Application Note

Instrument: GC-TOFMS and *ChromaTOF*® Sync Software

EMPOWERING RESULTS
Elemental Analysis | GC Mass Spectrometry | Metallography

Packaging Contaminants in the Headspace of Commercially Available Cola Beverages

LECO Corporation; Saint Joseph, Michigan USA

Key Words: Cola, GC-TOFMS, Mass Spectrometry, Deconvolution, Pooled Evaporative HS-SPME, ChromaTOF Sync 1D

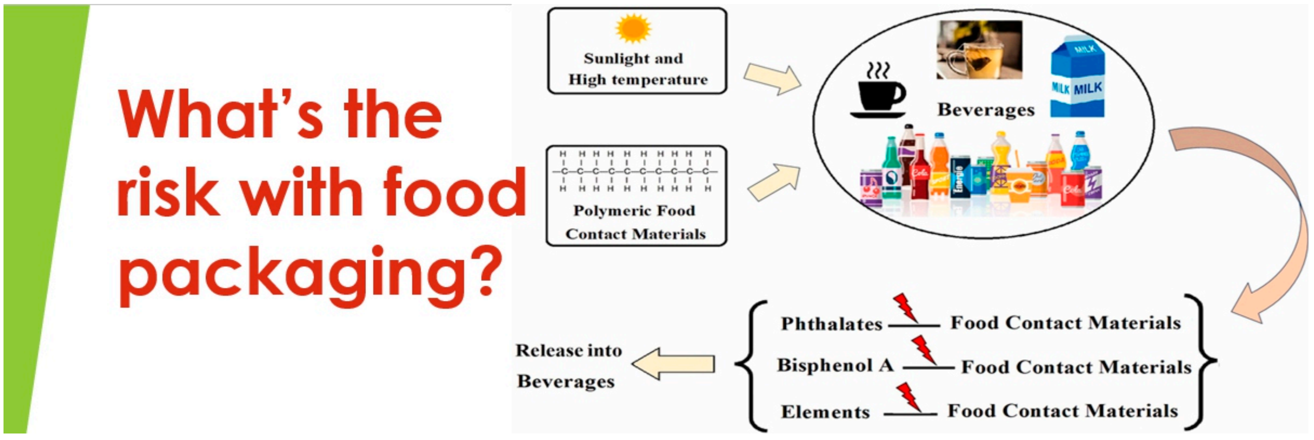
Introduction

Food packaging is an essential component in the food industry as it serves as a conveyancing material between food producers and consumers. During transit, storage, and handling, the packaging material protects the food product from encountering substances, pathogens, and contaminants that could potentially cause us harm or cause our foods to spoil prematurely.[1,2]

Food packaging is made from various types of materials such as plastics, glass, metal, paper, and their composites. These products also have additives such as plasticizers, monomers, and oligomers, and these chemicals could transfer contaminants to the food upon contact during processing or packaging. This interactive phenomenon could result in alterations in the quality and the safety of the food. The flavor may also change owing to both sorption of the food aroma (scalping) and to the transfer of undesirable components from the packaging material to the food (leaching). Thus, it is important to be able to screen for food packaging residues in edible products.[1,2]

Legislation is making manufacturers responsible for contaminants that are present in the final product as seen in the EU framework No. 1935/2004 and EU 2020/1245. Due to the complexity of these samples and the need to quickly identify potential threats, various analytical approaches have been developed over the years—such as gas chromatography (GC) and time-of-flight-mass spectrometry (TOFMS)—which is an excellent tool for non-targeted screening work. Software tools that streamline the non-targeted review and comparison of this data for multiple samples add to the power of this analytical technique. Thus, in this study, we have used *ChromaTOF* Sync, a data alignment tool that can simplify the data processing and provide strong support to the analyst in the quest for answers.

While the initial project was aimed at discovering trends of flavorants in the various colas, the presence of unexpected packaging contaminants was uncovered during data analysis. This highlights the power of GC-TOFMS for non-target work. In this application note we demonstrate an effective non-target screening for some packaging contaminants in commercially available Cola beverages. We also demonstrate the use of *ChromaTOF* Sync to easily review individual packaging contaminant trends present in the drinks analyzed.



