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# Volatile and semi-volatile compounds in flavoured hard seltzer beverages: Comparison of high-capacity sorptive extraction (HiSorb) methods

Lucy Hearn[a,*], Rebecca Cole[a], Natasha Damiana Spadafora[a,b], Rachael Szafnauer[a]

[a] Markes International Ltd, 1000B Central Park, Western Avenue, CF31 3RT, Bridgend, UK
[b] Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Via Luigi Borsari, n. 46, Ferrara 44121, Italy

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ABSTRACT

The change in consumer behaviour towards healthier lifestyles since the Covid-19 pandemic has seen a steep rise in popularity of low-calorie, low-sugar food and beverage alternatives, like flavoured hard seltzers. In this study, a fully automated, high-capacity sorptive extraction (HiSorb) technique, coupled with gas chromatography–mass spectrometry (GC–MS), was developed to investigate volatile and semi-volatile organic compounds (VOCs and SVOCs) used for flavouring of hard seltzers. As part of method optimisation we trialled various sample preparation protocols and compared extraction *via* direct immersion *vs.* extraction from the headspace. The best headspace and immersive techniques were then further analysed in a 'stacked' extraction, whereby extracts from both were collected onto a focusing trap and fired to the GC to produce a single chromatogram. HiSorb probes with 4 alternate phases were compared: Polydimethylsiloxane (PDMS), divinylbenzene/PDMS (DVB/PDMS), carbon wide range/ PDMS (CWR/PDMS) and a triple phase (DVB/CWR/PDMS), with the DVB/PDMS phase proving to extract the highest number of compounds. The DVB/PDMS probe was further applied to a study of four berry/cherry flavour hard seltzer drinks, produced by 4 different leading commercial brands, with 64 compounds extracted and identified. Chemometrics were able to distinguish each brand's flavour profile by detection of unique compounds, these having potential for use as quality and authenticity markers.

## 1. Introduction

In recent years, low calorie, ready-to-drink (RTD) spirits have risen exponentially in popularity, due primarily to a shift in consumer preference towards products considered healthier than traditional alternatives [1,2]. An example of such products, hard seltzers, made their debut in 2016 and gained traction in 2019, with annual sales of the most popular brand reaching $627.2 million in the United States (US) alone [2]. Concomitantly, beer sales fell by 1.6% in 2019, prompting many large brewing companies to release hard seltzer ranges to keep up with this emerging trend [3]. Aided by social media advertising, hard seltzers have since expanded internationally, with particular success in Europe. In 2020 global sales of hard seltzers totalled $5.6 billion with a predicted annual growth rate of 31.4% up to 2028 [4].

Over 800 different volatile organic compounds (VOCs) have been identified in the volatile fraction of RTD spirits, but only a small number of these have organoleptic properties [5]. Some such compounds can negatively influence the product's aroma and flavour, reducing the perceived quality. In contrast, the effects of desirable flavour-active VOCs can be enhanced by the presence of other VOCs. For example, ethyl hexanoate, in combination with ethyl butanoate and 2-methylbutanoate, enhances the perceived sweetness of apple juice substantially [6]. Similarly, vanilla flavouring such as vanillin has previously been used to induce subtle sweet tastes by enhancing olfactory interactions, as olfaction plays a key role with taste perception [7]. A strong mechanistic factor in compound aroma intensity is C–H bond number, with longer and less saturated molecules usually having more intense aromas[6]. Functional groups also play a pivotal role in flavour and aroma perception. For example, oxygenated groups are typically associated with pleasant flavour characteristics [6]. In beverages derived from fruit, the most important flavour compounds generally originate from metabolic pathways active during the harvest, ripening, post-harvest and storage stages [6]. Such compounds include alcohols, esters, aldehydes, fatty acids, monoterpenols, furanic compounds and volatile phenols. This complexity makes analysis of flavour-active compounds challenging, but comprehensive profiling is nevertheless very important to the industry [8]. Hence, there is a need for sensitive and nonspecific VOC and SVOC extraction from beverages.

* Corresponding author.
*E-mail addresses:* lhearn@markes.com (L. Hearn), rcole@markes.com (R. Cole), damiana.spadafora@unife.it (N.D. Spadafora), rszafnauer@markes.com (R. Szafnauer).

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**Abbreviations**

| | |
|---|---|
| GC–MS | Gas chromatography–mass spectrometry |
| VOCs | Volatile Organic Compounds |
| SVOCs | Semi-Volatile Organic Compounds |
| PDMS | Polydimethylsiloxane |
| DVB/PDMS | Divinylbenzene/PDMS |
| CWR/PDMS | Carbon Wide Range/ PDMS |
| DVB/CWR/PDMS | Triple phase |
| RTD | Ready-To-Drink |
| US | United States |
| LLE | Liquid-Liquid Extraction |
| SPME | Solid Phase Microextraction |
| SBSE | Stir-bar Sorptive Extraction |
| PCA | Principal Component Analysis |
| HPLC | High Performance Liquid Chromatography |
| AGREEprep | Analytical GREEnness Metric Approach |
| log $K_{(o/w)}$ | Octanol-water partition coefficient |
| ANOVA | Analysis of variance |
| IPCC | Intergovernmental Panel on Climate Change |

Traditional extraction techniques for flavour profiling of food and beverages include liquid-liquid extraction (LLE), solid phase microextraction (SPME) and stir-bar sorptive extraction (SBSE), typically with separation and detection by gas chromatography – mass spectrometry (GC—MS) [9]. LLE is a manual technique typically used for liquid samples, but can be modified for solids too. It involves exposing the matrix to an immiscible solvent with which the analytes of interest have a high affinity such that they partition from the matrix to the solvent, with subsequent clean-up steps [10]. The liquid phase extract is then typically injected directly to the GC column. LLE is notably time-consuming and generates large volumes of solvent waste, making it impractical, expensive and unsustainable to scale-up. SPME is the most commonly used extraction technique for the analysis of flavour compounds [11]. It uses a small volume of solid sorptive phase to extract analytes from the headspace of a sample, with heating and agitation of the sample both before and during extraction to increase the rate of analyte uptake [11]. Subsequent thermal desorption (TD) then transfers analytes to the GC. A significant advantage of SPME over LLE is its potential to be fully automated, although workflows are often interrupted by the breakage of delicate fibers, and the small volume of sorptive phase used limits extraction efficiency [12]. In some protocols, SPME fibers are immersed directly into liquid samples to extract analytes that do not readily partition to the headspace, however this limits fibre lifespan and often leads to carryover due to injector soiling and inadequate removal of matrix from the fibre between runs [12]. SBSE is conceptually similar to SPME in that it involves extraction of analytes onto a solid sorptive phase, though it uses a substantially larger phase volume to increase extraction efficiency [13]. In this case, the phase coats a magnetic stir-bar that provides agitation to the sample matrix during extraction. While SBSE overcomes SPME's main disadvantages, its scalability is limited by the fact that it cannot be automated, with stir-bars needing to be retrieved, washed, dried and inserted into TD tubes manually prior to desorption. Headspace sampling with SBSE, which may be desirable to detect VOCs contributing to the aroma of a product, requires extensive adaptation. Furthermore, phase options are limited to polydimethylsiloxane (PDMS) and PDMS/ethylene glycol, with no commercially available option for the common flavour profiling sorbents carbon wide range (CWR) and divinylbenzene (DVB) [14,15].

