

Automated aroma profiling of wine using sorptive extraction with GC×GC–TOF MS/SCD

This study demonstrates the use of headspace sorptive extraction coupled with GC×GC–TOF MS/SCD to provide comprehensive information on wine aroma and odour taints on a single, fully-automated, cryogen-free platform. We also show the added value of Tandem Ionisation[®] for confident identification of aroma-active species that can be challenging to identify by conventional 70 eV electron ionisation alone.



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.

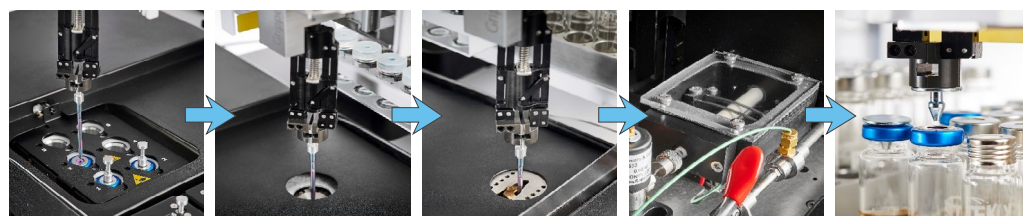
Sampling the headspace of wine using high-capacity sorptive extraction can help to tackle this issue, by increasing sample loadings through use of a larger volume of PDMS stationary phase (65 µL compared to ~0.5 µL for SPME). Used in conjunction with secondary refocusing, this approach offers excellent sensitivity, as well as the ability to re-collect a portion of the sample for repeat analysis in a fully automated workflow using the Centri[®] platform from Markes International.

Despite these advances, the aroma profiles of wine still present challenges to analysts because of their complexity, with important compounds (such as trace-level sulfur species that cause undesirable odour taints) frequently masked by higher-loading components. Fortunately, comprehensive two-dimensional gas chromatography (GC×GC) coupled with time-of-flight mass spectrometry (TOF MS) can help to tackle this problem, with the enhanced separation capacity of GC×GC complementing the ability of TOF MS to provide confident identification of targets and non-targets.

In this study we demonstrate the implementation of this approach on a multi-functional, cryogen-free platform, for definitive yet affordable aroma profiling of a range of red wines. In particular, we show how flow-modulated GC×GC enables simple configuration of parallel detection by both TOF MS and sulfur chemiluminescence detection (SCD), with the result being comprehensive aroma profiling and selective detection of sulfur odour taints in a single run. A further feature covered is how Tandem Ionisation® can be used to enhance confidence in identification by generating both hard and soft ionisation data simultaneously.

Experimental

The analytical workflow is shown in Figure 1.



The HiSorb probe is inserted into the vial for analyte equilibration.

The probe is removed from the vial, and taken to a wash/dry station to remove matrix.

The probe is thermally desorbed and vapours are transferred to a focusing trap.

The trap is rapidly heated to transfer the sample onto the GC as a narrow band (with optional split re-collection).

Vials can be re-sealed with special caps to avoid contamination of laboratory air.

Figure 1

Workflow for fully automated sorptive extraction on the Centri® platform.

Samples: Pinot Noir red wines from Chile (high-end, mid-range and low-cost varieties), South Africa and Australia were purchased. For sampling, 10 mL of wine was placed in a 20 mL headspace vial.

Sampling and preconcentration: Instrument: Centri® (Markes international). Headspace sorptive extraction: Inert-coated stainless steel HiSorb™ PDMS probe (Markes International); Vial temperature: 60°C; Agitation: 400 rpm; Equilibration: 5 min; Sampling: 30 min.

GC×GC: Flow modulator: INSIGHT® (SepSolve Analytical); Modulation period (P_M): 5.2 s.

TOF MS: Instrument: BenchTOF-Select™; Mass range: m/z 35–600; Acquisition speed: 100 Hz in Tandem Ionisation® mode at 70 and 14 eV.

