Application Note 535

Comparison of the aroma profiles of hops by TD–GC–TOF MS with Select-eV variable-energy electron ionisation

Summary

This Application Note demonstrates the high performance of a TD–GC–TOF MS system for the analysis of complex aroma profiles from hops. The use of complementary soft electron ionisation provides enhanced confidence in the identification of terpenoids, while the comparison of hop varieties is simplified by near-real-time analysis in the accompanying software package.

Introduction

Beer contains hundreds of organic ingredients, with concentrations spanning many orders of magnitude. Monoterpenes (C_{10}) and sesquiterpenes (C_{15}) are aroma-active hydrocarbons found in the essential oils of various plants. Most notably for the brewing industry, they are found in hops, which provide much of the characteristic 'bitterness' of the finished beer.[1]

Of the greatest importance for beer is the monoterpene β-myrcene, and the sesquiterpenes caryophyllene, β-farnesene and α-humulene. However, there are hundreds of other terpenes that may also be present and have an impact on the final aroma and flavour. Many of these compounds have very low odour thresholds, so a highly sensitive analytical approach is needed to assess the quality of the hops before brewing commences. A number of factors can affect VOC profiles of hops, including seasonal variations, packaging, storage and ageing, so it is essential that robust quality control is applied.

This study investigates the use of Markes' Micro-Chamber/Thermal Extractor™ (µ-CTE™) for dynamic headspace extraction of hop 'cones', with collection of vapours onto a sorbent tube and analysis by thermal desorption–GC–TOF MS.

The use of thermal desorption (TD) offers pre-concentration of the aroma compounds, while coupling to highly sensitive time-of-flight MS detection with Select-eV® variable-energy ionisation technology allows a comprehensive aroma profile to be investigated in a single sequence.

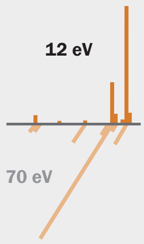
Background to BenchTOF systems

BenchTOF™ mass spectrometers are time-of-flight instruments designed specifically for gas chromatography. They are particularly appropriate for confident identification of trace-level compounds in hops for the following reasons:

- **Sensitivity:** Highly efficient direct-extraction technology allows BenchTOF systems to acquire full-range spectra with SIM-like sensitivity, allowing them to reliably detect trace-level targets and unknowns in a single run, which would be difficult or impossible on a quadrupole system.
- **Spectral quality:** The 'reference-quality' spectra produced by BenchTOF systems are a close match for those in commercial libraries such as NIST or Wiley. This enables quick and confident matching of analytes.
- **Speed:** The ability to record full-range mass spectral information to extremely high densities (10,000 transient spectral accumulations per second) enables advanced spectral deconvolution and 'data-mining' algorithms to extract maximum information from weak, matrix-masked signals.

Select-eV

Markes' flagship BenchTOF-Select™ instrument features Select-eV® ion-source technology as standard.



Select-eV breaks new ground by allowing ionisation energies to be reduced on a sliding scale from 70 eV to 10 eV, without impacting sensitivity, simply by changing a parameter in the method.

This low-energy (*i.e.* 'soft') electron ionisation reduces analyte fragmentation, which benefits a wide range of GC and GC×GC analyses by enhancing selectivity, sensitivity, and aiding structural elucidation – all while avoiding the inconvenience of reagent gases, ion source pressurisation, or changes in hardware setup typically associated with other soft ionisation techniques for GC–MS.

Place hops in microchambers.

Collect VOCs onto sorbent tubes.

Analyse sorbent tubes by automated TD–GC–TOF MS.

Process data in near-real-time.

Figure 1: Analytical procedure used for the characterisation of hop aroma.

Experimental

Figure 1 gives an overview of the analytical workflow.

Sampling:
Dynamic headspace sampling was performed for three varieties of hops ('Fuggle', 'Goldings' and 'Target') using the μ-CTE (Markes International). Hops (~1 g) were placed in individually sealed and temperature-controlled pots within the μ-CTE. Volatiles were then extracted by a dynamic headspace process for a period of 30 min at 30°C, and collected onto an inert-coated stainless steel sorbent tube packed with Tenax® TA. Tubes were then analysed by TD–GC–TOF MS using the conditions below.