In this study, we have investigated high capacity sorptive extraction with HiSorb probes for flavour analysis of hard seltzer beverages [16] . These probes carry sorptive phase (65 µL) mounted on inert stainless steel rods and are compatible with headspace and immersive extraction. The high volume of sorptive phase *vs.* SPME allows for greater extraction efficiency [17], while the availability of multiple sorbent combinations, including those with CWR and DVB, enhance the range of analytes detected compared with SBSE [17,18]. Furthermore, we leveraged the Centri extraction and enrichment platform to achieve full automation with sample preparation and extraction, probe washing and drying and probe desorption all taking place without manual automation. Upon desorption, analytes were transferred in a flow of carrier gas to a focusing trap containing multiple sorbent beds of differing sorptive strengths suitable for the full analyte range. The trap was then purged with carrier gas in the sampling direction to remove interferences such as residual water, solvents and air, before the gas flow was reversed and the trap rapidly heated to desorb analytes to the GC. The reversed gas flow is a crucial aspect of this method, as it ensures that low volatility compounds retained on weak sorbents do not come into contact with stronger sorbents to which they might undergo irreversible chemisorption.

To ensure profiling is comprehensive, the sorptive phase must be optimised to maximise the range of analytes extracted. PDMS is a relatively hydrophobic phase well-suited to the extraction of less polar analytes, with octanol/water coefficient ($\log K_{o/w}$) values > 2.6 [18–20]. As such, it is ideal for retaining large VOCs and SVOCs with long alkyl chains, which often have important flavour characteristics [6]. It is an absorptive phase, with analytes penetrating the polymer such that it is phase volume that determines extraction capacity [21] In contrast, adsorptive phases retain analytes on the sorbent surface only such that surface area is the critical variable [22] . Adsorptive phases, such as DVB and CWR, are beneficial for more volatile or polar compounds that can be missed with PDMS alone, hence these sorbents are often combined with PDMS in multi-phase SPME fibers, or indeed HiSorb probes [23,24].

Here, HiSorb probes were evaluated for the analysis of hard seltzers. Method optimisation is discussed, and multiple phase combinations (PDMS, DVB/PDMS, CWR/PDMS and DVB/CWR/PDMS) are compared to determine which is most suitable for this matrix. The DVB/PDMS probe produced the most comprehensive analyte profiles in this initial step of the investigation and was selected for flavour profiling of four hard seltzers, each from a different commercial brand. Data mining and chemometrics software allowed us to clearly differentiate between each product by principal component analysis (PCA), with tight clustering of replicates confirming the discriminatory power of the method.

## 2. Materials and methods

### 2.1. Samples

Four hard seltzer products described as having "cherry/berry" flavour, each manufactured by separate commercial brands, were bought from a local supermarket and anonymised as brands A, B, C and D.

### 2.2. Chemicals and reagents

HPLC grade water (Thermo Fisher Scientific, Waltham, MA,USA) was used to dilute the samples prior to placing on the autosampler. Anhydrous sodium chloride, ≥ 99% (Thermo Fisher Scientific, Waltham, MA, USA) was used to increase headspace sample concentration when investigating headspace HiSorb sampling mode by alteration of the ionic strength.

### 2.3. Automated immersive high-capacity sorptive extraction and desorption (HiSorb)

HiSorb probes (Markes International Ltd., Bridgend, UK) comprised of 75 mm inert-coated stainless steel rods with 65 µL sorptive phase applied. All aspects of the following workflow (summarised in Fig. 1) were

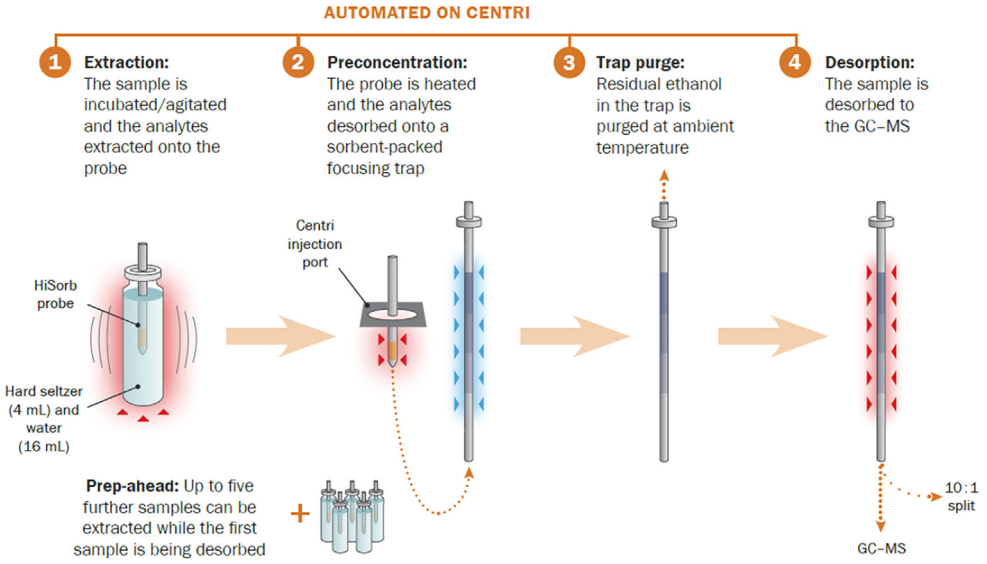


**Fig. 1.** A diagram of the fully automated sample extraction workflow using HiSorb probes via thermal desorption onto a focusing trap before further desorption for detection. Font: Calibri(Body).

**Table 1**
Six alternate extraction techniques, unmodified,1:4 dilution with water,1:4 dilution with water and salt addition, salt addition no dilution, 1:4 dilution with water and 1:4 dilution with water and salt addition with corresponding volumes of deionised water, hard seltzer and NaCl for each technique with the sampling type.

| Technique Number | Sample Preparation | HiSorb sampling mode | Hard Seltzer (mL) | Water (mL) | NaCl(g) |
|---|---|---|---|---|---|
| 1 | Unmodified | Headspace | 1 | – | – |
| 2 | 1:4 Dilution with water | Headspace | 1 | 4 | – |
| 3 | 1:4 Dilution with water and salt addition | Headspace | 1 | 4 | 1 |
| 4 | Salt Addition no dilution | Headspace | 1 | – | 1 |
| 5 | 1:4 dilution with water | Immersive | 4 | 16 | – |
| 6 | 1:4 dilution with water and salt addition | Immersive | 3 | 12 | 3 |

automated on a Centri extraction and enrichment platform (Markes International Ltd., Bridgend, UK). The sample vial was first pre-incubated at 40 °C with agitation at 300 rpm for 10 min to allow equilibration to extraction temperature. The HiSorb probe was subsequently inserted into the vial such that the sorptive phase was either immersed directly into the sample matrix (immersive sampling) or suspended above it (headspace sampling), and incubation continued for a further 10 min during which analyte extraction occurred. Following extraction, HiSorb probes were washed for 10 s in deionised water to remove residual sample matrix and then dried in a stream of air. Thermal desorption of the probes was then carried out at 260 °C for 15 min, with 50% of the efferent gas flow sent to vent and the remainder sent to a Peltier cooled (25 °C) focusing trap designed to retain analytes with boiling points between n-$C_4$ to n-$C_{32}$ ('Material emissions' multi-bed trap, Markes International Ltd., Bridgend, UK). Following probe desorption, the trap was purged with dry nitrogen in the sampling direction for 1 min to remove residual interferences such as water and ethanol, and the trap was then desorbed via rapid heating to 280 °C, maintained for 3 min (Fig. 1). During trap desorption, the trap was flushed with helium at 50 mL/min in the reverse direction, with 10 parts of the sample gas sent to vent for every 1 part injected to the GC. Splitting of the sample gas stream both during probe desorption and trap desorption was necessary to prevent system carryover and overloading of the MS detector with high-concentration analytes.

