Application Note 278

Flavour profiling of hard seltzers and identification of potential quality markers using HiSorb™

A fully automated workflow – from sample extraction to data analysis – was used for the flavour profiling of four different brands of hard seltzer. Using high-capacity polydimethylsiloxane/divinylbenzene (PDMS/DVB) HiSorb probes on the Centri® sample extraction and enrichment platform, followed by data mining and statistical analyses, we uncovered key flavour compounds that differed between brands, which could be used as markers of quality and authenticity.

Introduction

After the commercial success of hard seltzers in 2019, sales of the low-calorie alcoholic drinks increased further in 2020. Over 50 different brands have entered the competitive market, and restaurants, bars and celebrities are also selling hard seltzer products.

Consequently, flavour profiling is vital for maintaining brand authenticity, quality and consistency during bulk production to ensure longevity of the product in the market for leading brands.[1] A broad variety of chemical components, including ketones, lactones and aldehydes, contribute to the overall perceived flavour of seltzers, with some being unique to each brand.

One issue with profiling beverages is that low-volatility flavour-active compounds tend to remain in the liquid phase due to their lower vapour pressures. In this case, immersion techniques in which direct contact is made with the sample *via* the sorptive phase can be employed, allowing a more efficient extraction of these analytes. Traditional methods for flavour profiling typically involve solid-phase microextraction (SPME); however, when used for direct immersion, this can result in fouling of the fiber phase, leading to possible carryover and poor analyte extraction in subsequent analyses.[2] Alternative sorptive extraction techniques can improve immersive sampling by allowing washing and drying of the sorptive phase after each extraction to prevent carryover or contamination; however, this is traditionally a manual process.

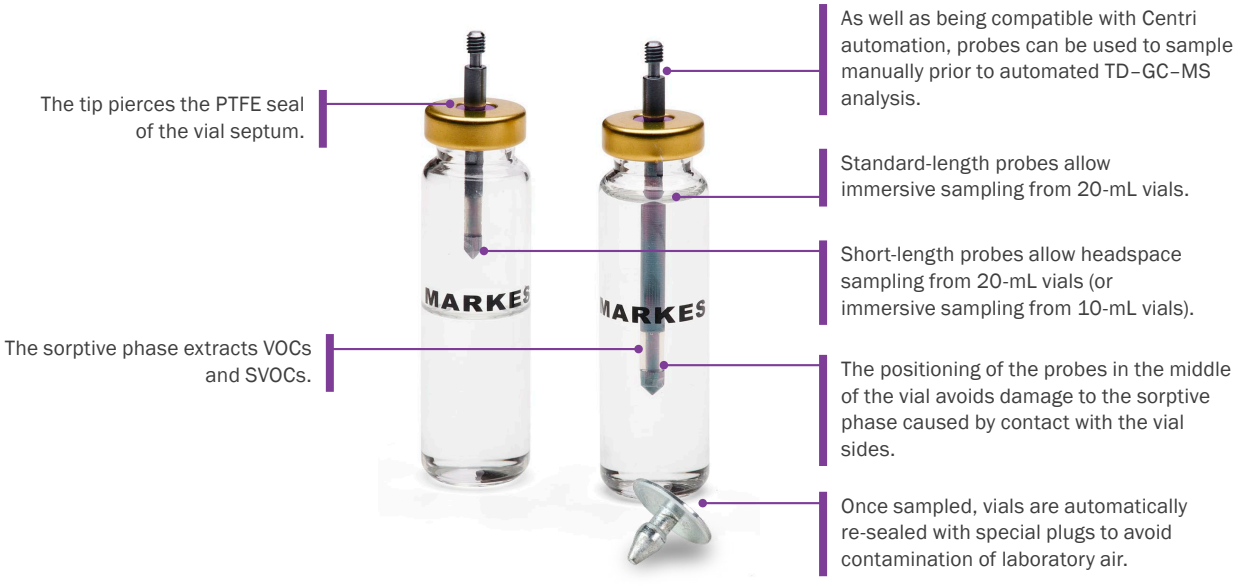

Figure 1: Headspace (left) and immersive (right) sampling with HiSorb probes.

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Therefore, an automated, efficient and robust method for immersive sample extraction is essential for comprehensive flavour profiling in the beverage industry to provide reliable results while placing fewer demands on the analyst.

