

Instrument: GC-TOFMS and ChromaTOF® Sync Software

Chemical Profiling of Commercially Available Cola Beverages Using GC-TOFMS and ChromaTOF Sync

LECO Corporation; Saint Joseph, Michigan USA

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Introduction

Cola is a flavored, carbonated soft drink that usually contains natural flavor ingredients such as vanilla extract, lime juice, essential oils of orange, lemon, cassia or cinnamon, coriander, nutmeg, and neroli, as well as other flavorings. The mixture of these ingredients and unknown trade secret ingredients are responsible for the uniqueness and complexity of the product. While the cola flavor itself is highly desirable, some of the volatile flavor (essential oils) components have been found to be unstable, undergoing acid-catalyzed degradation, leading to a reduction in product quality.¹ Thus, it is important for us to understand the chemical profiling of these beverages.

Gas Chromatography (GC) with Time-of-Flight Mass Spectrometry (TOFMS) is a powerful analytical tool that can reveal individual analyte components in complex samples. As there is a balance of numerous odorants that are responsible for the unique and characteristic aroma of cola flavored beverages, we will need to uncover a more complete picture of the chemical profile of cola to understand these products. This detailed data is also beneficial for us as we can answer many analytical questions such as the authenticity of the brand or product, specific chemicals that may contribute to the sensory observations, off-odor chemicals that may contribute to product quality, etc. In this study, HS-SPME was used to extract the Volatile Organic Compounds (VOCs) of 13 commercially available cola beverages for analysis by GC-TOFMS. The data were then processed using our data alignment tool *ChromaTOF Sync*, which compiles peak information through sets of samples by performing automated peak finding and deconvolution of the high quality TOFMS data on the set of samples. This allows quick identification of trends across complex samples and a streamlined workflow.



Experimental

A variety of commercially available cola beverages produced by eight brands were analyzed with the *Pegasus BT*. The sample preparation steps as well as the instrumental parameters are described in Table 1.

Table 1: GC-TOFMS sample and instrument conditions.

Sample Introduction	HS-SPME
Sample Volume	20 μ L in a 10 mL vial
SPME Fiber	Triple-phase Supelco SPME fiber (DVB/CAR/PDMS)
Incubation	1 min @ 60 °C
Extraction	10 min @ 60 °C
Desorption	3 min @ 250 °C
GC (in 1D mode)	Agilent 7890
Injection	3 min fiber desorption with inlet temp 250 °C, Split 10:1
Columns	1D: Rxi-SVOC, 20 m x 0.18 mm x 0.18 μ m (Restek) 2D: Rxi-17Sil MS, 1 m x 0.18 mm x 0.18 μ m (Restek)
Carrier Gas	Helium @ 1.01 mL/min
Oven Program	50 °C (0.3 min), ramp @ 15.6 °C/min to 280 °C (3.2 min)
Secondary Oven Offset	+5 °C (relative to primary oven temperature)
Modulator Offset	+15 °C (relative to secondary oven temperature)
Transfer Line	300 °C
MS	LECO <i>Pegasus BT</i> TOFMS
Ion Source Temperature	250 °C
Mass Range	35-400 m/z
1D Acquisition Rate	10 Spectra/s

Results and Discussion

Non-targeted sample characterization is important for competitive analysis because target analytes often provide an incomplete picture of the sample. The non-targeted volatile analysis of 13 commercially available cola drinks were performed in triplicate by HS-SPME and GC-TOFMS. The representative total ion chromatograms (TIC) from each sample are shown in Figure 1. The samples were processed with *ChromaTOF*'s automated Peak Finding and deconvolution algorithms with a minimum Signal-to-Noise (S/N) of 10. Approximately 150 peaks were detected that matched the NIST library databases with similarity scores ≥ 700 .

