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Comparison of headspace solid-phase microextraction high capacity fiber coatings based on dual mass spectrometric and broadband vacuum ultraviolet absorption detection for untargeted analysis of beer volatiles using gas chromatography

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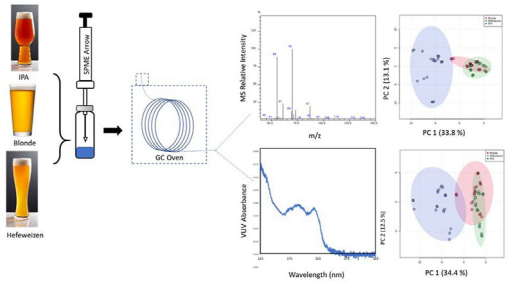
HIGHLIGHTS

- Parallel untargeted MS/VUV detection for GC separations of beer volatiles.
- VUV differentiated between beers using PCA as effectively as MS.
- Three different statistical approaches universally identified distinguishing components.
- Distinguishing components confirmed by spectral libraries and linear retention indices.

GRAPHICAL ABSTRACT



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ABSTRACT

Despite the same basic ingredients used in brewing, there is a significant variation in beer styles. With the rapid increase in craft brewing, beer styles have become even more numerous and complex in the recent past. A GC-MS/VUV (post-column split for dual detection) instrument with headspace high capacity SPME was used to investigate 21 different beers which represent three beer styles - India pale ales, blondes, and hefeweizens. Since results from untargeted studies can be affected by the sorbent material used, the extraction performances of three high capacity SPME fibers, *i.e.*, polydimethylsiloxane, polydimethylsiloxane/carbon wide range, and polydimethylsiloxane/carbon wide range/divinylbenzene, were evaluated. Good reproducibility (<10% RSD) was obtained for each high capacity fiber using both detectors. The tandem MS/VUV detection coupled with GC separation proved to be particularly valuable for compound identification, especially for isomers and compounds with similar structures. The evaluation of VUV detection for untargeted analysis led to similar performances as MS detection. Both the VUV and the MS were able to effectively differentiate between beer styles using principal component analysis. In addition, the use of 3 different statistical approaches, one-way ANOVA (p-value < 0.05), partial least square discriminant analysis, and random forest, universally identified 12 of the components most influential in distinguishing the three beer styles (e.g., β-myrcene, linalool, isopentyl acetate, 2,4-di-*tert*-

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butylphenol). This is the first reported evaluation of VUV detection and the first comparison of simultaneous VUV and MS detection for untargeted classification of complex mixtures using GC.

1. Introduction

Beer is one of the oldest and most popular alcoholic beverages in the world [1]. Although beers are brewed using the same four basic ingredients, malted grains, water, hops, and yeast for fermentation, beer styles can vary significantly in appearance, aroma, taste, and mouthfeel. Beer styles have become even more numerous and complex due to the recent renaissance of craft brewing. Throughout the brewing process, the ingredients introduce a wide variety of components into the finished product, including carbohydrates and volatile compounds, such as esters, ketones, aromatics, terpenes, alcohols, and organic acids. The combination of these volatiles contribute to the aromas of each style of beer [2]. Although many provide pleasant aromas to the beer, unwanted off-flavors, such as vicinal diketones and some phenolic compounds, can also be observed [2]. Quality control practices help to identify these compounds in an effort to avoid distributing tainted beers. While the diversity between styles can be determined through physical observations by the consumer, the differences on the chemical level are challenging to interpret due to the high complexity of beer [2]. Headspace analysis has proved to be an efficient technique to discern variations between styles through the fingerprinting of beer volatiles [3,4].

Headspace solid-phase microextraction (SPME) is one of the most frequently used extraction techniques for the analysis of volatile organic compounds (VOCs) in complex matrices [5,6]. Headspace SPME is a solvent-free extraction technique that can be used to concentrate a wide range of analytes; it avoids nonvolatile matrix interferences and is capable of being fully automated [6,7]. However, a lack of mechanical robustness for SPME devices has been reported in several studies [8,9]. Recently, a high capacity SPME (HCSPME) device has been developed to overcome this drawback and to further improve the extraction approach [10]. HCSPME fibers possess larger external diameter (1.1–1.5 mm for HCSPME vs. 0.6 mm for traditional SPME) and a conical solid tip. These features increase the mechanical strength of the device, decrease the applied resistance necessary to pierce the septa for sampling and injection, and limit contaminations from ambient air [10]. In addition, the larger extraction phase volume of the HCSPME increases sensitivity of the technique, which is particularly valuable for comprehensive volatile fingerprinting [11]. Recently, HCSPME have been manufactured to match most of the traditional SPME fiber sorbent coatings, maintaining the wide selectivity range of the technique [9]. Subsequently, the choice of the sorbent material can notably affect the results of untargeted studies and thus their reproducibility.

Gas chromatography coupled to mass spectrometry (GC-MS) is a method of choice for VOCs analysis in numerous fields [12,13]. MS is a well-established detection technique for VOCs fingerprinting since it provides specific fragmentation information, which can be used for compound identification. In addition, the elution pattern of the VOCs can be matched against libraries of linear retention indices (LRI), which provides additional criterion for analyte identification [13]. Despite the versatility of GC-MS, the identification of isomeric or isobaric compounds, or compounds presenting similar mass spectra, remains challenging [14,15]. The introduction of broadband vacuum ultraviolet (VUV) spectroscopy as a GC-compatible detector has addressed some of these limitations [15,16]. Indeed, the specificity of the absorbance spectra in the VUV region has been demonstrated, proving the power and added value of such a detector [14,16,17]. However, even with such a tool, only a certain degree of confidence can be achieved and uncertainties in compound identification remain an issue. Having multiple means to support compound identification is always desirable.

