White Paper 052
September 2022

Untargeted comparison of soft drink brands using immersive sorptive extraction, GC×GC–TOF MS and chemometrics

Immersive sorptive extraction coupled with comprehensive two-dimensional gas chromatography and BenchTOF2™ time-of-flight mass spectrometry (GC×GC—TOF MS) was used to compare flavour profiles from popular brand soft drinks with those of imitation products.

Introduction

Unlike counterfeit goods, replica and imitation products are legal, as they do not use the branded product's trademark. In the food and beverage industry, imitation products attempt to mimic the taste experience of popular branded products.

However, flavour profiles are extremely complex and consist of a broad range of chemical classes – the combination of which ultimately determines the consumer's preference for a particular brand. It is important to be able to confidently identify these volatiles during product development, as well as in quality and authenticity studies.

Traditional sample preparation methods, such as headspace and solid-phase microextraction (SPME), are widely used, but often limited in terms of sensitivity. Additionally, sampling is generally restricted to the headspace volatiles, meaning that there is a bias towards non-polar analytes. Here, we demonstrate the use of high-capacity sorptive extraction with novel trap-based focusing to provide enhanced sensitivity and improved chromatographic performance, as well as the ability to perform both headspace and immersive sampling for compatibility with a wider range of polar and semi-volatile analytes. This improved performance, coupled with improved separation by GC×GC and highly-sensitive detection by time-of-flight mass spectrometry (TOF MS), gains greater insight into sample composition.

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However, sampling, separation and detection is just the beginning – the resulting datasets must then be reduced to discover significant differences and ultimately allow meaningful conclusions to be reached. Here, we will demonstrate the use of a new chemometrics platform to transform complex datasets into usable results. Firstly, alignment of the raw data is applied to account for potential retention time drifts. Next, advanced feature discovery identifies key differentiators across sample classes using all of the raw data. This innovative approach ensures that trace peaks are not ignored and enables automated workflows to be adopted, minimising laborious pre-processing steps and accelerating analytical workflows.

We will show this efficient end-to-end workflow in action for the comparison of brand and imitation cola-flavoured soft drinks.

Experimental

A schematic of the workflow used in this study is provided in Figure 1.

Samples: Five store-bought cola-flavoured soft drinks from different suppliers. Sampling and analysis were performed in triplicate for each soft drink.

Immersive sampling: HiSorb™ high-capacity sorptive extraction probes (PDMS/CWR/DVB). Fully automated on the Centri® extraction and enrichment platform (Markes International).

GC×GC: INSIGHT® flow modulator (SepSolve Analytical); Modulation period (P_M) = 2.5 s.

TOF MS: BenchTOF2™ mass spectrometer (SepSolve Analytical); Mass range: m/z = 40–350; Ionisation energy: Tandem Ionisation® at 70 eV and 14 eV.

Software: ChromSpace® software (SepSolve Analytical) for full instrument control and processing, with chemometric comparisons by ChromCompare+ (SepSolve Analytical).

Please contact SepSolve for full analytical parameters.

Figure 1

Overview of the analytical workflow used in this study.

Results and discussion

HiSorb high-capacity sorptive extraction probes not only offer a larger volume of sorptive phase compared to traditional SPME for higher sample loadings, but their robust design also enables immersive sampling, fully automated on the Centri platform. Coupling this high-sensitivity extraction with GC×GC–TOF MS ensures that a wide range of chemical classes is efficiently separated and confidently identified.

The additional chemical detail provided on the composition of soft drinks is clearly evident in Figure 2. Four compounds are highlighted that would have co-eluted in a conventional 1D analysis, but have been separated in the second dimension and identified confidently using the spectral quality and mass accuracy of the BenchTOF2 mass spectrometer (Figure 3).