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

Please contact SepSolve for full analytical parameters.

Results and discussion

1. Separation of complex aroma profiles

The enhanced separation of the GC×GC system is clearly evident in Figure 2, with over 300 peaks separated in the aroma profile of the high-end Chilean wine. The expanded region of the GC×GC–TOF MS chromatogram shows how a co-elution that would have occurred in 1D GC is avoided thanks to the additional separation in the second dimension.

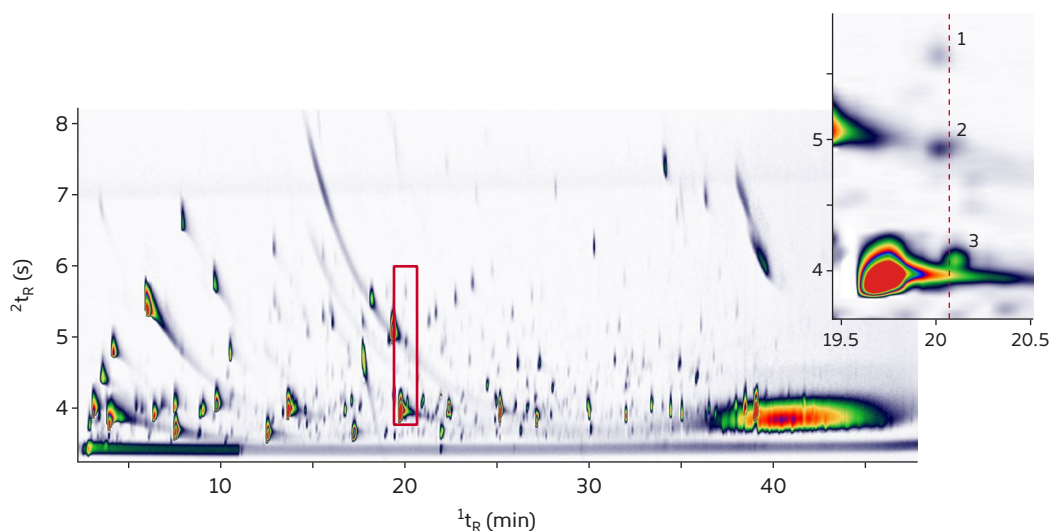


Figure 2

GC×GC–TOF MS chromatogram showing the separation of aroma compounds in the high-end Chilean Pinot Noir. The inset shows separation of three components that would have co-eluted in 1D GC.

The associated spectra (Figure 3) show the confident identification achievable using the Tandem Ionisation[®] capability of BenchTOF™. The 70 eV ionisation spectra produce excellent matches to reference libraries such as NIST and Wiley, while the soft EI spectra (14 eV in this case) provide confirmation of analyte identity, as a result of the reduced degree of fragmentation and enhanced signals from molecular ions and other structurally-significant fragments.

