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Comprehensive analysis of coffee bean extracts by GC×GC–TOF MS

This study shows that GC×GC and time-of-flight mass spectrometry provides a high-performance solution for screening complex coffee extracts for key aroma compounds.

Introduction

Almost 1000 compounds have been identified in roast coffee extracts, with chemical composition varying due to a number of factors, such as coffee bean origin and degree of roasting.[1] The overall flavour and aroma of coffee results from the combined presence of chemicals from a number of classes, including hydrocarbons, aldehydes, acids, esters as well as sulfur- and nitrogen-containing compounds. Nitrogen-containing compounds – including pyrazines, pyridines and thiazoles – are of particular importance to the aroma of roasted coffee.

Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS) is ideal for the analysis of such complex samples, because the enhanced separation capacity allows analysts to screen the entire composition in a single analysis, with confident identification of compounds that would ordinarily co-elute.

In this study, we show how this capability enables companies in the drinks industry to ensure that their products meet all quality assurance criteria, and that the taste and aroma expected by consumers is consistently maintained.

Experimental

Samples: Three green coffee samples (obtained from different regions of South America and roasted in Italy) were analysed in this study. Extracts were prepared using liquid–liquid extraction from the brewed coffee into dichloromethane.

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GC×GC: 2D column set: 1st dimension: DB-50™, 30 m × 0.25 mm × 0.25 µm; 2nd dimension: Stabilwax®, 0.6 m × 0.10 mm × 0.10 µm; Temperature program: Main oven: 40°C (1.0 min), 3°C/min to 250°C (20 min); Secondary oven: No offset; Thermal modulation period: 6 s; Total run time: 68 min.

TOF MS: Instrument: BenchTOF-HD™ (Markes International); Filament voltage: 1.8 V; Ion source: 250°C; Transfer line: 240°C; Mass range: m/z 35–400; Data rate: 50 Hz.

Software: Data analysis: ChromSpace® (Markes International).

Please contact SepSolve for full analytical parameters.

Results and discussion

The coffee samples were analysed using a semi-polar to polar column set for optimal separation of the various polar constituents, including numerous classes of heterocyclics. The resulting GC×GC–TOF MS colour plots for the three coffee extracts are displayed in Figure 1. Extracts A and B are very similar, while Extract C has noticeable differences in composition, with drastically reduced complexity.

As expected, the caffeine peak was most prevalent in each of the three samples, with vanillin and 5-hydroxymethylfurfural (HMF) also in high abundance in Extracts B and C. The extreme complexity of these three samples would have resulted in numerous co-elutions in conventional GC–MS analyses, but GC×GC–TOF MS ensured that as much information as possible was collected, enabling greater confidence in product quality.

To overcome the difficulty of finding trace-level aroma components in these complex samples, simple scripts were created to identify key sulfur- and nitrogen-containing compounds, including pyrroles, pyridines and pyrazines. As an example, the script shown in Figure 2 was used to identify 2-acetyl-3-methylpyrazine, which is a key contributor to a hazelnut aroma in coffee.[2] The compounds identified using scripting were then added to a template, enabling fast application to the remaining samples.