TD:

Instrument:	TD-100™ (Markes International)
Dry purge:	1 min at 50 mL/min
Pre-purge:	1 min at 20 mL/min to split
Tube desorb:	10 min at 280°C and 50 mL/min trap flow (no split)
Focusing trap:	Tenax TA
Pre-trap fire purge:	1 min at 50 mL/min
Trap low:	25°C
Trap high:	280°C
Trap heating rate:	Max, hold 5 min
Split flow:	150 mL/min, collected onto a clean Tenax TA sorbent tube

GC:

Column:	HP-5ms™, 30 m × 0.25 mm × 0.25 μm
Carrier gas:	Helium, 1.2 mL/min
Oven temp.:	40°C for 5 min, then 4°C/min to 280°C (hold 5 min)
Total run time:	70 min

TOF MS:

Instrument:	BenchTOF-Select™ (Markes International)
Data rate:	5 Hz
Mass range:	m/z 35–500
Ion source:	230°C
Transfer line:	280°C
Filament voltage:	1.8 V

Software:
The comprehensive TOF-DS™ (Markes International) software package was used for both instrument control and data processing.

Results and discussion

The aroma profiles for three hop varieties were collected by μ-CTE–TD–GC–TOF MS (Figure 2). As expected, β-myrcene, caryophyllene and α-humulene dominated in all three samples. However, 'Fuggle' was the only variety that contained β-farnesene, which suggests it may have a more floral aroma.[2]

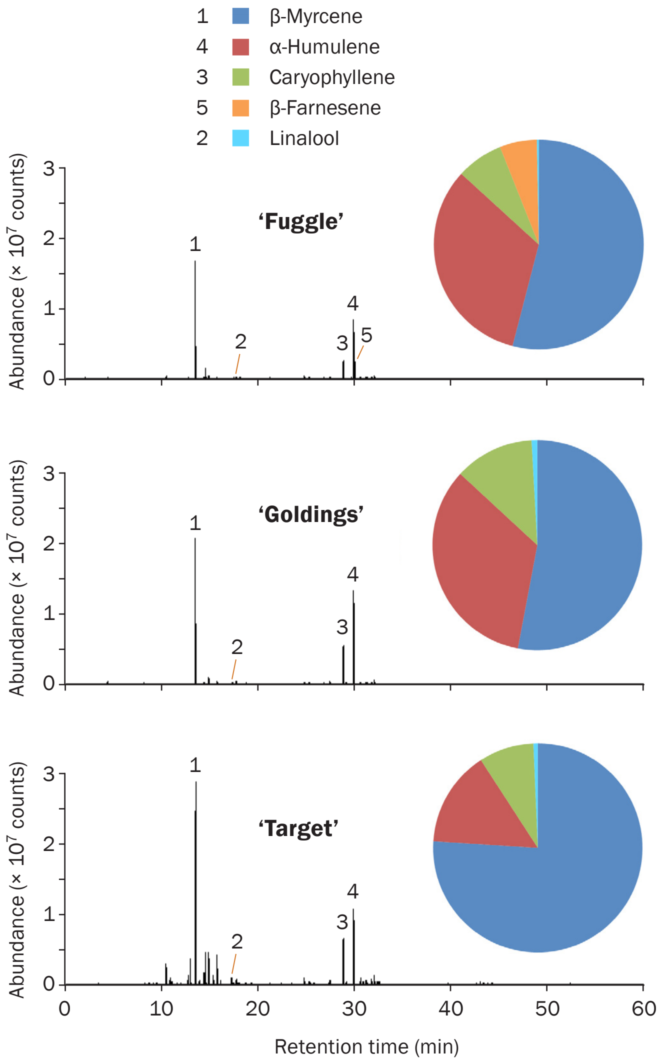


Figure 2: TD–GC–TOF MS (TIC) chromatograms for each hop variety, and pie-charts showing the relative abundances of five key aroma compounds.