### 2.4. Sampling preparation optimisation

Samples were prepared in industry standard 20 mL headspace vials. We used brand A to assess the effect of manipulating the ionic strength of the sample through addition of sodium chloride and dilution with water for both headspace and immersive sampling (Table 1).

We further used brand A to assess the performance of four phase combinations in triplicate – PDMS, DVB/PDMS, CWR/PDMS and DVB/CWR/PDMS – using technique 5 above.

### 2.5. Sample stacking

In sample stacking, two samples are analysed consecutively as per techniques 2 and 5 (Table 1), except that the trap is not desorbed following the first extraction. Instead, analytes from this (immersive) extraction are retained on the trap while the probe used for the second (headspace) extraction is desorbed, so that ultimately analytes from both extractions are "stacked" on the trap together. The trap is then desorbed and the combined analytes are sent to the GC for analysis. Thus, if the range of analytes captured by headspace and immersive sampling differ, both ranges should be captured in one analysis via sample stacking.

We performed sample stacking for Brand A in triplicate, comparing the results with techniques 2 and 5 alone. Compounds identified with a NIST match factor >800 were tabulated and peak areas compared (table S1) [25].

### 2.6. Gas chromatography-mass spectrometry detection (GC–MS)

GC–MS analysis was carried out on a Trace 1310 gas chromatograph fitted with an ISQ LT single quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). A DB-WAX Ultra Inert 60 m length column with internal diameter 0.25 mm and phase thickness of 0.25 µm (Agilent Technologies, California, USA) was used with an oven program of 35 °C (initial temperature) held for 5 min, then a ramp rate of 10 °C/min to 240 °C and a final hold of 10 min. The overall GC run time was 35.5 min. Helium was used as carrier gas at a flow rate of 2 mL/min. The MS transfer line temperature was set at 250 °C, the ion source held at 200 °C, and the scan range was 35–350 Da.

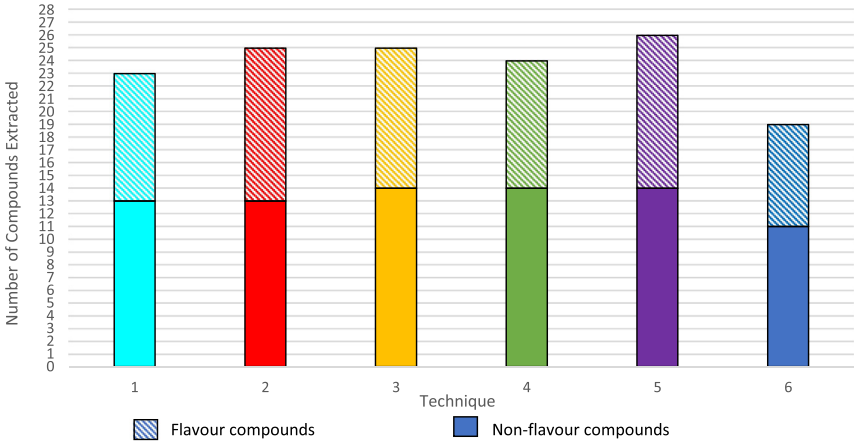


**Fig. 2.** A bar chart indicating the number of non-flavour compounds (block colour) and flavour compounds (striped) for each sample technique. The techniques include 1: unmodified,2: 1:4 dilution with water, 3: 1:4 dilution with water and salt addition, 4: salt addition no dilution, 5: 1:4 dilution with water and 6: 1:4 dilution with water and salt addition (more details in Table 1). Mean results over three replicates per technique are shown.

### 2.7. Data processing and elaboration

We generated a dataset comprising five replicates per hard seltzer brand (1 outlier excluded from brand A). ChromCompare+® chemometric software (SepSolve analytical, Peterborough, UK) was used for data mining, generation of a principal component analysis (PCA) plot and identification of key discriminatory features between brands.

For comparisons of sample preparation, extraction mode and phase combination we carried out visual inspections of chromatograms in ChromSpace (SepSolve Analytical, Peterborough, UK). Peaks were subsequently integrated via ChromSpace's proprietary deconvolution algorithm, wherein co-eluting peaks can be resolved on the basis of mass spectra. Integrated peaks were identified *via* comparison with the NIST20 library with a threshold match factor of 800, and the average peak area over three replicates was determined for each identified compound [25]

## 3. Results and discussion

### 3.1. Sampling method evaluation

Six sample preparation and extraction regimens (Table 1) were assessed to determine which provided the most comprehensive sample profile when applied to brand A. PDMS-only probes were used in each case as PDMS was present in all the other phase combinations and hence these probes were likely to be broadly representative. As measured by number of compounds detected, the best technique was technique 5, a 1:4 hard seltzer: water dilution (no salt addition) with immersive extraction (Fig. 2). However, techniques 2 and 3, which both use headspace extraction, detected almost as many compounds (25 *vs.* 26 for technique 5). Indeed, while immersive extraction without salt addition gave the best results, immersive extraction with salt addition performed worst (18 compounds). Headspace extraction relies on the formation of an equilibrium first between the sample matrix and the headspace, then the headspace and the sorptive phase, and is expected to be more efficient for more volatile compounds. In contrast, immersive extraction requires the formation of an equilibrium directly between the matrix and the phase and is expected to better extract less volatile compounds that do not readily reach the headspace [26]. Therefore, that the two methods extracted such similar numbers of compounds was unexpected, and we sought to determine whether the ranges of compounds detected were qualitatively different.

We further analysed Brand A via techniques 2 and 5, determining the mean peak area ($n = 3$) for each compound in each case (Table 2). As expected, the range of compounds detected by each technique only partially overlapped, with 16 compounds unique to the immersive technique and 15 unique to the headspace technique. Important flavour compounds featured prominently amongst these unique compounds, with

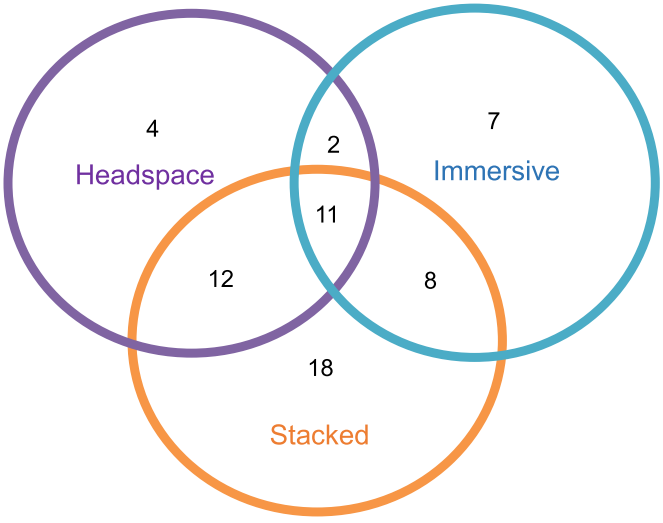


**Fig. 3.** Venn diagram indicating extracted compounds via three different extraction types.

ethyl hexanoate, an ester with strawberry, pineapple and fruity notes, being extracted only immersively, while headspace extraction was required to detect benzaldehyde diethylacetal, providing almond aromas, and 4-ethylbenzaldehyde, contributing cherry scents. While these headspace-extracted compounds are generally considered aroma compounds, rather than taste compounds *per se*, aroma strongly influences human perception of flavour [27] and is an important factor in overall consumer experience.