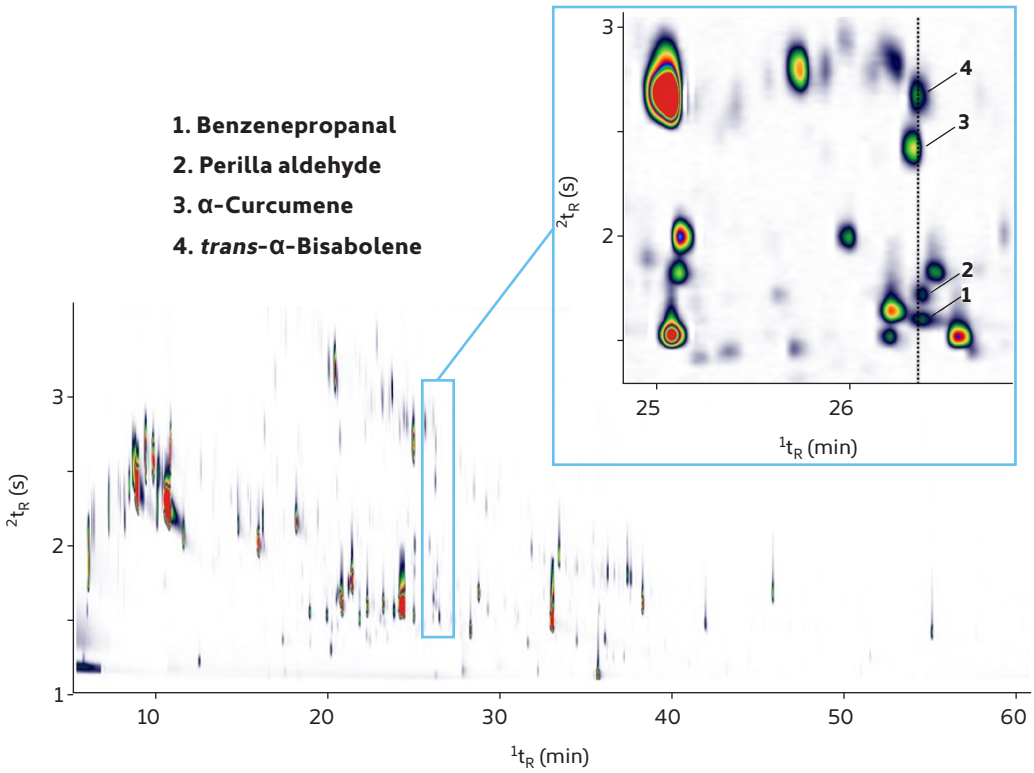

Figure 2

Example GC×GC—TOF MS chromatograms for the analysis of five brands of soft drink. Spectral examples are provided in Figure 3.

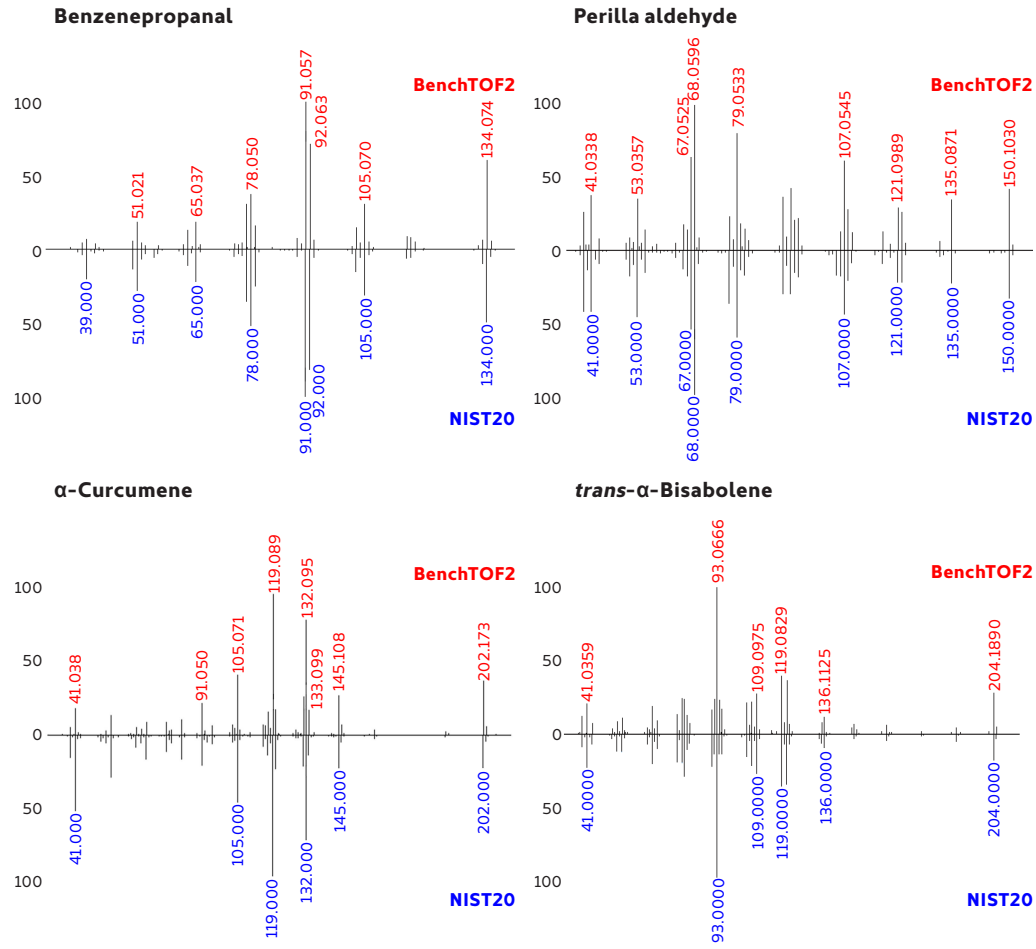

Figure 3

Identification of the four components in Figure 2 using comparison of BenchTOF2 spectra against the NIST 20 commercial library with additional confirmation through mass accuracy of the system, with all examples <10 ppm mass accuracy in this case.