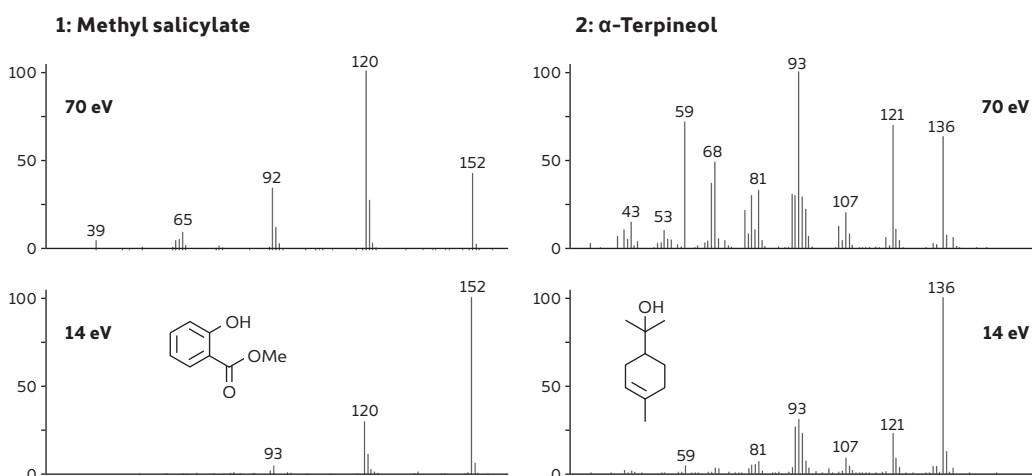
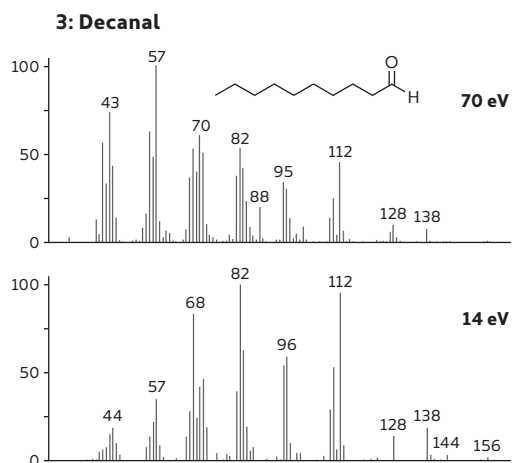


Figure 3

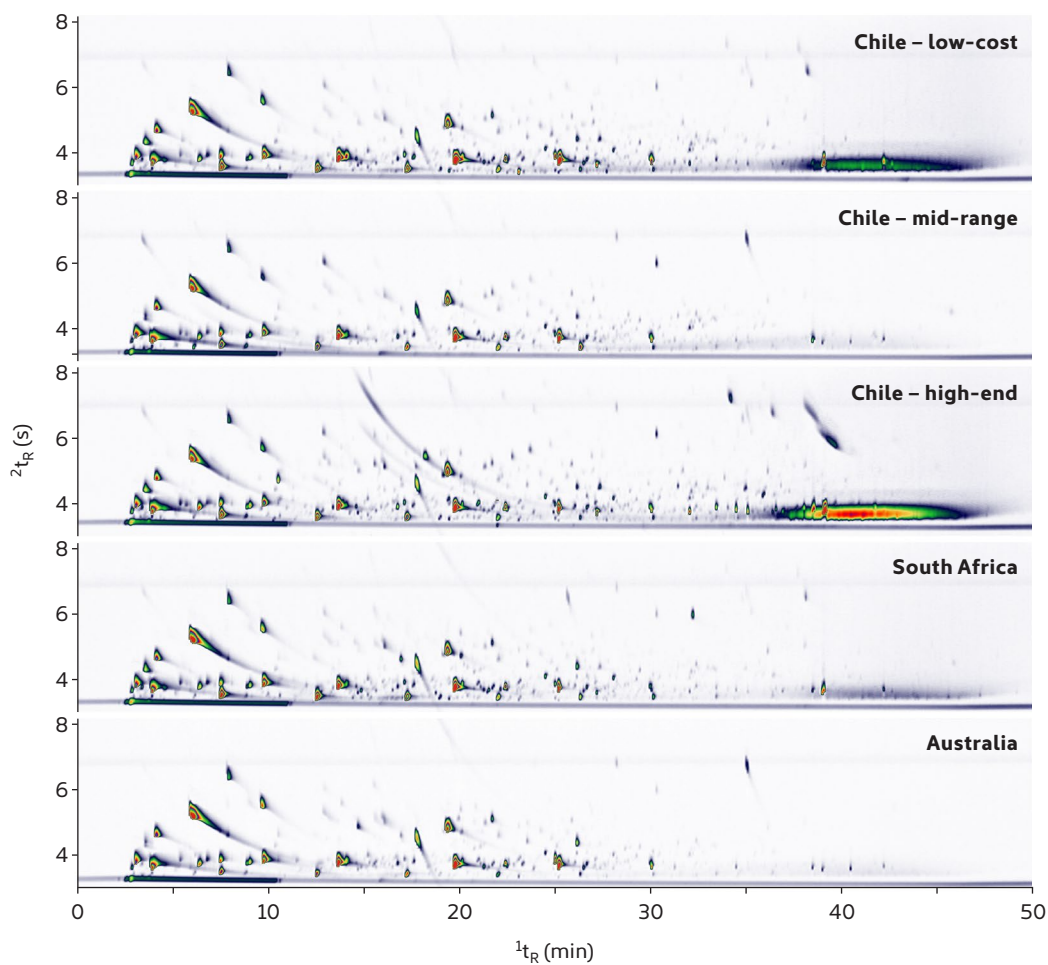
Spectral comparisons at 70 eV and 14 eV for the three analytes highlighted in Figure 2. (Continued on next page)