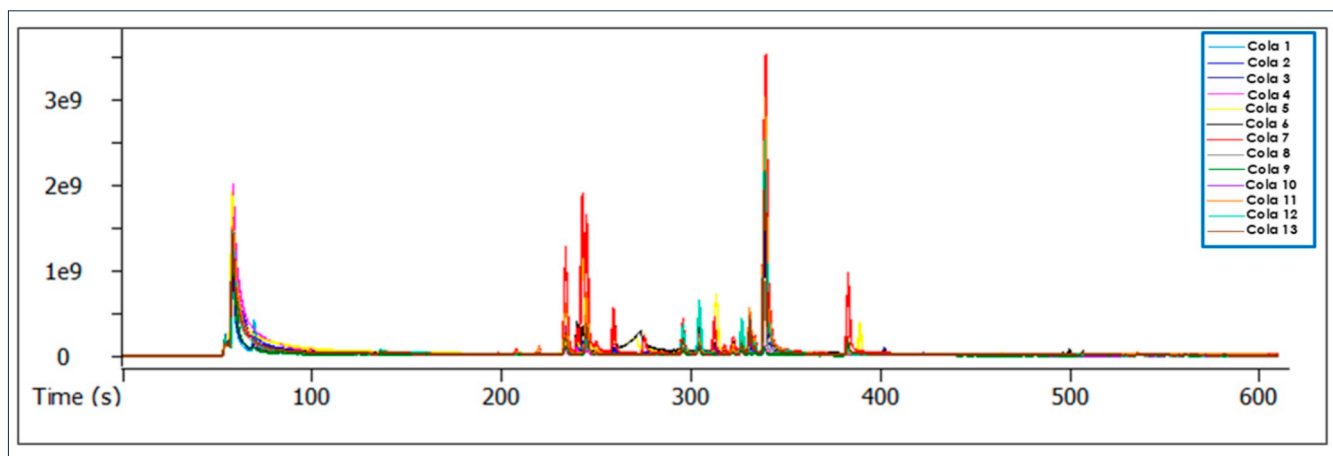


Figure 1. Overlaid Total Ion Chromatograms (TIC) of the 13 cola beverage samples analysed (in triplicate).

Identifications for these untargeted analytes can often be determined by matching the observed mass spectral (MS) and retention index (RI) data to library databases, which can provide insight to the products. For example, many of the volatile flavorings in cola are not stable under acidic conditions, and this leads to degradation of potent odorants resulting in flavor changes and reduced product quality. As an example, the overlaid TIC chromatograms of the analytes p-cymene (an off-odor, degradation product of γ -terpinene and/or limonene), Limonene (flavorant), and Eucalyptol (flavorant) are shown in Figure 2.

