Application Note 023

Enhancing aroma profiling of wine with GC–TOF MS

Summary

This Application Note shows that BenchTOF time-of-flight mass spectrometers, coupled with GC, can provide a high-performance solution for the detection and quantitation of aroma compounds in wine extracts. In particular, we show how the ChromCompare data-mining module of BenchTOF software allows rapid identification of minor differences between samples. Results also demonstrate how the Select-eV variable-energy ionisation capability of the flagship BenchTOF-Select enhances both the sensitivity and selectivity of analyses.

Introduction

Aroma profiles, such as those for wine, contain a wide variety of components at a range of concentrations. Detection and identification of key compounds with low odour thresholds and compounds responsible for off-odours is a challenging prospect.

Gas chromatography coupled with time-of-flight mass spectrometry (GC–TOF MS) is an ideal choice for such analyses. BenchTOF time-of-flight mass spectrometers offer low detection limits, sub-amu selectivity, full-range (reference-quality) spectra and fast acquisition speeds, which allow trace components, including adulterants, to be identified even within the most challenging of matrices.

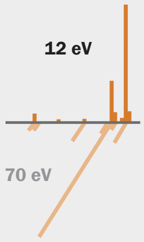
This Application Note describes how these benefits are complemented by two unique technologies: ChromCompare data-mining software for the pairwise comparison of complex chromatograms; and Select-eV variable-energy ionisation technology. Select-eV enhances compound identification by generating repeatable, low-energy mass spectra containing structurally-significant fragment ions and an improved molecular ion signal.

Background to BenchTOF instruments

ALMSCO BenchTOF™ time-of-flight mass spectrometers are designed specifically for gas chromatography. They are particularly appropriate for the analysis of aroma profiles, because they offer exceptional sensitivity, spectral quality and speed:

- **Sensitivity**: Highly efficient direct-extraction technology allows BenchTOF to acquire full-range spectra at sensitivity levels that are similar to or better than SIM detection limits on quadrupole instruments. This allows it to reliably detect and identify trace-level target analytes and unknowns in a single run.
- **Spectral quality**: The ‘reference-quality’ spectra produced by BenchTOF are a close match for those in commercial libraries such as NIST or Wiley. This enables quick and confident matching of both targets and unknowns.
- **Speed**: The acquisition rate of 10,000 full-range spectra per second enhances the performance of the latest automated spectral deconvolution and ‘data-mining’ algorithms.

Select-eV



The flagship BenchTOF-Select™ instrument also features Select-eV® ion-source technology, which allows the energy of electron ionisation to be reduced without impacting sensitivity. Energies can be reduced on a sliding scale from standard 70 eV to 10 eV, by simply changing a parameter in the method.

This low-energy (*i.e.* ‘soft’) electron ionisation reduces analyte fragmentation, which has benefits for a wide range of analyses. However, Select-eV technology breaks new ground by avoiding the inconvenience of reagent gases, ion source pressurisation, or changes in hardware setup typically associated with other soft ionisation techniques for GC–MS.

As well as featuring full system control and a comprehensive data-analysis package, the TOF-DS™ software for BenchTOF also features ChromCompare® – an innovative module that saves time and effort when comparing chromatographic profiles.

ChromCompare distils the information-rich chromatographic data sets generated by BenchTOF into ‘H-Plots’ or line chromatograms that are understandable at a glance. Each line in the H-Plot represents an identified component, and its height is proportional to the peak area. Libraries of H-Plots can be created to characterise future unknown samples.

ChromCompare improves productivity and data confidence, both when comparing GC–MS profiles and when screening large numbers of chromatograms for target compounds.

Experimental

Sample preparation: Solid-phase micro-extraction (SPME) was used to extract target analytes from wine samples. Each 20 mL headspace sample vial contained the following: wine (1 mL), water (4 mL), sodium chloride (2 g) and internal standard (40 µL) containing ISTDs to cover a range of chemical classes (namely D_8-ethyl acetate, D_{11}-ethyl hexanoate, D_{10}-butanol, 5-methylhexanol, D_3-acetic acid, D_{11}-hexanoic acid and 1,3-dimethylphenol).