Experimental Conditions

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: Sample Introduction and Instrument Conditions of the *Pegasus* BT

Sample Introduction	**HS-SPME**
Sample Volume	20 µL in a 10 mL vial
SPME Fiber	Triple-bed 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 µm (Restek) **2D:** Rxi-17Sil MS, 1 m x 0.18 mm x 0.18 µm (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 *Pegasus* BT TOFMS**
Ion Source Temperature	250 °C
Mass Range	35-400 m/z
1D Acquisition Rate	10 Spectra/s

Results and Discussion

Representative GC-TOFMS chromatograms for all thirteen cola samples are shown in Figure 1. These are complex samples with many analyte peaks. Initially, the sample files were data processed using peak find in *ChromaTOF* with a S/N ratio of 10, which found approximately 400 peaks per sample. This provided detailed information, allowing a thorough non-target review of each sample. Connecting this much peak information from separate peak tables to observe analyte trends across the sample set, though, can be challenging. For example, this means that across thirteen samples, an analyst would be reviewing approximately 5200 peaks; a long, time-consuming process. To streamline this sample comparison, the cola beverages were also processed with *ChromaTOF* Sync, which involved peak finding and data alignment, to produce a combined peak table with approximately 600 peaks compiled across all the samples. Many expected aroma compounds were observed, as well as some non-target food packaging-related analytes.

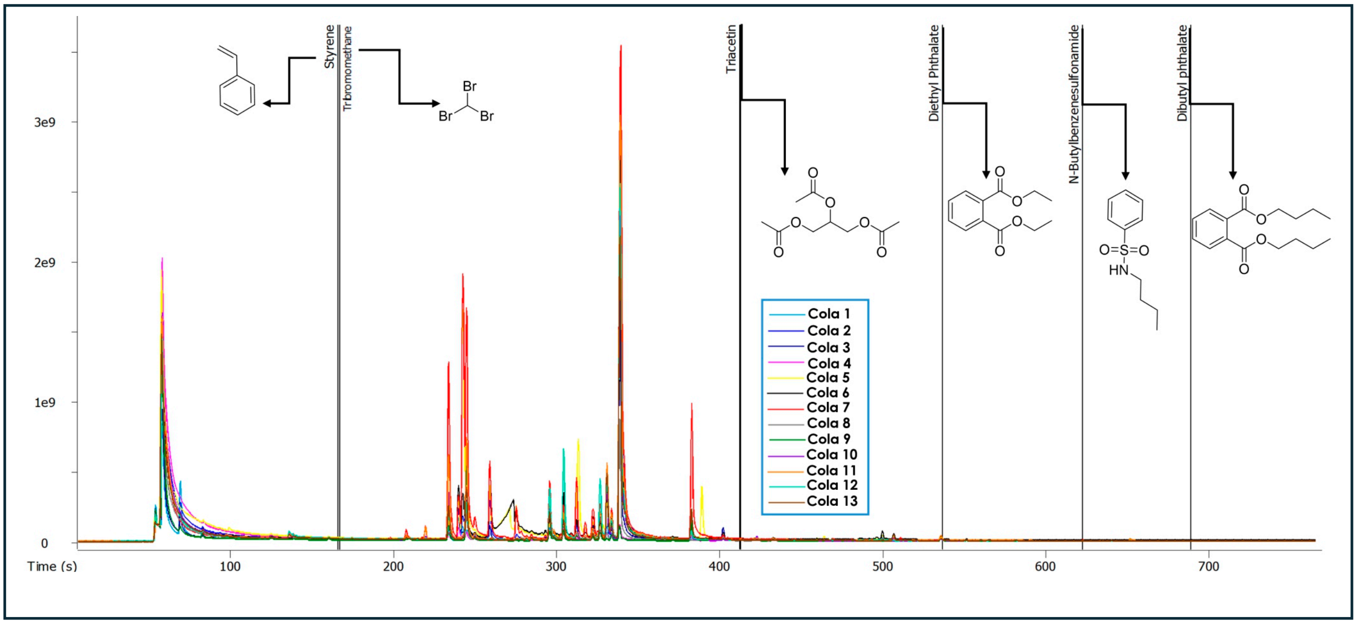
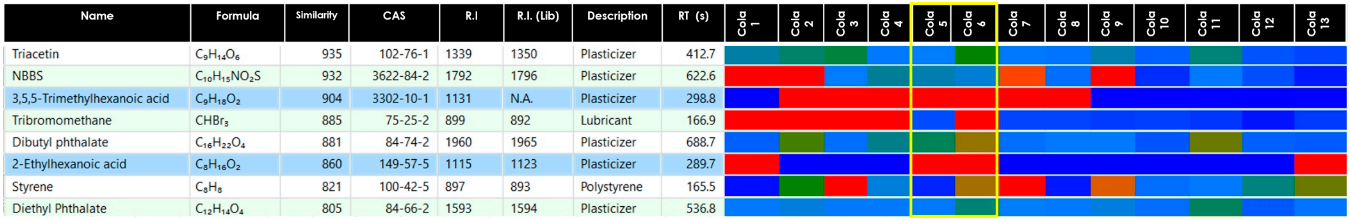


