In the "spirit" of VOCs: sampling automation and concentration strategy for aroma profiling of alcoholic beverages

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Introduction

Monoterpenes (C_{10}) and sesquiterpenes (C_{15}) are aroma-active hydrocarbons found in the essential oils of various plants, including the hops used during the brewing of beer.

The terpene composition of the hops – and their relative proportions – has a major impact on the aroma and flavour of the finished beer. However, a number of factors can affect the levels of terpenes in hops, including seasonal variations, packaging, storage and ageing, so it is essential that robust quality control is applied.

In addition, many of these aroma compounds have very low odour thresholds, so a highly sensitive analytical approach is needed to assess the quality of the hops before brewing commences.

Here, we employ high-capacity sorptive extraction and secondary refocusing (using thermal desorption) to enhance sensitivity for hop terpene analysis, compared to conventional techniques such as SPME and headspace. Furthermore, we apply robust, repeatable and affordable flow-modulated GC×GC with parallel detection by both TOF MS and SCD, for comprehensive screening and highly-specific detection of sulfur species, all in a single run.

Experimental

The instrument setup is shown in Figure 1.

Samples: Three varieties of American hops (Citra, Mosaic and Amarillo) (2 g in a 20 mL headspace vial, sampled using headspace), and an American pale ale (10 mL in a 20 mL headspace vial, sampled immersively).

Sampling and preconcentration: Instrument: Centri® (Markes International); Sorptive extraction: Inert HiSorb™ PDMS sampler (Markes International). Equilibration time: 60 min; Temperature: 35°C; Agitation: 400 rpm.

GC×GC: Flow modulator: INSIGHT® (SepSolve Analytical).

Detection: Parallel detection using a three-way splitter to: *TOF MS*: Instrument: BenchTOF-Select™; Mass range: m/z 35–600; *SCD*: Base temp.: 250 °C; Burner temp.: 800°C; H_2 flow: 38 mL/min (upper), 8 mL/min (lower); O_2 flow: 11.5 mL/min; *FID*: H_2 flow: 30 mL/min; Air flow: 300 mL/min; Temperature: 300°C.

Software: Full instrument control and data processing by ChromSpace®.

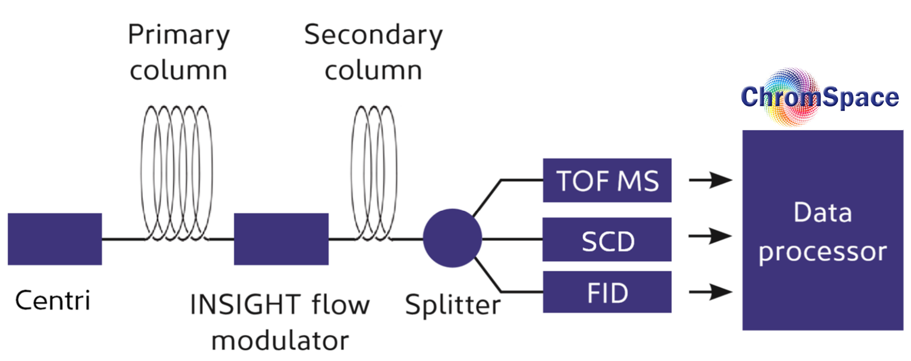


Figure 1: Schematic and photograph of the analytical system used in this study.

Results and discussion

Screening of high-loading species by FID

Monoterpenes and sesquiterpenes in beer are often found at concentrations many orders of magnitude greater than other components, making it a challenge to quantify these species in a single GC–MS run while also investigating trace-level species. The ability to perform GC×GC with parallel detection allows the use of FID to capture these high-loading species.

Compound	Composition (%)			Aroma
	Amarillo	Mosaic	Citra	
α-Pinene	1.02	0.56	0.44	Herbal
β-Farnesene	0.28	0.00	0.01	Woody
β-Myrcene	88.01	95.95	96.67	Spicy
β-Pinene	3.39	0.30	0.38	Herbal
β-Caryophyllene	1.20	0.38	0.39	Spicy
α-Humulene	2.62	0.85	0.46	Woody
Limonene	3.00	1.85	1.52	Citrus
Linalool	0.47	0.11	0.13	Floral