Sample vials were heated at 60 °C for 2 min, then extracted, with agitation, at 60 °C for 15 min using a DVB/carboxen/PDMS fiber (50/30 µm). The SPME fiber was conditioned at 260 °C for 30 min between samples.

GC:

Instrument:	Agilent 7890A
Column:	HP-Innowax (50 m × 200 µm × 0.4 µm).
Carrier gas:	Helium
Oven temp.:	40 °C for 1.0 min, then 2 °C/min to 60 °C, 3 °C/min to 150 °C, 10 °C/min to 200 °C, 25 °C/min to 250 °C (hold 10 min).
Mode:	Splitless
Inlet temp.:	260 °C

MS:

Instrument:	BenchTOF-Select (Markes International)
Filament voltage:	1.8 V
Ion source:	200 °C
Transfer line:	260 °C
Mass range:	30–450 m/z
Data rate:	4 Hz with 2500 spectra per data point

Results and discussion

Chemical comparison of wine aroma profiles using ChromCompare

Four wine samples (two white, two red) were analysed using the experimental parameters described. Example GC–TOF MS chromatograms obtained for a white wine and a red wine are shown in Figure 1.

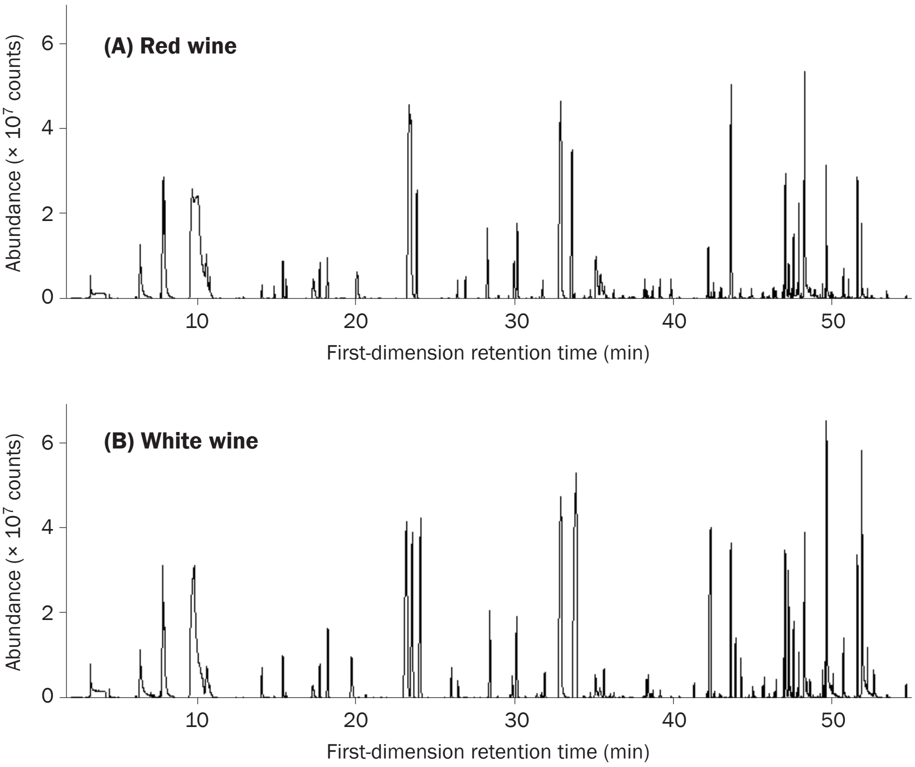


Figure 1: GC–TOF MS chromatograms of **(A)** a red wine and **(B)** a white wine, after dynamic background compensation.

	wine 1	wine 2	wine 3	wine 4
▸ wine 1	-	982	787	564
wine 2	982	-	741	496
wine 3	787	741	-	754
wine 4	564	496	754	-

Figure 2: Histogram 'transforms' aid the visual comparison of wines analysed by GC–TOF MS. Top: Comparison of two red wines (#1, black; #2, red). Middle: Comparison of a red wine (#1, black) and a white wine (#4, red). Bottom: Match factors generated.

