Application Note 277

Evaluation of new high-capacity sorptive extraction (HiSorb™) phases for flavour profiling of hard seltzers

New high-capacity phase combinations for HiSorb probes have been evaluated to enable selection of the most suitable phase type for flavour profiling hard seltzers, popular new alcoholic drinks. Three phases for HiSorb were tested and compared to the traditional single-phase polydimethylsiloxane (PDMS) HiSorb probe: divinylbenzene (DVB)/PDMS, carbon wide range (CWR)/PDMS and the triple phase combination of DVB/CWR/PDMS. Volatile and semi-volatile organic compounds (VOCs and SVOCs) over a wide volatility range were extracted and further in-depth analyses revealed differences in extraction between the phase combinations. Also, some components that contribute to the overall flavour were missed with PDMS alone and were only extracted when a multi-phase probe was used.

Introduction

In 2019, hard seltzers – carbonated, low calorie alcoholic drinks – grew in popularity in the US beverage market. Over a year, US sales had increased by 193% and were reported to have reached $4 billion in 2020.[1] Many beer manufacturers across the globe now produce hard seltzer ranges and are competing to be ahead of the trend. In the UK, for example, sales are expected to reach £75 million by 2023.[2] This means that companies that produce hard seltzers need to ensure the quality of their products is maintained to stay ahead in the market and protect brand reputation. Understanding the diverse range of chemical compounds that contribute towards the flavour of their products is key and many companies employ analysts to extract and analyse these compounds using techniques such as sorptive extraction.

Phase selection

When using sorptive extraction, an appropriate phase to extract analytes from a sample must be selected. Polydimethylsiloxane (PDMS) is well-known for extracting a wide range of compounds and is ideal for flavour and aroma analysis; however, several volatile and more polar compounds either remain in the sample or are not extracted well by PDMS alone. Therefore, there is a need for a broader range of absorptive and adsorptive phases.

Compounds will partition from a sample into the sorptive phase based on their chemical characteristics; therefore, each compound has its own partition coefficient (log $K_{(o/w)}$), also known as distribution coefficient. For more detail on partition coefficients, see references.[3,4]

With the addition of more selective phases, such as divinylbenzene (DVB) and carbon wide range (CWR), to the PDMS phase, more components, including those known for their organoleptic properties, such as ketones, aldehydes and esters, showed improved extraction and were more confidently identified in this study.

Immersive sample extraction

Immersion of the phase into the sample provides a greater insight into sample composition. Compounds that have low vapour pressures tend to remain in the liquid phase, and so immersive sampling improves extraction when compared to headspace sampling for these compounds. This is because the sample matrix is in direct contact with the phase, eliminating the liquid–gas (matrix–headspace) equilibrium that needs to be achieved for successful analyte extraction from the vial headspace.

When using immersion techniques, residue from the sample matrix can remain on the phase, a common issue experienced by analysts, which requires additional washing and drying steps prior to analysis. With traditional methods such as solid-phase microextraction (SPME), fouling of the fiber and quick saturation during immersive sampling can often lead to poor analyte extraction and the need to change the fiber more frequently. For high-capacity sorptive extraction techniques, cleaning the extraction device by washing and drying is traditionally performed manually. The Centri® platform allows HiSorb sorptive extraction to be fully automated for the first time – washing and drying the sorptive phase (as part of the automated workflow) after each extraction prevents contamination and enables

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completely unattended operation, placing fewer demands on the analyst and improving productivity (Figure 1).

Here, robust immersive sampling with multi-phase HiSorb probes is demonstrated for efficient extraction of a broad range of compounds, highlighting those that would have been missed if using PDMS alone. High sensitivity was achieved and numerous analytes that contribute to the overall flavour profile of the sample were confidently identified. The most suitable phase type was selected for further analysis on a variety of seltzers (see Application Note 278: Flavour profiling of hard seltzers and identification of potential quality markers using HiSorb™ [5]).