Ideally, volatile headspace-extracted compounds and less volatile immersive-extracted compounds would be analysed simultaneously to generate a single, comprehensive aroma/flavour profile. Hence, we trialled sample "stacking", in which analytes from both an immersive and a headspace extraction are desorbed from the focusing trap to the GC column simultaneously, providing an augmented extraction. We applied this technique to brand A and compared the results with discrete headspace and immersive extractions. We identified a total of 62 compounds across all techniques (table S1), with the range of compounds extracted qualitatively differing (Fig. 3). Stacked sampling extracted substantially more compounds than either headspace or immersive sampling, at 49 vs. 29 and 28 respectively. Eighteen of the compounds extracted by stacked sampling were unique, and of these, 6 were flavour active. Even amongst compounds that were not unique to stacked sampling, many showed a higher response with this technique than with headspace or immersive sampling alone, such as the "creamy" flavour-active compound 4-methoxybenzaldehyde.

**Table 2**
A compound list of extracted compounds screened with a match factor of >800 variable to either headspace or immersive extraction. The average peak area of 3 replicates was recorded [28].

| Compound | Retention Time (mins) | Log(Kow) | Average Peak area ($n$ = 3) | |
|---|---|---|---|---|
| | | | Headspace | Immersive |
| Acetone | 4.7914 | −0.1 | 8.60E+08 | 5.24E+08 |
| (2-Aziridinylethyl)amine | 5.1627 | −0.9 | | 5.03E+06 |
| Ethyl Acetate | 6.1703 | 0.7 | 8.78E+08 | |
| 1,1-diethoxyethane | 6.3063 | 0.8 | 2.23E+08 | |
| 2,3-Butanedione | 8.175 | −1.3 | | 8.18E+07 |
| Acetonitrile | 8.6824 | 0 | 6.91E+07 | |
| Hexanal | 10.452 | 1.8 | 4.09E+09 | 4.93E+08 |
| Undecane | 10.723 | 5.6 | | 1.77E+07 |
| ethyl pentanoate | 11.5395 | 1.9 | 1.13E+08 | |
| p-Xylene | 11.6149 | 3.2 | | 3.20E+07 |
| 2-methyl, 2-Pentenal | 11.9952 | 1.4 | 1.98E+10 | 1.93E+09 |
| 2-Hexenal | 13.0771 | 1.5 | | 2.85E+08 |
| 1,1-diethoxyhexane | 13.2851 | 3.2 | 3.73E+08 | |
| ethyl hexanoate | 13.3084 | 2.4 | 8.55E+08 | |
| Tetrahydro-2,2-dimethyl-5-(1-methyl-1-propenylfuran | 13.4992 | 2.7 | 8.75E+08 | |
| 2-propenyl hexanoate | 15.4496 | 2.7 | 7.06E+09 | 7.06E+08 |
| 3-Hexen-1-ol, (E)- | 15.6452 | 1.3 | 8.76E+09 | 7.10E+08 |
| Nonanal | 15.8247 | 3.3 | 3.03E+09 | 2.04E+08 |
| Acetic acid | 16.5289 | −0.2 | 3.27E+09 | 1.40E+09 |
| Furfural | 16.7842 | 0.4 | 1.62E+09 | 4.52E+09 |
| Decanal | 17.2865 | 3.8 | 1.52E+09 | |
| Benzaldehyde | 17.7022 | 1.5 | 2.25E+11 | 7.03E+10 |
| 5-methyl,2-Furancarboxaldehyde | 18.2765 | 0.7 | | 3.87E+08 |
| Propylene Glycol | 18.3891 | −0.9 | 5.21E+08 | 3.66E+09 |
| Hexadecane | 18.4461 | 8.3 | 4.78E+08 | |
| Benzaldehyde diethylacetal | 18.6548 | 2.3 | 6.71E+08 | |
| Ethyl benzoate | 19.4721 | 2.6 | 6.93E+08 | |
| $\alpha$-Terpineol | 19.7819 | 1.8 | | 1.41E+08 |
| Dodecanal | 19.8863 | 4.9 | 3.16E+08 | |
| 4-ethylbenzaldehyde | 20.0253 | 2.4 | 2.01E+08 | |
| , 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)- 2-Buten-1-one, (E)- | 21.2923 | 3.2 | 1.38E+09 | |
| 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl propanoate | 21.6629 | 3.1 | 8.54E+09 | |
| 2,5-Furandicarboxaldehyde | 22.8674 | 0.6 | | 4.47E+08 |
| 3-methyl, 2-phenylethyl butanoate | 23.044 | 3.6 | 5.36E+09 | 2.13E+09 |
| Furyl hydroxymethyl ketone | 23.3041 | −0.1 | | 8.31E+08 |
| 4-methoxybenzaldehyde | 23.4858 | 1.8 | 7.59E+09 | 8.13E+09 |
| 3-phenyl, methyl 2-propanoate | 23.9648 | 2.6 | | 1.29E+08 |
| 5-hexyldihydro-2(3H)-Furanone | 24.6598 | 2.7 | | 4.19E+09 |
| 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one | 25.7596 | −0.4 | | 2.12E+09 |
| 4-(4-methoxyphenyl) 2-Butanone | 25.9829 | 2.1 | 3.09E+09 | 9.73E+09 |
| 1-Hexadecanol | 26.5711 | 7.3 | 2.62E+10 | |
| Triethyl citrate | 27.5835 | 0.1 | | 1.66E+10 |
| 5-Hydroxymethylfurfural | 28.0278 | −0.6 | | 2.69E+10 |
| Tetradecanoic acid | 30.6682 | 5.3 | | 6.07E+08 |

Intriguingly though, 13 compounds that were present in headspace or immersive extractions were absent from the stacked extraction, including flavour active compounds such as 4-(4-methoxyphenyl)- 2-butanone (berry, fruity and raspberry notes) and (E)−3-Hexen-1-ol (juicy, fruity notes) (Fig. 4). This suggests a possible loss of volatile analytes from the focusing trap while the second extraction is being loaded onto the trap.

### 3.2. Phase comparison and selection

Brand A was further used for a comparison of phase combinations (DVB/PDMS, CWR/PDMS, DVB/CWR/PDMS and PDMS) in triplicate (Table 3). Initial evaluation of the data indicated that the multi-phase HiSorb probes generally extracted more compounds with high polarities, such as piperonal and 2-methylbenzaldehyde, compared with single-phase PDMS. Further analysis into peak responses of identified analytes indicated that DVB/PDMS was the most suitable sorptive phase combination, retaining analytes with a wider polarity range and generating larger peaks than other phase combinations.