The use of GC separation with VUV in tandem with MS (GC-MS/VUV) for the detection of VOCs significantly improves compound identification capabilities [18,19]. To increase the robustness in metabolite identification, the Metabolomics Standard Initiative (MSI) has defined a ranking based on specific criteria for identification [20], which can be easily translated to any untargeted analysis. The highest and most rigorous level is achieved (Level 1) when a compound is identified using at least two orthogonal analytical techniques, spectral similarity with available libraries, and the injection of a neat standard corresponding to the targeted compound [20,21]. A GC-MS/VUV system enables one to reach a confidence level of 2 in compound identification by collecting three orthogonal sources of identification (mass spectra, VUV absorption spectra, and linear retention indices). The increased identification power of GC-MS/VUV tandem detection has been previously demonstrated in targeted analysis [18,19], but not for untargeted analysis.

In this work, an optimized sampling technique using HCSPME was evaluated for fingerprinting the volatiles of 21 craft beers. The selectivity and extraction performances of three different sorbent coatings (polydimethylsiloxane (PDMS), polydimethylsiloxane/carbon wide range (PDMS/CarWR), and polydimethylsiloxane/carbon wide range/divinylbenzene (PDMS/CarWR/DVB)) were compared. In addition, a GC-MS/VUV system configured in parallel dual detection mode, combined with multivariate statistical analysis, was used to analyze and identify VOCs specific to each style of beer. The performances of the two detectors for the differentiation between the beer styles were highlighted, and the complementarity of the two approaches was pointed out. This is the first report of untargeted analysis using VUV detection; the combination of VUV and MS detection for untargeted analysis is also compared for the first time. The increased identification power obtained through the combined use of GC, MS, and VUV, as well as the complementarity of the approaches, are emphasized.

2. Materials & methods

2.1. Chemicals and standards

Calibration standards used to assess the linearity range of both detectors were β-myrcene, isopentyl acetate, and styrene (Sigma Aldrich, St Louis, MO; 98%+ purity); fluorobenzene was used as an internal standard (Alfa Aesar, Ward Hill, MA) with a minimum purity of 99%. ACS grade sodium chloride (Macron, Randor, PA) was used to enrich the volatiles in the headspace. Linear alkanes (C7–C40, Supelco, Bellefonte, PA) were used for the linear retention index (LRI) determination.

2.2. Beer samples

Twenty-one craft beers from three different styles, (7 types of India pale ale (IPA), 7 types of blonde, and 7 types of hefeweizen) were selected from a local liquor store (Table S1). Following degassing by sonication for 10 min at 4 ^{O}C, 20 mL headspace vials were filled with 5 mL of beer and 2 g of sodium chloride. The vials were sealed with magnetic caps, which contained a PTFE/silicone septum. The internal standard (IS), fluorobenzene, was added to a final concentration of 20 ppm. Since the analysis of all the samples took approximately 3 days for each fiber, samples not immediately analyzed were stored at 4 ^{O}C for short time periods until closer to their analysis time.

The experimental design, found in Table S2, included four quality controls: an IPA QC (QC_IPA), a blonde QC (QC_Bl), a hefeweizen QC (QC_H), and a general QC (QC_All) containing all the beers. The IPA, blonde, and hefeweizen QCs were prepared by mixing equal portions of each IPA, blonde, and hefeweizen beer used, respectively. The QC_All was prepared by mixing equal portions of all the beer samples used in the study. The QC mixtures were transferred to headspace vials and prepared in a manner identical to the individual samples (5 mL of mixture in a 20 mL headspace vial, plus 2 g of NaCl, and the IS to a final concentration of 20 ppm). All individual beer samples and QC samples were analyzed in triplicate.

2.3. HCSPME extraction optimization

The volatile profile of the beer samples was extracted using headspace HCSPME. Three different SPME Arrow coatings were used for the analysis: 100 μm polydimethylsiloxane (PDMS), 120 μm polydimethylsiloxane/carbon wide range (PDMS/CarWR) and 120 μm polydimethylsiloxane/carbon wide range/divinylbenzene (PDMS/CarWR/DVB). All of them had a diameter of 1.1 mm and were supplied by Restek Corporation (Bellefonte, PA, USA).

The sampling conditions (incubation time, extraction time, extraction temperature, and desorption time) were optimized on the PDMS fiber using a central composite design (CCD). The incubation time, extraction time, extraction temperature, and desorption time ranged from 0-60 min, 0.5–60 min, 30–90 °C, and 0.5–10.5 min, respectively. For the AOC-6000, the incubation temperature and extraction temperature are the same. The central point of the CCD was 30.5 min incubation, 30.5 min extraction at 60 °C, and a desorption time of 5.5 min. The HCSPME fiber was thermally desorbed in the GC injector at 250 °C in split mode (1:5). During incubation and extraction, the samples were under constant agitation at 500 rpm. Subsequent fibers were evaluated using equivalent conditions as those established as optimal for PDMS.