Figure 1. Packaging contaminants found in the overlaid Total Ion Chromatograms (TIC) of the thirteen cola beverage samples.

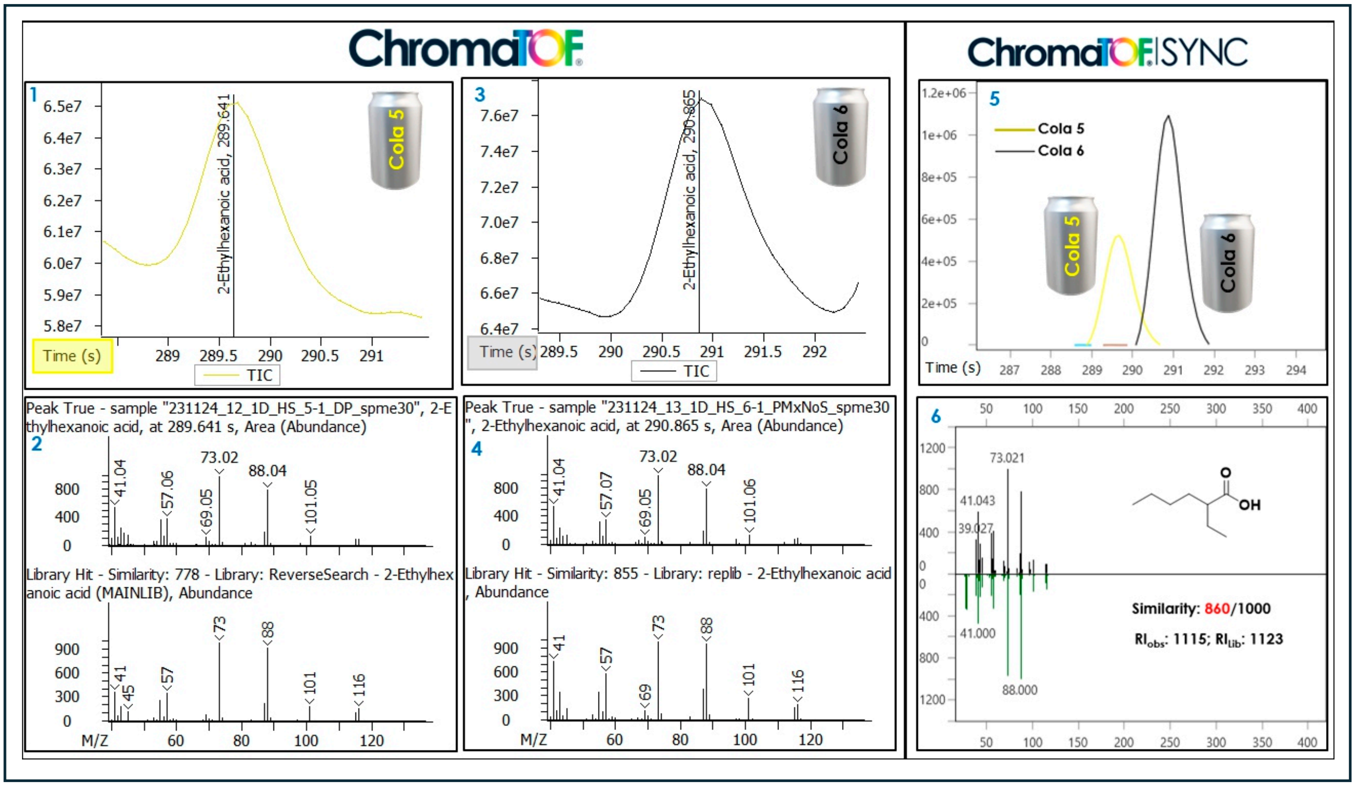
A subset of the aligned table showing some of these packaging contaminants is shown in Table 2. These were observed at elevated levels in the cola samples relative to the method blank. *ChromaTOF* Sync compiled peak areas for all samples and has a heat map feature which makes it easier to visualize the trends in the sample set. In Table 2, it can easily be seen that there are elevated levels of 2-ethylhexanoic acid and 3,5,5-trimethylhexanoic acid in Cola samples 5 and 6, both unsweetened products from the same manufacturer.

