

Introduction

Beer contains hundreds of organic ingredients, with concentrations spanning many orders of magnitude. Amongst these are monoterpenes (C_{10}) and sesquiterpenes (C_{15}), which are aroma-active hydrocarbons found in the essential oils of various plants, including hops. Some of these compounds have very low odour thresholds, making them challenging to detect.



The ability to apply robust quality control to the raw ingredients offers desirable cost and time savings to breweries. Furthermore, the ability to obtain aroma fingerprints for different hop varieties can speed up the development of new beer flavourings.

This study investigates the use of the Micro-Chamber/Thermal Extractor™ for dynamic headspace extraction of hop 'cones', with collection of vapours onto a sorbent tube and analysis by thermal desorption (TD)–GC–TOF MS. The use of TD offers pre-concentration of the aroma compounds, while highly sensitive time-of-flight MS detection ensures that trace-level targets and unknowns can be analysed. This capability is enhanced further by flow-modulated GC×GC for enhanced separation of complex hop oils, and by novel soft ionisation technology for collection of comprehensive flavour profiles in a single run.

Experimental

TD–GC–TOF MS of hops

Dynamic headspace sampling was performed for two types of hops using a Micro-Chamber/Thermal Extractor™ (µ-CTE™). Hops were placed in individually sealed and temperature-controlled pots within the µ-CTE (Figure 1). Volatiles were then extracted for 30 min at 30°C, and collected onto an inert-coated stainless steel sorbent tube packed with Tenax[®] TA prior to analysis by TD–GC–TOF MS. Experimental conditions can be found in S. Koschinski et al., LCGC: The Column, April 2016, pp. 16–23.



Figure 1: Analytical workflow for aroma profiling of hops by microchamber TD–GC–TOF MS.

GC×GC–TOF MS of hop oils

Sample preparation: 10% (v/v) dilutions of two hop oils were prepared in hexane.

GC: Injector: Split/splitless; Injection volume: 0.5 µL; Split: 100:1; Temperature: 280°C; Carrier gas: Helium (0.6 mL/min). 2D column set: 1st dimension: BPX5, 20 m × 0.18 mm × 0.18 µm; 2nd dimension: DB1701, 2 m × 0.25 mm × 0.15 µm; Temperature program: 40°C (1 min), 3°C/min to 260°C (10 min). Modulator: Insight™ flow modulator (SepSolve Analytical); Loop dimensions: 0.53 mm i.d. × 230 mm (loop volume: 50 µL); Fill time: 3600 ms; Flush time: 200 ms; Р_м: 3.8 s.

TOF MS: Instrument: BenchTOF-HD[™] (Markes International); Filament voltage: 1.7 V; Ion source: 300°C; Transfer line: 280°C; Mass range: m/z 40–300; Data rate: 50 Hz. **Software:** Image processing: ChromSpace[®] for TOF-DS[™].

WORLD BREWING CONGRESS 2016 Comparison of the aroma profiles of hops using thermal desorption and time-of-flight mass spectrometry

Laura McGregor¹, Stefan Koschinski², Matthew Edwards¹, Wade Bontempo³ and Gareth Roberts¹

¹Markes International Ltd, Gwaun Elai Medi-Science Campus, Llantrisant, RCT, Wales, UK. ²Markes International, Inc., 11126-D Kenwood Road, Cincinnati, Ohio 45242, USA.

Figure 2: TD–GC–TOF MS chromatograms comparing the aroma profiles of two hop samples.

As expected, the terpenes β -myrcene, caryophyllene and humulene were the most abundant compounds in both hop samples. However, 'Target' had a higher volatile content, with high-intensity peaks for pinene isomers and isobutyl isobutanoate eluting before the large β -myrcene peak.

Sulfur compounds are an undesirable component of the aroma profile of hops, because they can result in an odour-taint in the final beer product. In the case of 'Target', both Smethyl 3-methylbutanethioate and S-methyl-2-methylpropanethioate were identified, which have been described as having a 'cheesy'/'cooked vegetable' aroma.¹ Neither compound was detected in 'Goldings'.



Analysis of hop oil by GC×GC–TOF MS

Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS) has become the technique of choice for the separation of complex oils. The enhanced separation capacity offered by the coupling of two columns of different selectivity, combined with highly sensitive mass spectral identification, provides a high-performance approach to quality control.

This study investigates the application of flow-modulated GC×GC–MS to enhance the separation of hop oils. Unlike thermal devices, flow modulation enables efficient modulation of volatiles boiling below C_5 and additional options including heart-cutting, splitting for simultaneous detection and backflushing.

A chromatogram resulting from flow-modulated GC×GC–MS of a hop oil is shown in Figure 4, with an expansion in Figure 5. As is common in GC×GC, the identification process is simplified because different chemical classes elute together in bands (the 'roof-tiling' effect). Both show the enhanced separation achieved by GC×GC – enabling confident identification of compounds that would ordinarily coelute in a 1D GC–MS system







Figure 5: Above: Expanded region of Figure 4. Right: BenchTOF spectra (top, red) and NIST 14 spectra (bottom, blue) for two compounds that would have co-eluted in a one-dimensional separation.



Despite the wealth of information supplied by GC×GC–TOF MS, confident detection of trace compounds with weak molecular ions and/or similar 70 eV spectra can be difficult. Markes International's Tandem Ionisation[®] technology resolves this by simultaneously generating both conventional 70 eV spectra and soft electron ionisation spectra (down to 10 eV). The latter provide enhanced molecular ions and reduced numbers (and relative intensities) of fragment ions, but with no inherent loss in sensitivity. Figure 6 shows spectral comparisons of six terpenes found in hops, analysed at 70 eV and 12 eV. In each case, the molecular ion and structurally-significant fragments were enhanced at 12 eV, making it easier to detect trace levels of such compounds in complex matrices, as well as improving spectral discrimination.





Furthermore, Tandem Ionisation produces complementary spectra with simplified fragmentation patterns and enhanced molecular ions. This provides another dimension of sample information to aid confident identification of aroma profiles.



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TOF MS detection with Tandem Ionisation

Figure 6: Spectral comparisons for six key hop aroma compounds at 70 eV and 12 eV.

Conclusions

This study has shown that thermal desorption and TOF MS are powerful tools for identifying trace-level components in complex aroma profiles. We have also shown that GC×GC–TOF MS with flow modulation offers enhanced separation and confident identification of compounds that would ordinarily co-elute on a 1D GC–MS system. Moreover, this type of GC×GC screening enables key compositional differences to be established quickly and routinely, without the need for expensive liquid cryogen.