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The use of the TOF-DS software suite enabled near-real-time data-processing to be employed during analysis. This allowed the chromatograms to be background-subtracted, integrated, deconvolved and library-searched while the samples were still acquiring, significantly reducing the amount of time spent on data analysis. All samples were screened against the NIST 14 library as acquisition proceeded, and the resulting peak tables were compared (all identifications have a match factor >750).

Expansions of the chromatograms (Figure 3) give a selection of these identifications. It is clear that ‘Fuggle’ and ‘Goldings’ are extremely similar in content, while ‘Target’ shows considerable differences. However, all three varieties differ in the relative abundances of these compounds – a factor that likely results in their characteristic aromas.

Full details of the aroma compositions of the three hop varieties are given in Table 1, which indicates the differences in aroma that may arise due to the presence of specific compounds.

1 2-Methylpropyl 2-methylpropanoate
2 *trans*-1,3-Nonadiene
3 α-Pinene
4 β-Pinene
5 α-Myrcene
6 β-Myrcene
7 α-Thujene
8 3-Methylbutyl 2-methylpropanoate
9 2-Methylbutyl 2-methylpropanoate
10 Sylvestrene
11 *trans*-β-Ocimene
12 α-Ocimene
13 γ-Terpinene
14 δ-Terpinene (Terpinolene)
15 Linalool
16 Undecan-2-one
17 α-Copaene
18 Caryophyllene
19 α-Humulene
20 β-Farnesene
21 γ-Muurolene
22 δ-Cadinene