A range of compound classes with important flavour characteristics including acids, alcohols, aldehydes, furanic compounds, and lactones were extracted (Fig. 5), with DVB/PDMS again providing the most com-

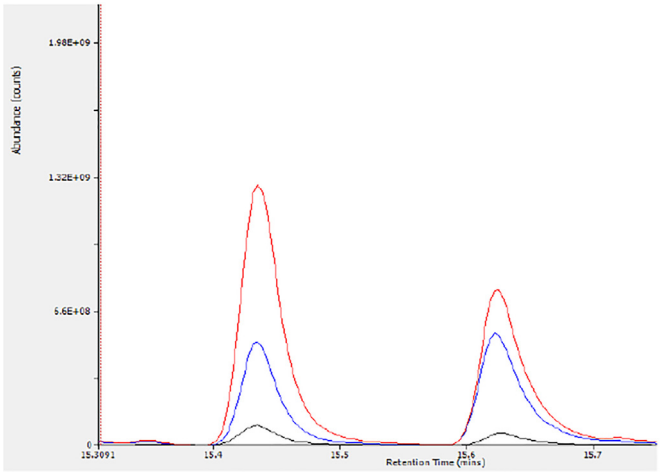


**Fig. 4.** A TIC chromatogram demonstrating improved extraction of Ethyl hexanoate and (E) 3-hexen-1-ol, using a stacked (red) extraction technique compared with immersive (black) and headspace (blue) extractions.

**Table 3**
A custom library of 34 compounds extracted across four phase combinations, with corresponding flavour profiles sourced from an aroma and flavour compound database [28,29].

| Compounds | RT (min) | Log($k_{o/w}$) | Average Peak Area (x$10^8$) ($n$ = 3) PDMS | DVB/PDMS | CWR/PDMS | DVB/CWR/PDMS | Flavour Profile* |
|---|---|---|---|---|---|---|---|
| **Acids** | | | | | | | |
| Acetic acid | 17.32 | −0.2 | | 37.9 | 37.9 | | pungent, sour, overripe fruit |
| Oxalic acid | 18.16 | −0.3 | | | 13.6 | | |
| 3-methyl butanoic acid | 22.57 | 1.2 | | 19.5 | | 16.9 | sweet, waxy, berry |
| Decanoic acid | 25.60 | 4.1 | | 3.45 | | | soapy, waxy fruity |
| Benzoic acid | 27.51 | 1.9 | 19.6 | | | 8.07 | Faint, balsam, urine |
| Dodecanoic acid | 27.75 | 4.1 | | | | 4.94 | creamy, coconut, fruity |
| Octadecanoic acid | 29.76 | 7.4 | | 19.8 | 4.79 | | Food Additive |
| Tetradecanoic acid | 30.66 | 5.3 | | 20.1 | 26.8 | 11.6 | waxy, fatty, creamy |
| **Alcohols** | | | | | | | |
| 3-hexen-1-ol | 15.88 | 1.9 | 6.91 | 15.2 | 8.63 | 30.1 | citrus, anise, floral |
| 2-methyl 1-hexanol | 16.31 | 2.3 | 1.42 | | | | Citrus, sweet, fruity |
| 1-hexadecanol | 26.62 | 7.3 | 2.42 | 10.4 | 4.59 | 6.07 | Waxy, clean, laundered |
| 1-octadecanol | 29.50 | 8.4 | | 8.63 | 11.0 | 11.0 | |
| **Aldehydes** | | | | | | | |
| 3-methyl Butanal | 6.87 | 1 | | | 0.55 | 0.96 | Fruity, green, nutty. |
| hexenal | 10.48 | 1.5 | 6.38 | 6.97 | | 8.49 | |
| 2-methyl2-pentanal | 11.96 | 1.4 | 8.27 | 7.91 | 6.76 | 5.32 | jammy, fruity, sweet |
| Decanal | 16.38 | 3.8 | 2.49 | 16.6 | | | Citrus, green, melon |
| Undecanal | 17.27 | 4.3 | | | 0.44 | | citrus, waxy, aldehydic |
| Benzaldehyde | 17.45 | 1.5 | 1010 | 1150 | 1230 | 1010 | 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 | 23.50 | 1.8 | 106 | 195 | 134 | 129 | 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 | 0.58 | | | | 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 | 0.511 | 0.359 | | | sweet, fresh, fruity. |
| **Lactones** | | | | | | | |
| Anisylactone | 26.03 | 2.1 | 165 | 137 | 98.9 | 88.1 | raspberry, fruity, berry |
| $\gamma$-decalactone | 24.62 | 3.8 | 6.53 | 53.9 | 27.8 | 11.8 | fruity creamy peach |
| **Extra Compounds** | | | | | | | |
| Benzene | 7.32 | 2.1 | 2.66 | 0.54 | 2.42 | 2.66 | |
| 1-Ethenyl 3-ethyl benzene | 15.75 | 3.5 | 4.00 | | | | |
| 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 25.81 | 0.8 | | 15.1 | | 33.0 | |
| 5-hydroxymethylfufural | 28.11 | −0.6 | | 207 | 4.44 | 142 | Sweet, caramellic, brown |
| Furfural | 16.48 | 0.4 | 8.26 | 47.5 | 19.2 | 32.0 | waxy, aldehydic, with a citrus note |
| Phenol | 23.12 | 1.5 | 2.53 | | | | |

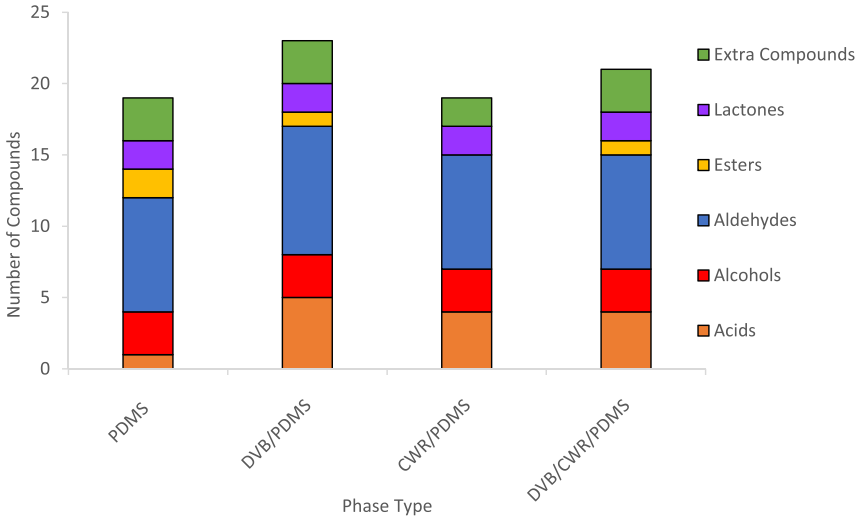


**Fig. 5.** A bar chart indicating the number of alternate functional groups extracted by each phase type. These include acids (orange), alcohols (red), aldehydes (blue), esters (yellow), lactones (purple) and extra compounds (green).