Table 2: *ChromaTOF* Sync Heat Map Feature of the Identified Packaging Contaminants



Name	Formula	Similarity	CAS	R.I	R.I. (Lib)	Description	RT (s)	Cola 1	Cola 2	Cola 3	Cola 4	Cola 5	Cola 6	Cola 7	Cola 8	Cola 9	Cola 10	Cola 11	Cola 12	Cola 13
Triacetin	$C_9H_{14}O_6$	935	102-76-1	1339	1350	Plasticizer	412.7	🟦	🟩	🟩	🟦	🟦	🟩	🟦	🟦	🟦	🟦	🟩	🟦	🟦
NBBS	$C_{10}H_{15}NO_2S$	932	3622-84-2	1792	1796	Plasticizer	622.6	🟥	🟥	🟦	🟦	🟦	🟦	🟧	🟦	🟥	🟦	🟦	🟦	🟦
3,5,5-Trimethylhexanoic acid	$C_9H_{18}O_2$	904	3302-10-1	1131	N.A.	Plasticizer	298.8	🟦	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟦	🟦	🟦	🟦	🟦
Tribromomethane	$CHBr_3$	885	75-25-2	899	892	Lubricant	166.9	🟥	🟥	🟥	🟥	🟦	🟥	🟦	🟦	🟦	🟦	🟦	🟦	🟦
Dibutyl phthalate	$C_{16}H_{22}O_4$	881	84-74-2	1960	1965	Plasticizer	688.7	🟦	🟩	🟦	🟦	🟩	🟫	🟦	🟦	🟦	🟦	🟫	🟦	🟦
2-Ethylhexanoic acid	$C_8H_{16}O_2$	860	149-57-5	1115	1123	Plasticizer	289.7	🟥	🟦	🟦	🟦	🟥	🟥	🟦	🟦	🟦	🟦	🟦	🟦	🟥
Styrene	C_8H_8	821	100-42-5	897	893	Polystyrene	165.5	🟦	🟩	🟥	🟦	🟦	🟫	🟥	🟦	🟧	🟦	🟦	🟩	🟫
Diethyl Phthalate	$C_{12}H_{14}O_4$	805	84-66-2	1593	1594	Plasticizer	536.8	🟦	🟦	🟦	🟦	🟦	🟩	🟦	🟦	🟦	🟦	🟩	🟦	🟦

ChromaTOF Sync can also accommodate retention time shifts that may occur across a multitude of samples. For example, in Figure 2, *ChromaTOF* Sync automates the identification of 2-Ethylhexanoic acid, which has shifted by 1.2 seconds in the cola 6 beverage.