2.4. GC-MS/VUV tandem detection

Samples were analyzed using a GCMS-TQ8030 gas chromatograph - triple quadrupole mass spectrometer (Shimadzu Scientific Instruments, Inc., Columbia, Maryland) connected in parallel to a VGA-101 VUV/UV spectroscopic absorption detector (VUV Analytics, Inc., Cedar Park, TX). For simultaneous detection, the GC effluent was directed into a SilFlow GC 3 Port Splitter (Trajan, Victoria, Australia). The exits of the splitter directed the flow towards the MS and the VUV through two fused silica uncoated capillary columns (Restek Corporation, Bellefonte, PA, USA). Practically, 1.5 m × 0.25 mm column directed the effluent to the MS and 0.3 m × 0.32 mm column was connected to the VUV. The resulting initial GC effluent split was 20% to the MS and 80% to the VUV (1:4), due to the MS detector being more sensitive than the VUV detector. The system was equipped with an AOC-6000 autosampler system (Shimadzu) for automated headspace HCSPME extraction and desorption.

The GC separation was performed on a non-polar Rtx-5MS (30 m × 0.25 μm x 0.25 mm) column from Restek. Helium was used as the carrier gas at a flow rate of 12.3 mL/min, which corresponded to a constant linear velocity of 45 cm/s. The oven temperature program was set to begin at 40 °C (isothermal hold for 1 min) then ramped at 10 °C/min to 200 °C, and finally to 300 °C at a rate of 20 °C/min (isothermal hold for 2 min).

For the MS detection, the transfer line and the ion source were set at 200 °C. The TQ8030 was operated in Q3 scan mode with an electron ionization energy of 70 eV. A scan range from 45 to 500 *m/z* was collected at an event time of 0.05 s. For VUV detection, the transfer line and the flow cell temperatures were set at 275 °C. For all the analyses, the VUV wavelength detection range was 125–430 nm and the data collection rate was set to 4 Hz. Nitrogen was used as the post-column make-up gas at a pressure of 0.5 psi.

2.5. Data processing and statistical analysis

The data were acquired and processed using GCMSsolutions v4.45 (Shimadzu) and VUVision v3.2.1 (VUV Analytics) for the MS and VUV data, respectively. The MS data were baseline corrected and a signal-to-noise (S/N) ratio of 10 was used as a threshold for peak detection. Artifacts from the HCSPME and column bleed, such as siloxanes, were removed from the final peak list.

Library searches were performed combining the GC, MS, and VUV information. Compounds were identified using LRI calculated from the GC with a ±10 unit deviation from the library LRI. The mass spectra were matched to the NIST 2014 library using an 85% score threshold. The VUV absorption spectra were matched to the available VUV spectral library (v2.08) using a combined criteria of match score $\geq$ 70% and the highest correlation coefficient (R^2). The R^2 value corresponds to the correlation between the measured spectrum and the library spectrum over the full range of spectral data points, while the match score takes into account a reduced number of spectral data points.

The final peak list containing the relative peak area, measured from the total ion chromatogram (TIC), of the detected compounds for each sample was used to conduct multivariate analysis on each beer style. The experimental design was created using Design Expert 12 and response surface plots were obtained using the response-surface methods (rsm) package in R (v3.3.2) to extract the optimized sampling parameters. Principal component analysis (PCA), hierarchical cluster analysis (HCA), and partial least squares discriminant analysis (PLS-DA) were performed using MetaboAnalyst 4.0 online (Quebec, Ca). Prior to statistical analysis, the data were normalized using the IS signal and auto-scaled. No further data transformation was performed.

3. Results & discussion

3.1. Optimization of the HCSPME sampling

The extraction conditions were optimized on the PDMS fiber using a central composite design (CCD) with four variables, specifically incubation time, extraction time, temperature, and desorption time. All the variables were evaluated within a wide analytical range, resulting in 25 different sampling points analyzed. The efficiency was evaluated using the peak areas measured from the TIC of the MS of 22 compounds that were present in all the samples while covering most of the chemical families.

Surface response plots (Fig. 1A) were used to evaluate the best extraction range for each compound. These are reported as a Gantt plot in Fig. 1B. Practically, the extraction range was defined as the