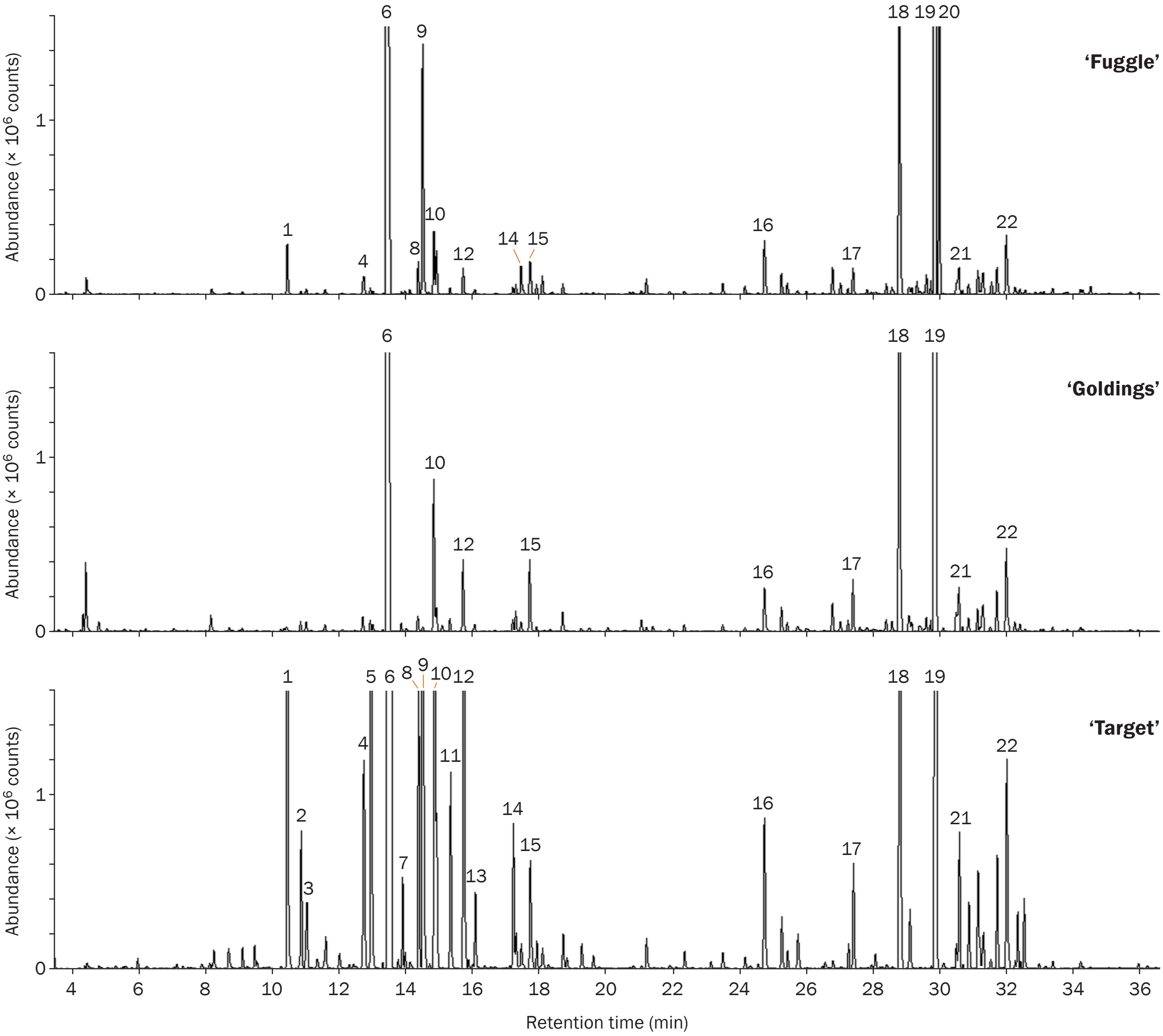

Figure 3: Expanded views of the TD–GC–TOF MS chromatograms comparing the aroma profiles of three varieties of hops, with key compounds shown.