In this study, we demonstrate the extraction potential of HiSorb, a high-capacity sorptive extraction technique, using divinylbenzene (DVB) with polydimethylsiloxane (PDMS) as the sorptive phase. This phase combination is suitable for the extraction of an array of volatile and semi-volatile organic compounds (VOCs and SVOCs) over a wide polarity range.[3] During the method development step, different HiSorb phase combinations (PDMS, PDMS/DVB, PDMS/CWR and DVB/CWR/PDMS) were evaluated and analyte extraction was compared. PDMS/DVB performed the best overall, extracting key flavour-active components that were not well extracted, or not extracted at all, when using the other phases (demonstrated in Application Note 277: Evaluation of new high-capacity sorptive extraction (HiSorb™) phases for flavour profiling of hard seltzers[4]).

The metal-core probes supporting the sorptive phase provide improved robustness compared to traditional SPME fibers, and with automation on Centri, manual washing and drying of the phase (to remove residual matrix) are eliminated, providing completely unattended operation (Figure 2). Automated data mining using ChromCompare+ (SepSolve Analytical) quickly highlighted key differences between brands, enabling distinct compounds within the intricate flavour profiles to be identified.

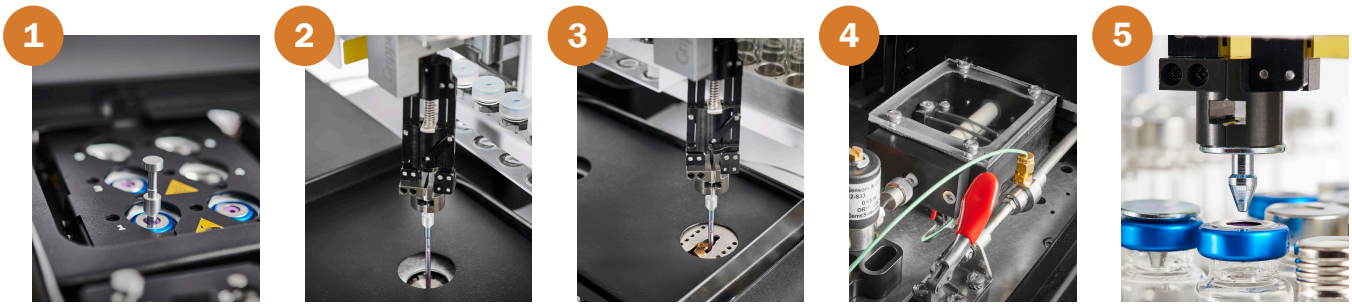
Background to Centri®

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.

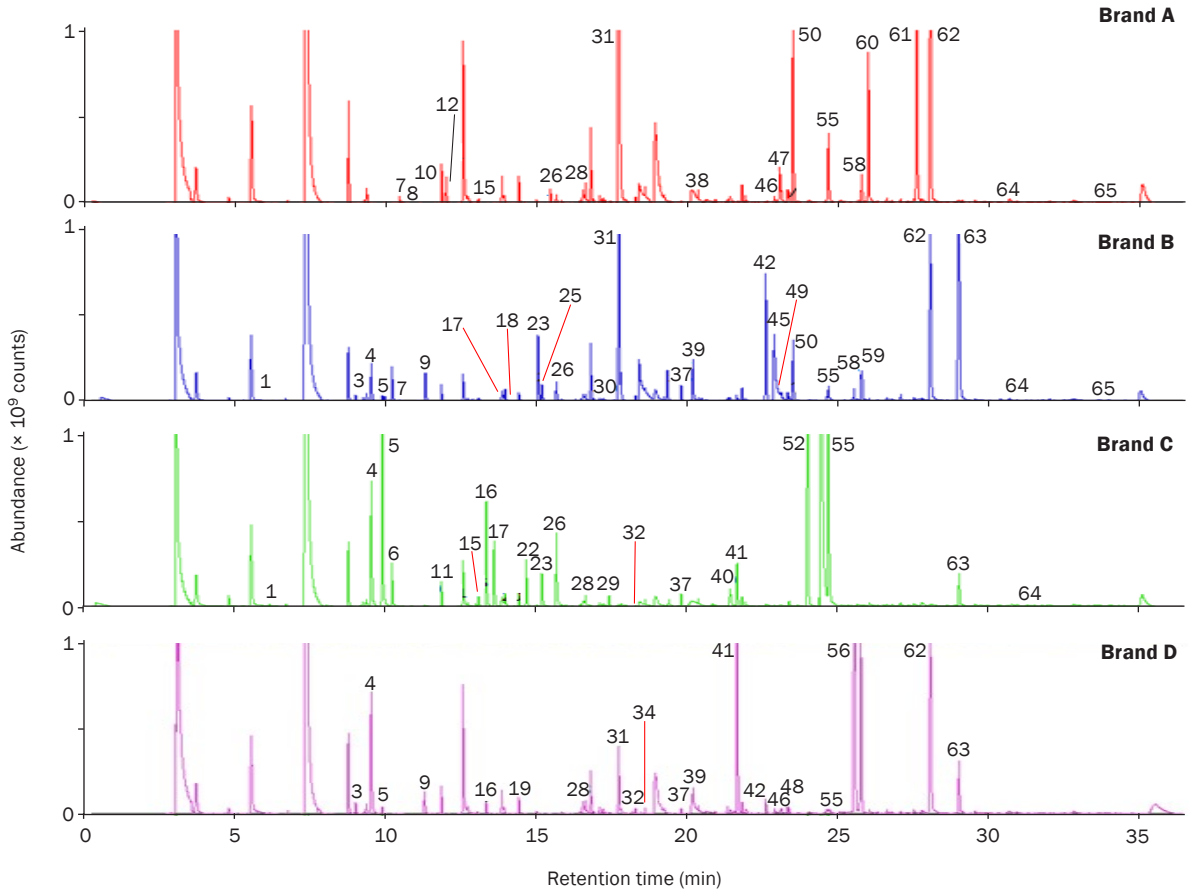
In addition, Centri allows samples from any injection mode to be split and re-collected onto clean sorbent tubes, avoiding the need to repeat lengthy sample extraction procedures and improving security for valuable samples, amongst many other benefits.