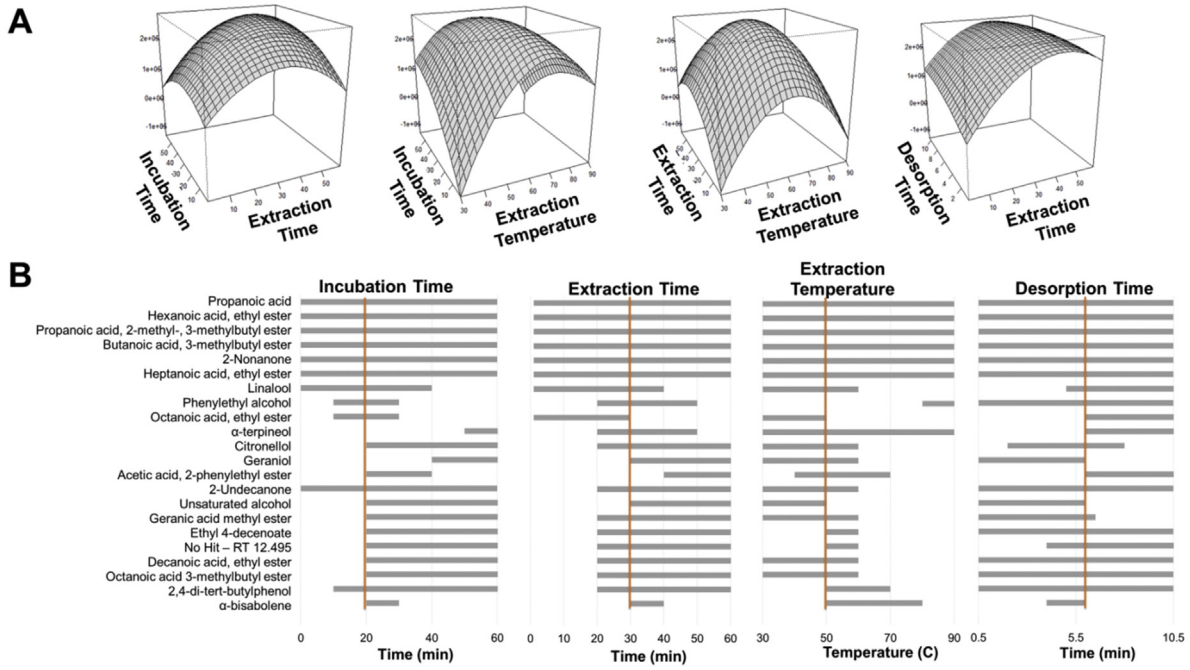

Fig. 1. **A**) Surface response plot of 2,4-di-*tert*-butylphenol from the HCSPME Polydimethylsiloxane (PDMS) fiber optimization using central composite design. **B**) Gantt diagram representing the range of the maximum area obtained for each peak (grey lines) and the optimal parameters chosen (orange vertical line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

region surrounding the maxima for which the intensity of the compounds was not affected.

From the surface response plots, such as those shown in Fig. 1, the delineation of maxima in responses for the different variables can be observed, showing that the wide parameter ranges evaluated enabled to find optimal extraction conditions for all the variables. The combination of high incubation and extraction time did not result in higher extraction yield. Therefore, a longer HCSPME exposure to the sample would not increase the extracted amount of analytes. Artifact formation was observed when extraction temperatures over 60 °C were used. These likely resulted from the chemical oxidation of the beer. In general, longer desorption time (from 4 to 6 min) resulted in an increased signal response without affecting the peak width and thus, the peak capacity. Due to the larger sorption phase volume of the HCSPME, a longer desorption time was to be expected [10,22]. In addition, no carry-over was observed, which insured the complete desorption of the compounds in the GC injection port.

The study resulted in the optimal sampling conditions, using an extraction time of 30 min, extraction temperature of 50 °C, incubation time of 20 min, and a desorption time of 6 min. These parameters resulted in the best compromise to maximize analyte extraction while ensuring high analytical turnover.

3.2. Untargeted analysis using parallel MS and VUV detection

The volatile profile of the beer styles is displayed in Fig. 2. The MS and VUV detection provided similar information, although the number of analytes detected by the MS was slightly higher. Following artifact removal, 80 features were detected using the MS and 58 features using VUV. When using the VUV as the detector, the analysis of the ethanol content within the beer was possible since no solvent cut-off time is required to protect the detector.

Unsupervised principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used to evaluate the discrimination between the beer samples using the three different HCSPME fiber chemistries on the MS and VUV detectors. The unsupervised PCA was generated using 58 features for the MS, corresponding to the features present in at least 2 of the replicates, and 58 features for the VUV. Fig. 3 presents the observed clustering between the different beer types when using the high capacity PDMS/CarWR fiber. Similar clustering was observed when using the other fiber chemistries, indicating similar extraction performances for the three fiber types (Figs. S1 and S2). The relative composition of different chemical classes between the beer types, as visualized based on extracted and detected compounds using the three different HCSPME fibers, can be found in Table S3A. More importantly, the classification performances were similar when the detection was performed using the MS or the VUV as detectors. This demonstrates that the VUV is equally as effective as the MS for differentiating between sample classes, with a total variance expressed within the dataset using only the two first principal components (PC) of 46.9% (Fig. 3).

The first PC, accounting for ~34% of the total variance using both the MS and the VUV, enabled the discrimination of the IPAs from the blonde and hefeweizen beer types. The IPAs presented the richest and most distinct volatile profile. This can be seen in the HCA in Fig. 3 and is confirmed from the analysis of the chromatograms (Fig. 2). Due to the different raw materials and production processes used for brewing, high variability was observed between the beers within each style. Despite this, the unsupervised PCA enabled the differentiation of the three beer styles. Indeed, when displaying the four QCs, representing the mixture of each beer style, in the unsupervised PCA score plot, the clustering of each QC with the corresponding beer type is displayed and more importantly, the clear discrimination between the QCs and thus the three beer styles is highlighted (Fig. S3). In addition, small clustering ($n = 3$) can be observed within each beer type. These clusters correspond to the grouping of the three replicates analyzed for each beer, which indicates that the methodology used can discriminate