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In this study, five 'cola' soft drinks from different manufacturers were analysed using this workflow (Figure 4). To determine the differences in composition between each brand of soft drink, a non-target, tile-based workflow was then applied in ChromCompare+ software to compare all of the raw data. The Feature Discovery tool then enabled the top 50 most significant differences between the sample classes to be identified automatically.

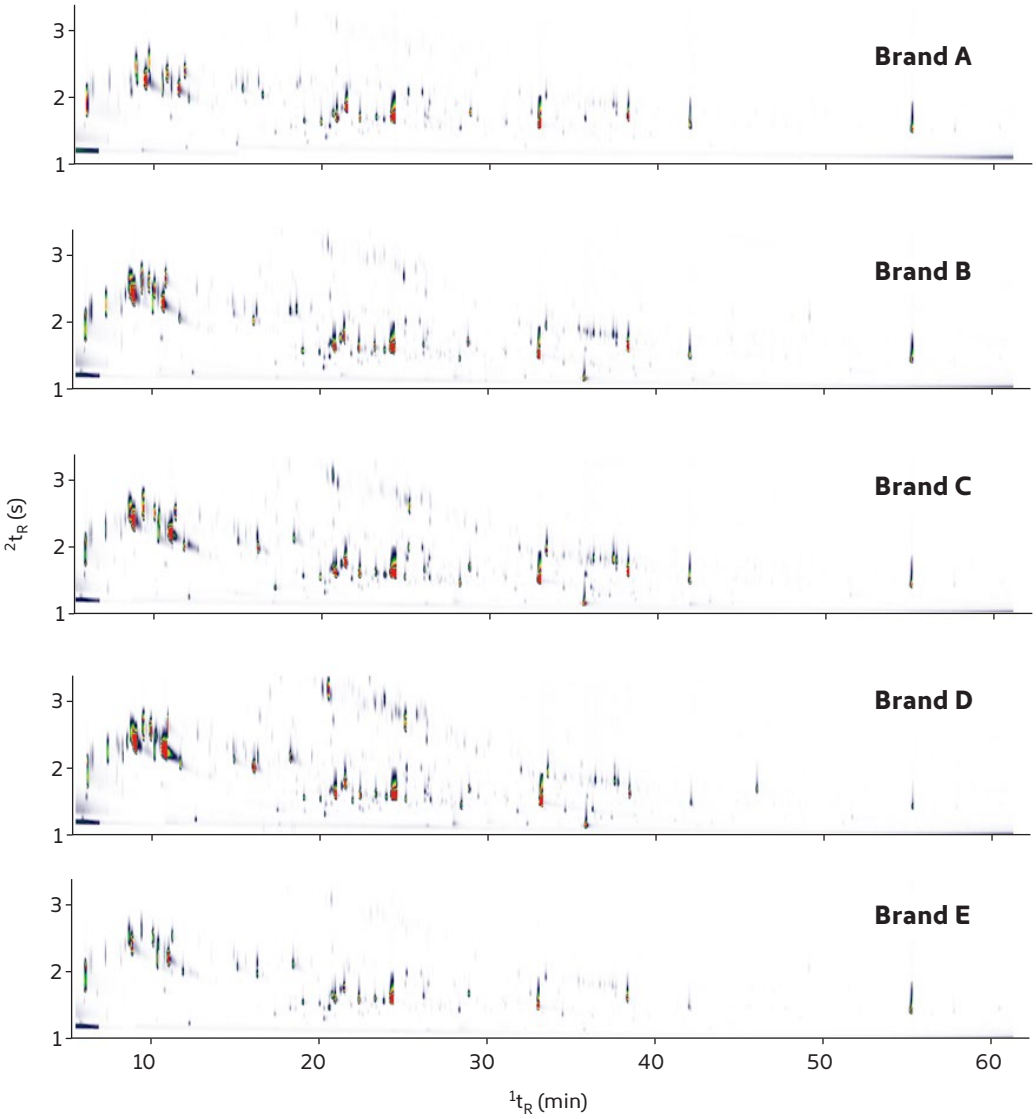

Figure 4

Example GC×GC—TOF MS chromatograms for analysis of five brands of soft drink.