Name	Retention time (min)	Peak area			Aroma
		'Fuggle'	'Goldings'	'Target'	
Acids					
2-Methylpropanoic acid	4.818	—	7.92×10^{5}	1.78×10^{5}	Rancid butter[3]
Ketones					
Butan-2-one	2.475	—	2.85×10^{4}	—	Chocolate, cheese, butter, ethereal, gas[1]
Methyl isopropyl ketone	3.057	6.58×10^{4}	—	—	Sweet[4]
Acetoin	3.821	1.29×10^{5}	1.36×10^{5}	—	Dairy sweet, buttery[1]
Methyl isobutyl ketone	4.495	1.66×10^{5}	—	—	Sharp, solvent-like with green, herbal, fruity[1]
Nonan-2-one	17.455	2.47×10^{6}	1.02×10^{6}	2.01×10^{6}	Varnish[4]
Decan-2-one	21.207	1.26×10^{6}	2.89×10^{5}	2.73×10^{6}	Citrus, orange-like[1]
Undecan-2-one	24.743	5.68×10^{6}	4.32×10^{6}	1.56×10^{7}	Fruity, musty, dusty, green[1]
Tridecan-2-one	31.178	—	—	2.07×10^{6}	Spicy, herbaceous[1]
Sulfur compounds					
Dimethyl disulfide	4.555	1.09×10^{4}	—	—	Sulfury, cabbage, putrid[1]
S-Methyl 2-methylpropanethioate	7.865	—	—	2.97×10^{5}	Cheesy, estery, cooked vegetable[5]
S-Methyl 3-methylbutanethioate	11.328	—	—	8.90×10^{5}	Cheesy, estery, cooked vegetable[5]
Monoterpenes					
α-Pinene	11.025	3.75×10^{5}	—	3.49×10^{6}	Terpenic[1]
Camphene	11.590	3.35×10^{5}	5.35×10^{5}	3.04×10^{6}	Pine, oily, herbal[1]
β-Pinene	12.719	1.04×10^{6}	1.22×10^{6}	1.40×10^{7}	Musty, green, sweet, pine[1]
α-Myrcene	12.938	4.41×10^{5}	8.12×10^{5}	5.38×10^{7}	
β-Myrcene	13.442	2.83×10^{8}	3.56×10^{8}	9.38×10^{8}	Musty, sweet, lemon, spicy, woody[1]
α-Phellandrene	13.870	—	1.70×10^{5}	—	Terpenic, citrus, lime, green[3]
α-Thujene	13.888	—	—	5.89×10^{6}	
Sylvestrene	14.844	5.24×10^{6}	1.35×10^{7}	6.55×10^{7}	
trans-β-Ocimene	15.328	4.75×10^{5}	1.01×10^{6}	1.65×10^{7}	Green, tropical, woody, floral[3]
α-Ocimene	15.721	2.22×10^{6}	6.02×10^{6}	5.96×10^{7}	
γ-Terpinene	16.068	3.74×10^{5}	4.95×10^{5}	6.26×10^{6}	Citrus-like, herbaceous, fruity, sweet[1]
δ-Terpinene (Terpinolene)	17.205	5.15×10^{5}	9.14×10^{5}	1.20×10^{7}	Sweet, pine, citrus[3]
Linalool	17.719	1.53×10^{6}	5.89×10^{6}	8.33×10^{6}	Green, floral, lemon, lavender[1]
Sesquiterpenes					
Ylangene	27.247	3.37×10^{5}	7.41×10^{5}	1.85×10^{6}	Fruity[1]
α-Copaene	27.395	2.27×10^{6}	4.39×10^{6}	9.06×10^{6}	Woody, earthy[1]
Caryophyllene	28.784	3.77×10^{7}	8.24×10^{7}	1.05×10^{8}	Oily, fruity, woody[1]