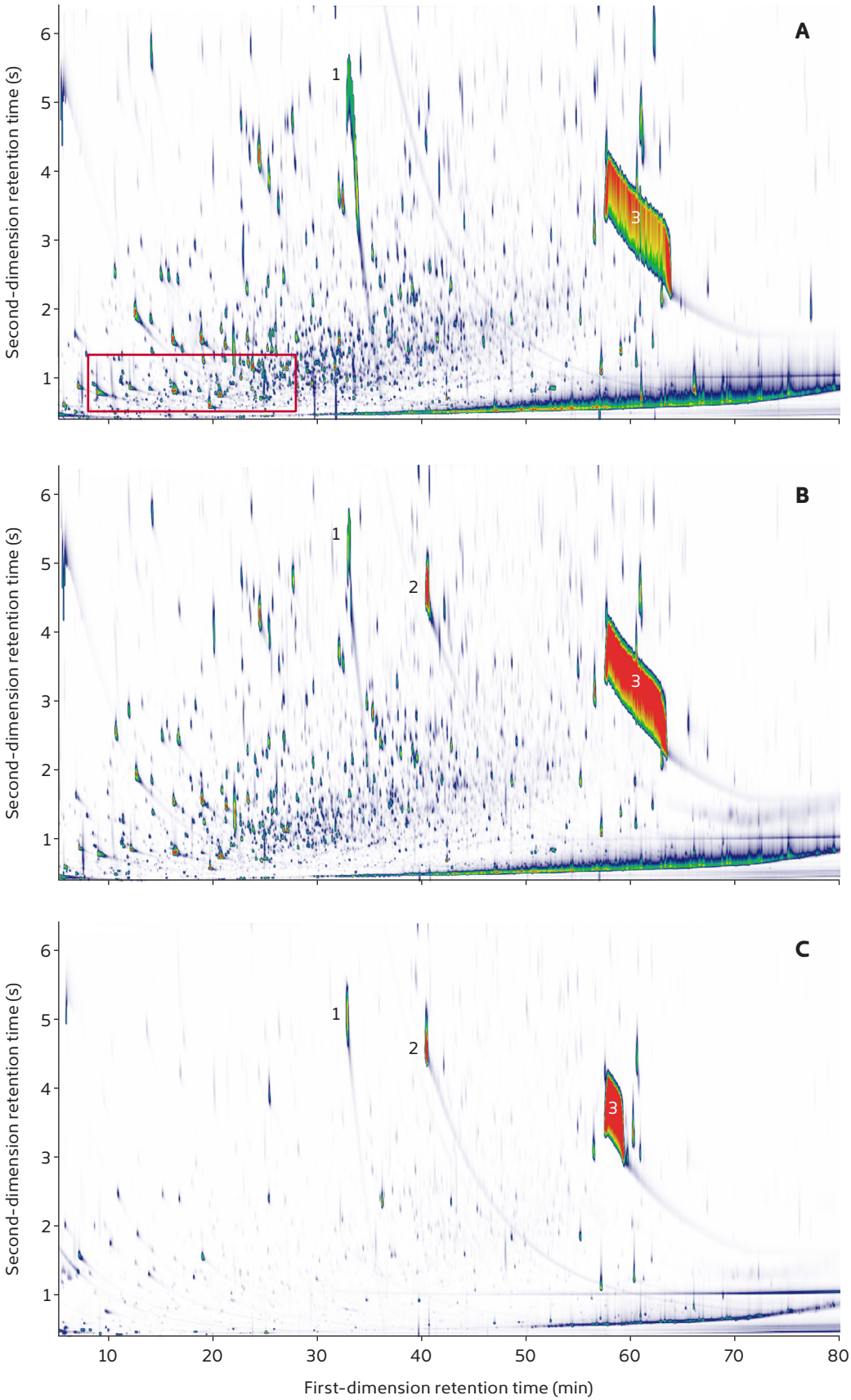

Figure 1

GC×GC–TOF MS (TIC) colour plots of Extracts A, B and C. The numbers indicate HMF (1), vanillin (2) and caffeine (3), and the box in the top panel shows the region expanded in Figure 3.

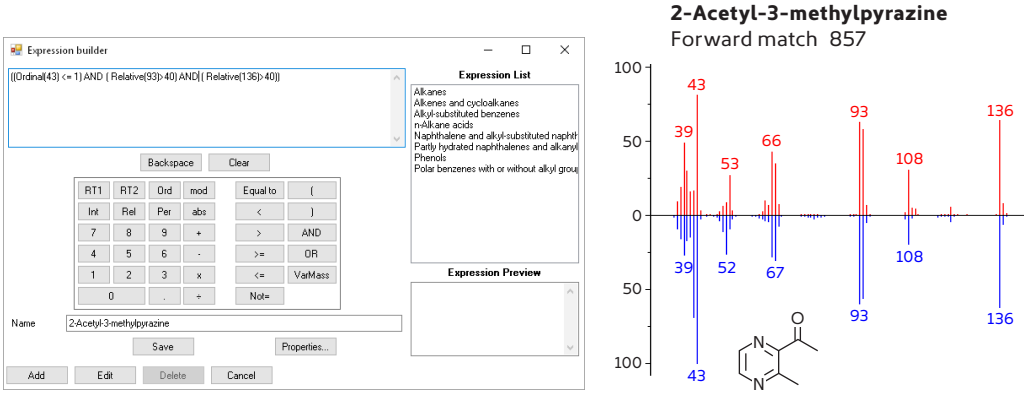

Figure 2

Left: A simple scripting function using mass spectral qualifiers for the identification of 2-acetyl-3-methylpyrazine. Right: Comparison of spectra for 2-acetyl-3-methylpyrazine in Extract A (top, red) with the NIST 14 library spectrum (bottom, blue).