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



1. The robot inserts the probe into the vial and the assembly is incubated/agitated for analyte extraction.
2. The probe is removed from the vial and a wash/dry station removes residual sample matrix.
3. The probe is thermally desorbed and vapours transferred to the focusing trap.
4. The trap is thermally desorbed at up to 100°C/s to inject the sample into the GC–MS as a narrow band.
5. The vials are re-sealed with special caps to avoid contamination of laboratory air.

Figure 2: Automated HiSorb workflow on Centri: Streamlined sample extraction, eliminating manual handling.



1 Ethyl acetate
3 Isobutyl acetate
4 Ethyl butanoate
5 2-Methylethyl butanoate
6 3-Methylethyl butanoate
7 Hexanal
8 Undecane
9 3-Methyl-1-butyl acetate
10 *o*-Xylene
11 3-Carene
12 2-Methyl-2-pentenal
15 (*E*)-2-Hexenal
16 Ethyl hexanoate
17 3-Methylbutyl butanoate
18 Hexyl acetate
19 Octanal
22 (*Z*)-3-Hexen-1-ol acetate
23 1-Hexanol
25 2-Propenyl hexanoate
26 (*E*)-3-Hexen-1-ol
28 Acetic acid
29 Ethyl (2*E*,4*E*)-2,4-hexadienoate
30 Oxalic acid
31 Benzaldehyde
32 Linalool
37 α-Terpineol
38 4-Ethylbenzaldehyde
39 Phenylmethyl acetate
40 Hexanoic acid
41 α-Ionone
42 *trans*-β-Ionone
45 Maltol
46 2,5-Furandicarboxaldehyde
47 3-Methyl-2-phenylethyl butanoate
48 Methyl 2-furoate
49 Furyl hydroxymethyl ketone
50 4-Methoxybenzaldehyde
55 5-Heptyldihydro-2(3H)-furanone
56 Piperonal
58 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one
59 4-Methoxybenzenemethanol
60 4-(4-Methoxyphenyl)-2-butanone
61 Triethyl citrate
62 5-Hydroxymethylfurfural
63 Vanillin
64 Tetradecanoic acid
65 n-Hexadecanoic acid

Figure 3: Full TIC flavour profiles of all brands, in which a total of 65 compounds were discriminated by a match factor (MF) > 800 (major compounds are labelled; a full list is provided in Table A1 (see Appendix)).

Experimental

Samples:
Four different brands of hard seltzer, each with a cherry/berry flavour (labelled A–D hereafter), were purchased from a local supermarket. Samples were prepared in a standard 20-mL vial containing 4 mL of hard seltzer and 16 mL of HPLC-grade water. Each sample was analysed five times.

Instrument: Centri (Markes International)

Immersive high-capacity sorptive extraction:

Probe:	PDMS/DVB, inert-coated, standard-length (H3-AXAAC)
Pre-incubation:	10 min
Sample extraction:	40°C for 10 min at 300 rpm
Probe desorption:	260°C for 15 min
Inlet split flow:	50 mL/min

Preconcentration:

Flow path:	180°C
Focusing trap:	'Material emissions' (U-T12ME-2S)
Purge flow:	50 mL/min for 1 min
Trap low:	25°C
Trap high:	280°C (3 min)
Outlet split flow:	8 mL/min
Overall split ratio:	10:1

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:
Data mining and chemometrics in ChromCompare+ (SepSolve Analytical).

