Application Note 254

Identifying low-level odorants in wine by automated high-capacity sorptive extraction with GC–MS

This study shows that immersive sorptive extraction using high-capacity HiSorb probes, automated on the new Centri sample extraction and enrichment platform for gas chromatography–mass spectrometry (GC–MS), can be used to screen wine for ppb levels of two yeast-derived spoilage compounds, as well as a range of other compounds.

Introduction

The yeast *Brettanomyces* (*Dekkera*) *bruxellensis* ('Brett') is part of the natural microflora of wine, but its growth can result in the production of 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG, also known as 4-ethyl-2-methoxyphenol). These compounds impart unpleasant 'medicinal', 'phenolic' or 'horse sweat' olfactory notes to wine (known as 'Brett character'), and also exert a masking effect on the desirable 'fruity' aromas.

Historically, a wide variety of sampling methods have been used to extract volatiles from wine, with a key driver being the need to improve upon inefficient solvent-extraction methods. HiSorb™ high-capacity sorptive extraction is a new and highly efficient sampling approach for a wide range of applications. It involves use of robust, inert metal probes fitted with a relatively large volume of PDMS sorptive phase (Figure 1), allowing high sensitivity to be achieved. Following headspace or immersive extraction, the probes are rinsed, dried and desorbed, with the analyte vapours then concentrated on a focusing trap prior to GC–MS injection.

Figure 1: A standard-length (75 mm) HiSorb high-capacity sorptive extraction probe, showing the PDMS phase on the left.

In this study, we use this extraction approach immersively, in conjunction with GC–MS, to identify a range of VOCs in red wine, including 4-EP and 4-EG. The entire process of HiSorb extraction and trap-based analyte preconcentration is automated by the Centri® system from Markes International, and here we show the numerous advantages of this approach for analysts tasked with monitoring wine aroma profiles. These include detecting analytes across a wide volatility range, extending dynamic range by varying the split ratio, and eliminating problems caused by ethanol overloading.

We also describe how automated sample splitting and re-collection on Centri can streamline method development and speed up workflows, by allowing a single sample to be analysed more than once in a fully automated sequence.

Background to Centri®

Markes International's Centri system for GC–MS is the first platform to offer high-sensitivity unattended extraction and enrichment of VOCs and SVOCs in solid, liquid and gaseous samples.

Centri allows full automation of immersive and headspace extraction using HiSorb™, high-capacity sorptive extraction probes. It also offers full automation of headspace, SPME and tube-based thermal desorption with enrichment. Leading robotics and analyte-trapping technologies are used to improve sample throughput and maximise sensitivity for a range of applications – including profiling of foods, beverages and fragranced products, environmental monitoring, clinical investigations and forensic analysis.

In addition, Centri allows samples from any injection mode to be split and re-collected onto clean sorbent tubes, avoiding the need to repeat lengthy sample extraction procedures and improving security for valuable samples, amongst many other benefits.

For more on Centri, visit www.markes.com.

Experimental

Sample:
Spanish red wine (12% v/v alcohol) was dispensed into a 20 mL round-bottom headspace vial (Markes International part no.

C-HSVC20-100). A volume of 19 mL was used to help minimise loss of analytes into the headspace.

High-capacity sorptive extraction and trapping:

Instrument:	Centri® (Markes International)
Probe:	Standard-length stainless steel HiSorb™ probe (part no. H1-XXAAC)
Incubation:	30°C (60 min) at 600 rpm
Desorption:	280°C (12 min)
Flow path:	200°C
Focusing trap:	'Material emissions' (part no. U-T12ME-2S)
Purge flow:	50 mL/min (1 min)
Trap low:	25°C
Trap high:	290°C (5 min)
Split flow:	High split: 50 mL/min Low split: 5 mL/min

Sample re-collection:

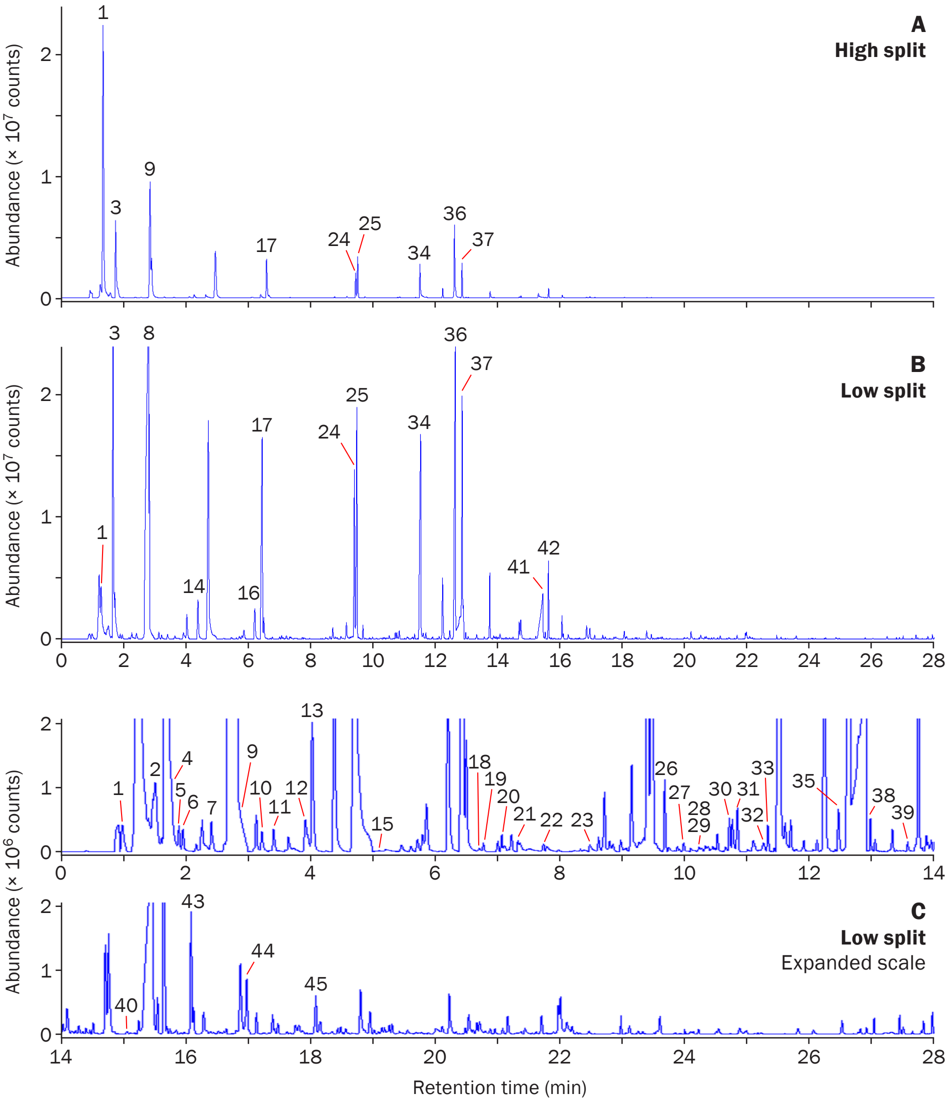
Sorbent tube:	Tenax® TA stainless steel sorbent tube (part no. C1-AAXX-5003)
Tube desorption:	300°C (10 min)

GC–MS:

Column:	MEGA®-5 MS, 30 m × 0.25 mm × 0.25 µm
Constant flow:	Helium, 2.0 mL/min
Oven program:	40°C (5 min), then 10°C/min to 250°C, then 35°C/min to 300°C (2.5 min)
Transfer line:	310°C
Ion source:	250°C
Quad:	200°C
Mass range:	m/z 15–350

Software:

TargetView™ GC–MS software (Markes International) was used to selectively remove unwanted background noise and to deconvolve analyte peaks, improving the identification of lower-level analytes during subsequent automated comparison against a customised library generated from spectra in the NIST MS Search 2.0 database.