**Figure 3**

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2. Comparison of wine aromas

Figure 4 shows the GC×GC–TOF MS chromatograms for the five wines, and the numerous aroma-active species identified are listed in Table 1. The high-end Chilean Pinot Noir had the widest-ranging composition, and interestingly was also found to contain a higher abundance of saturated fatty acids, such as myristic acid and palmitic acid, which are thought to raise blood cholesterol.

**Figure 4**

GC×GC–TOF MS colour plots for the five Pinot Noir wines.

Key aroma ^[1]	Compound	¹ t _R (min)	² t _R (s)	Library RI	Peak area (counts)				
					Chile (high)	Chile (mid)	Chile (low)	South Africa	Australia
Ethereal	Ethyl acetate	3.9294	3.7447	612	1.33×10 ⁷	1.17×10 ⁷	1.01×10 ⁶	1.07×10 ⁷	1.07×10 ⁷
	2-Methylpropan-1-ol	4.1335	4.7527	625	2.23×10 ⁶	2.21×10 ⁶	2.52×10 ⁶	2.49×10 ⁶	2.85×10 ⁶
Fruity	Ethyl propanoate	5.3955	3.9912	710	9.59×10 ⁵	6.14×10 ⁵	5.46×10 ⁵	6.02×10 ⁵	7.78×10 ⁵
	Ethyl isobutanoate	6.3520	3.8376	756	1.46×10 ⁶	8.09×10 ⁵	9.85×10 ⁵	1.06×10 ⁶	8.89×10 ⁵
	Isobutyl acetate	6.8019	3.9695	771	2.29×10 ⁵	1.94×10 ⁵	2.99×10 ⁵	2.44×10 ⁵	2.64×10 ⁵
	Ethyl butanoate	7.4894	3.9593	802	1.65×10 ⁶	1.64×10 ⁶	2.11×10 ⁶	1.58×10 ⁶	1.43×10 ⁶
	Ethyl lactate	7.8936	6.5164	815	4.21×10 ⁶	3.73×10 ⁶	4.16×10 ⁶	4.93×10 ⁶	4.90×10 ⁶
	Ethyl 2-methylbutanoate	8.8757	3.8578	849	6.18×10 ⁵	2.98×10 ⁵	4.35×10 ⁵	4.61×10 ⁵	3.99×10 ⁵
	Ethyl 3-methylbutanoate	9.0133	3.8527	854	9.16×10 ⁵	4.96×10 ⁵	6.85×10 ⁵	8.06×10 ⁵	7.28×10 ⁵
	Isoamyl acetate	9.7346	3.9525	876	2.94×10 ⁶	2.95×10 ⁶	4.92×10 ⁶	4.47×10 ⁶	5.04×10 ⁶
	Ethyl pentanoate	10.4161	3.9704	887	7.58×10 ⁴	—	—	6.35×10 ⁴	—
	Benzaldehyde	12.8603	6.1228	962	1.24×10 ⁵	4.03×10 ⁴	6.55×10 ⁴	1.17×10 ⁵	6.32×10 ²
	2-Pentylfuran	13.3673	3.8349	993	2.55×10 ⁵	2.28×10 ⁴	7.84×10 ⁴	8.21×10 ³	4.11×10 ⁴
	Ethyl hexanoate	13.6447	3.8345	1000	5.13×10 ⁶	5.00×10 ⁶	6.45×10 ⁶	5.50×10 ⁶	4.89×10 ⁶
	Hexyl acetate	14.0300	3.8870	1011	3.94×10 ⁵	5.54×10 ⁵	1.82×10 ⁶	9.28×10 ⁵	6.46×10 ⁵
	Ethyl hex-2-enoate	15.1483	4.0731	1037	8.34×10 ⁴	5.00×10 ⁴	1.38×10 ⁵	1.03×10 ⁵	1.06×10 ⁵
	Ethyl 2-hydroxy-4-methylpentanoate	15.5440	5.1413	1060	4.92×10 ³	1.08×10 ⁴	4.30×10 ⁴	8.21×10 ⁴	6.40×10 ⁴