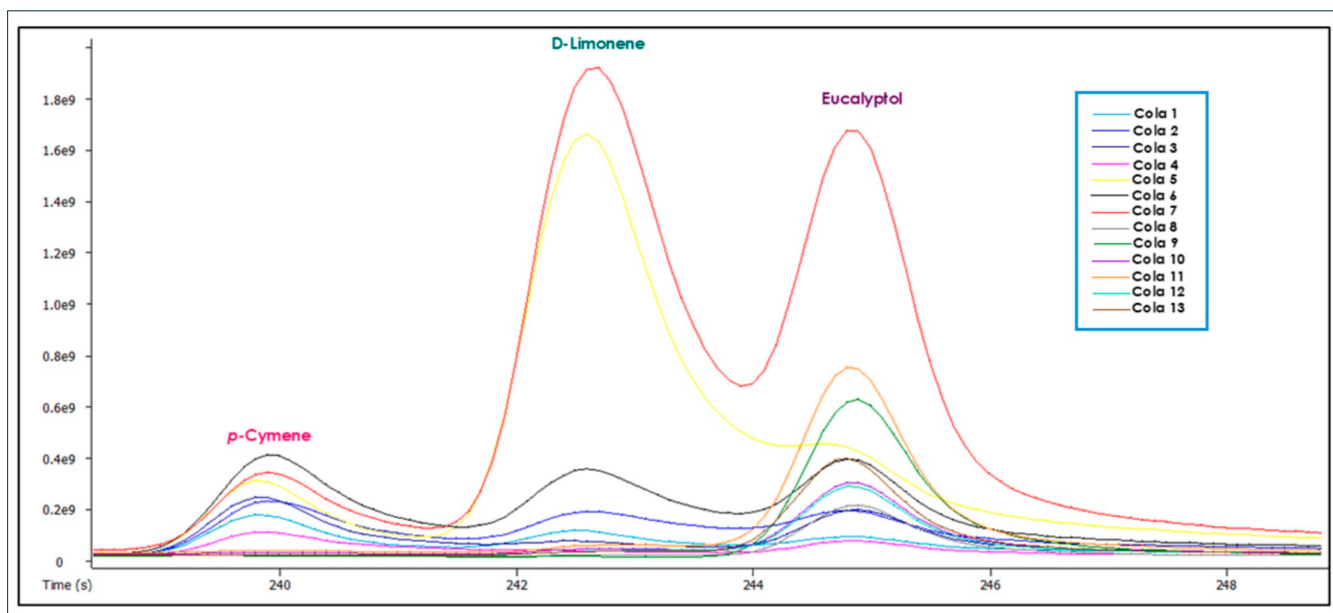


Figure 2. Overlaid Total Ion Chromatograms (TIC) of p-Cymene, D-Limonene, and Eucalyptol.

The identifications of p-Cymene, D-Limonene, and Eucalyptol were determined with spectral matching to the NIST library database (Figure 3). The spectral similarity scores of p-Cymene were observed as 916/1000, D-Limonene was 863/1000 and Eucalyptol was 816/1000. These results were further supported by matching RI values, p-Cymene: RI_{obs} = 1028 and RI_{lib} = 1025; D-Limonene: RI_{obs} = 1033 and RI_{lib} = 1031; and Eucalyptol: RI_{obs} = 1037 and RI_{lib} = 1032.

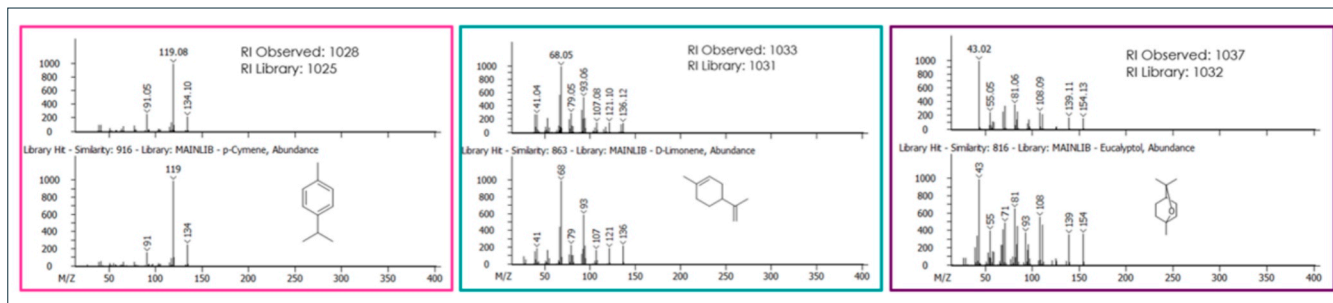


Figure 3. p-Cymene, D-Limonene and Eucalyptol have been identified with library searching of the full m/z data and supported with RI matching to library.

However, the ability to generate more data can lead to a burden of more time-consuming data processing. To maximize the benefit of the data generated by these techniques, ChromaTOF Sync was used to streamline our workflow, and process the complete set of 1D data files together to compile analyte information and determine trends quickly and easily in the data.