Results and discussion

Overall flavour profile

The resulting total ion chromatograms (TIC) and compound identification for the four different brands of cherry/berry-flavoured hard seltzers are shown in Figure 3. The compound classes extracted from each brand cover a broad range. Many aldehydes and esters are present in the samples, which is expected because they provide fruity and fresh notes. One of the main aldehydes present is benzaldehyde (#31), typically known for its almond, cherry flavour. A variety of ketones were also present, which are generally used in flavouring due to their sweet undertones. These chemical components vary in concentration (abundance) from brand to brand (shown in Table A1 in the Appendix), significantly affecting the perceived flavours, creating a distinctive taste for each product and so influence consumer preference.

Identification of flavour compounds allowing for brand association and quality assessment

It is important to look at the entire sample composition during quality and authenticity assessments to uncover significant differences between samples. Many flavour producers perform a statistical analysis, typically a principal components analysis (PCA), which allows them to easily recognise similarities and differences between sample varieties. This enables a further understanding of what compounds (and how these compounds) influence consumer perception.

From the data comparison in Figure 3, it is relatively easy to identify the major components and assess the differences between them. However, some flavour-active components that significantly contribute to the overall flavour of the product may only be present at low levels, making detection and confident identification more challenging for the analyst, especially for untargeted (profiling) investigations.

Automated data mining and chemometrics software ChromCompare+ (SepSolve Analytical) easily enabled a rapid investigation of differences between brands evaluated here, using all the raw data to minimise the risk of important details from being overlooked. Figure 4 demonstrates a PCA plot of the four seltzer brands.

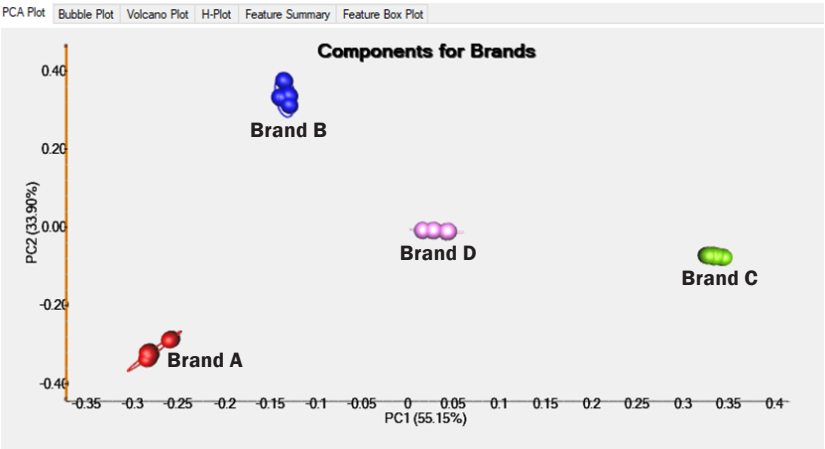


Figure 4: Two-dimensional principal components analysis (PCA) plot for the top 14 most characteristic compounds (detailed in Table A2 in the Appendix) found in the comparison of each brand with tight replicate clustering.