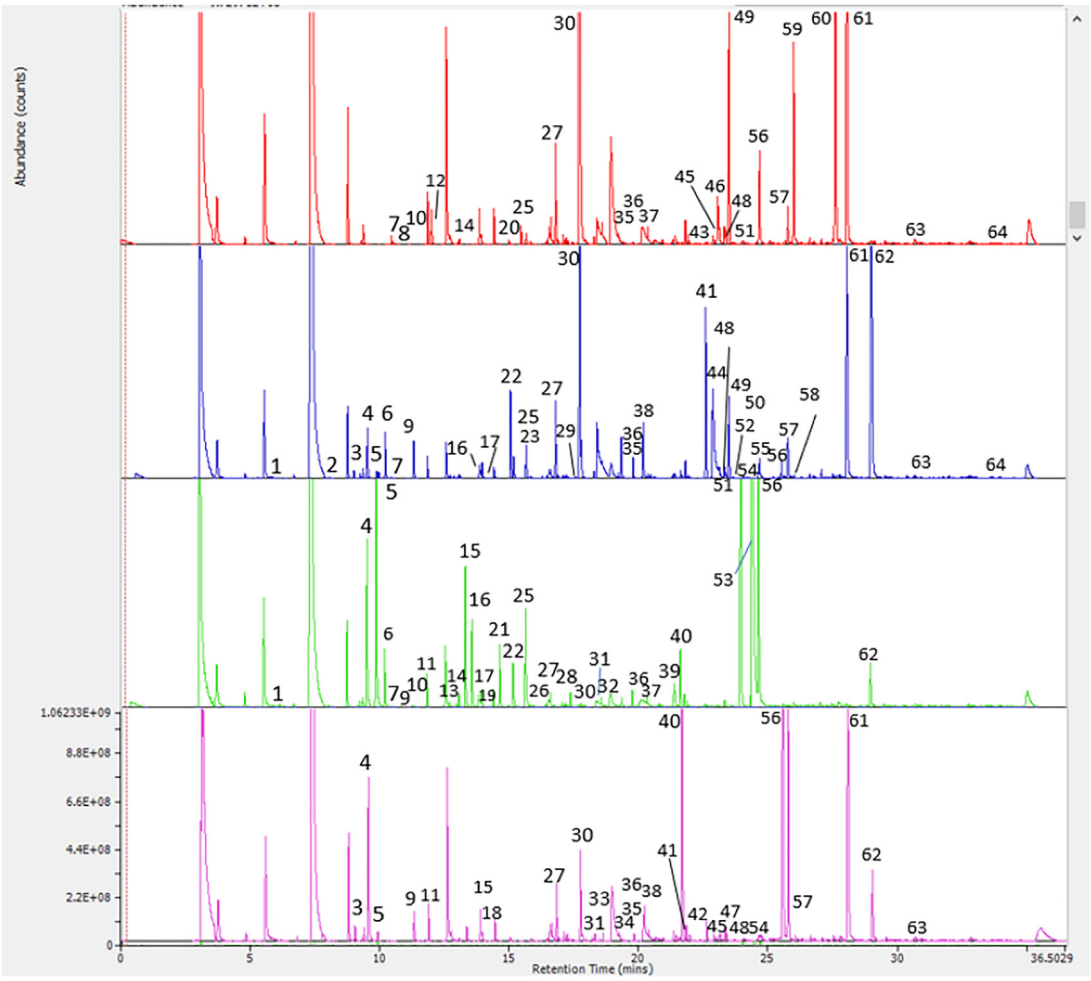


**Fig. 6.** Total ion chromatograms (TICs) of all phase extractions from the hard seltzer sample: PDMS (red), PDMS/CWR (blue), DVB/CWR/PDMS (green) and PDMS/DVB (black) with corresponding labelled compounds.

| Peak Number | Compound | Peak Number | Compound |
|---|---|---|---|
| 1 | Ethyl Acetate | 33 | Propylene Glycol |
| 2 | ethenyl acetate | 34 | 2-Furanmethanol |
| 3 | Isobutyl acetate | 35 | Acetophenone |
| 4 | Ethyl Butanoate | 36 | α-Terpineol |
| 5 | 2-methylethyl Butanoate | 37 | 4-ethyl-Benzaldehyde |
| 6 | 3-methylethyl Butanoate | 38 | , phenylmethyl acetate |
| 7 | Hexanal | 39 | Hexanoic acid |
| 8 | Undecane | 40 | α-Ionone |
| 9 | Isoamyl acetate | 41 | trans-β-Ionone |
| 10 | o-Xylene | 42 | 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-Buten-2-one |
| 11 | 3-Carene | 43 | 2,2′-oxybis ethanol |
| 12 | 2-methyl,2-Pentenal | 44 | Maltol |
| 13 | Eucalyptol | 45 | 2,5-Furandicarboxaldehyde |
| 14 | 2-Hexenal, (E)- | 46 | 3-methyl2-phenylethyl butanoate |
| 15 | Ethyl hexanoate | 47 | Methyl 2-furoate |
| 16 | 3-methylbutyl Butanoate | 48 | Furyl hydroxymethyl ketone |
| 17 | hexyl acetate | 49 | 4-methoxybenzaldehyde |
| 18 | Octanal | 50 | Octanoic acid |
| 19 | Methyl acetate | 51 | 3-phenyl-, ethyl 2-Propenoate |
| 20 | 1-hydroxy-2-Propanone | 52 | Nonanoic acid |
| 21 | 3-Hexen-1-ol, acetate, (Z)- | 53 | Eugenol |
| 22 | 1-Hexanol | 54 | Piperonal |
| 23 | 2-hydroxyethyl propanoate | 55 | n-Decanoic acid |
| 24 | 2-propenyl hexanoate | 56 | 5-heptyldihydro-2(3H)-Furanone |
| 25 | 3-Hexen-1-ol, (E)- | 57 | 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one |
| 26 | 3-Hexen-1-ol, (Z)- | 58 | 4-methoxybenzenemethanol |
| 27 | Acetic acid | 59 | 4-(4-methoxyphenyl)-2-Butanone |
| 28 | ethyl 2,4-Hexadienoate (2E,4E)- | 60 | Triethyl citrate |
| 29 | Oxalic acid | 61 | 5-Hydroxymethylfurfural |
| 30 | Benzaldehyde | 62 | Vanillin |
| 31 | Linalool | 63 | Tetradecanoic acid |
| 32 | Menthyl acetate | 64 | n-Hexadecanoic acid |

**Table 4**
Means of percentage relative abundance with standard error and significant differences ($P \leq 0.001$) of top 14 volatile compounds detected in the headspace of hard seltzer brands. Values are expressed as percentage of the total volatiles detected per sample. Superscript letters in the same row indicate differing levels of significance for each respective brand (ANOVA Tukey's HSD test; $P \leq 0.05$). All flavour components were sourced from good scents database [29].

| VOC | Brand A | | Brand B | | Brand C | | Brand D | | Flavour Notes* |
|---|---|---|---|---|---|---|---|---|---|
| | Average | SE | Average | SE | Average | SE | Average | SE | |
| 3-Hexen-1-ol, acetate, (Z)- | 0.012[a] | 0.003 | 0.011[a] | 0.003 | 0.202[b] | 0.001 | 0.015[a] | 0.002 | Green, fruity, tropical |
| 2-hydroxy-, ethyl propanoate | 0.017[a] | 0.005 | 0.278[b] | 0.005 | 0.009 [a] | 0.003 | 0.020[a] | 0.003 | Sweet, fruity pineapple |
| 4-(4-methoxyphenyl)−2-Butanone | 0.336 [a] | 0.006 | 0.018[b] | 0.005 | 0.011 [b] | 0.003 | 0.024 [b] | 0.004 | Floral, fruity, berry |
| ethyl hexanoate | 0.004[a] | 0.001 | 0.004[a] | 0.001 | 0.320[b] | 0.003 | 0.092[c] | 0.012 | Sweet, pineapple, banana |
| Triethyl citrate | 0.453[a] | 0.010 | 0.024[b] | 0.006 | 0.014 [b] | 0.004 | 0.032 [b] | 0.005 | |
| 4-methoxy-benzaldehyde | 0.376[a] | 0.008 | 0.241[b] | 0.005 | 0.008[c] | 0.002 | 0.018[d] | 0.003 | Creamy, vanilla, marshmallow |
| 2-methyl-, ethyl butanoate | 0.004[a] | 0.001 | 0.080[b] | 0.003 | 0.514[c] | 0.008 | 0.077[b] | 0.010 | Fruity, berry, cherry |
| 2-propenyl hexanoate | 0.104[a] | 0.003 | 0.005[b] | 0.001 | 0.003[b] | 0.001 | 0.007[b] | 0.001 | Sweet, fresh, pineapple |
| Maltol | 0.025[a] | 0.007 | 0.451[b] | 0.015 | 0.014[a] | 0.004 | 0.031[a] | 0.005 | Jammy, fruity, berry notes |
| 3-methyl-, butyl butanoate | 0.015[a] | 0.004 | 0.014[a] | 0.004 | 0.257[b] | 0.005 | 0.019[a] | 0.003 | Apple, fruity, berry |
| 3-methyl-, 2-phenylethyl butanoate | 0.158[a] | 0.005 | 0.008[b] | 0.002 | 0.005[b] | 0.001 | 0.011[b] | 0.002 | Berry, peachy notes, sweet |
| 4-methoxy-benzenemethanol | 0.009[a] | 0.002 | 0.157[b] | 0.006 | 0.005[a] | 0.001 | 0.011[a] | 0.002 | Cherry, vanilla, creamy |
| Eucalyptol | 0.003[a] | 0.001 | 0.003[a] | 0.001 | 0.056[b] | 0.001 | 0.004[a] | 0.001 | Minty, eucalyptus, cooling |
| $\alpha$-Terpineol | 0.031[a] | 0.009 | 0.129[b] | 0.001 | 0.102[c] | 0.002 | 0.064[d] | 0.007 | Citrus, lemon and lime, woody |