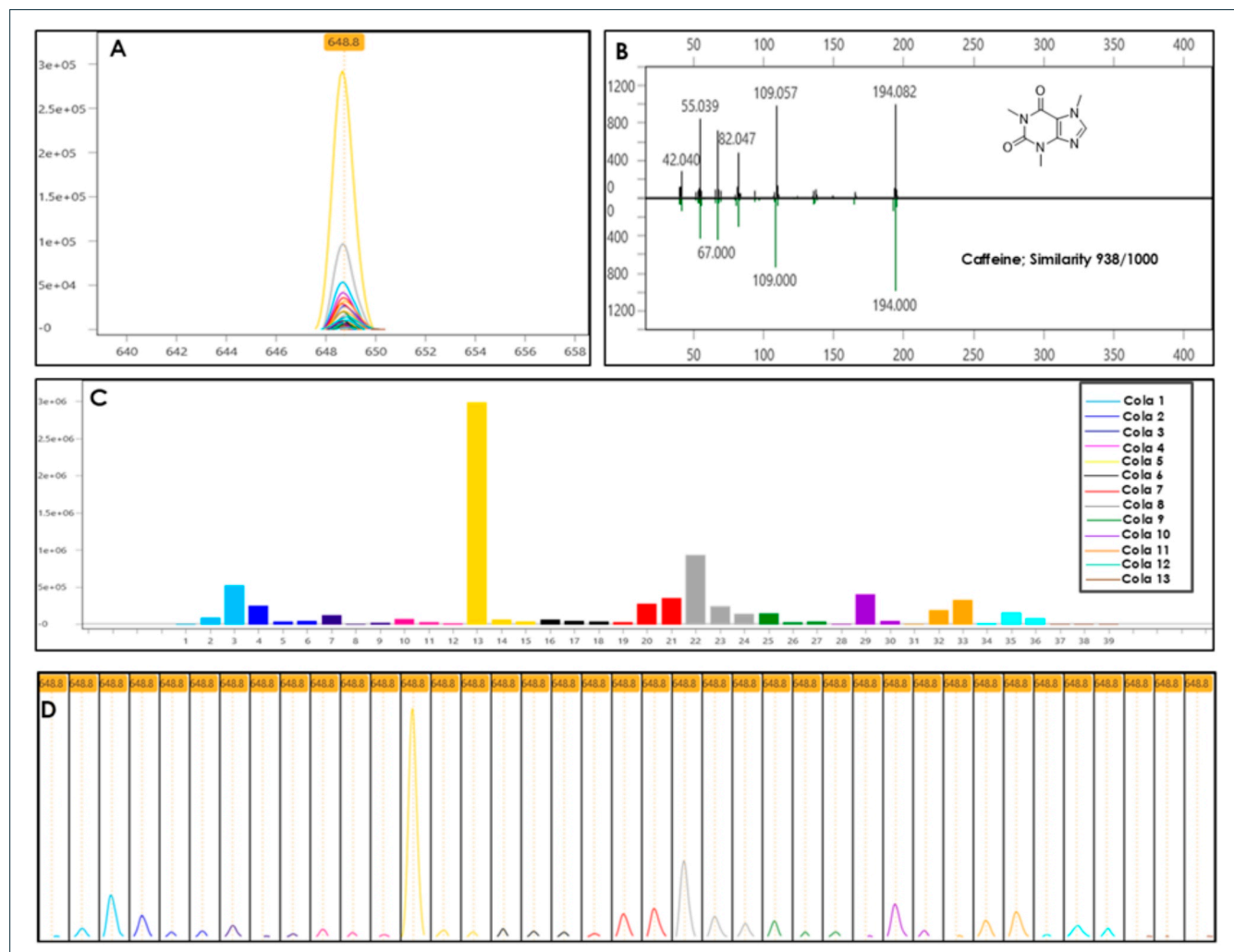


Figure 4. Investigation of caffeine across the 13 cola samples. (A) overlaid Total Ion Chromatograms (TIC) of caffeine; (B) observed spectrum of caffeine (black) matched to the NIST library database (green); (C) bar chart; (D) side-by-side plot.

Individual features can be explored from the results compiled by the *ChromaTOF Sync* software. For example, caffeine was found at $R_t = 648.8$ s in all the cola beverages (Figure 4A). The observed mass spectrum (Figure 4B) was matched to caffeine in the NIST library database with a similarity score of 938/1000. This identification was further supported with RI information. The calculated RI for this peak was 1857, showing good agreement with the library RI value of 1837. It was expected that Cola 7 would have more caffeine present because this product is generally marketed as an energy drink. Interestingly enough, it was the Cola 5, an unsweetened beverage, that contained the highest amount of caffeine. The trend of this analyte across the 13 samples can be observed in the overlaid chromatogram plot (Figure 4A), the side-by-side plot (Figure 4D), and the bar chart (Figure 4C).

ChromaTOF Sync also has a heatmap feature which allows for visualizing trends. This facilitates fast navigation through the peak table and the ability to easily spot the differences in the various samples. Terpenes and esters play an important role in aroma and flavor contributions, and are also hypothesized to have therapeutic properties. A selection of features (monoterpenes and terpenoids, sesquiterpenes, and esters) that were found to be abundant in the different cola beverages are shown in Tables 2, 3, and 4. Many of the terpenes and esters that are elevated are found in Cola 5 and 6 (unsweetened beverages from two different manufacturers) and Cola 7 (energy drink cola).