Peak True - sample "231124_12_1D_HS_5-1_DP_spme30", 2-Ethylhexanoic acid, at 289.641 s, Area (Abundance)

Library Hit - Similarity: 778 - Library: ReverseSearch - 2-Ethylhexanoic acid (MAINLIB), Abundance

Peak True - sample "231124_13_1D_HS_6-1_PMxNoS_spme30", 2-Ethylhexanoic acid, at 290.865 s, Area (Abundance)

Library Hit - Similarity: 855 - Library: replib - 2-Ethylhexanoic acid, Abundance

Similarity: 860/1000

RI_{obs}: 1115; RI_{Lib}: 1123

Figure 2. (1) TIC of 2-Ethylhexanoic acid in Cola 5; (2) Mass Spectrum of 2-Ethylhexanoic acid in Cola 5; (3) TIC of 2-Ethylhexanoic acid in Cola 6; (4) Mass Spectrum of 2-Ethylhexanoic acid in Cola 6; (5) Overlaid TIC of 2-Ethylhexanoic acid in Cola 5 and Cola 6; (6) Observed Mass Spectrum of 2-Ethylhexanoic acid (black) matched to the NIST library database (green).

Individual analytes can also be reviewed to determine whether there are any trends in the samples tested. Phthalates are expected contaminants from food packaging as they are used to ensure a plastic's flexibility and durability. However, at high concentrations, they are also hormone disruptors and may cause health problems, thus it is important to keep track of such contaminants. As can be seen in Figure 3, Diethyl phthalate and Dibutyl phthalate were tentatively identified in all the cola beverages and confirmed with both spectral matching (similarity score of 805 and 881) and RI confirmation (observed RI of 1593 and 1960 compared to library RI of 1594 and 1965, respectively). Higher levels of Diethyl phthalate and Dibutyl phthalate were found present in Cola 2, Cola 6, and Cola 11 (Figure 3, sections 3 & 6).