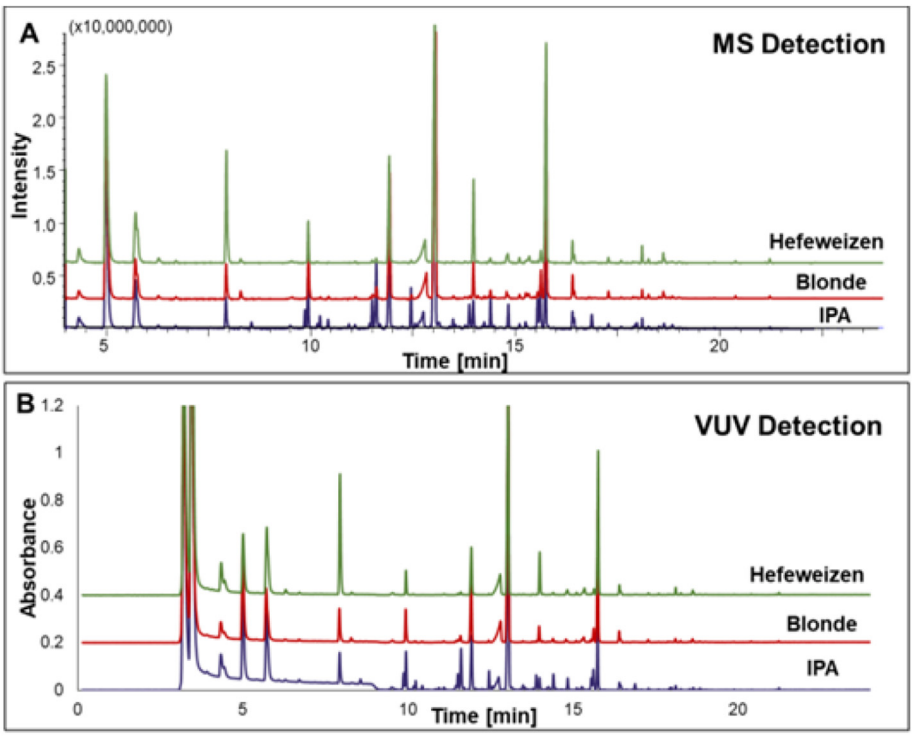

Fig. 2. A) GC-MS and **B)** GC-VUV chromatograms of the QC samples for each beer style from the analysis with the high capacity PDMS/CarWR fiber; IPA (blue), blonde (red), hefeweizen (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

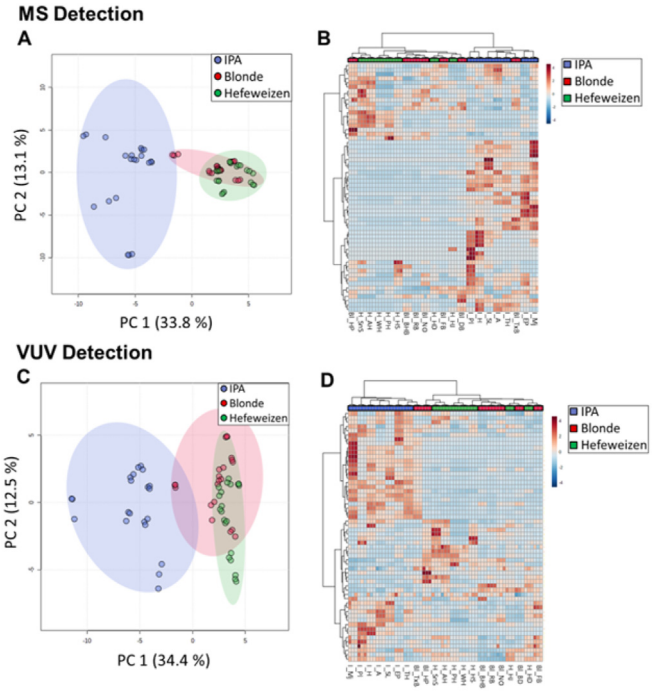

Fig. 3. Principal component analysis of the beer samples evaluated by GC-MS using 58 features **(A)** and VUV using 58 features **(C)** using the high capacity PDMS/CarWR. The x-axis represents principal component 1 (PC1) and y represents principal component 2 (PC2). **B** and **D** represent a heatmap of the samples and the metabolites for MS and VUV, respectively.

the beers to a deeper extent based on their labels.

The relative composition of the samples in terms of chemical classes, determined by MS detection, is depicted in Fig. 4 and represents the PCA loadings. The response used corresponds to the areas of the analytes measured from the TIC. For the majority of the compounds, good reproducibility (<5% RSD) was achieved. Only 10% of the compounds (mainly low-level acids and esters) were characterized by a >15% RSD. From the HCA of Fig. 3 and the PCA loadings of Fig. 4, IPAs are characterized by a higher content of terpenes, terpenoids, and ketones compared to the blondes and hefeweizens. This contrast between IPAs and the two other beer types could be expected as IPAs are typically brewed with higher amounts of hops, preferably added at a later stage of the boil to preserve their characteristic aromas and to produce a very distinct bitter flavor [23–26]. Interestingly, Bl_TxB beer clusters with the IPAs because it shares a richer volatile profile. The elevated concentration of terpenes and terpenoids detected in Bl_TxB compared to the other blondes (Fig. 4B) is the main reason for this unexpected clustering.

Blondes and hefeweizens are light-bodied beers and share a more similar profile, characterized by a higher acid content compared to the IPAs. This higher acid content may be due to the yeast and/or grain used during the fermentation process [2]. Nevertheless, their volatile profile was partially resolved by the second PC, especially when using VUV detection (Fig. 2C).