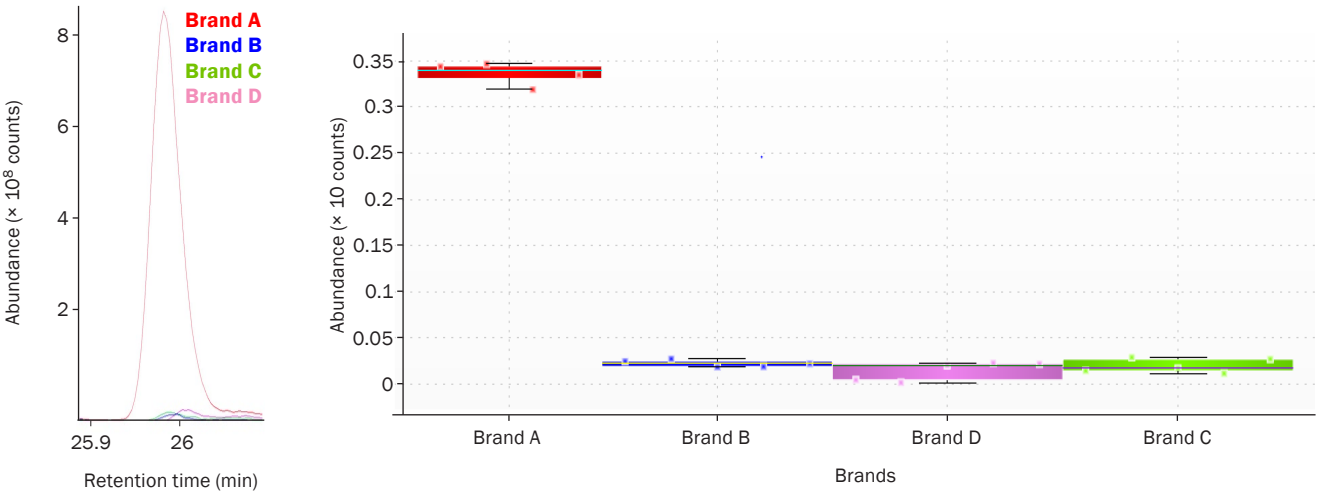


Figure 5: TIC overlay of *p*-anisylacetone (or 4-(4-methoxyphenyl)-2-butanone) (left) and a box and whisker plot (right) indicating high abundance, specifically in brand A.

Tight clustering of the replicates (n = 5) confirmed reproducibility of the study, while clear separation of these clusters for each brand demonstrated successful brand association. Key differentiators between brands were automatically highlighted by the software using the box and whisker plot, allowing easier and quicker evaluation of the chromatography thereafter (Figure 5).

Figure 6 shows high abundances of benzaldehyde identified (top) in brands A and B (red and blue, respectively), a smaller amount in brand D (pink), but little-to-no detection in brand C (green). In contrast, ethyl-2-methylbutanoate (bottom), typically known for its fruity, sweet and cherry notes, was identified in brand C mainly. It's worth noting that benzaldehyde is widely used by the food industry[5] as an 'essential component' that provides cherry flavouring[6] and is generally considered as safe for use. However, research suggests that degradation of benzaldehyde by light exposure during storage of the pure flavouring (prior to addition to a product) or during final product storage can result in benzene 'contamination'.[6] Due to the long-term harm to human health from exposure to benzene, this could be a serious cause of concern for producers and consumers and requires close monitoring for product quality and good laboratory practice to be maintained.

Figure 7 indicates the top 14 characteristic features (*i.e.*, compounds) for each brand compared in this study. Analysis of these features indicates that brands A, B and C have higher abundances of discriminatory compounds, most providing cherry/berry flavours compared to brand D, which has more citrus, banana notes leading to a possible increased customer preference with brands A–C compared with D.[7]

Brand A has unique berry and fruity notes, provided by *p*-anisylacetone (shown as the box plot with the highest abundance in Figure 5), 4-methoxybenzaldehyde and allyl hexanoate. This brand is one of the most popular hard seltzers, and this study shows that it has higher abundances

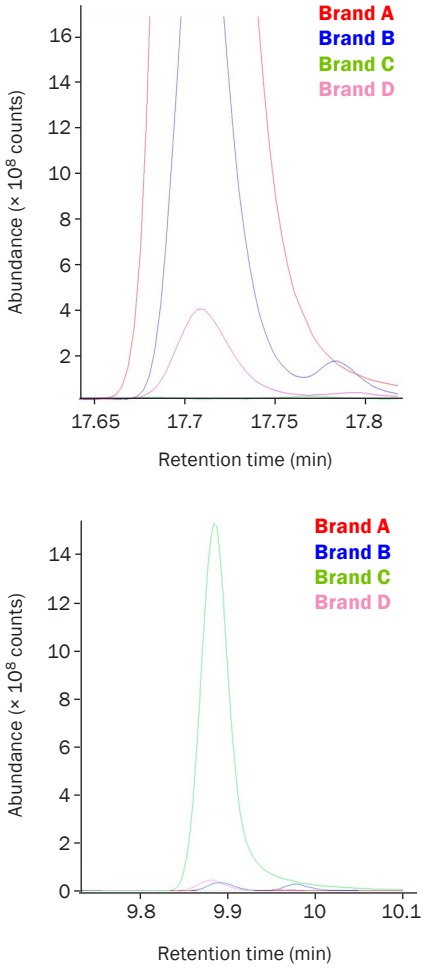


Figure 6: TIC overlay of benzaldehyde (top) and ethyl-2-methylbutanoate (bottom).