α-Humulene	29.847	1.72×10^{8}	2.28×10^{8}	1.83×10^{8}	Musty, spicy, woody[1]
β-Farnesene	29.978	3.00×10^{7}	—	—	Oily, fruity, citrus-like, woody[1]
γ-Muurolene	30.570	1.69×10^{6}	2.83×10^{6}	1.04×10^{7}	Oily, herbaceous[1]
β-Selinene	30.853	8.14×10^{5}	1.10×10^{6}	5.98×10^{6}	
α-Selinene	31.125	—	—	9.78×10^{6}	Pepper-like, orange[1]
γ-Cadinene	31.703	1.69×10^{6}	3.57×10^{6}	9.69×10^{6}	
cis-Calamenene	31.971	8.30×10^{5}	7.26×10^{5}	1.53×10^{6}	Weak spicy, weak floral[1]
δ-Cadinene	31.987	5.14×10^{6}	7.54×10^{6}	1.90×10^{7}	Wood, herbaceous[1]
α-Cadinene	32.394	3.27×10^{5}	5.41×10^{5}	—	Dry wood, weak medicinal[1]
α-Calacorene	32.553	—	1.16×10^{5}	3.25×10^{5}	Woody, fruity, sweet, pine[1]
Caryophyllenyl alcohol	33.369	4.79×10^{5}	2.94×10^{5}	2.33×10^{5}	Warm, moss-like, spicy[3]
Esters					
2-Methylpropyl propanoate	8.242	—	—	1.43×10^{5}	Sweet, fruity, bitter[3]
2-Methylbutyl acetate	9.110	1.46×10^{5}	1.86×10^{5}	1.57×10^{6}	Herbaceous, ethereal, rum, fruity[1]
2-Methylpropyl 2-methylpropanoate	10.453	3.93×10^{6}	4.71×10^{4}	3.97×10^{7}	Pineapple[3]
3-Methylbutyl 2-methylpropanoate	14.372	2.00×10^{6}	—	5.98×10^{7}	
2-Methylbutyl 2-methylpropanoate	14.512	2.14×10^{7}	–	6.82×10^{7}	
Methyl octanoate	18.705	8.59×10^{5}	1.63 × 106	2.98×10^{6}	Orange, fruity, green[1]
2-Methylbutyl 3-methylbutanoate	18.089	1.48×10^{6}	—	1.54×10^{6}	Herbaceous, fruity, sweet[3]
Ethyl octanoate	21.397	—	2.90×10^{5}	—	Fruity, floral, apricot-like[3]
Methyl nonanoate	22.342	1.90×10^{5}	5.00×10^{5}	1.46×10^{6}	Fruity, nut-like, coconut-like[1]
Methyl geranate	25.733	1.84×10^{5}	2.87×10^{5}	2.95×10^{6}	Green, fruit, floral[1]
Octyl 2-methylpropanoate	26.468	—	—	7.99×10^{4}	Fruity, fatty, grape[3]
Alcohols					
2-Methylpropan-1-ol	2.702	1.25×10^{5}	8.06×10^{4}	—	Disagreeable, wine-like[3]
3-Methylbutan-2-ol	3.323	—	1.12×10^{5}	—	Fruity, fresh[3]
3-Methylbutan-1-ol	4.344	—	9.80×10^{5}	1.29×10^{5}	Whisky, pungent, balsamic, alcohol[1]
2-Methylbutan-1-ol	4.436	1.16×10^{6}	5.33×10^{6}	4.10×10^{5}	Malty, balsamic, wine, ripe onion[1]
Pentan-1-ol	5.287	—	—	1.49×10^{5}	Fruity, green, sweet, pungent[1]
(*Z*)-Hex-3-en-1-ol	8.189	4.02×10^{5}	1.33×10^{6}	1.77×10^{5}	Green, herbaceous[3]
Benzyl alcohol	15.094	—	5.10×10^{5}	—	Aromatic, floral, fruity[1]

