Enhanced separation and identification of fungicides and other components in wine by headspace SPME–GC×GC–TOF MS

Summary
This Application Note describes the use of solid-phase micro-extraction (SPME) coupled with GC×GC–TOF MS for the detection of trace-level fungicides and compounds responsible for undesirable attributes ('organoleptic faults') in wine. Of particular interest are the fungicides, which because of their trace levels are difficult to detect using conventional quadrupole GC–MS without resorting to selected ion monitoring mode.

Introduction
Vineyards use fungicides to control various fungal diseases, such as moulds and mildews, in order to maintain crop quality. Despite regulations prohibiting fungicide application in the period before harvest, fungicides can and do end up in the wine, necessitating continued monitoring to minimise any potential risk to human health.

Fungicides, however, represent a relatively small number of the many compounds that are of interest to the wine analyst. Of particular concern are compounds causing undesirable smells, tastes or aromas (so-called 'organoleptic faults'), especially those where trace levels have a disproportionate impact upon the characteristics of the final product.

Currently, fungicides and compounds giving rise to organoleptic faults are screened by headspace SPME with GC–quadrupole MS. However, the major drawback of this technique is the poor sensitivity in full-scan mode, which is a particular problem when detecting trace-level components in complex wine matrices. This issue can be addressed by using selected ion monitoring (SIM) mode, but in this mode it is not possible to detect unknowns.

Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS) is an ideal choice for such analyses, because the enhanced peak capacity afforded by the coupling of two columns of different selectivity means that more information on sample composition can be obtained.

This Application Note describes how the enhanced separation offered by GC×GC can be coupled with the exceptionally sensitive BenchTOF-HD mass spectrometer to enable the screening of wine for trace-level fungicides, as well as compounds giving rise to organoleptic faults.

Background to BenchTOF instruments
Markes’ BenchTOF™ mass spectrometers are bench-top time-of-flight instruments designed specifically for gas chromatography. They are particularly appropriate for the GC×GC analysis of complex samples, such as the aroma profiles of food and drink, for the following reasons:

• Sensitivity: Highly efficient direct-extraction technology allows BenchTOF instruments 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 compound identification.

• Speed: The ability to record full-range mass spectral information to extremely high densities (10,000 transient spectral accumulations per second) enables BenchTOF instruments to handle the narrowest peaks encountered in well-optimised GC×GC couplings.

Experimental
Sample preparation:
SPME was used to extract target analytes from samples of Chianti wine. Each 20 mL headspace sample vial contained wine (1 mL), water (4 mL), sodium chloride (2 g) and internal standard (40 µL) containing internal standards from a range of chemical classes (ethyl acetate-d₈, ethyl hexanoate-d₁₁, butanol-d₁₀, 5-methylhexanol, acetic acid-d₃, hexanoic acid-d₁₁ and 1,3-dimethylphenol).

Sample vials were sealed and heated at 60°C for 2 min, then the headspace was extracted, with agitation, at 60°C for 15 min using a DVB/carboxy/PDMS fibre (50/30 µm). The SPME fibre was conditioned at 275°C for 30 min between samples.

GC:
Instrument: GC with a Zoex ZX1 Thermal Modulator
Carrier gas: Helium, 1 mL/min
Mode: Splitless
Inlet temp.: 275°C
**2D column set:**
1st dimension: BPX5, 30 m × 0.25 mm × 0.25 µm
2nd dimension: BPX50, 2.00 m × 0.1 mm × 0.1 µm
Modulator delay loop: As for second dimension (1.00 m)

**Temperature programme:**
Main oven: 40 °C (1.0 min), 5 °C/min to 315 °C (5 min)
Secondary oven: No offset
Hot jet: 165 °C (2.0 min), 3.5 °C/min to 400 °C (hold time matched to total run time)
Modulation period: 4.0 s, hot-jet pulse 350 ms
Total run time: 61 min

**TOF MS:**
Instrument: BenchTOF-HD™ (Markes International)
Filament voltage: 1.9 V
Ion source: 250 °C
Transfer line: 300 °C
Mass range: m/z 40–500
Data rate: 50 Hz (200 spectral accumulations per data point)

**Software:**
Image processing: GC Image™ (Zoex, Inc.)

**Results and discussion**
The GC×GC–TOF MS colour plot produced from the analysis of Chianti wine headspace is shown in Figure 1. Of note are the vast number of compounds separated, many of which would co-elute in 1D GC–MS analyses.

![Figure 1: Top: GC×GC–TOF MS colour plot of the headspace of a Chianti wine. Bottom: Expansions highlight compounds listed in the table and discussed in the text.](image-url)}
The GC×GC–TOF MS data generated from the Chianti headspace was searched for the presence of a number of compounds of potential interest, including fungicides and compounds giving rise to organoleptic faults. Ten examples (A–J) from across the dynamic range are indicated in Figure 1, and spectra for each are shown in Figure 2.

**Figure 2:** Comparison of BenchTOF-HD spectra (top, red) and NIST library spectra (bottom, blue) of three fungicides (A–C), two potential toxins (D,E), three compounds causing common organoleptic faults (F–H) and two desirable aroma compounds (I,J), identified in Chianti headspace.
Three common fungicides were detected. Pyrimethanil (A) and fenhexamide (B) are used to treat Bunch Rot (Botrytis cinerea), while mefenoxam (C) is the active ingredient in fungicides used to treat Downy Mildew (Plasmopara viticola).

Two other compounds of potential concern were also identified. Ethyl paraben (D), as for other parabens, is widespread in cosmetics, pharmaceuticals and foodstuffs, and has given causes for concern because of its potential as an endocrine disruptor. Benzothiazole (E) is present at low levels in all wines as the natural product of yeast or microbial action, but at higher levels may be indicative of contamination from materials such as rubber.1

Three compounds giving rise to common organoleptic faults were detected. One of these, 4-ethylguaiacol (F), is produced by spoilage bacteria yeasts of the genus Brettanomyces (Dekkera) from precursors present in wine, and gives rise to an undesirable ‘leathery’ or ‘cattleshed’ aroma.2 Two other compounds, guaiacol (G) and p-cresol (H), have been related to smoky attributes in wines made from smoke-affected grapes.3

Two compounds detected are, in contrast, considered to be positive attributes. Whiskey lactone (I) is characteristic of barrel-aged wines and spirits,4 while phenylethyl alcohol (J) lends a rose/floral note5 and is generally referred to as the ‘rose scent’ in red wines.

Conclusions
In this Application Note, we have shown that GC×GC–TOF MS using a BenchTOF-HD instrument as the detector provides the sensitivity, spectral quality and chromatographic resolving power necessary to screen wine for a variety of compounds, including potentially harmful trace-level compounds such as fungicides as well as compounds of organoleptic relevance. A particular feature is the confident characterisation of both high-loading and trace components from a complex sample in a single analytical run.

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References

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