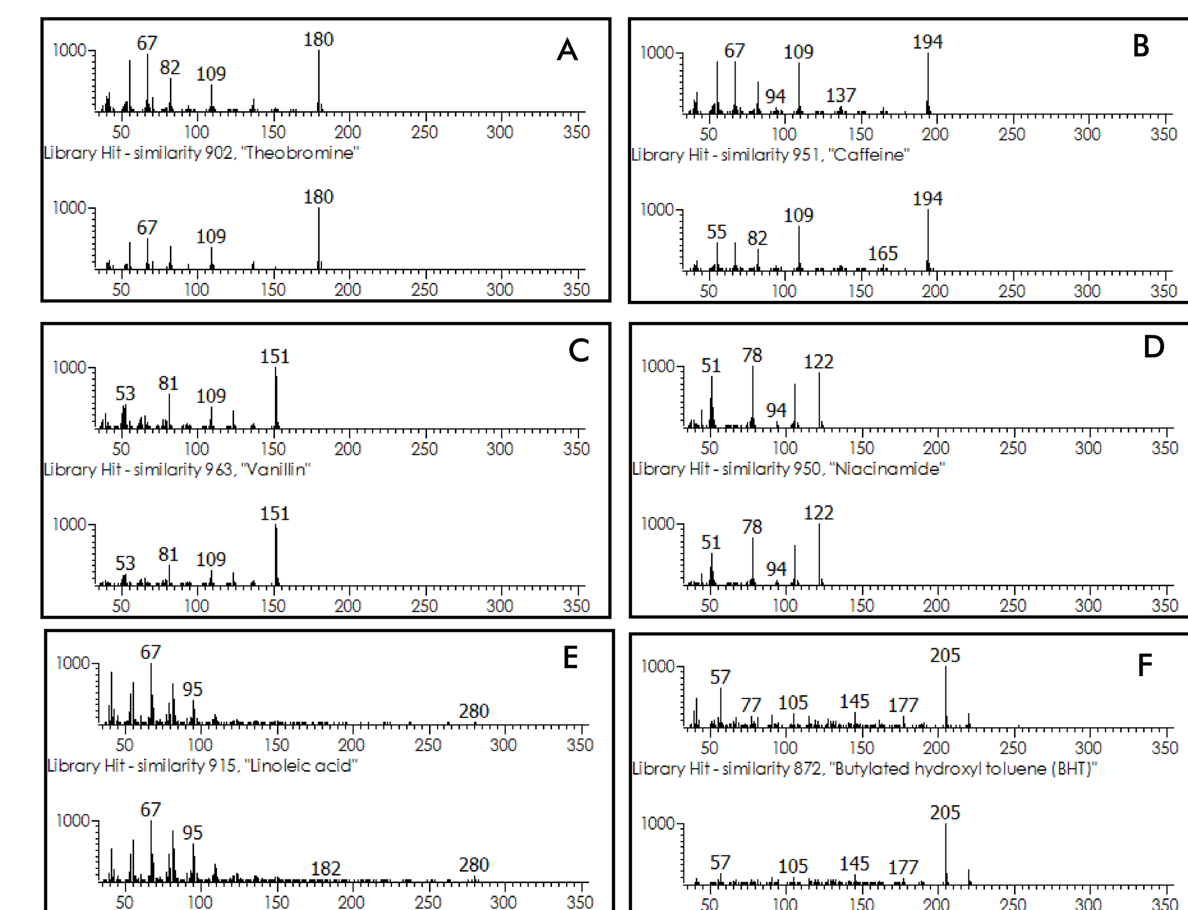