1	Ethanol
2	3-Methylfuran
3	Ethyl acetate
4	2-Methylpropan-1-ol
5	3-Methylbutanal
6	Benzene
7	n-Propyl acetate
8	1,1-Diethoxyethane
9	Pentan-1-ol
10	Toluene
11	2-Methylpropyl acetate
12	Hexanal
13	Ethyl butanoate
14	Ethyl 2-hydroxypropanoate
15	Furfural
16	Hexan-1-ol
17	3-Methylbutyl acetate
18	Styrene
19	Non-1-ene
20	Heptanal
21	γ-Butyrolactone
22	3-Methylbutan-1-ol
23	Benzaldehyde
24	Hexanoic acid
25	Ethyl n-hexanoate
26	n-Hexyl acetate
27	2-Ethylhexan-1-ol
28	1-Phenylpropyne
29	Benzeneacetaldehyde
30	Acetophenone
31	Octan-1-ol
32	Linalool
33	Nonanal
34	Phenylethyl alcohol
35	2-Ethylphenol
36	Diethyl butanedioate
37	Ethyl n-octanoate
38	Decanal
39	Ethyl phenylacetate
40	Ethyl phenylpropanoate
41	n-Decanoic acid
42	Ethyl n-decanoate
43	Ethyl 3-methylbutyl butanedioate
44	n-Dodecane
45	Ethyl n-dodecanoate

Figure 2: HiSorb analysis (TIC) of red wine: (**A**) High-split run; (**B**, **C**) Low-split run.

Results and discussion

1. Overall aroma profile

Figure 2 shows the red wine profile obtained by immersive extraction using HiSorb probes. A range of aroma and flavour compounds were identified, including aldehydes, acids, alcohols, esters, aromatics and alkanes, with some in the C_{10}–C_{14} range. Such extraction of lower-volatility analytes is a consequence of direct contact of the sorptive phase with the sample when immersed; an equivalent headspace procedure would be expected to provide very limited responses for these compounds.

Figure 2 also demonstrates the option to fine-tune the sample loading on-column, by varying the split ratios at the trap desorption stage. Figure 2A shows the result of using a high split, in which a relatively small proportion of the sample is sent to the GC–MS. This is best in the initial stages of method development for determining the concentrations of the more abundant analytes without problems from overloading.

In contrast, Figure 2B and 2C show the outcome of using a low split, in which the proportion of the sample sent to the GC–MS is much larger. A low split value enables significantly more compounds to be detected without degrading chromatographic peak shape, and it also minimises any problems with overloading from residual ethanol (see Section 4).

A key feature of the Centri system used here is that it allows split flows to be 're-collected' onto a clean, industry-standard thermal desorption tube, rather than being lost through the split line. This means that the same sample can be analysed multiple times, so greatly reducing the amount of time spent on sample preparation. For example, in the above case an initial high split can be used to obtain a clear idea of the overall analyte loading, followed by a low-split run of the re-collected sample to investigate trace-level compounds. All operations can be configured to run in a single automated sequence using Centri and its control software.

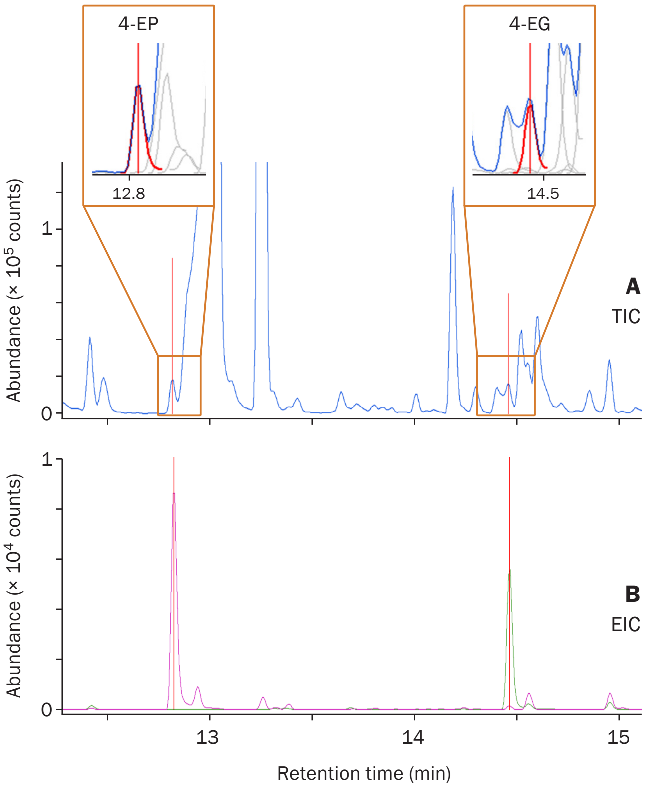


Figure 3: HiSorb analysis of red wine, showing detection of 4-EP and 4-EG (red bars): (**A**) TIC, with insets show the co-eluting analytes (grey); (**B**) EIC at m/z 107 (magenta) + 137 (green).