3.3. Identification of features of merit

The most influential compounds able to discriminate the beer types were selected using three different statistical approaches, namely one-way ANOVA (p-value < 0.05), partial least squares discriminant analysis (PLS-DA), and random forest (RF). While the ANOVA is a univariate approach that does not consider correlation among features, PLS-DA and RF are multivariate approaches that can be used with collinear features. The compounds shared by the three statistical approaches are listed in Table 1. The combination of these 12 compounds enabled the complete separation of the three

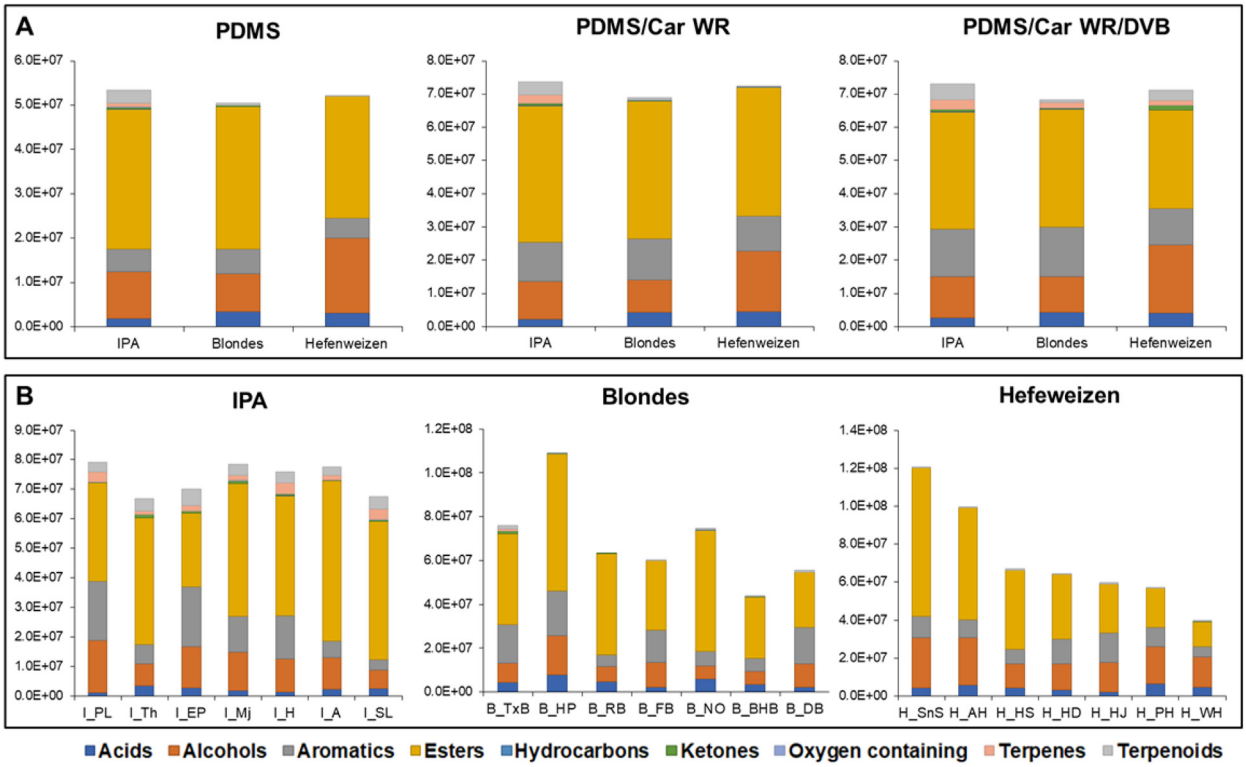

Fig. 4. A) Bar graphs representing the sum of the peak areas measured from the TIC of the MS data for each compound class identified in each analyzed beer style (IPA, blondes, hefeweizen). Results from the PDMS (left), PDMS/CarWR (middle), and PDMS/CarWR/DVB (right) high capacity fiber. **B)** The bar graph represents the average peak area of the replicates of the volatile compounds class identified for each beer style, IPA (left), blonde (middle), and hefeweizen (right), using the PDMS/CarWR high capacity fiber for HS extraction.

Table 1
Most influential compounds for the discrimination between the beer styles, selected by one-way ANOVA (p-value < 0.05), PLS-DA, and random forest.

Class	Name	RT (min)	LRI exp.	LRI lib.	MS	VUV		High Capacity Fiber Type		
					Match (%)	R^2	Match (%)	Monophasic	Biphasic	Triphasic
IPA	β-Myrcene	9.901	991	991	93	0.96	97	x	x	x
	2-Nonanone	11.484	1090	1091	96	0.82	86	x	x	x
	Linalool	11.628	1099	1098	94	0.99	99	x	x	x
	Citronellol	13.53	1228	1228	94	0.84	89	x	x	x
	Geraniol	13.934	1256	1258	95	0.97	98	x	x	x
Hef	Isopentyl acetate	7.958	874	878	98	0.99	99	x	x	x
	2-phenylethyl acetate	14.006	1263	1256	96	0.98	98	x	x	x
Hef &	Octanoic acid	12.773	1176	1175	96	0.99	99		x	x
Blonde	Decanoic acid	15.359	1363	1372	92	0.94	97		x	x
Blonde	Styrene	8.294	895	883	97	0.93	98		x	x
	Ethyl 9-decenoate	15.625	1386	1389	76	–	–	x	x	x
	2,4-di-*tert*-butylphenol	17.31	1520	1515	91	0.96	98	x	x	x