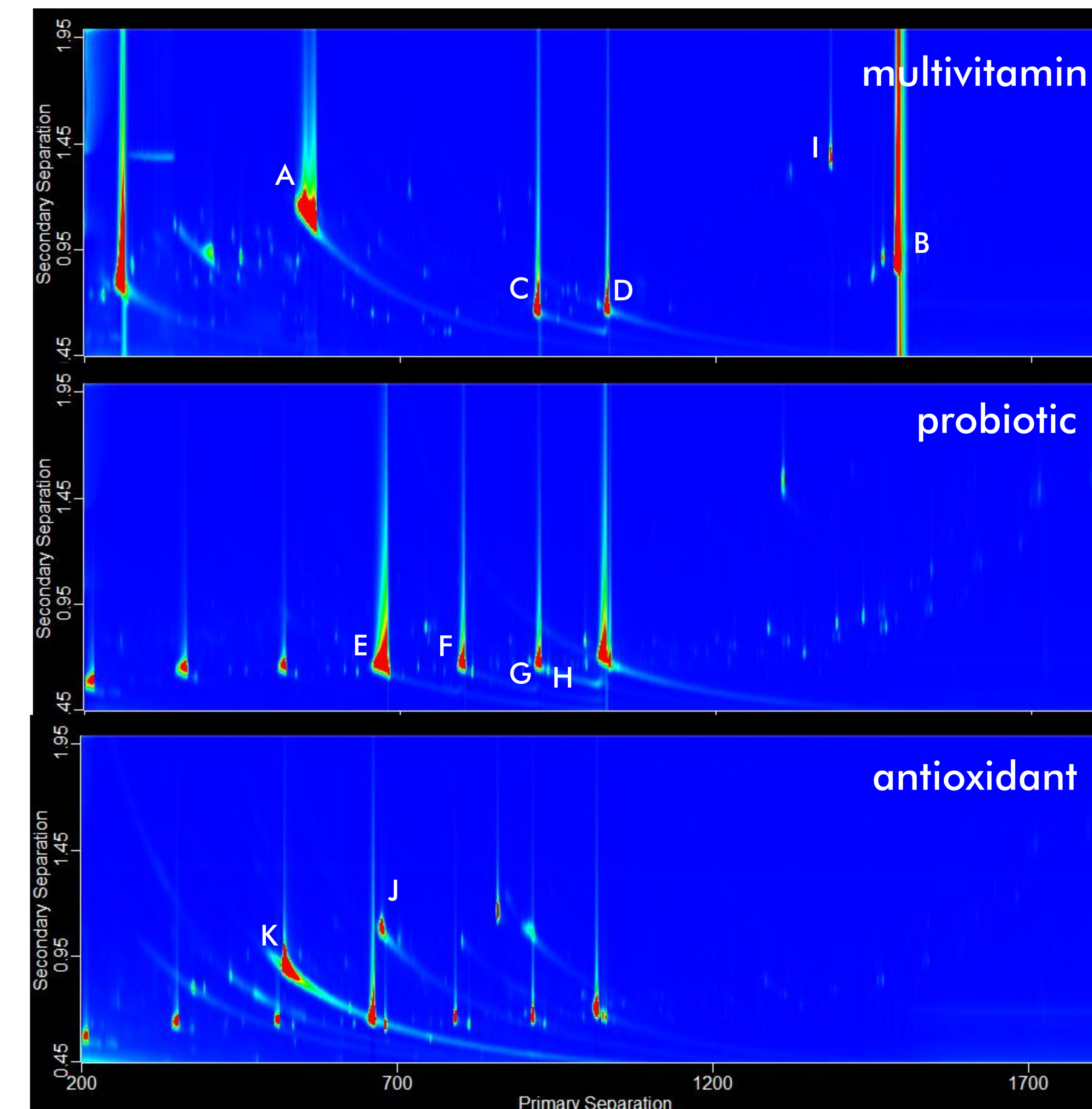