Experimental

Sample: Hard seltzer labelled as having a 'cherry/berry' flavour. Samples were prepared in a standard 20-mL vial containing hard seltzer (4 mL) with HPLC-grade water (16 mL). Replicates for extraction by each phase type were prepared in triplicate.

Instrument: Centri (Markes International)

Immersive high-capacity sorptive extraction:

Probe:	Four phase combinations on standard-length inert-coated probes: PDMS (H1-AXAAC), PDMS/CWR (H2-AXAAC), PDMS/DVB (H3-AXAAC) and DVB/CWR/PDMS (H4-AXAAC).
Incubation/agitation:	35°C (10 min) at 300 rpm
Desorption:	260°C (10 min)
Flow path:	180°C

Preconcentration:

Focusing trap:	'Material emissions' (part no. U-T12ME-2S)
Purge flow:	50 mL/min
Trap low:	25°C
Trap high:	260°C (3 min)
Split flow:	8 mL/min (5:1)

Background to Centri®

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

Centri allows full automation of sampling using HiSorb™ high-capacity sorptive extraction, headspace(–trap), SPME(–trap), and tube-based thermal desorption. 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.

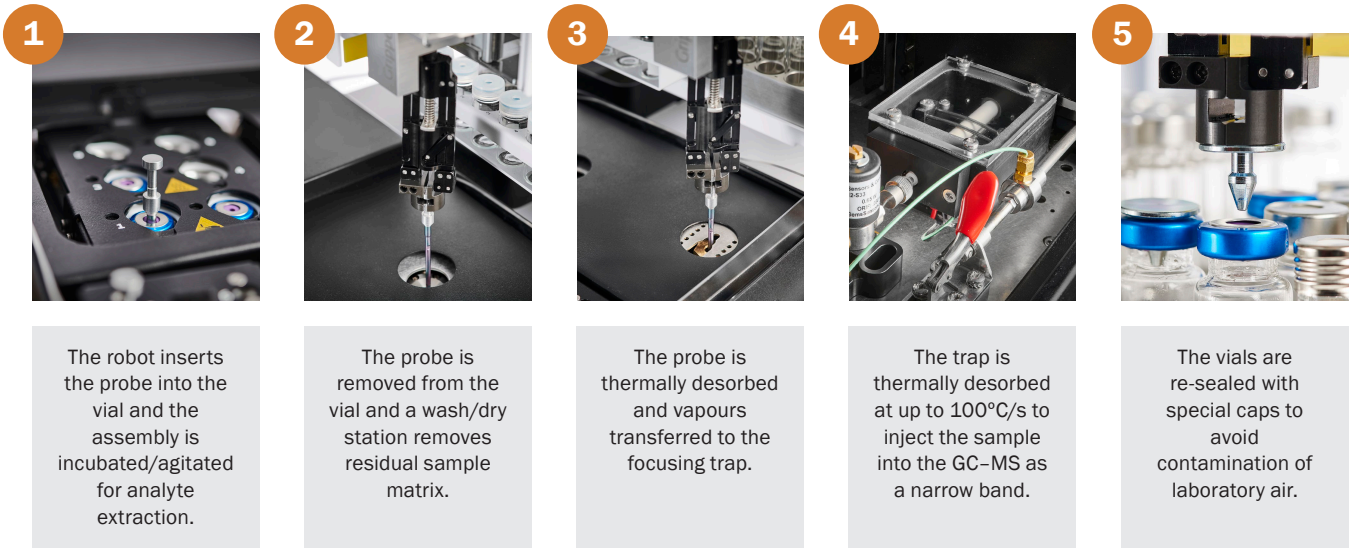


Figure 1: Automated HiSorb workflow on Centri: Streamlined sample extraction, eliminating manual handling.