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The resulting principal components analysis (PCA) score plot (Figure 5) shows distinct clustering of the different brands. Interestingly, brands B and E are both 'diet' soft drinks from different manufacturers and cluster separately from the 'zero sugar' brands.

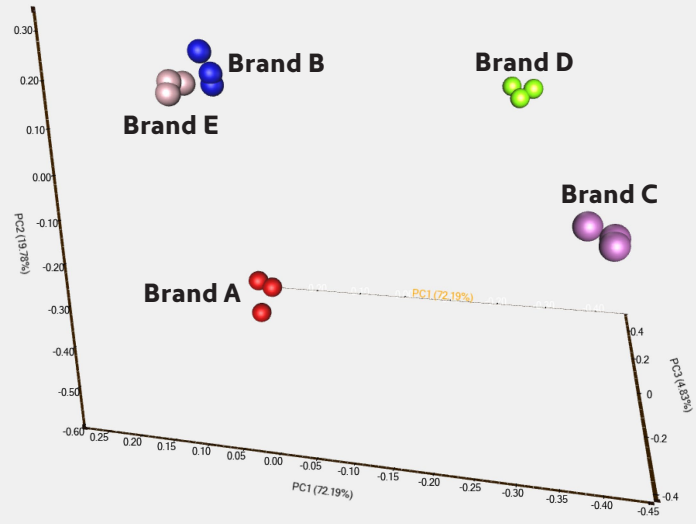


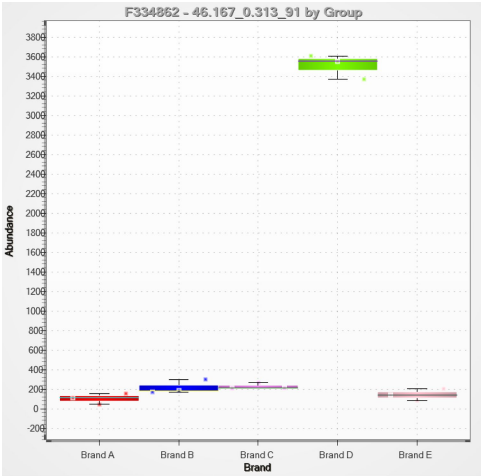
Figure 5

Principal components analysis (PCA) score plot of the top 50 significant differences between soft drink brands.

Benzyl benzoate was found to be a key differentiator of brand D (Figure 6, left) and likely contributes a balsamic, fruity flavour. Additionally, *trans*-cinnamaldehyde (Figure 6, right) was found to differentiate the 'diet' and 'zero sugar' classes.

Volcano plots in ChromCompare+ were also used to directly compare the imitation colas against popular brand products – an example of which is shown in Figure 7. Figure 7 also shows the reference-quality spectra of the BenchTOF2. This helps to ensure confident identification of potential markers of brand and/or imitation cola products. It is also important to note that Tandem Ionisation data was acquired in this study at 70 eV and 14 eV. The tandem data was used to confirm positive hits during Feature Discovery, improving the discovery of subtle, trace differences by reducing false positives.

Benzyl benzoate
Balsamic, fruity[1]



***trans*-Cinnamaldehyde**
Spicy, sweet, cinnamon[1]

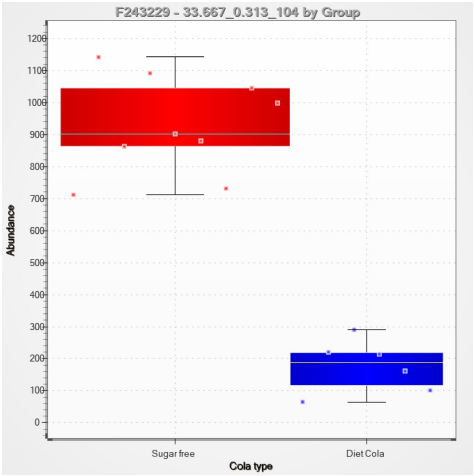


Figure 6

Box and whisker plots showing two key differentiators of the soft drink brands.