beer styles, as can be visualized on the PCA score plot of Fig. S4. All the analytes, except octanoic acid, decanoic acid, and styrene, were extracted from the samples using each of the three high capacity fibers. β-myrcene, linalool, citronellol, and geraniol have been previously reported as prominent aroma compounds in IPAs [26]. Isopentyl acetate and 2-phenylethyl acetate are known for the sweet, fruity, and floral flavor characteristics they impart on hefeweizen beers [27]. Finally, ethyl-9-decenoate and 2,4-di-*tert*-butylphenol have been characterized as fatty, woodlands, and fruity compounds [27,28].

The identification of 8 of these 12 specific compounds reached the level 2 of identification according to the MSI guidelines [20], thanks to the orthogonal and complementary information provided by the GC, MS, and VUV. Three compounds, β-myrcene, isopentyl acetate, and styrene, were confirmed with standards, yielding a level 1 identification. Only ethyl-9-decenoate reached a level 3 of confidence, since its spectrum was not available in the VUV spectral library. Fig. 5 demonstrates this unique advantage of the use of dual detection provided for the identification of β-myrcene and isopentyl acetate. The specificity of VUV enabled the unequivocal identification of β-myrcene (Fig. 5A). The mass fragmentation pattern of many terpenes is almost identical, with only small dissimilarities regarding the ion intensity ratios, as can be observed for β-myrcene and β-pinene. Therefore, the mass spectrum alone is insufficient for specific terpene identification, resulting in inconclusive MS library matches for such structural isomers. However, VUV spectra of these isomers resulted in highly distinguishable features in the 165–230 nm spectral region. Indeed, β-myrcene is

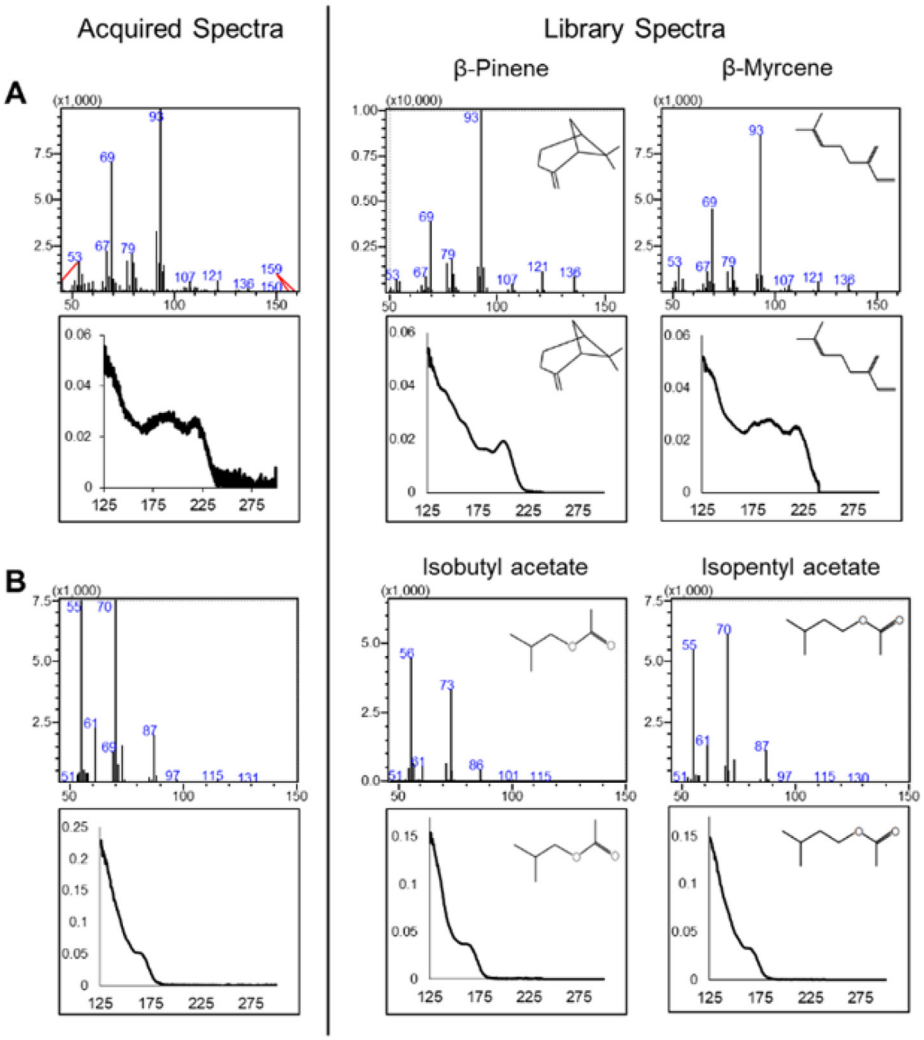

Fig. 5. Acquired (left) and library (right) spectra using MS-detection (top-right) and VUV-detection (bottom-right) of **A)** β-myrcene and **B)** isopentyl acetate.

characterized by an additional absorption maximum around 190 nm, together with different slopes and shapes at 165–230 nm. The identification of β-myrcene was further corroborated by the LRI experimental value obtained from the GC separation. Indeed, the NIST database reports LRI values of 991 for β-myrcene and 964 for β-pinene, which increased the level of confidence of the identification.