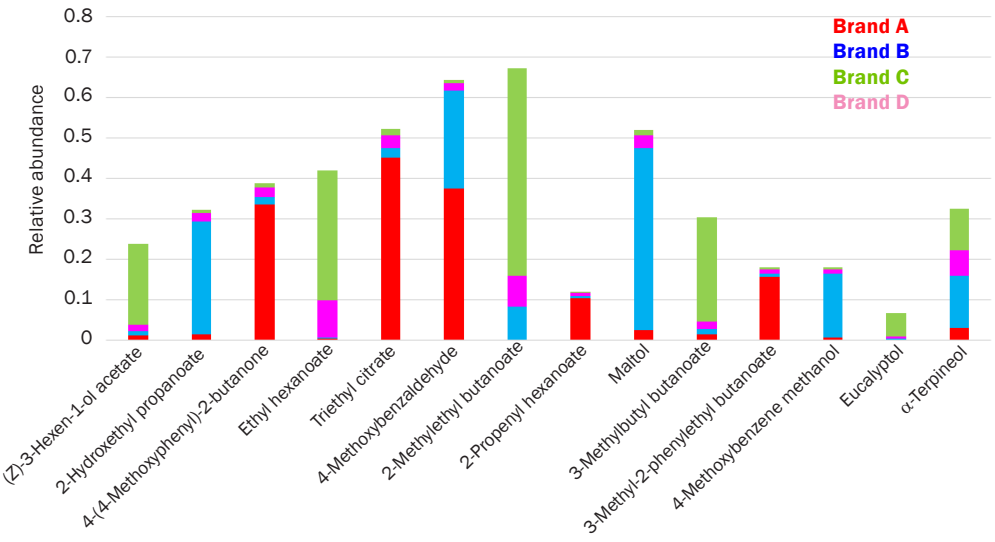


Figure 7: The top 14 discriminators between the four brands discovered using ChromCompare+.

of berry/cherry flavour components compared to the other brands evaluated. These unique compounds found in brand A may be the most desirable to consumers, significantly influencing its popularity in the market.

Brand B is one of the newest seltzers on the market, manufactured by a commercial giant in the carbonated beverage industry. The distinct flavour components in this brand provide fruity and berry notes, with similarities in perceived flavour to brand A, indicating that it may become a popular beverage. One of the flavour compounds key to its fruity, berry flavour is *trans*-β-ionone (Figure 8).

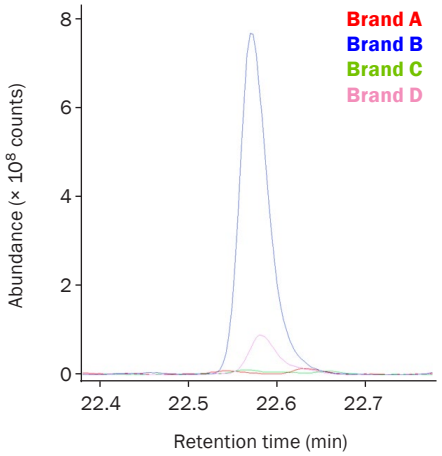


Figure 8: A TIC overlay indicating the high abundance of *trans*-β-ionone in brand B compared to brands A, C and D. *trans*-β-Ionone provides fruity, berry notes to the flavour profile.

Interestingly, in brand C, the main distinguishing compounds gave tropical, citrus and fruity nuances to the sample. The compounds responsible were (*Z*)-3-hexen-1-ol acetate and eucalyptol, the former providing the main tropical notes with the latter bestowing hints of mint and cooling flavours.

Brand D showed a profile consisting of banana and citrus notes from ethyl hexanoate (Figure 9) and α-terpineol. These compounds were also present in brand C but were detected in lower abundances in brand D.

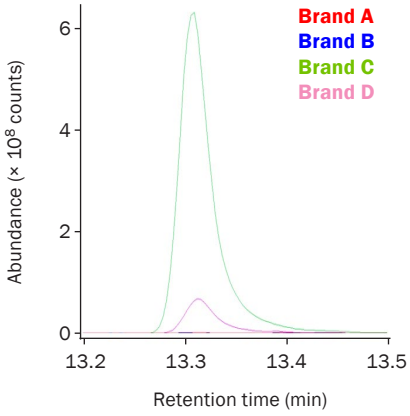


Figure 9: TIC overlay of ethyl hexanoate (13.3 min) providing banana notes in brands C and D, and present in lower concentrations in brands A and B.

Conclusion

The flavour profiles, comprising a broad range of compounds, of four hard seltzer brands have been successfully extracted using immersive HiSorb with a PDMS/DVB phase, generating useful insights into their unique flavour compositions.

Full automation and 'prep-ahead' functionality (multiple samples are prepared ahead of time) for HiSorb meant that sample run time was substantially reduced. Automated washing and drying steps allowed completely unattended operation whilst significantly reducing probe fouling, leading to improved extraction and analysis of key flavour-active components from the samples. Using automated data mining and statistical analyses, reproducibility between samples was confirmed with tight clustering of replicates, as well as clear separation of each brand cluster.

The results indicate that this extraction technique could be an excellent method for quality control in the beverage industry. Using the feature summary tool in the software, unique flavour components of each brand could be rapidly determined. These have the potential to be used as markers to confirm product quality and brand authenticity, in addition to correlation to consumer preference to ensure longevity in the market.