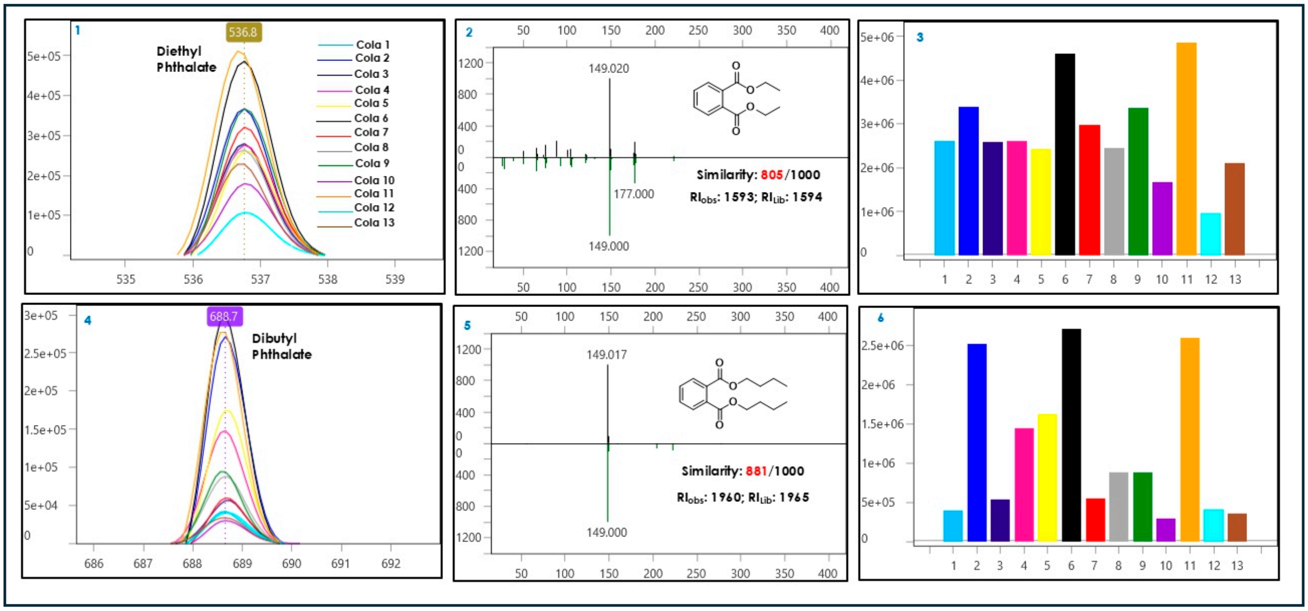


Figure 3. (1) Overlaid TIC of Diethyl Phthalate; (2) Observed Mass Spectrum of Diethyl Phthalate (black) matched to the NIST library database (green); (3) Bar Chart view of Diethyl Phthalate; (4) Overlaid TIC of Dibutyl Phthalate; (5) Observed Mass Spectrum of Dibutyl Phthalate (black) matched to the NIST library database (green); (6) Bar Chart view of Dibutyl Phthalate.

Styrene is another common packaging contaminant that is carcinogenic in high concentrations. The European Food Safety Authority allows a maximum permissible limit for styrene in food of 0.6 mg/kg whereas World Health Organization (WHO) and the World Food Organization allow a Tolerable Daily Intake of 40 mg/kg-body weight per day.[2,3,4] As shown in Figure 4, higher levels of styrene were tentatively identified in Cola 7 and confirmed with spectral matching (similarity score of 821) and with an observed RI of 897 compared to library RI of 893. This initial non-target screen uncovers analytes like these, which could then be targeted for quantification in future work in order to compare to these known exposure thresholds.

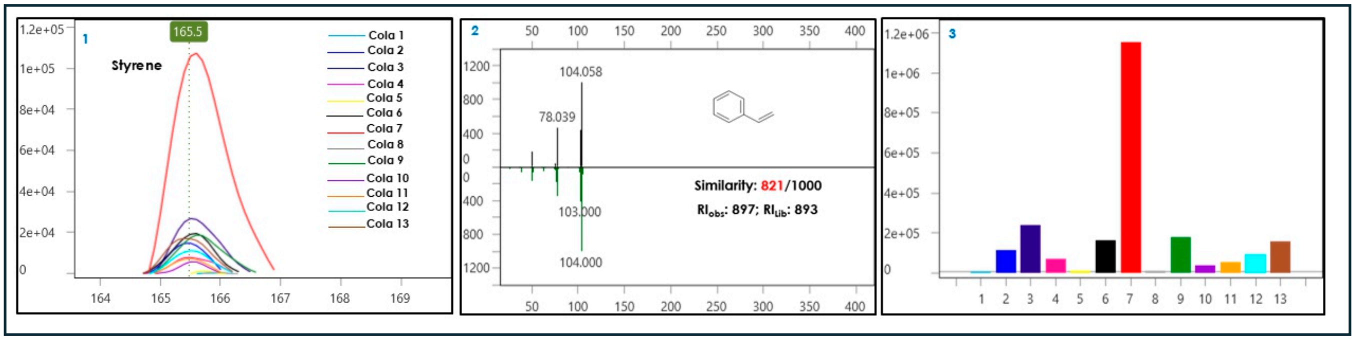


Figure 4. Elevated levels of Styrene in Cola 7, when compared to the other twelve Cola samples. (1) Overlaid Total Ion Chromatograms (TIC) of Styrene; (2) Observed Spectrum of Styrene (black) matched to the NIST library database (green); (3) Bar Chart of the Styrene levels found in the thirteen beverage samples analyzed.

The PCA scores plot (Figure 5) shows that the thirteen cola beverages are plotted separately in opposite quadrants of the principal component axes. Interestingly, these results help us to answer the age-old question of a cola war between two popular cola manufacturers. Thus, these preliminary results would suggest that Cola 3 and Cola 4, both original beverages, are very similar in its chemical profile even though they are manufactured by two different companies. Another interesting observation is that the chemical profiles differ more in the unsweetened beverages, as can be seen comparing Cola 1 and 2 to Cola 5 and 6.