	Nonan-2-one	16.6238	4.0179	1092	4.89×10 ⁴	2.37×10 ⁴	3.72×10 ⁴	6.60×10 ⁴	2.65×10 ⁴
Ethyl heptanoate	16.7451	3.8243	1097	4.82×10 ⁵	2.77×10 ⁵	2.44×10 ⁵	2.24×10 ⁵	1.90×10 ⁵	
Ethyl succinate	19.3448	4.8625	1182	8.98×10 ⁶	7.28×10 ⁶	8.91×10 ⁶	9.21×10 ⁶	8.14×10 ⁶	
Citrus	Limonene	14.6310	3.6335	1030	9.02×10 ⁴	2.62×10 ⁴	8.47×10 ⁴	2.81×10 ⁴	8.61×10 ⁴
	2-Ethylhexan-1-ol	14.6749	4.9347	1030	1.31×10 ⁵	2.52×10 ⁴	7.35×10 ⁴	5.30×10 ⁵	3.14×10 ⁴
Tropical	β-Cyclocitral	20.7016	4.2359	1217	2.53×10 ⁴	1.21×10 ⁴	2.16×10 ⁴	1.44×10 ⁴	2.53×10 ⁴
Green	Heptanol	12.8963	5.3747	970	3.17×10 ⁵	4.62×10 ⁵	2.30×10 ⁵	3.84×10 ⁴	1.17×10 ⁵
	Benzene-acetaldehyde	15.4963	6.2160	1045	5.84×10 ⁴	3.96×10 ⁴	6.08×10 ⁴	1.97×10 ⁴	4.89×10 ⁴
	Nerol oxide	18.5217	4.0142	1153	1.54×10 ⁴	7.15×10 ³	8.23×10 ³	4.96×10 ³	2.97×10 ⁴
Sweet	Furfuryl ethyl ether	10.5066	4.6592	917	1.87×10 ⁶	1.02×10 ⁵	7.25×10 ⁴	1.12×10 ⁵	—
Floral	Acetophenone	16.1885	5.9602	1065	4.37×10 ⁴	—	2.80×10 ⁴	4.31×10 ³	2.60×10 ⁴
	Linalool	16.9214	4.5385	1099	2.51×10 ⁴	2.86×10 ⁴	6.59×10 ⁴	7.21×10 ⁴	3.08×10 ⁵
	Phenylethyl alcohol	17.7271	4.5187	1116	6.76×10 ⁶	3.75×10 ⁶	6.30×10 ⁶	8.61×10 ⁶	8.43×10 ⁶
	Benzyl acetate	19.0046	5.4397	1164	4.39×10 ⁴	4.79×10 ³	2.42×10 ⁴	7.01×10 ³	3.43×10 ³
Herbal	Hexanol	9.6520	5.6463	868	1.71×10 ⁶	9.35×10 ⁵	1.82×10 ⁶	2.61×10 ⁶	2.41×10 ⁶
	Eucalyptol	14.8200	3.6817	1032	1.11×10 ⁴	6.00×10 ³	2.65×10 ⁴	1.24×10 ⁴	6.22×10 ⁴
Minty	Methyl salicylate	20.0011	5.4481	1192	9.41×10 ⁴	2.69×10 ⁴	3.57×10 ⁴	6.87×10 ⁴	1.13×10 ⁵
Aldehydic	3-Methylbutanal	4.5611	3.8274	652	1.26×10 ⁵	2.21×10 ⁴	1.36×10 ⁵	5.00×10 ⁴	1.14×10 ⁵
	Nonanal	17.0950	3.9515	1104	1.87×10 ⁵	4.95×10 ⁴	2.58×10 ⁵	5.03×10 ⁴	1.52×10 ⁵
	Decanal	20.1067	3.8883	1206	4.85×10 ⁴	2.58×10 ⁴	8.66×10 ⁴	3.93×10 ⁴	5.91×10 ⁴
Terpenic	γ-Terpinene	15.5804	3.6883	1060	2.07×10 ⁵	4.40×10 ⁴	9.34×10 ⁴	—	9.01×10 ⁴
	α-Terpineol	20.0285	4.7476	1189	3.32×10 ⁴	1.22×10 ⁴	2.42×10 ⁴	2.11×10 ⁴	6.59×10 ⁴
Waxy	Octan-1-ol	16.0266	5.0569	1071	1.80×10 ⁵	7.96×10 ⁴	1.23×10 ⁵	1.00×10 ⁵	1.92×10 ⁵
	Methyl octanoate	17.5214	3.8520	1126	2.30×10 ⁵	2.35×10 ⁵	5.61×10 ⁵	2.14×10 ⁵	2.35×10 ⁵
	Octanoic acid	19.5667	7.2402	1180	6.66×10 ⁶	6.78×10 ⁶	9.04×10 ⁶	6.85×10 ⁶	6.83×10 ⁶
Fermented	Isopentanol	5.9653	5.3527	736	9.16×10 ⁶	8.47×10 ⁶	1.05×10 ⁷	8.60×10 ⁶	1.21×10 ⁷