**Figure 5. TIC chromatograms for a chocolate and vanilla protein powder mix are shown. A collection of representative analytes are labeled (A-F), and listed in the associated table. Corresponding MS information is shown. BHT, an additive banned in some countries, was observed in the chocolate protein mix.**

## Vitamin Characterization

A daily vitamin regimen, consisting of a multivitamin, probiotic, and antioxidant, was analyzed. These samples contained several vitamins, fatty acids, fatty acid methyl and ethyl esters, flavor compounds, and polyphenols. Some representative examples are shown below in Figure 6.




| | Name and (CAS) | Similarity | R.T. (s) | Notes | Observed in |
|---|---|---|---|---|---|
| A | Niacinamide (98-92-0) | 966 | 564, 1.1 | Vitamin | Multi & Pro |
| B | Vitamin E Acetate (58-95-7) | 749 | 1486, 0.98 | Vitamin | Multi |
| C | Palmitinic acid (57-10-3) | 910 | 916, 0.70 | Fatty Acid | All |
| D | Stearic acid (57-11-4) | 924 | 1028, 0.71 | Fatty Acid | All |
| E | Lauric Acid (143-07-7) | 934 | 670, 0.71 | Fatty Acid | Pro |
| F | Myristic acid (544-63-8) | 920 | 800, 0.70 | Fatty Acid | All |
| G | Methyl palmitate (112-39-0) | 923 | 894, 0.64 | Fatty Acid Methyl Ester | All |
| H | Ethyl palmitate (628-97-7) | 879 | 934, 0.64 | Fatty Acid Ethyl Ester | Pro & Anti |
| I | Piperine (94-62-2) | 911 | 1380, 1.40 | Pepper extract flavor | Multi |
| J | 1,3,5-benzenetriol (108-73-6) | 956 | 674, 1.10 | Polyphenol | Anti |
| K | 1,2,3-benzenetriol (87-66-1) | 958 | 520, 0.96 | Polyphenol found in coffee | Anti |

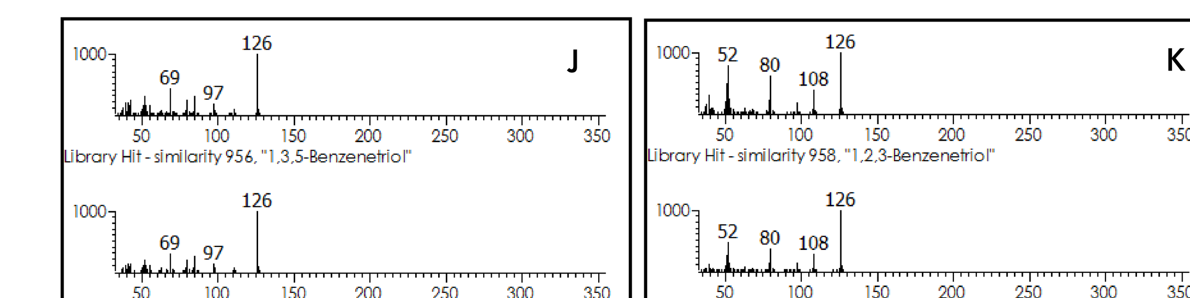
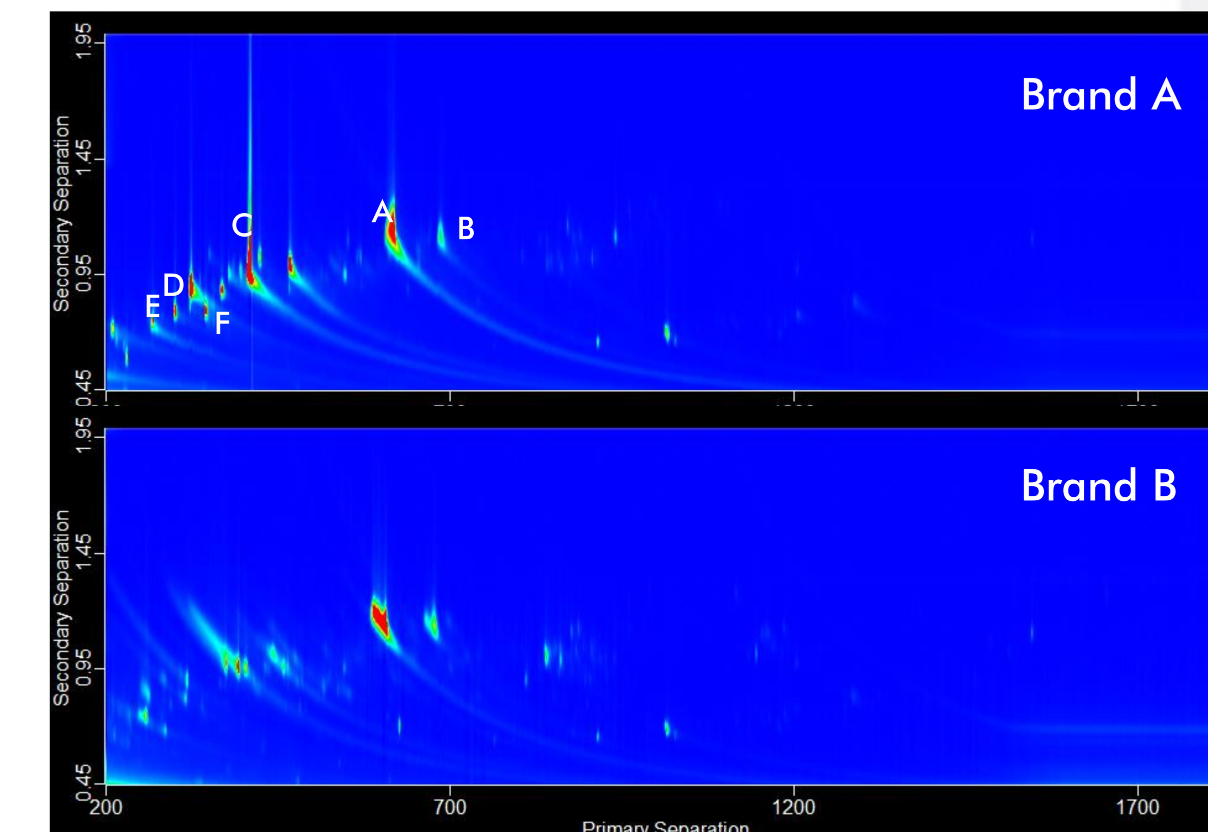