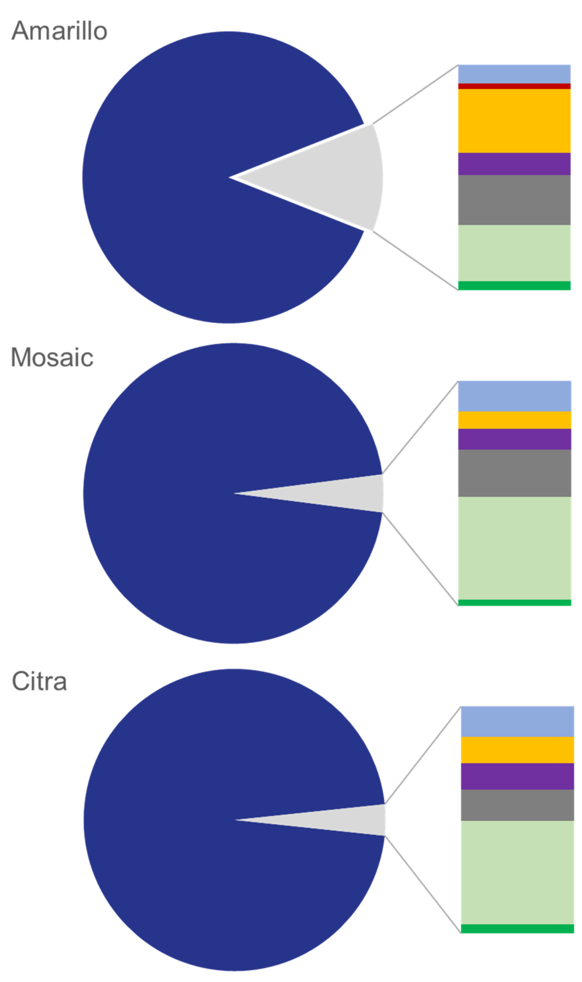
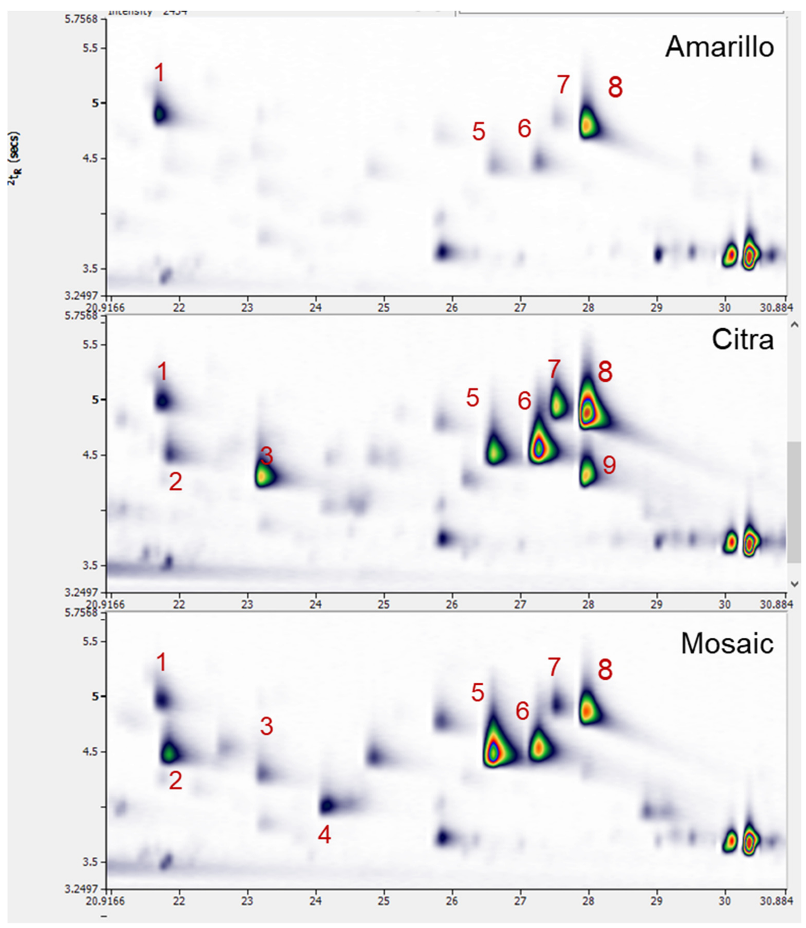


Figure 2: Percentage composition of the key mono- and sesquiterpenes in hops.

Confident identification of minor components by TOF MS

To investigate the remaining aroma-active species, the TOF MS data was examined. Figure 3 shows an expansion of highly complex region in the hop data, demonstrating the enhanced separation achieved. As well as avoiding co-elutions that would have occurred with 1D GC, the enhanced separation enables differences between the complex aroma profiles to be spotted more readily.