GC:

Column type:	DB-WAX Ultra Inert, 60 m × 0.25 mm × 0.25 µm
Column flow:	2 mL/min (constant flow)
Oven program:	35°C (5 min), 10°C/min to 240°C (10 min)

Quadrupole MS:

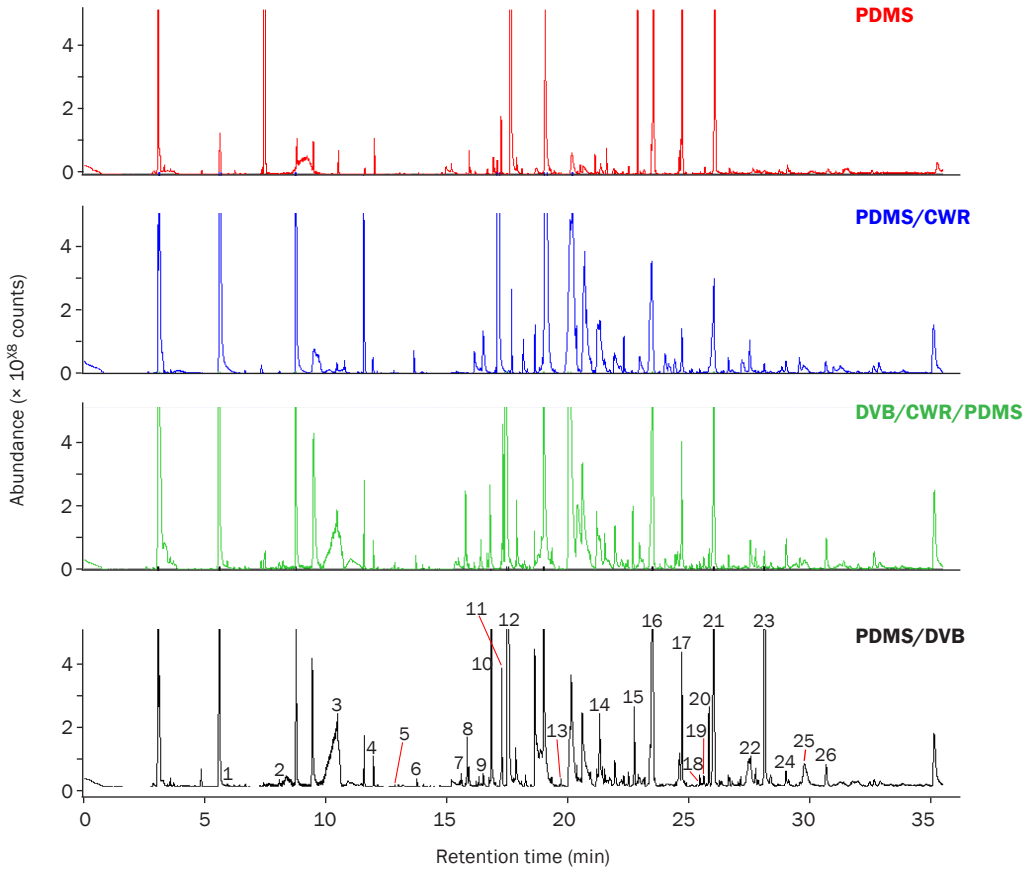
Transfer line:	250°C
Ion source:	200°C
Mass range:	m/z 35–350

Software:
ChromSpace® (SepSolve Analytical) using the deconvolution tool with a match factor >800.

Results and discussion

Overall profile of the 'cherry/berry' seltzer

Samples of the hard seltzer were analysed using the different phases – PDMS, PDMS/CWR, PDMS/DVB and DVB/CWR/PDMS – by immersing the probes into the samples. The resulting chromatograms (Figure 2) show that multi-phase probes extract a wider range of compounds than single-phase PDMS.