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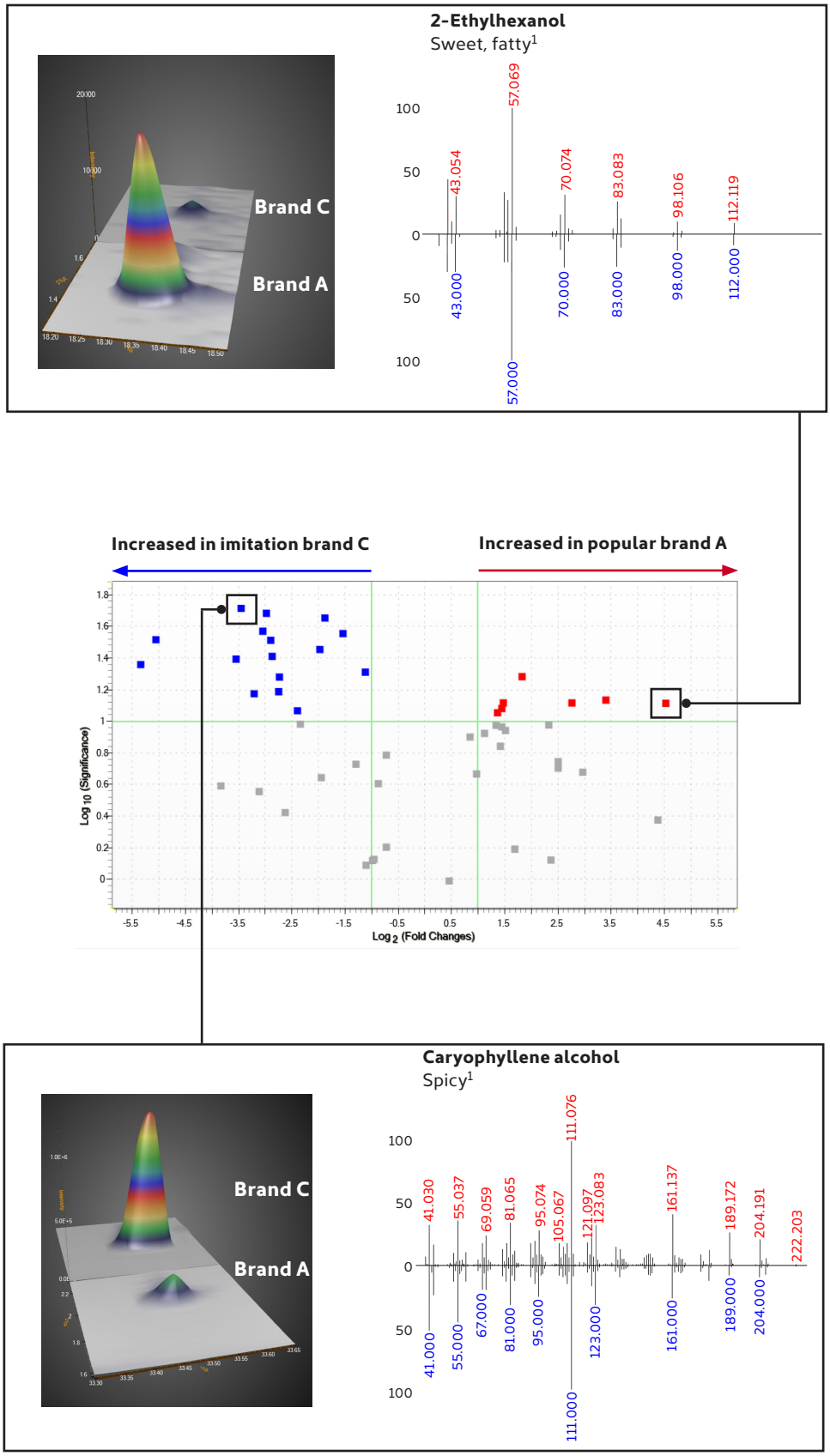

Figure 7

Volcano plot in ChromCompare+ showing comparison of an imitation cola (brand C) against a popular brand (brand A) with two significant differences highlighted and identified.

Conclusions

This white paper has demonstrated an end-to-end untargeted workflow for aroma profiling of food and beverages, namely:

- Immersive sampling of wide-ranging aroma-active volatiles using HiSorb high-capacity sorptive extraction probes, fully automated on the Centri platform.
- Enhanced separation by GC×GC using INSIGHT consumable-free flow modulation to obtain comprehensive aroma profiles.
- Sensitive detection and confident identification of analytes using the excellent spectral quality and mass accuracy of the BenchTOF2 mass spectrometer.
- ChromCompare+ provides easy-to-use, automated workflows for alignment and comparison of complex chromatograms.

For more information on this application, or any of the techniques or products used, please contact SepSolve.

References

[1] The Good Scents Company Information System (search facility), www.thegoodscentscompany.com/search2.html (accessed on 20th June 2022).

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