Table 2: Monoterpene and terpenoid trends of individual analytes present in the commercially available cola beverages.

[illegible]

Table 3: Sesquiterpene trends of individual analytes present in the commercially available cola beverages.

[illegible]

Table 4: Ester profiles of individual analytes present in the commercially available cola beverages.

[illegible]

Principal Component Analysis (PCA) was also performed to determine more trends in the data. The generated scores plot (Figure 5) represents each sample replicate as a data point. The samples that are closest to each other are more similar and those further from each other are more different. As an example, the PCA shows that Cola 3 and Cola 4 are quite similar to each other. This is very interesting since Cola 3 and Cola 4 are two of the most widely consumed cola-flavored soft drinks globally. While each is marketed with distinct branding and flavor claims, their underlying chemical compositions are remarkably similar, which contributes to their comparable taste profiles.

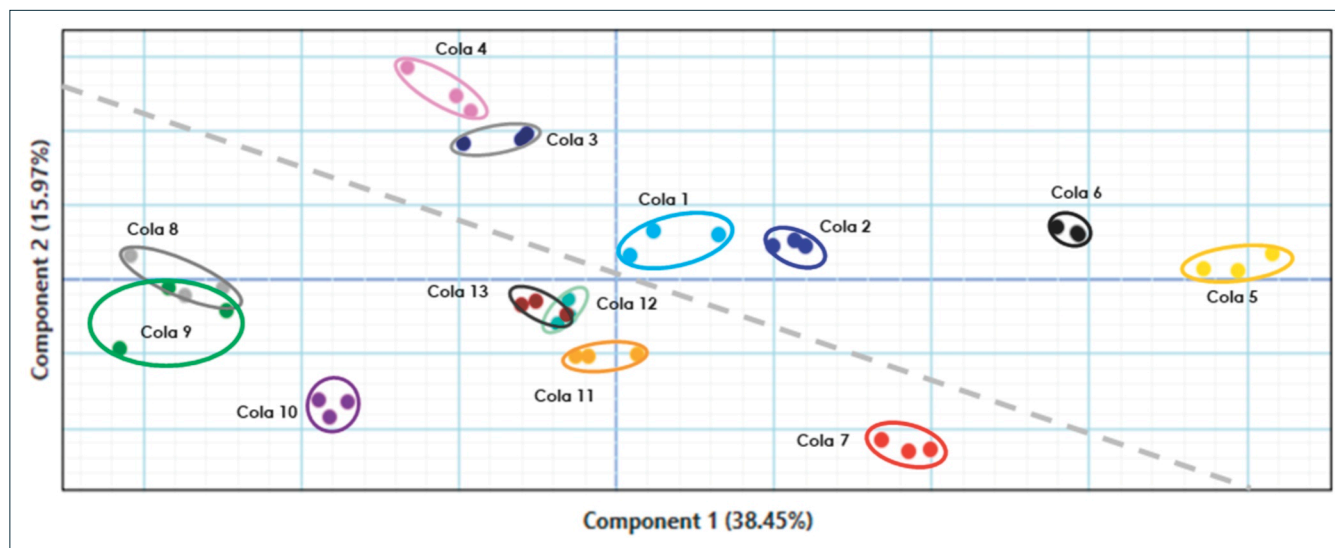


Figure 5. PCA scorings of the 13 cola beverages with a Similarity >500 and RI match with ± 30 .

Conclusion

This analysis demonstrates the value of advanced analytical technologies in revealing the subtle chemical nuances that define cola beverages. By comparing 13 different products using GC-TOFMS and ChromaTOF Sync, we uncovered detailed insights into flavor compounds, degradation markers, and formulation trends. One of the key findings was the high degree of chemical similarity between Cola 3 and Cola 4. While each is positioned uniquely in the market, their core flavor profiles are closely aligned. These insights are a powerful reminder of the importance of both product consistency and the brand story. With tools like GC-TOFMS and ChromaTOF Sync, companies can not only ensure quality and authenticity—but also better understand their market position, differentiate with confidence, and innovate with clarity.

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References

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