Chemical profiling of four wine samples was performed using ChromCompare (Figure 2). This takes the spectrally deconvolved component intensities, established in the previous quantitative processing step, and normalises them to an internal standard to enable precise comparison and classification of samples. Visualisation of the differences between samples is aided by histograms of the centroids of the deconvolved component peaks reconstructed into 'H-Plots'. Weighting factors can be applied to increase the importance of individual compounds in the matching process.

Each H-Plot is thus composed solely of identified analytes. Results of any pair-wise comparison are automatically generated, yielding a very reliable numerical match factor. This provides a robust and objective measure of similarity between complex mixtures. Match factors are displayed as a matrix, allowing comparison of all samples (Figure 2).

Differentiation of isomers with Select-eV

Additional complications in wine aroma profiling are the identification of compounds with weak molecular ions and the differentiation of isomeric compounds.

Markes' variable-energy ion-source technology, Select-eV, aims to solve such problems by allowing electron ionisation energies to be tuned from 70 eV to 10 eV, without compromising sensitivity. This enables enhancement of the molecular ion and a reduction in the number and relative intensity of fragment ions. Figure 3 shows three mass spectral comparisons of compounds identified in wine, analysed at 70 eV and 14 eV. In each case, at 14 eV absolute intensities for the molecular ion were enhanced, and relative fragmentation was reduced. This means that quantitation can easily be performed using the molecular ion (or a distinct structurally-significant ion) rather than one common to many compounds.