**Figure 6. TIC chromatograms for a multivitamin, antioxidant, and probiotic supplement are shown. A collection of representative analytes are labeled (A-K), and listed in the associated table. Corresponding MS information is shown.**

## Sports Drink Characterization

Two sports drink powders were also analyzed. A variety of sugars and flavor analytes were observed. Some representative examples are shown below in Figure 7.




| | Name and (CAS) | Similarity | R.T. (s) | Notes | Observed in |
|---|---|---|---|---|---|
| A | Levoglucosan (498-07-7) | 917 | 618, 1.14 | Sugar | Both |
| B | 1,6-Anhydro-β-D-glucofuranose (74252-74-3) | 872 | 686, 1.12 | Sugar | Both |
| C | 5-Hydroxymethylfurfural (67-47-0) | 864 | 410, 1.01 | Flavor | Brand A |
| D | Methyl 3-furoate (13129-23-2) | 881 | 300, 0.80 | Flavor | Brand A |
| E | Levulinic acid (123-76-2) | 940 | 268, 0.76 | Flavor (sweet, acidic) | Brand A |
| F | Butanedioic acid (110-15-6) | 855 | 346, 0.74 | Flavor (sour, acidic) | Brand A |

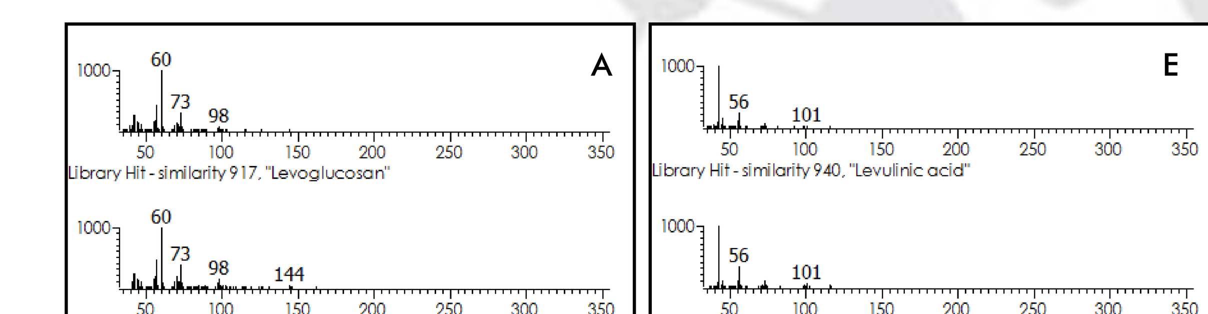



**Figure 7. TIC chromatograms for two sports drink mixes are shown. A collection of representative analytes are labeled (A-F), and listed in the associated table. Corresponding MS information is shown.**

## Conclusions

The experiments described in this poster demonstrate a non-targeted characterization of various nutritional supplement products. A selection of commercially available protein powders, sports drink mixes, and vitamins were prepared through a general QuEChERS extraction and analyzed by GC and GC×GC paired with TOFMS detection. GC×GC allowed for the chromatographic separation of analytes that coelute in a one-dimensional separation and the detection of analytes with low S/N that were below the thresholds with GC. TOFMS detection provided identifications through library matching and gave further insight to these complex supplements. A variety of vitamins, fatty acids, fatty acid ethyl and methyl esters, sugars, lactones, flavor compounds, and additives were observed.