Table 1: Aroma profiles of the three hop varieties, ordered by compound class then by retention time. Reported aromas are indicated.

Some notable variations are mentioned below:

- Undecan-2-one is found in higher abundance in ‘Target’. This correlates with the findings presented by Lermusieau and Collin[6] in which ‘Target’ was differentiated from other European hops by a high abundance of this compound.
- Two sulfur-containing compounds, S-methyl 2-methylpropanethioate and S-methyl 3-methylbutanethioate, were both also only found in ‘Target’, and may contribute an undesirable ‘cooked vegetable’ or ‘cheese-like’ aroma.
- 2-Methylpropanol was detected in both ‘Fuggle’ and ‘Target’, and gives a disagreeable, wine-like aroma.

Increased confidence with Select-eV

Despite the excellent spectral quality of BenchTOF instruments, it can be challenging to identify individual terpenoids, due to weak molecular ions and/or similar spectra using conventional (70 eV) ionisation.

To address this, the split flows from TD analysis of each sample were re-collected onto fresh sorbent tubes, and the analysis repeated using Select-eV soft ionisation at 12 eV – a selection of spectral comparisons are provided in Figure 4. Soft ionisation gave both increased intensity for the molecular ion and reduced fragmentation, resulting in simplified, more selective spectra.

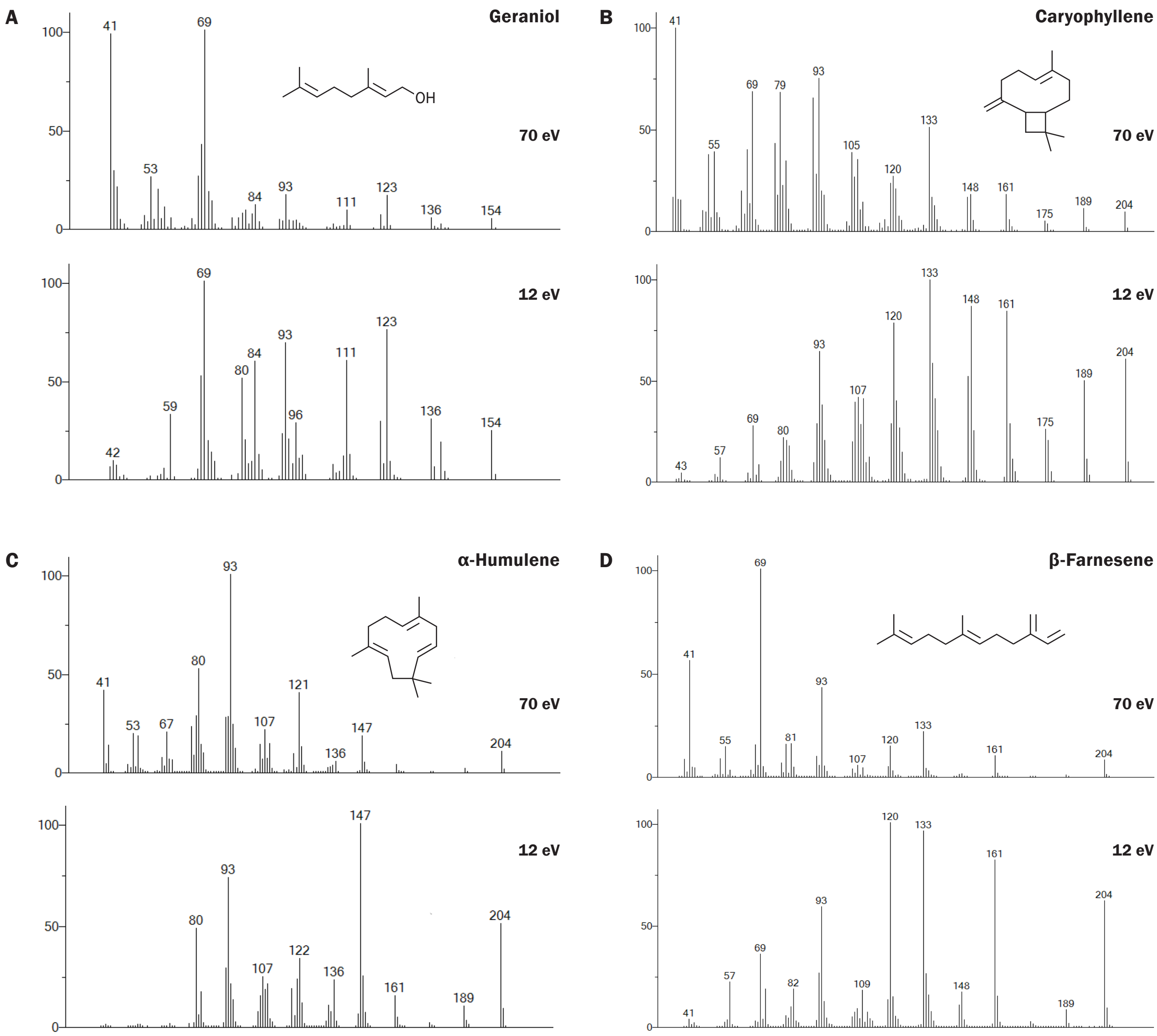

Figure 4: Spectral comparisons at 70 and 12 eV for a selection of the mono- and sesquiterpenoids that are important for contributing the characteristic aroma of hops to beer. *(Continued on next page)*