1 3-Methylbutanal
2 Benzene
3 Hexanal
4 2-Methyl-2-pentenal
5 2-Hexenal
6 Allyl hexanoate
7 1-Ethenyl-3-ethylbenzene
8 3-Hexen-1-ol
9 Decanal
10 Furfural
11 Acetic acid
12 Benzaldehyde
13 4-Ethylbenzaldehyde
14 1-Dodecanol
15 3-Methylbutanoic acid
16 4-Methoxybenzaldehyde
17 γ-Decalactone
18 Piperonal
19 Decanoic acid
20 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one
21 *p*-Anisylacetone
22 Dodecanoic acid
23 5-Hydroxymethylfurfural
24 1-Octadecanol
25 Octadecanoic acid
26 Tetradecanoic acid

Figure 2: Total ion chromatograms (TICs) of all phase extractions from the hard seltzer sample: PDMS (red), PDMS/CWR (blue), DVB/CWR/PDMS (green) and PDMS/DVB (black).

Compounds	log $K_{(o/w)}$	RT	Flavour notes
Hexanal	1.5	10.53	Green, woody, apple, citrus
2-Methyl-2-pentenal	1.7	12.01	Fruity, sweet, jammy, sharp
2-Hexenal	1.5	12.99	Green, fruity, fresh
3-Hexen-1-ol	1.9	15.88	Citrus, anise, floral
Furfural	0.4	16.48	Waxy, aldehydic, citrus
Acetic acid	-0.2	17.24	Sour, pungent, fruit, overripe
Benzaldehyde	1.5	17.63	Fruity, sweet, almond, cherry
4-Methoxybenzaldehyde	1.76	23.52	Creamy, powdery, vanilla, marshmallow
γ-Decalactone	2.7	24.62	Fruity, creamy, peach, apricot
p-Anisylacetone	1.6	26.03	Berry, floral, woody, raspberry

Table 1: Data corresponding to the major compounds present in the hard seltzer sample covering a range of log $K_{(o/w)}$ values.

Major components (Table 1) in the hard seltzer samples were easily detected by all the HiSorb phases. Most contribute a fruity flavour, including the aldehyde benzaldehyde (which also contributes cherry and almond flavours). 4-Methoxybenzaldehyde provides creamy, powdery, vanilla and marshmallow flavours. Another major component is 2-methyl-2-pentenal, which provides fruity, sweet and jammy notes.

Ketones also have a large influence on the perceived flavour – *p*-anisylacetone and γ-decalactone provide fruity, berry and creamy notes. These compounds had longer retention times and higher boiling points, indicating the benefit of immersing the sorptive phase to extract more SVOCs from the samples.

Another example of an SVOC extraction is piperonal, an arenecarbaldehyde that bestows cherry and vanilla nuances. This compound was only detected using the multi-phase HiSorb probes, as shown in Figure 3.

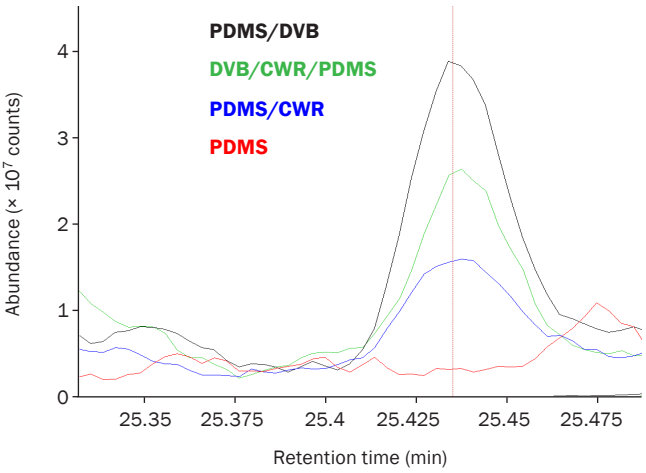


Figure 3: A TIC chromatogram of piperonal (25.45 min) indicating improved extraction efficiency of the flavour compound using multi-phase HiSorb probes compared with PDMS-only HiSorb. PDMS/DVB (black), DVB/CWR/PDMS (green), PDMS/CWR (blue) and PDMS (red).

Selecting a suitable phase type

ChromSpace software enabled key differences in analyte extraction among the phase types to be identified. The deconvolution tool, used with a minimum match factor of 800, allowed co-eluting compounds to be separated into individual analyte peaks, enhancing identification.