Table 1

Aroma-active compounds identified in the Pinot Noir wine samples.

Of all the aroma categories, 'fruity' contained the largest number of components, which is in agreement with references to "cherries" and "berries" in the tasting notes for each of the wines.

Some further points of interest were:

- ▶ Furfuryl ethyl ether ('sweet, nutty') was only found in the high-end Chilean wine.
- ▶ 4-Ethylguaiacol (an odour taint produced by the spoilage yeast *Brettanomyces*) was detected in all of the wines except the South African variety.
- ▶ Eucalyptol was more abundant in the Australian Pinot Noir compared to the other varieties. In other studies, this has been ascribed to the proximity of grapevines to eucalyptus trees in Australian vineyards.

In addition, a number of potential odour taints were also identified (Figure 5). The high capacity of HiSorb™ sorptive extraction probes, combined with the inherent sensitivity of BenchTOF, ensure that odour taints are reliably detected, even when present in trace amounts. Interestingly, the most expensive wine was found to have the highest abundances of these components.

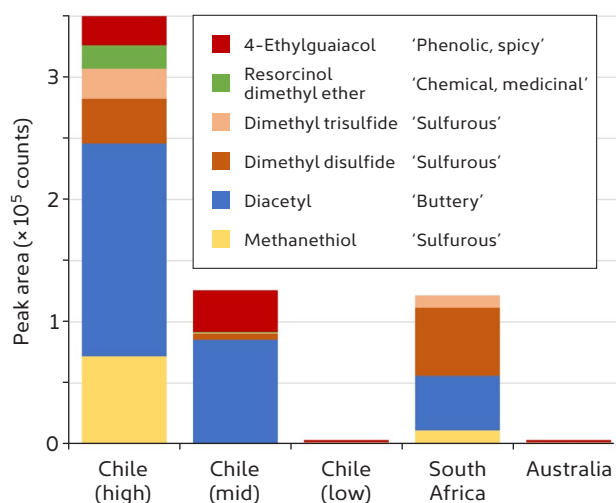


Figure 5

Comparison of peak areas for six potential odour taints in the Pinot Noir samples.

3. Sensitive detection of sulfur odour taints

Parallel detection by both TOF MS and sulfur chemiluminescence detection (SCD) further improves the ability to detect undesirable sulfur components. Using the SCD data to pinpoint sulfur compounds allowed them to be targeted in the TOF MS data and so identified confidently. Figure 6 shows some key sulfur compounds (and their associated aroma traits) detected and identified in this study using GC×GC–TOF MS/SCD.

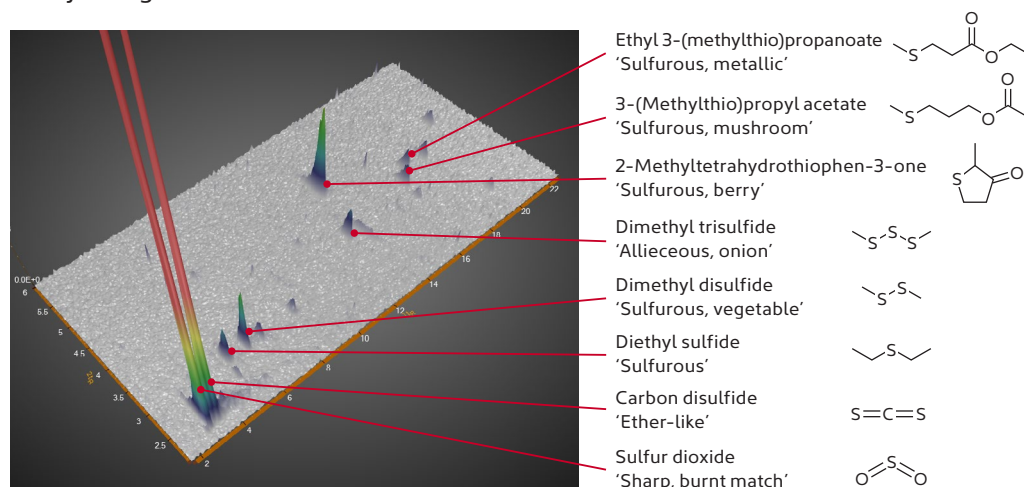


Figure 6

GC×GC–SCD surface chart showing important sulfur species identified in the headspace of the high-end Chilean Pinot Noir.

Conclusions

This study has shown that headspace sorptive extraction, in conjunction with flow-modulated GC×GC–TOF MS/SCD, provides:

- ▶ Robust and sensitive sampling, using HiSorb™ high-capacity sorptive extraction probes with preconcentration on the Centri® platform.
- ▶ Comprehensive aroma profiles through enhanced GC×GC separation of wine headspace.
- ▶ Cryogen-free and solvent-free preconcentration and INSIGHT® flow modulation, for substantial cost savings.
- ▶ Enhanced confidence in identification, through use of the Tandem Ionisation® capability of the BenchTOF™ instrument to provide hard and soft EI spectra in a single run.
- ▶ Highly sensitive SCD screening for odour taints caused by trace sulfur species.
- ▶ Fully-automated workflows for unattended operation, with full instrument control and simple, unified data processing in ChromSpace® software.

Reference

- [1] The Good Scents Company Information System (search facility), www.thegoodscentscompany.com/search2.html (accessed on 18 September 2019).

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