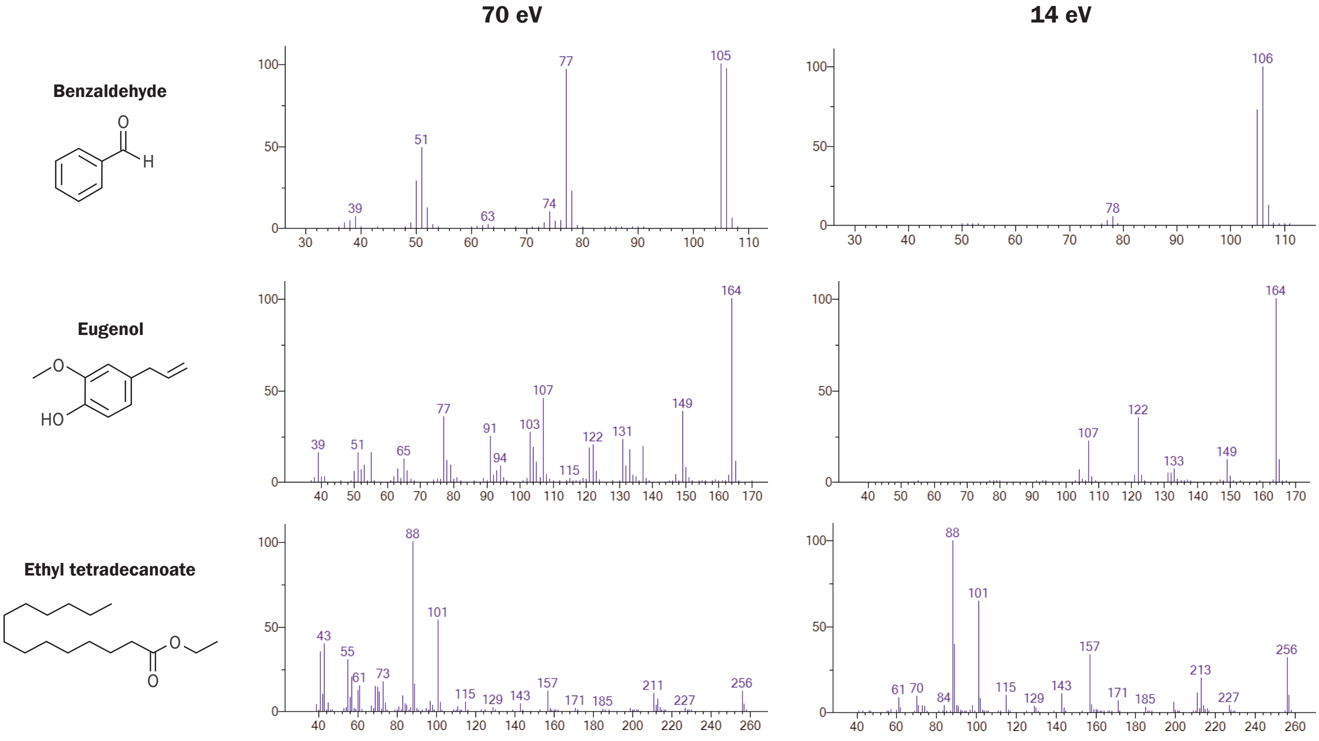


Figure 3: Comparison of mass spectra obtained at ionisation energies of 70 eV (left) and 14 eV (right) for three analytes in the wine samples.

To illustrate this, a variety of flavour/fragrance compounds, covering five chemical classes (alcohols & terpenes, aldehydes & ketones, acids, esters and lactones), were quantified in the wine samples at 14 eV. A wine matrix mimic was spiked with calibration standards at various levels (0.08–230 ppb) and extracted by SPME as previously described.

Figure 4 displays the resulting calibration curves for a selection of standards, with comparisons of the signal-to-noise ratio for the quantitation ion at 70 eV and 14 eV (using the lowest calibration standard) displayed in the accompanying table. Select-eV increased the signal-to-noise ratio for the quant ion in all cases, resulting in more robust quantitation and better limits of detection.

Target analyte	Quant ion	R^2	Lowest calibration standard (ppb)	S/N of molecular ion	
				70 eV	14 eV
Benzaldehyde	106	0.9947	0.46	524	1798
Eugenol	164	0.9946	0.08	111	419
Ethyl tetradecanoate	256	0.9997	0.44	425	2679
Hexanoic acid	87	0.9996	4.62	175	539

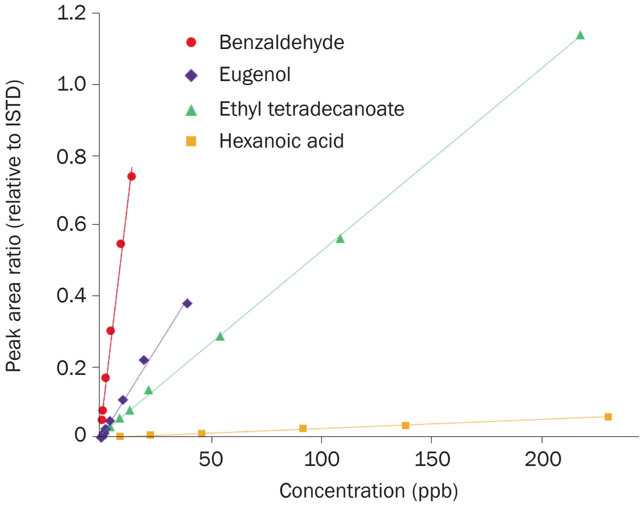


Figure 4: Top: Correlation coefficients and signal-to-noise comparisons for four analytes in the wine samples. Bottom: Calibration curves at 14 eV ionisation, showing high linearity.

Conclusions

In this Application Note, we have illustrated the power and efficiency of GC–TOF MS for the reliable aroma profiling of wines. We have also shown how ChromCompare can be used to achieve fast and objective comparison of complex chromatograms, speeding up the identification of differences in aroma profiles. In addition, the ability to switch between hard and soft ionisation using Select-eV ion-source technology generates complementary spectra for enhanced compound identification, and improves the detection levels of target compounds in complex matrices.

Acknowledgement

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