Figure 3 shows the large number of sulfur- and nitrogen-containing compounds present in Extract A. Even though the inset shown covers a small proportion of the entire chromatogram, it is clear that a large number of peaks would have co-eluted with these target compounds in a conventional one-dimensional system. Therefore, if GC×GC had not been employed, key differences in sample composition would likely have been missed.

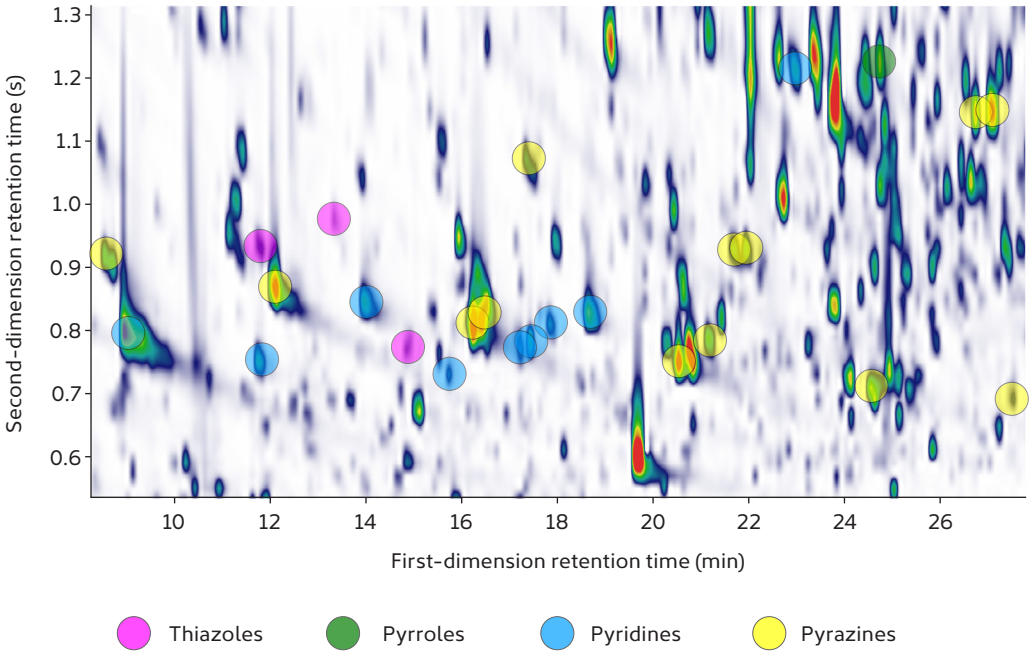

Figure 3

GC×GC–TOF MS colour plot for the expanded region of Extract A, with overlaid circles highlighting the variety of sulfur- and nitrogen-containing compounds present in the sample.

After application of the template to all three samples, it was found that Extract C had a very low relative abundance of pyrazines compared with Extracts A and B (Figure 4). Pyrazines generally impart a pleasant nutty, earthy, or roasted aroma to coffee, and in certain cases can have extremely low odour thresholds (<0.1 ppb in the case of 2,3-diethyl-5-methylpyrazine).

In contrast, phenolic compounds are typically considered to be undesirable in high amounts in coffee, as they impart a medicinal, clove-like taste. This class of compounds was found in the highest abundances in Extract C, indicating that this extract may give inferior results if presented to a tasting panel.

A large number of oxygen-containing compounds were also identified in the coffee extracts, by searching against the NIST 14 database. The examples in Figure 5 show excellent spectral matches, which is a consequence of the 3 kV floating ion source used in BenchTOF mass spectrometers. This minimises differences in ion impact velocities at the detector, so ensuring that 'classical' EI spectra are generated that can be directly compared to commercial libraries such as NIST. Note in particular the preservation of the weak molecular ion for 5-acetoxymethyl-2-furaldehyde (Figure 5A), which would most likely be missed using other TOF instruments.

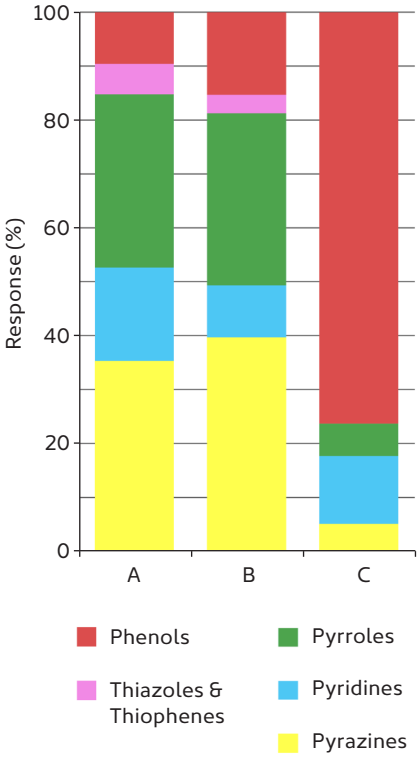

Figure 4

The differences in percentage contribution of some important aroma compounds across the three coffee samples.