The results show differences in the extracted VOCs, due to the varying chemical natures of the sorptive phases investigated. Table 2 lists the compounds that were extracted from the sample (grouped by chemical class) using the different phase types. A high number of aldehydic and acidic compounds were identified as well as several compounds with a log $K_{(o/w)}$ less than 3, largely identified when using a multi-phase combination. An example of this is 4-ethylbenzaldehyde (provides cherry notes), which was only detected using the PDMS/DVB probe. Extracted ion chromatograms (EIC) for both PDMS-only and PDMS/DVB extractions (using the ion 134) are compared in Figure 4, which shows that the multi-phase extraction is more efficient.

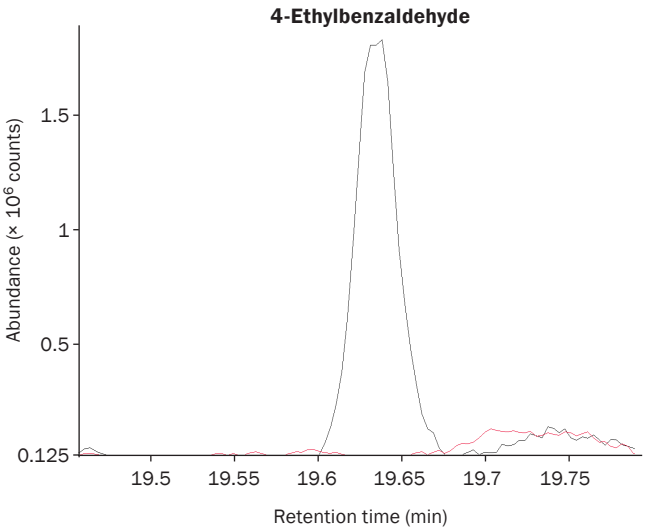


Figure 4: An extracted ion chromatogram (EIC) at m/z 134 of 4-ethylbenzaldehyde (19.70 min) extracted using PDMS/DVB (black) compared with PDMS (red).