plete profile. The main benefit of adding DVB to PDMS appeared to be a substantial improvement in the extraction of acids. Addition of CWR to PDMS did not appear to be beneficial overall in the case of hard seltzers, as while extraction of acids was improved, extraction of esters was ablated. Many acids have log $K_{(o/w)}$ below what would be readily extracted by PDMS only (log $K_{(o/w)} > 2.6$), hence it is unsurprising that the addition of other phases enhanced their extraction. An example of these highly polar, low log $K_{(o/w)}$ acids is acetic acid, which is a known by-product of fermentation [8] and confers sour, pungent and overripe tastes. In this case it may be an undesirable malodour compound.

### 3.3. Brand comparison

We assessed four hard seltzer brands immersively using sample preparation technique 5 (Table 1). Across all brands, a total of 11 aldehydes and 19 ketones were found to be present, providing fruity and fresh notes to the samples. Chromatograms for each of the brands are shown in Fig. 6 with corresponding compounds below. Upon visual inspection, the brands appeared to produce similar numbers of peaks, though the identity of these peaks differed significantly. Benzaldehyde, typically known for cherry, almond notes (compound 30) is shown to be in high abundance in brands A, B and D but lower in brand C. Brand C however did have high abundance in ethyl 3-phenylprop-2-enoate, an ester with fruity, berry, sweet notes (compound 51) that is only present in low concentrations in brand A and not present in B and D, indicating a more 'berry' taste for brand C.

We further analysed five replicates per hard seltzer brand. One replicate of brand A was found to be an outlier and was discarded, but the remaining 19 chromatograms were provided to ChromCompare+ and PCA was performed, allowing us to identify compounds with the power to discriminate between brands. The dataset was then filtered to include only the top 14 most discriminatory compounds, and PCA was performed again. Principal components (PCs) 1, 2 and 3 were found to explain most of the variance in the sample (total of 99.79%), and upon plotting these, we found that the replicates formed tight, non-overlapping clusters (Fig. 7). This indicates that brands can be readily distinguished on the basis of VOC / SVOC profile.

We performed ANOVA and Tukey's test on the 14 most discriminatory VOCs. In each case, ANOVA was highly significant ($P \leq 0.001$). More prominent flavour compounds were noted in brands A-C compared with brand D. Based on ANOVA and Tukey's test a total of 7 distinct discriminatory VOCs are significantly different in brand A and in

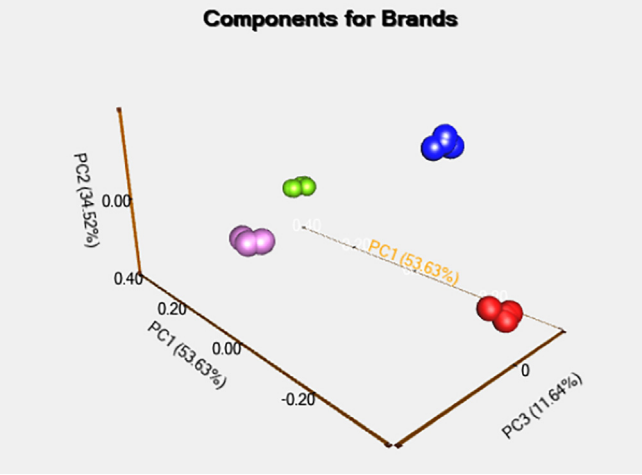


**Fig. 7.** A Principal component analysis (PCA) plot of 4 alternate hard seltzer brands with tight clustering of 5 replicates for each brand (1 replicate excluded from brand A). Brand A (red), brand B (blue), brand C (green) and brand D (pink).

brand C, followed by brand B that differs significantly for 5 VOCs while Brand D differs significantly from 2 in an overall comparison (Table 4). amongst these discriminatory compounds, the most abundant were ethyl esters, such as ethyl hexanoate and 2-methylethyl hexanoate, which are added to beverages to enhance flavour [5]. However, each individual brand had distinguishable VOC compounds that highlighted different key flavour notes.

Brand A is one of the most popular hard seltzers currently on the market, indicating that its flavour profile may be the most palatable to consumers. 4-(4-methoxyphenyl)−2-butanone, an ester also known as raspberry ketone methyl ether, provides raspberry, berry, fruity notes to the flavour profile and is in high abundance in brand A compared with the other brands (Fig. 8A). Brand B, a recent addition to the hard seltzer market, had a high abundance of compounds providing sweet, floral and fruity notes to the overall flavour such as 2-hydroxypropanoate (Fig. 8B) and maltol. In contrast, Brand C had more tropical, fruity flavour notes with hints of pineapple, sweet and peach provided by 3-methylbutyl butanoate (Fig. 8C). Brand D contained a range of flavour-active compounds, but none that were unique in comparison with the other brands.