References

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

Appendix

No.	Compound	Compound class	Retention time (min)	Average peak area (n = 5) x10^7			
				Brand A	Brand B	Brand C	Brand D
1	Ethyl acetate	Ester	6.1586	–	7.91	–	15.78
2	Ethenyl acetate	Ester	8.1946	–	13.40	–	–
3	Isobutyl acetate	Ester	9.0066	–	49.94	–	99.94
4	Ethyl butanoate	Ester	9.5265	–	292.22	993.61	966.60
5	2-Methylethyl butanoate	Ester	9.8863	–	39.89	2.44	56.48
6	3-Methylethyl butanoate	Ester	10.2247	–	212.18	315.92	–
7	Hexanal	Aldehyde	10.4521	47.98	8.07	–	5.88
8	Undecane	Alkane	10.7229	2.04	–	–	–
9	3-Methyl-1-butyl acetate	Ester	11.2936	–	135.46	13.86	236.60
10	*o*-Xylene	Aromatic	11.6153	4.04	–	2.00	–
11	3-Carene	Aromatic	11.7748	–	–	1124.31	3.12
12	2-Methyl-2-pentenal	Aldehyde	11.9952	161.51	–	–	–
13	Eucalyptol	Monoterpenoid	12.9694	–	–	24.38	–
14	2-Methylfuran	Furan	13.0246	–	–	–	9.82
15	(*E*)-2-Hexenal	Aldehyde	13.0771	31.30	–	70.61	–
16	Ethyl hexanoate	Ester	13.3165	–	–	774.65	76.51
17	3-Methylbutyl butanoate	Ester	13.4183	–	29.40	37.18	–
18	Hexyl acetate	Ester	13.5704	–	86.71	90.29	–
19	Octanal	Aldehyde	14.2344	–	–	–	5.42
20	Methyl acetate	Ester	14.4213	–	–	3.05	–
21	1-Hydroxy-2-propanone	Ketone	14.444	15.67	–	–	9.30
22	(*Z*)-3-Hexen-1-ol acetate	Ester	14.6521	–	–	305.54	–
23	1-Hexanol	Alcohol	15.3174	–	145.34	243.53	–
24	2-Hydroxyethyl propanoate	Ester	15.3461	–	478.52	–	–
25	2-Propenyl hexanoate	Ester	15.4496	91.50	–	–	–
26	(*E*)-3-Hexen-1-ol	Alcohol	15.6452	70.03	209.91	542.28	–
27	(*E*)-2-Hexen-1-ol	Alcohol	15.9342	–	–	12.01	–
28	Acetic acid	Acid	16.5289	177.40	180.37	70.34	112.09
29	Ethyl (2*E*,4*E*)-2,4-hexadienoate	Ester	17.4005	–	–	70.27	–
30	Oxalic acid	Acid	17.5408	–	54.67	–	–
31	Benzaldehyde	Aldehyde	17.7022	9418.13	3031.12	15.11	453.37
32	Linalool	Monoterpenoid	17.7877	–	–	16.20	26.27
33	Menthyl acetate	Aldehyde	18.1734	–	–	–	18.42
34	Propylene glycol	Alcohol	18.3891	509.55	–	–	–
35	2-Furanmethanol	Alcohol	19.2324	–	26.56	–	–
36	Acetophenone	Ketone	19.31	8.47	203.77	–	7.21
37	α-Terpineol	Monoterpenoid	19.782	14.04	104.06	77.39	40.30
38	4-Ethylbenzaldehyde	Aldehyde	20.0085	27.67	5.87	–	6.12
39	Phenylmethyl acetate	Ester	20.1628	–	264.42	–	119.25
40	Hexanoic acid	Acid	21.3612	–	–	204.41	–
41	α-Ionone	Ketone	21.6277	–	–	293.48	1847.03
42	*trans*-β-Ionone	Ketone	22.5801	–	779.94	–	99.19
43	4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	Ketone	22.5835	–	–	–	100.59
44	2,2'-Oxybis(ethanol)	Alcohol	22.7874	4.23	–	–	–

Table A1: A table of compounds present in hard seltzer brands using a match factor of >800 (*continued on next page*).