2. Detection of 'Brett' odorants

The two odorants 4-EP and 4-EG listed in the target library were automatically identified in the red wine sample using TargetView, as indicated by the red bars in Figure 3A. Both compounds co-eluted with other analytes, but the software's deconvolution capabilities provided a high degree of confidence, with NIST match factors of 921 (4-EP) and 908 (4-EG). The peaks due to these odorants are also clearly shown in the extracted-ion profiles (Figure 3B).

3. Quantitation of 'Brett' odorants

The sensory threshold levels for 4-EP and 4-EG vary between tasters and the wine matrix, but 'Brett-like' characters are typically apparent above 300–600 ppb (µg/L) for 4-EP, and 50 ppb for 4-EG. The ratio of 4-EP to 4-EG in wines also varies, with values from 3 : 1 to 22 : 1 having been reported.[1]

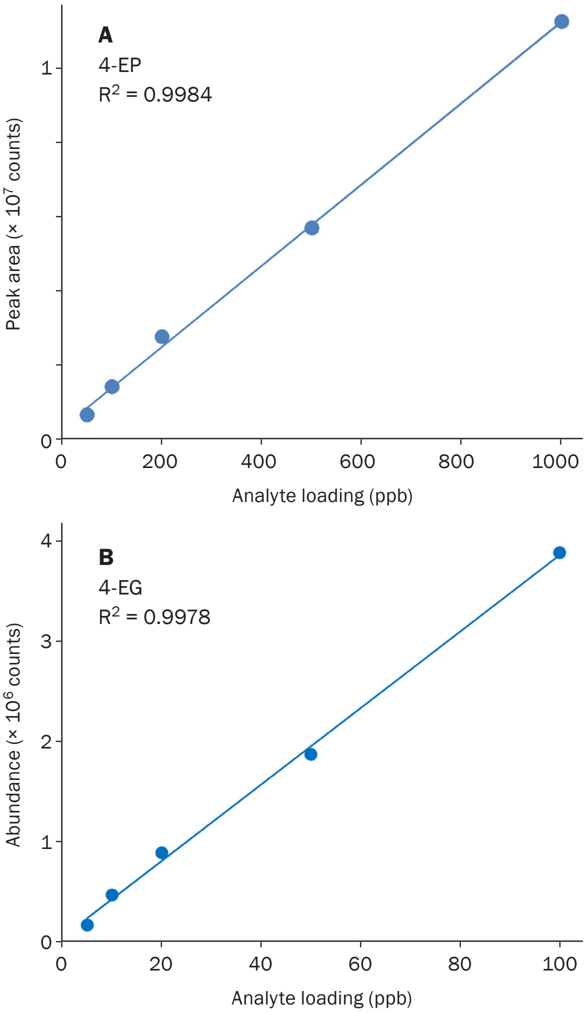


Figure 4: Calibration curves for (**A**) 4-EP and (**B**) 4-EG.

With this in mind, to determine the concentrations present in the wine samples, five-point calibration curves were generated (Figure 4) in the ranges 50–1000 ppb (for 4-EP) and 5–100 ppb (for 4-EG), all in 12% v/v ethanol–water. R^2 values greater than 0.995 were achieved for both compounds.

These calibrations indicated a concentration of 112 ppb for 4-EP and 25 ppb for 4-EG in the wine sample. Although these levels are somewhat lower than the threshold levels indicated above and so would not be of immediate concern, it would likely be useful to repeat the analysis with a sample taken at a later time, to see if concentrations were increasing.