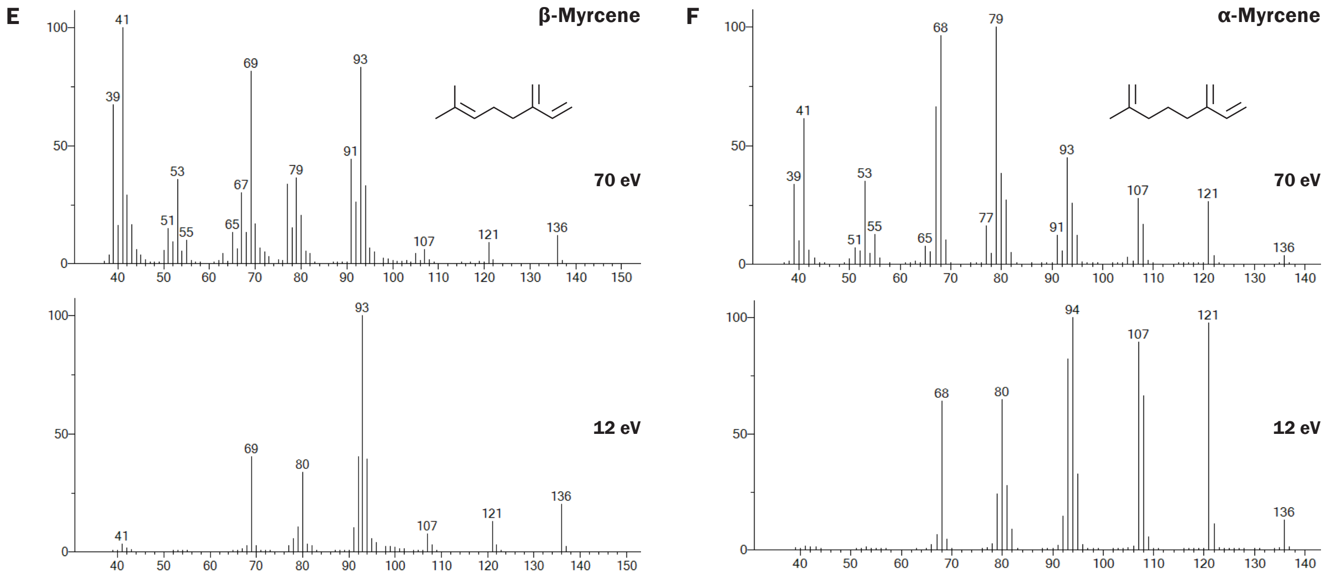

Figure 4: Spectral comparisons at 70 and 12 eV for a selection of the mono- and sesquiterpenoids that are important for contributing the characteristic aroma of hops to beer. *(Continued from previous page)*

The intensity improvement for the important 'diagnostic' ions provides enhanced detection limits and increased confidence in the identification of similar components. Moreover, unlike other soft ionisation techniques, Select-eV retains a degree of fragmentation, aiding structural elucidation and allowing easy library-matching.

Conclusion

In this Application Note, we have shown that TD–GC–TOF MS can be successfully applied to the analysis of strongly aromatic plant materials such as hops. The 'reference-quality' spectra provided by BenchTOF enabled comprehensive characterisation of VOCs in the three hop varieties, while Select-eV provides enhanced confidence in the identities of the most challenging analytes, such as mono- and sesquiterpenes. These capabilities enable robust comparison between samples, and consequently make this approach valuable for quality control in the brewing industry.

References

1. J.L. Gonçalves, J.A. Figueira, F.P. Rodrigues, L.P. Ornelas, R.N. Branco, C.L. Silva and J.S. Câmara, A powerful methodological approach combining headspace solid phase microextraction, mass spectrometry and multivariate analysis for profiling the volatile metabolomic pattern of beer starting raw materials, *Food Chemistry*, 2014, 160: 266–280, http://dx.doi.org/10.1016/j.foodchem.2014.03.065.

2. F. Van Opstaele, T. Praet, G. Aerts and L. De Cooman, Characterization of novel single-variety oxygenated sesquiterpenoid hop oil fractions via headspace solid-phase microextraction and gas chromatography-mass spectrometry/olfactometry, *Journal of Agricultural and Food Chemistry*, 2013, 61: 10555–10564, http://dx.doi.org/10.1021/jf402496t.

3. G.A. Burdock, *Fenaroli's Handbook of Flavor Ingredients* (5th edition), CRC Press, 2004.

4. G. Eyres and J. Dufour, Hop essential oil: Analysis, chemical composition and odor characteristics (ch. 22), in: *Beer in Health and Disease Prevention*, ed. V.R. Preedy, Academic Press, 2009.

5. T.L. Peppard, Volatile organosulphur compounds in hops and hop oils: A review, *Journal of the Institute of Brewing,* 1981, 87: 376–385, http://dx.doi.org/10.1002/j.2050-0416.1981.tb04054.x.

6. G. Lermusieau and S. Collin, Hop aroma extraction and analysis (ch. 5), in: *Analysis of Taste and Aroma* (*Molecular Methods of Plant Analysis*, vol. 21), ed. J.F. Jackson and H.F. Linskens, Springer, 2002, pp. 69–88.

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Trademarks