Compounds	Retention time	log $k_{(o/w)}$	Average peak area (n = 3) x10^8				Flavour profile[6]
			PDMS	DVB/PDMS	CWR/PDMS	DVB/CWR/PDMS	
Acids							
Acetic acid	17.24	-0.2	–	37.90	37.90	–	Pungent, sour, overripe fruit
Oxalic acid	18.16	-0.3	–	–	13.60	–	–
3-Methyl butanoic acid	22.57	1.2	–	19.50	–	16.90	Sweet, waxy, berry
Decanoic acid	25.60	4.1	–	3.45	–	–	Soapy, waxy, fruity
Benzoic acid	27.51	1.9	19.60	–	–	8.07	Faint, balsam, urine
Dodecanoic acid	27.75	4.1	–	–	–	4.94	Creamy, coconut, fruity
Octadecanoic acid	29.76	7.4	–	19.80	4.79	–	Food additive
Tetradecanoic acid	30.66	5.3	–	20.10	26.80	11.60	Waxy, fatty, creamy
Alcohols							
3-Hexen-1-ol	15.88	1.9	6.91	15.20	8.63	8.63	Citrus, anise, floral
2-Methyl 1-hexanol	16.31	2.3	1.42	–	–	–	Citrus, sweet, fruity
Phenol	23.12	1.5	2.53	–	–	–	–
1-Hexadecanol	26.62	7.3	2.42	10.40	7.40	7.40	Waxy, clean, laundered
1-Octadecanol	29.50	8.4	–	8.63	11.00	11.00	–
Aldehydes							
3-Methyl butanal	6.87	1	–	–	0.55	0.96	Fruity, green, nutty
Hexanal	10.53	1.5	6.38	6.97	–	8.49	–
2-Methyl-2-pentanal	12.01	1.4	8.27	7.91	6.76	5.32	Jammy, fruity, sweet
Decanal	16.38	3.8	2.49	16.60	–	–	Citrus, green, melon
Furfural	16.48	0.4	8.26	47.5	19.20	32.00	Waxy, aldehydic, with a citrus note
Undecanal	17.27	4.3	–	–	0.44	–	Citrus, waxy, aldehydic
Benzaldehyde	17.63	1.5	1010.00	1150.00	1230.00	1010.00	Almond, cherry, nutty
2-Methylbenzaldehyde	18.26	2.1	–	–	0.37	–	Berry, cherry, fruity
4-Ethylbenzaldehyde	19.70	2.4	–	2.77	–	–	Cherry, almond, berry
4-Methoxybenzaldehyde	23.52	1.8	106.00	195.00	134.00	129.00	creamy, vanilla, marshmallow
Piperonal	25.50	1.1	–	1.47	–	1.53	Cherry, vanilla, maraschino cherry
2-Hexenal	12.99	1.5	0.47	0.59	0.38	0.55	Green, fruity, fresh
2-Hydroxybenzaldehyde	19.60	1.8	0.57	–	–	–	Spicy, cinnamon, cooling
Esters							
Ethyl acetate	6.24	0.7	1.68	–	–	1.07	Fruity, sweet, with a grape and cherry nuance
Allyl hexanoate	14.71	2.7	0.51	0.36	–	–	Sweet, fresh, fruity
Ketones							
p-Anisylacetone	26.03	2.1	165.00	137.00	98.90	88.10	Raspberry, fruity, berry
γ-Decalactone	24.62	3.8	6.53	53.90	27.80	11.80	Fruity, creamy, peach
Aromatic compounds							
Benzene	7.32	2.1	2.66	0.54	2.42	2.66	–
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	25.81	0.8	–	15.10	–	33.00	–
5-Hydroxymethylfurfural	28.11	-0.6	–	207.00	4.44	142.00	Sweet, caramellic, brown

Table 2: Components identified from a hard seltzer sample using different types of HiSorb probes, screened by a match factor of >800. All values are represented as the mean value of three replicates (n = 3) x10^8.