The EIC profiles of the wine sample in Figure 3B show signal-to-noise values of ~100 : 1 for 4-EP and ~300 : 1 for 4-EG. These indicate that the probe-based immersive sorptive extraction technique described here could be used to reliably detect low-ppb levels of 4-EP, and sub-ppb levels of 4-EG. For wineries creating very young wines without a history of ‘Brett’ off-odours, detection of such low levels could be valuable to flag a potential issue and so enable early intervention to prevent off-odour development.

4. Minimising ethanol interference

Interference due to water is a common problem when analysing volatiles from beverages, but this is avoided in this study because of the use of the relatively hydrophobic PDMS phase on the HiSorb probes. However, the abundant ethanol present in wine remains a difficulty, because it is sampled by PDMS, and can result in an overloaded peak that can mask other early-eluting compounds.

The capabilities of Centri enable a three-fold approach to this problem:

- Use of hydrophobic sorbents in the focusing trap (and ambient-temperature trapping) help prevent the trapping of excessive amounts of ethanol.
- Purging the focusing trap with ambient-temperature carrier gas prior to desorption further reduces the amount of ethanol on the trap.
- Use of a low split ratio helps to eliminate any problems with overloading from residual ethanol.

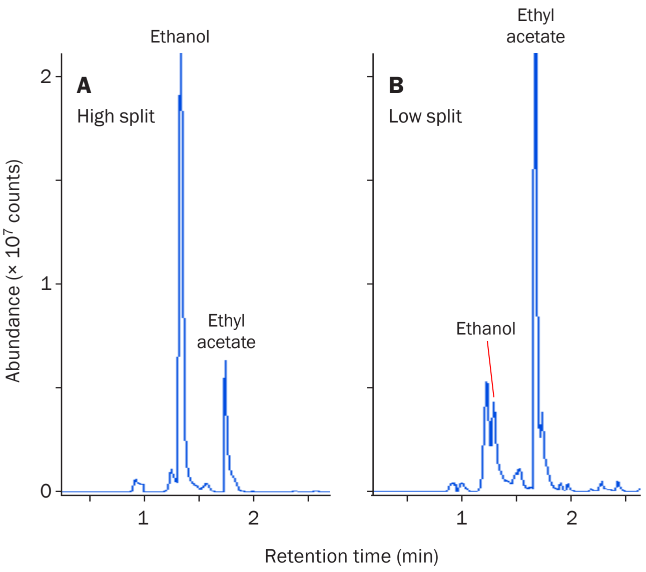


Figure 5: HiSorb analysis of red wine, showing the effect of optimised analytical conditions on the response of ethanol: (**A**) High-split run; (**B**) Low-split run on a re-collected sample.

The outcome of using the above approaches is demonstrated in Figure 5, which shows the responses of ethanol under the two splitting conditions.

Figure 5A shows the result of using a high split. Even under these conditions, the use of hydrophobic sorbents and the purging process mean that the ethanol response is only 4–5 times greater than the nearby ethyl acetate peak, and substantially lower than what would be expected from the 12% alcohol content of the wine.

Figure 5B shows the result of using a low split on a re-collected sample. Although the column loading has increased by a factor of 10, the repeated processes of sorbent sampling and purging have led to a further reduction in ethanol response, and in addition ethyl acetate and some smaller components are more prominent.

Conclusions

This study has demonstrated that automation of probe-based immersive HiSorb sorptive extraction on the Centri platform, in conjunction with GC–MS, allows the detection of wine volatiles including the ‘Brett’ odorants 4-EP and 4-EG.

These odorants were detected at levels substantially below typical sensory threshold values, demonstrating the sensitivity resulting from the sorptive extraction and preconcentration processes. This would enable early identification of a ‘Brett’ spoilage issue, and allow prompt remedial action to be taken.

A key feature of the Centri system employed is its ability to split and re-collect samples onto clean TD tubes, which allows fully automated sequencing of multiple runs of the same sample. In this study, this capability was used to extend the dynamic range through use of different split ratios, and it also has numerous other benefits across a wide range of applications – such as allowing preservation of valuable samples, and avoiding repetition of lengthy sample preparation protocols.

Reference

1. M. Malfeito-Ferreira, Two decades of “horse sweat” taint and *Brettanomyces* yeasts in wine: Where do we stand now?, *Beverages*, 2018, 4: 32, http://doi.org/10.3390/beverages4020032.