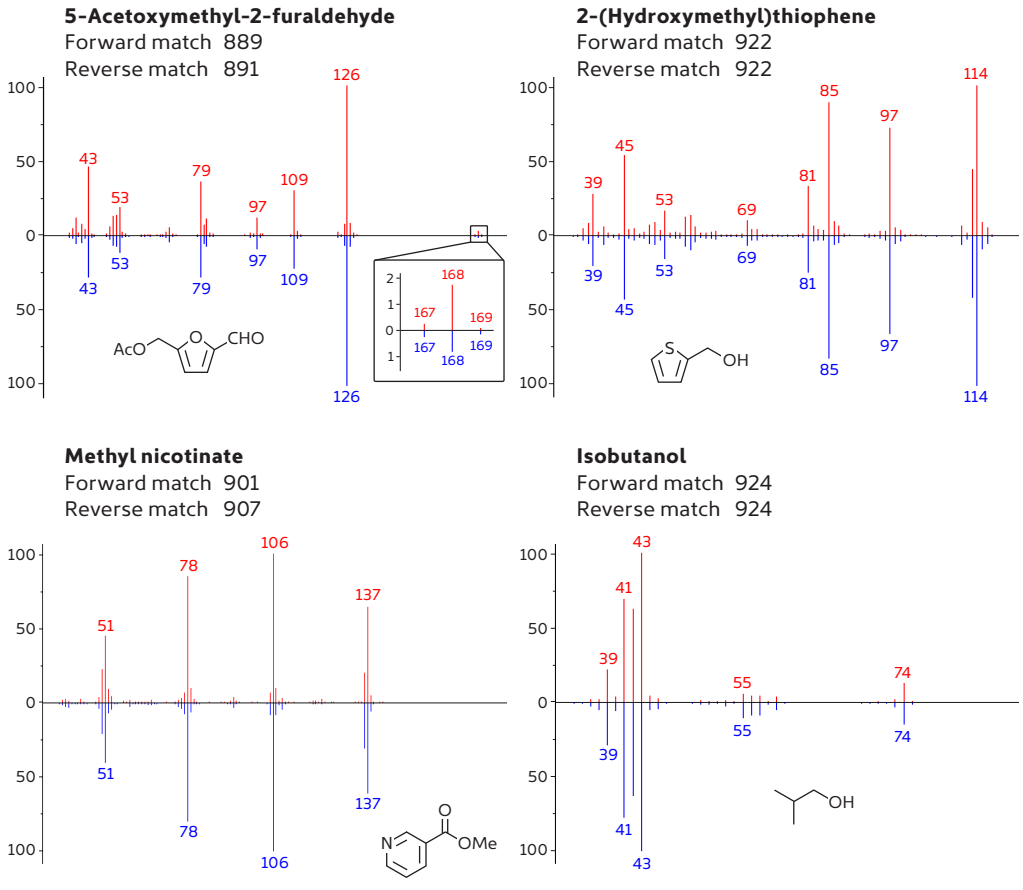

Figure 5

Comparison of spectra for a selection of compounds from Extract A (top, red) with the NIST 14 library spectra (bottom, blue). Note the preservation of the weak molecular ion for 5-acetoxymethyl-2-furaldehyde.

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Conclusions

In this study, we have shown that GC×GC with BenchTOF offers the detailed sample characterisation needed to ensure that the quality of coffee products is maintained from batch to batch, and that undesirable deviations are quickly flagged.

In particular, compound separation was far better than would be possible with one-dimensional GC–MS, drastically reducing co-elutions and so allowing confident identification of the widest possible range of key aroma components. Also, in addition to providing the high acquisition speed needed to deal with second-dimension peak widths of 200 ms, BenchTOF provided 'reference-quality' spectra that match those in the NIST 14 library – vital for achieving confident identifications of compounds with similar mass spectra or weak molecular ions.

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

References and notes

[1] H.-D. Belitz, W. Grosch and P. Schieberle, *Food Chemistry* (4th edition), Springer-Verlag, 2009, ch. 21, pp. 938–969.

[2] G.A. Burdock, *Fenaroli's Handbook of Flavor Ingredients* (5th edition), CRC Press, 2004, p. 23.

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