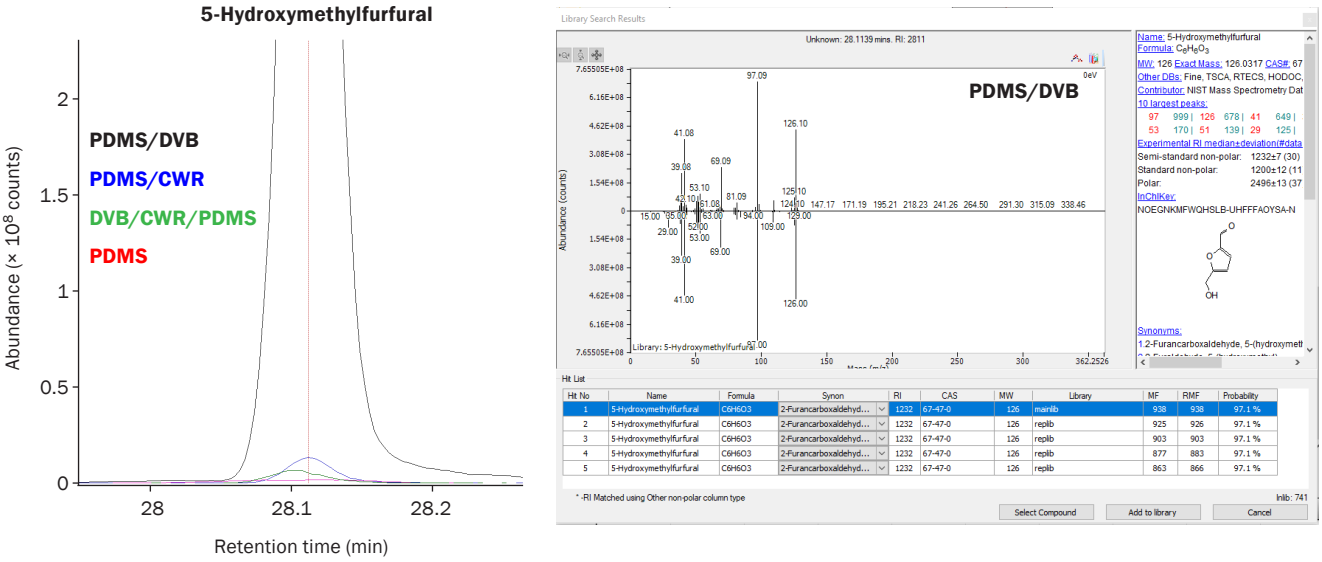


Figure 5: An EIC of 5-hydroxymethylfurfural (m/z 97) with NIST data (right) indicating the match factor and probability when using the multi-phase PDMS/DVB (MF = 938, 97.1%) providing a more efficient extraction compared with PDMS (MF = 578, 41.99%). PDMS/DVB (black), PDMS/CWR (blue), DVB/CWR/PDMS (green) and PDMS (red).

Many compounds correlating to the 'sweet' taste of the sample were extracted, such as 5-hydroxymethylfurfural, also providing caramel notes. Figure 5 demonstrates the enhanced extraction of this compound using the PDMS/DVB phase compared with the other phases.

These types of compounds demonstrate the ability of the DVB phase for extracting compounds over a broader polarity range (down to log $K_{(o/w)}$ values of -0.6 in this study) from the sample when compared to PDMS alone. Many of these compounds have organoleptic properties and contribute to the flavour of the product.

Overall, a broad range of compounds were putatively identified using the different phase types. PDMS and PDMS/CWR probes extracted the lowest number of compounds from the seltzer sample. The PDMS/DVB probes extracted the greatest number, with improved peak area response when compared to the other phase types with which they were extracted. Therefore, PDMS/DVB was chosen as the most suitable phase type for further analysis of a variety of seltzers.

Conclusions

HiSorb sorptive extraction provided high sensitivity and efficiency in the detection of a hard seltzer's flavour components. With the use of ChromSpace software, compounds were promptly detected in each sample and the deconvolution tool enabled several co-eluting peaks to be separated and identified, leading to the discovery of more key components that contribute to the overall flavour of the hard seltzer sample than before.

HiSorb probes are robust enough to be immersed in liquid samples, and direct contact of a sample with the sorptive phase results in analysis of less volatile components such as the ketones identified in this study. The process was fully automated on the Centri platform, and a wash-and-dry step prior to probe desorption reduced both sorptive-phase and system contamination.

Probes with multi-phase combinations extracted analytes over a wider volatility and polarity range than traditional PDMS, delivering a more detailed profile for the 'cherry/berry' flavour. Ultimately, the different HiSorb phase types extracted varying ranges of compounds due to the chemical natures of both the compounds and the sorptive phases themselves.

The PDMS/DVB phase extracted the highest number of flavour compounds with good responses from the sample and, therefore, was selected for more extensive research on a variety of hard seltzer brands (see Application Note 278: Flavour profiling of hard seltzers and identification of potential quality markers using HiSorb™ [5]).

References

1. https://www.nytimes.com/2021/05/30/business/white-claw-hard-seltzer-sales.html.
2. https://www.thespiritsbusiness.com/2021/01/hard-seltzer-sales-could-reach-75m-in-uk-by-2023/.
3. Octanol–Water Partition Coefficient – an overview, ScienceDirect Topics, https://www.sciencedirect.com/topics/chemistry/octanol-water-partition-coefficient.
4. Partition Coefficient – an overview, ScienceDirect Topics, https://www.sciencedirect.com/topics/earth-and-planetary-sciences/partition-coefficient.
5. Application Note 278: Flavour profiling of hard seltzers and identification of potential quality markers using HiSorb™.
6. http://www.thegoodscentscompany.com/.

Applications were performed under the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.