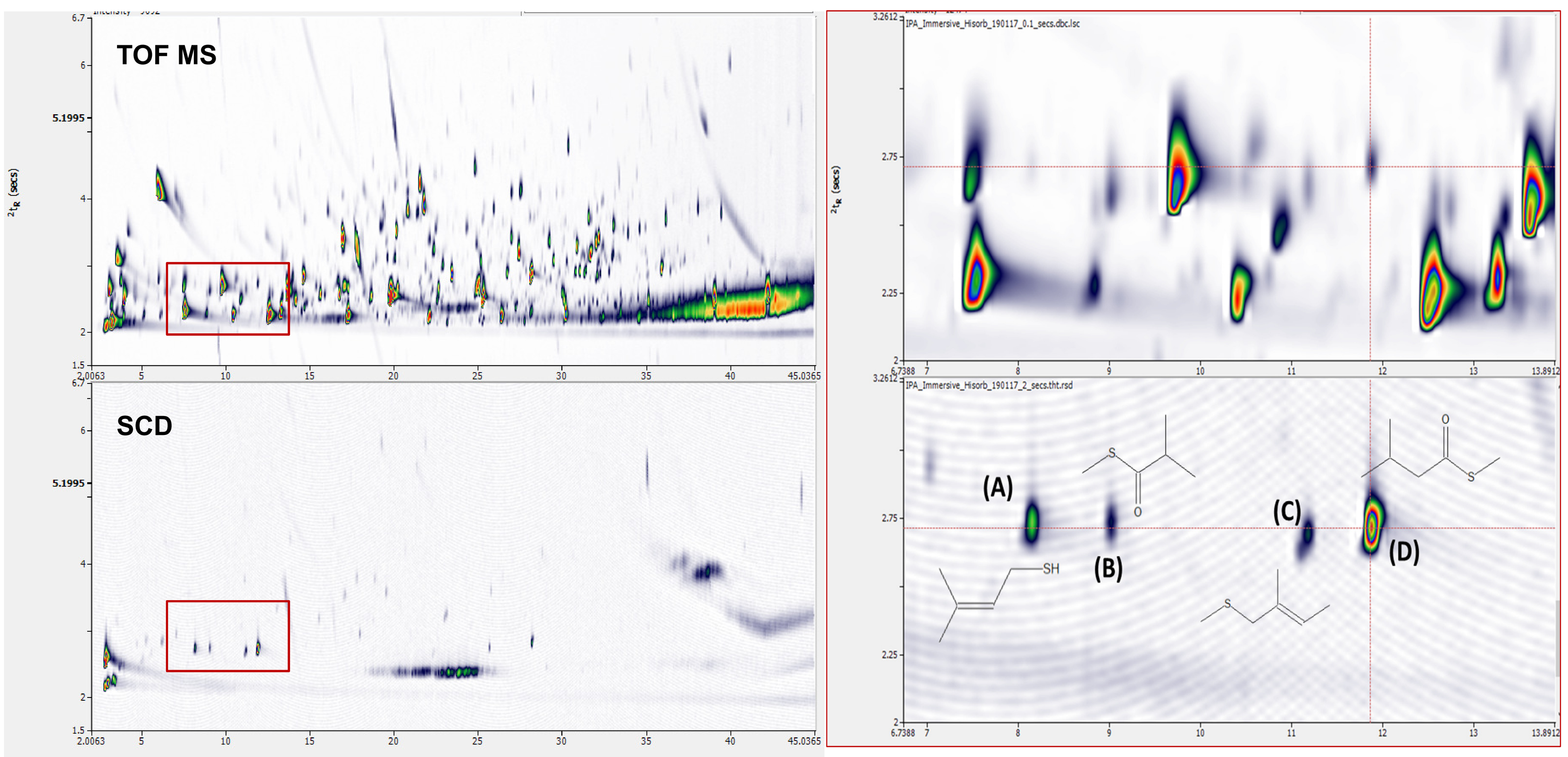


1 Neral
2 Decan-2-one
3 Methyl nonanoate
4 Heptyl isobutanoate
5 Undecan-2-one
6 Methyl dec-4-enoate
7 Unsaturated ester ($C_{11}H_{18}O_2$)
8 Methyl geranate
9 Methyl decanoate

Figure 3: Expanded GC×GC–TOF MS color plots for the headspace aroma profile of the three hop varieties.

Sensitive detection of sulfur species by SCD

Sulfur chemiluminescence detection (SCD) provides highly selective and sensitive analysis of sulfur species, which often cause undesirable odour taints in food and beverages. As shown in Figure 4, the SCD data assists the analyst in finding trace-level sulfur species in the TOF MS data – where they may otherwise have been overlooked or hidden by higher-loading peaks.



Label	Compound	Aroma
A	3-Methyl-2-butene-1-thiol	Sulfurous, skunk, smoky, onion
B	S-Methyl 2-methylpropanethioate	Sharp, fruity
C	1-(Methylthio)-2-methylbut-2-ene	Meaty, cooked, roasted
D	S-Methyl 3-methylbutanethioate	Cheesy, sharp, ripe, sulfurous

Figure 4: GC×GC plots for TOF MS (top) and SCD (bottom) for immersive sorptive extraction of a pale ale. The expanded region (right) shows identification of some key sulfur species.

The BenchTOF MS used in this study provides highly sensitive detection and reference-quality spectra, enabling the sulfur compounds to be identified confidently, while also screening the entire aroma profile (>600 peaks detected).

In this case, the identification of 3-methylbut-2-ene-1-thiol (3-MBT) is of particular importance. This compound is associated with an undesirable '*lightstruck*' or '*skunky*' character in beer, and with flavour thresholds in the low ppt range, highly-sensitive instrumentation is vital for detection.

Conclusions

This study has demonstrated:

- The performance of an analytical system for comprehensive aroma-profiling of beverages and their ingredients (in this case, beer and hops)
- Highly sensitive screening for odour taints caused by trace sulfur species.
- Robust and sensitive sampling (in both headspace and immersive modes) using HiSorb high-capacity sorptive extraction probes.
- Fully-automated workflows for unattended operation, with full instrument control and simple, unified data processing in ChromSpace software.
- Cryogen-free, solvent-free operation for substantial cost savings.