No.	Compound	Compound class	Retention time (min)	Average peak area (n = 5) x10^7			
				Brand A	Brand B	Brand C	Brand D
45	Maltol	Alcohol	22.8353	–	1285.84	–	–
46	2,5-Furandicarboxaldehyde	Aldehyde	22.8674	68.68	–	–	56.96
47	3-Methyl-2-phenylethyl butanoate	Ester	23.044	205.41	–	–	–
48	Methyl 2-furoate	Furan	23.299	–	–	–	81.22
49	Furyl hydroxymethyl ketone	Ketone	23.3041	92.57	60.37	–	66.64
50	4-Methoxybenzaldehyde	Aldehyde	23.4858	1164.43	358.39	–	–
51	Octanoic acid	Acid	23.5497	–	18.39	–	–
52	3-Phenylethyl-2-propenoate	Ester	24.4488	12.73	–	79.10	–
53	Nonanoic acid	Acid	24.5787	–	33.01	–	–
54	Eugenol	Alcohol	24.7033	–	–	28.70	–
55	5-Heptyldihydro-2(3H)-furanone	Ketone	24.7595	403.71	83.16	1499.63	1087.55
56	Piperonal	Aldehyde	25.511	–	75.94	–	8634.73
57	n-Decanoic acid	Acid	25.5612	–	23.85	–	–
58	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	Ketone	25.7595	202.61	161.65	–	188.50
59	4-Methoxybenzene methanol	Alcohol	25.7723	–	150.74	–	–
60	4-(4-Methoxyphenyl)-2-butanone	Ketone	25.9829	936.43	–	–	–
61	Triethyl citrate	Ester	27.5835	1645.20	–	–	–
62	5-Hydroxymethylfurfural	Furan	28.0278	2951.98	2026.48	–	1818.96
63	Vanillin	Aldehyde	28.9798	–	4439.33	287.96	445.81
64	Tetradecanoic acid	Acid	30.6685	63.69	58.45	–	18.96
65	n-Hexadecanoic acid	Acid	35.0658	462.00	451.75	–	–

Table A1: A table consisting of compounds present in all hard seltzer brands using a match factor of >800 (*continued from previous page*).

Compound	Retention time (min)	Average abundance (n = 5)				Flavour profile[8]
		Brand A	Brand B	Brand C	Brand D	
(*Z*)-3-Hexen-1-ol acetate	14.65	0.012 ± 0.003	0.011 ± 0.002	0.202 ± 0.001	0.015 ± 0.002	Fresh, tropical, fruity
2-Hydroxyethyl propanoate	15.04	0.017 ± 0.005	0.278 ± 0.005	0.009 ± 0.003	0.020 ± 0.003	Sweet, caramellic, pineapple
4-(4-Methoxyphenyl)-2-butanone	26.02	0.336 ± 0.005	0.018 ± 0.005	0.011 ± 0.003	0.024 ± 0.004	Raspberry, berry, fruity
Ethyl hexanote	13.347	0.004 ± 0.001	0.004 ± 0.001	0.320 ± 0.003	0.092 ± 0.012	Pineapple, fruity, banana
Triethyl citrate	27.56	0.453 ± 0.01	0.024 ± 0.006	0.014 ± 0.004	0.032 ± 0.005	Fruity, bitter, floral
4-Methoxy-benzaldehyde	23.502	0.376 ± 0.007	0.241 ± 0.005	0.008 ± 0.002	0.018 ± 0.002	Creamy, powdery, marshmallow
2-Methylethyl butanoate	9.88	0.004 ± 0.001	0.080 ± 0.003	0.514 ± 0.008	0.077 ± 0.011	Berry, cherry, mango
2-Propenyl hexanoate	15.456	0.104 ± 0.003	0.005 ± 0.001	0.003 ± 0.001	0.007 ± 0.001	Apply, berry, bitter
Maltol	23.139	0.025 ± 0.007	0.451 ± 0.015	0.014 ± 0.004	0.031 ± 0.005	Jammy, fruity, berry
3-Methyl-butyl butanoate	13.58	0.015 ± 0.004	0.0138 ± 0.004	0.257 ± 0.005	0.019 ± 0.003	Peach, pineapple, fruity
3-Methyl-2-phenylethyl butanoate	23.05	0.158 ± 0.005	0.008 ± 0.002	0.005 ± 0.001	0.011 ± 0.002	Berry, honey, fruity
4-Methoxy-benzenemethanol	25.77	0.009 ± 0.002	0.157 ± 0.006	0.005 ± 0.001	0.011 ± 0.002	Cherry, vanilla, creamy
Eucalyptol	12.97	0.003 ± 0.001	0.003 ± 0.001	0.056 ± 0.001	0.004 ± 0.001	Minty, cooling, camphoreous
α-Terpineol	19.78	0.031 ± 0.009	0.129 ± 0.001	0.102 ± 0.002	0.064 ± 0.007	Citrus, lemon, lime

Table A2: Top 14 discriminatory compounds between brands distinguished in ChromCompare+.