In contrast, VUV was unable to give a definitive identification of isopentyl acetate. Its VUV spectrum was matched to several other esters, including isobutyl acetate, pentyl acetate, and hexyl acetate, with the same R^2 (0.99) and match factor (99) (Fig. 5B). Although minor differences can be observed within the spectrum, *i.e.*, the slope in the 125–135 nm region and the peak shape of the main absorbing peak at 160 nm, these features are not sufficient to fully discriminate these homologous compounds. However, the mass spectrum provided specific features at *m/z* 55, 70, and 87 for the identification of isopentyl acetate, highlighting the specificity of MS-detection towards esters. Worthy to note is the value of the GC LRI data. Those can be correlated to established libraries to reinforce confidence in identification. The identification of octanoic acid and decanoic acid relying solely on the mass and VUV spectra was unsuccessful. These long chain acids presented similar absorbance and fragmentation patterns (Fig. S5). In addition, the molecular ion signal was lost due to the hard electron ionization at 70 eV. In that case, the LRI experimental values (1176 for the octanoic acid and 1363 for the decanoic acid) enabled discrimination and identification of these compounds.

3.4. Extraction performance of the HCSPME

The reproducibility of the sampling using each HCSPME for each beer style was evaluated using the area response of the internal standard (IS) measured from the TIC, as shown in Fig. S6. The inter-day reproducibility was evaluated in three consecutive days using the 75 analyses consisting of the twenty-one craft beers and the four QCs analyzed in triplicate. The PDMS/CarWR fiber exhibited the highest reproducibility with an average relative standard deviation (RSD) of 5.2% using the MS for detection and 6.4% using the VUV. When using the high capacity PDMS fiber, 7.6 %RSD and 7.1 % RSD were obtained using MS and VUV detection, respectively. Similarly, the PDMS/CarWR/DVB fiber presented a 7.1 %RSD when using MS for detection and 6.6 %RSD with the VUV. Linearity was evaluated for β-myrcene, isopentyl acetate, and styrene for each of the fibers. The linearity from the mass spectral data was $R^2 > 0.995$ or greater for all the compounds on all the fibers evaluated (Table S4).

The extraction yields of the different HCSPME were determined using the pooled QC sample of each beer type. For most of the compound classes, higher extraction yields were obtained using the diphasic and triphasic fibers (Table S3B). The overall extraction of aromatic compounds within all beer types was on average 2.5 times higher when using the biphasic and the triphasic high capacity fibers, compared to the monophasic. Terpenes were also extracted in significantly higher amount using the biphasic and triphasic fibers. However, the amount of hydrocarbons extracted from IPA and blonde decreased by 20% when using the triphasic. Additionally, the amount of oxygen-containing compounds extracted from IPA, blonde, and hefeweizen decreased by 21%, 37%, and 70%, respectively, when using the triphasic fiber. Although the performances of the high capacity PDMS/CarWR and PDMS/CarWR/DVB are comparable, the PDMS/CarWR seemed to be the most efficient for volatile extraction.

Similar discrimination efficiency between the beer types was obtained when using the three HCSPME fibers. However, the discrimination of the beer types was slightly improved when using the PDMS/CarWR and the PDMS/CarWR/DVB fibers. Considering together the reproducibility, the overall extraction yields for a wide range of volatile compounds, and the discrimination efficiency, the high-capacity PDMS/CarWR fiber seemed to be the most suited fiber for untargeted analysis of beer volatiles.

4. Conclusions

The use of GC-MS/VUV with headspace HCSPME extraction of volatile compounds enabled the differentiation between three beer styles. IPAs were more easily discriminated due to their rich terpene and terpenoid profile, while blondes and hefeweizens had greater similarity. This is the first time that GC-VUV or GC-MS/VUV has been used for untargeted analysis. The ability of the VUV to differentiate between beers as effectively as MS indicates that it is a robust tool for untargeted analysis, an attribute which can continue to be explored and utilized in numerous other application fields. The tandem MS/VUV detection coupled with GC separation proved to be powerful and particularly valuable for compound identification, especially for isomers and compounds with similar structures. The expansion and development of the VUV spectral library will only reinforce the use of such coupling in untargeted analysis.

Good reproducibility (<10% RSD) was obtained for each of the HCSPME using both detectors. The best reproducibility (5.2% RSD for MS detection and 6.4% RSD for VUV detection) and the best overall extraction yields for all compound classes were obtained using the high capacity PDMS/CarWR fiber for analyte extraction.

Such an approach could be further extended to all type of beverages to identify adulteration or unwanted off-flavors. In addition, the effect of the storage conditions on the volatile profile could be investigated. More generally, the dual MS/VUV detection potential could be used in the flavor and fragrance industry, where a high number of isomers and isobars are usually detected.

Disclaimer

KAS is a member of the scientific advisory board for VUV Analytics, Inc.

CRediT authorship contribution statement

Delphine Zanella: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Hailee E. Anderson:** Validation. **Talena Selby:** Resources. **Robert H. Magnuson:** Resources. **Tiffany Liden:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Kevin A. Schug:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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