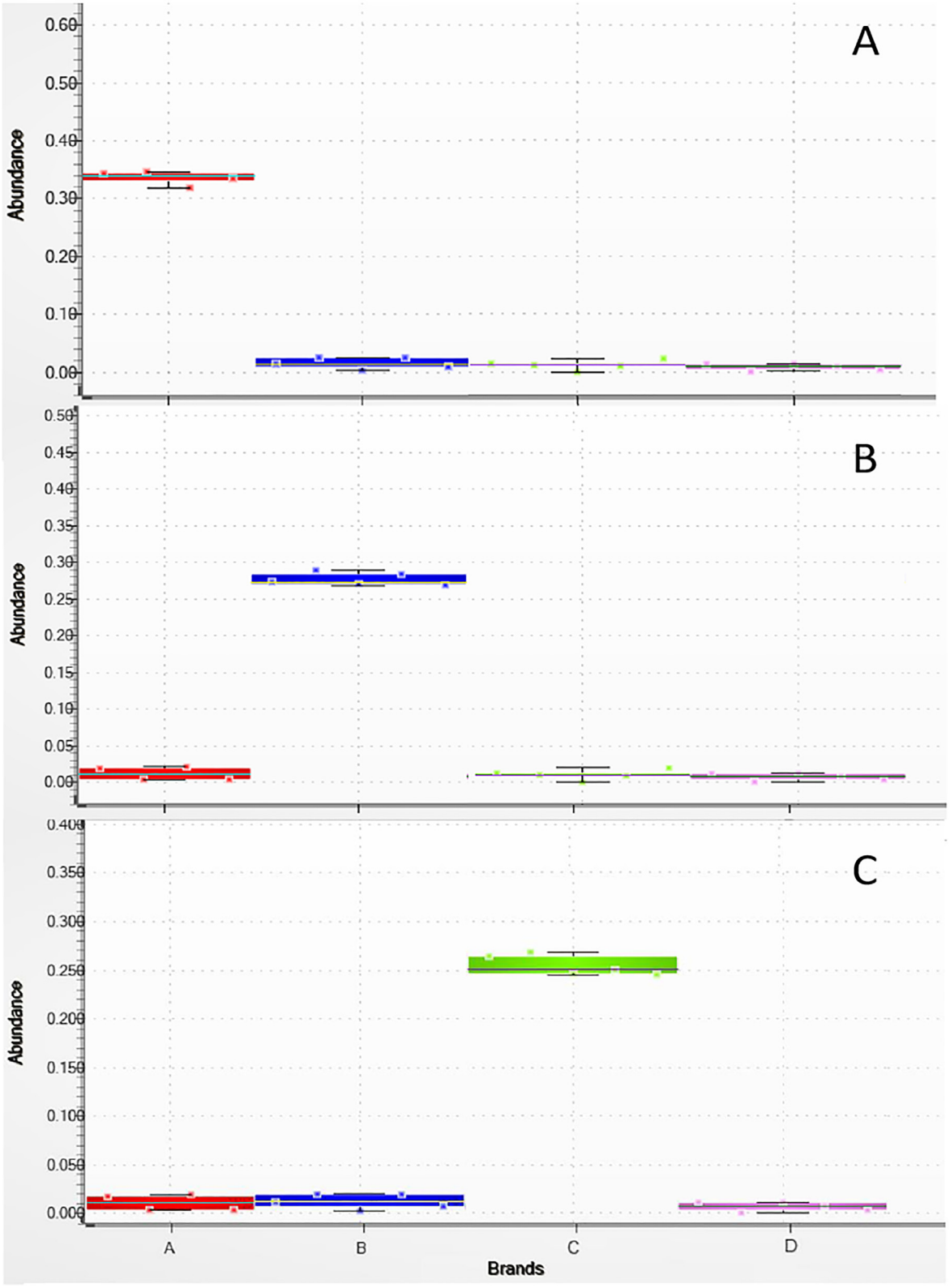


**Fig. 8.** Box plots determining key characteristic VOCs measuring abundances in each brand. A: 4-(4-methoxyphenyl)−2-butanone B: ethyl, 2-hydroxypropanoate and C: 3-methylbutyl butanoate.

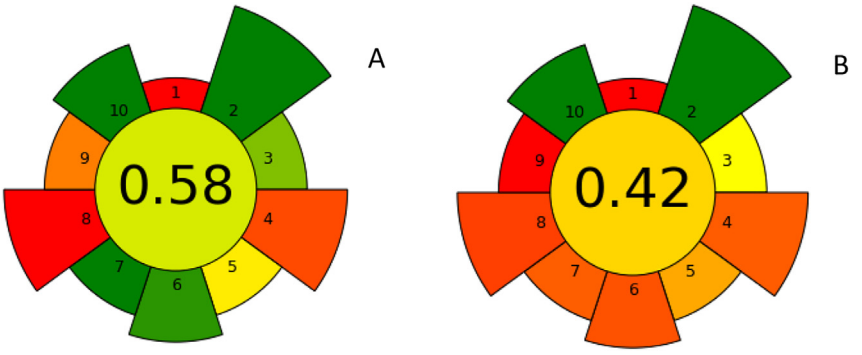


**Fig. 9.** The results of AGREEprep assessment of procedures for the current method of flavour profiling of hard seltzers using HiSorb (a) compared with a Stirrer bar Sorptive extraction (SBSE) method for profiling for Graciano Vitis vinifera wine variety (b). [31,32].

### 3.4. Green metrics

An April 2022 statement from the United Nations scientific body Intergovernmental Panel on Climate Change (IPCC) insisted that the global temperature increase target of 1.5 ˚C set by the Paris climate agreement in 2015 would not be met with current energy policies [30]. Given the urgent nature of improving sustainability in all sectors, the chemical industry must adapt by reducing energy consumption. Further, there is a need to reduce hazardous waste disposal and solvent use in order to prevent pollution.

Therefore, we assessed the greenness of the current method via the AGREEprep,(Analytical GREEnness Metric Approach) metric [31]. Under this approach, the impact of a sample preparation method is scored from 0 (not green) to 1 (ideal) based on the scores of each of 10 sub-sections. These sub-sections cover a range of variables such as solvent volume and use, waste generation, materials and reagents, energy consumption, sample volume, automation and throughput [25]. The AGREEprep score for HiSorb was 0.58 (Fig. 9A). The strongest detractor of this value was energy consumption (section 8), however this is expected for a fully automated system. Full automation and 'prep-ahead' functionality for HiSorb meant that multiple samples could be prepared ahead of time and loaded onto the sample tray for unattended analysis, scoring highly for sample throughput (section 7). The amount of chemical waste produced (Section 4) was also high at up to 20 mL per sample, however this consisted primarily of water with a small component of ethanol and other hard seltzer ingredients, so was not deemed hazardous. Due to the simplicity of sample preparation and sustainability of the components used, a high volume of samples could be prepared with an estimated 50 samples prepared in one hour, therefore scoring highly in Sections 3 and 6. Comparison with a previously published SBSE method for wine analysis [32] indicated that HiSorb with Centri is the 'greener' method according to this metric (Fig. 9), primarily due to full automation with HiSorb.

## 4. Conclusion

We developed a straightforward, extensively automated method for the flavour profiling of hard seltzers using HiSorb probes, exploring extraction from the headspace, extraction *via* direct immersion of the probe, and sample "stacking", wherein extracts from both sampling techniques were combined in a single analysis. We subsequently applied the optimised immersive method to four hard seltzer brands, generating useful insights into their unique flavour compositions. Principal component analysis was performed in ChromCompare+, demonstrating tight clustering of replicates with clear separation of brands on the basis of chemical components and identifying which components were most responsible for this separation. These discriminatory compounds could potentially be used as markers of product quality and brand authenticity, and allow correlation to consumer preference to ensure longevity in the market. Using the AGREEprep metric, the 'greenness' of sample preparation was measured, comparing favourably with a more popular SBSE technique with aroma profiling.

## Declaration of Competing Interest

The authors RS,LH, and RC are employees of Markes International, an instrument manufacturer specialising in thermal desorption instruments such as Centri that was used in this study.

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## CRediT authorship contribution statement

**Rachael Szafnauer:** Conceptualization; Data curation; Investigation; Methodology; Project administration; Resources; Supervision; Roles/Writing—original draft. **Lucy Hearn:** Data curation; Investigation; Methodology; Roles/Writing—original draft; **Rebecca Cole:** Data curation; Investigation; Methodology; Visualization; Roles/Writing—original draft. **Natasha Spadafora** Conceptualization; Methodology; Project administration; Resources; Software; Supervision; Writing—review & editing. 521

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