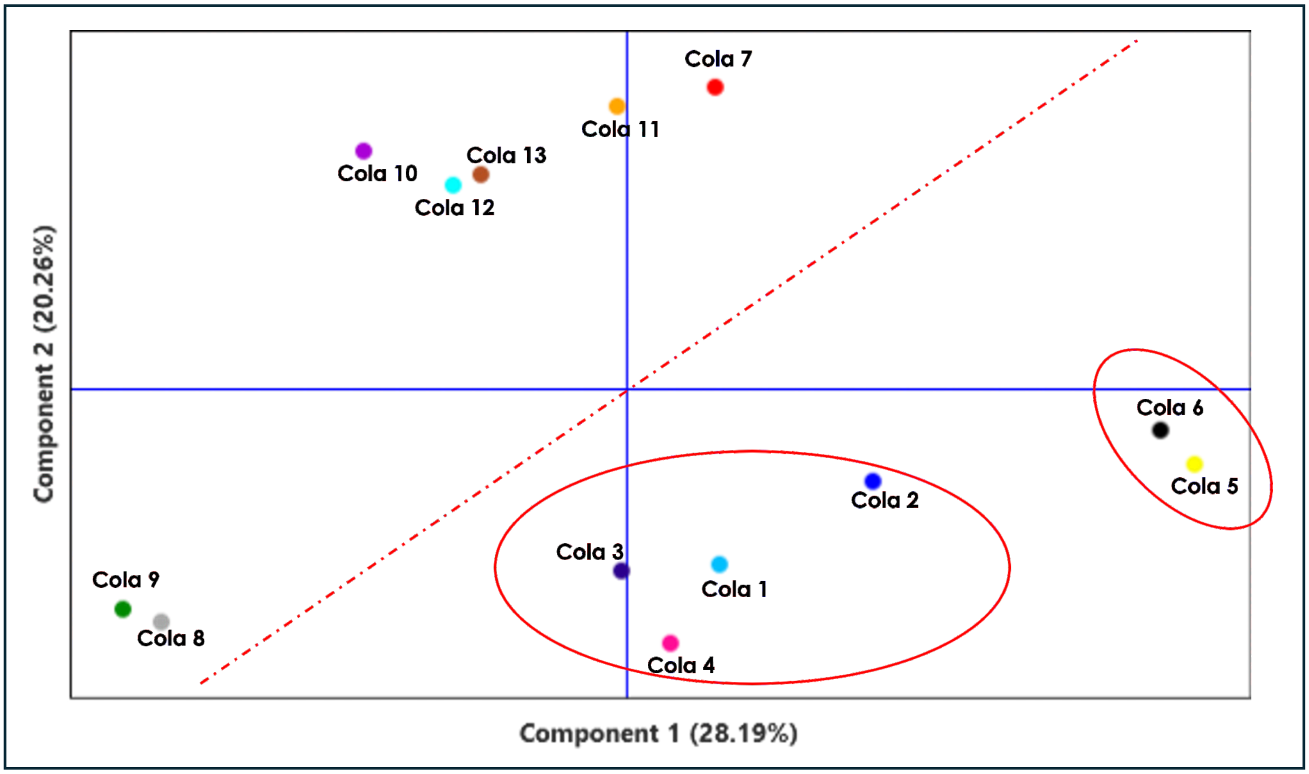


Figure 5. PCA scores plot from compiled peak information shows the cola beverages are distinguished on PC1.

Conclusion

GC-TOFMS was used to accurately separate and detect trace contaminants in the headspace of the thirteen cola beverages. While the initial project was aimed at exploring the flavorants in the various colas, *ChromaTOF* Sync also uncovered the presence of unexpected packaging contaminants, highlighting the power of the system for non-target surveillance. Using this data alignment software, it was possible to compile multiple samples into a single peak table for review and to extract minute differences and features that are difficult to detect by manual chromatogram comparison. Advanced data comparisons in *ChromaTOF* Sync, like PCA, also assisted in revealing trends in the data and samples. The results of this study suggest that not all colas are equal and that the relative abundance of known food packaging contaminants vary based on the manufacturer, packaging material, and on the type of cola consumed. Further quantitative studies are required to establish whether these levels are any cause for concern and in order to comply with the EU framework No. 1935/2004 and EU 2020/1245.

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