Evaluation of PDMS-based Extraction Techniques and GC-TOFMS for the Analysis of Off-flavor Chemicals in Beer

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ABSTRACT


Various formats employing polydimethylsiloxane (PDMS) as an extraction medium prior to gas chromatography/time-of-flight mass spectrometry (GC-TOFMS) analysis were investigated to measure off-flavors in aged beer. The techniques included stir bar sorptive extraction (SBSE), headspace sorptive extraction (HSSE), purge and trap, and closed-loop stripping (CLS). Although all PDMS extraction methods worked well, SBSE appeared to provide the most accurate quantitation and was capable of detecting the most odor-active compounds. The peak deconvolution capability of the Leco Pegasus TOFMS was critical to the detection and accurate quantitation of key off-flavor chemicals. Compared with fresh control beer, increases in furfural; furfuryl ethyl ether; furfuryl hydroxymethyl ketone; 2,4-dodecadienal, (E,E)-; benzeneacetic acid ethyl ester; β-damascenone; and 3-pyridinecarboxylic acid ethyl ester (also known as nicotinic acid ethyl ester) were observed in beer samples incubated for 12 weeks at 30°C, and increases in dimethyl disulfide, dimethyltrisulfide, and benzeneacetaldehyde occurred in beer samples exposed to sunlight for 8 hr.

Keywords: Beer, Gas chromatography/time-of-flight mass spectrometry (GC-TOFMS), Headspace sorptive extraction (HSSE), Off-flavors, Polydimethylsiloxane (PDMS), Stir bar sorptive extraction (SBSE)

RESUMEN

Varios formatos que emplean el polidimetilsiloxano (PDMS) como un medio de extracción antes del análisis de la cromatografía de gases/espectrometría de masas por tiempo-de-vuelo (GC-TOFMS) fueron investigados para medir sabores desagradables en cerveza envejecida. Los métodos incluidos fueron la extracción por absorción en barra agitadora (SBSE), extracción por absorción del espacio libre (HSSE), purgación y trampa, y el separador de ciclo cerrado (CLS). Aunque todos los métodos de la extracción PDMS trabajaron bien, SBSE aparecía proporcionando la cuantificación más exacta y era capaz de detectar los compuestos de olor más activos. La capacidad máxima de desconvolución del Leco Pegasus TOFMS era crítica a la detección y cuantificación exacta de los productos químicos del sabor extraño dominantes. Comparado con la cerveza control fresca, aumentos en el furfural; furfural etil éter; furfural hidroximetil ketona; 2,4-dodecadienal, (E,E)-; ácido benzencético etil éster; β-damascenona; y el 3-piridina-carboxilico ácido etil éster (también conocido como etil éster del ácido nicotínico) fueron observados en las muestras de la cerveza incubadas por 12 semanas en 30°C, y los aumentos en dimetil disulfuro, dimetiltrisulfuro, y benzenoetilaldehído ocurrieron en las muestras de la cerveza expuestas a la luz del sol para 8 hr.

Palabras claves: Cerveza, Cromatografía de gases/espectrometría de masas por tiempo-de-vuelo (GC-TOFMS), Extracción por absorción del espacio libre (HSSE), Extracción por absorción en barra agitadora (SBSE), Polidimetilsiloxano (PDMS), Sabores desagradables

Carbonyl compounds (particularly aldehydes), furfuryl derivatives, and other types of organic chemicals are thought to play a role in the development of off-flavors in aged beer samples. 3-Methyl-2-buten-1-thiol and other thiols and organic sulfur compounds contribute skunky off-notes to light-exposed beer. Numerous GC-MS methods have been conducted to study off-flavor problems in beer. Sample preparation or extraction techniques that commonly have been used in the past for studying beer off-flavors include steam distillation (15), purge and trap (P&T) on Tenax GR (12), extraction with XAD-2 resin (10), solid-phase microextraction (5), solid-phase microextraction with on-fiber derivatization (16), and Freon extraction (13).

The numerous important advantages of polydimethylsiloxane (PDMS) phase as an extraction medium have been described previously (1). PDMS-coated magnetic stir bars (Twister, GERSTEL GmbH Co.) can be applied in different formats, including placement in the beer sample (stir bar sorptive extraction [SBSE]) or in the headspace above the beer sample (headspace sorptive extraction [HSSE]). PDMS foam mounted in thermal desorption tubes, a new PDMS format introduced by GERSTEL, was investigated as an extraction sorbent for a (P&T) technique and a closed-loop stripping (CLS) method.

The goal of our work was to develop a solventless, quantitative, sensitive, and relatively simple analytical method for studying off-flavor development in beer. Beer contains dozens of odor-active chemicals in concentrations ranging from percents to parts per billion and lower. Developing an analytical technique to cover such a wide range of volatiles and concentration levels is challenging. In addition to PDMS extraction, a second critical component to the analytical strategy employed in this work was the application of gas chromatography/time-of-flight mass spectrometry (GC-TOFMS) incorporating sophisticated peak deconvolution software.

EXPERIMENTAL

Analytical Strategy to Evaluate Four PDMS Extraction Techniques with GC-TOFMS

Determination of accuracy and precision commonly is used as a way of assessing the acceptability of new analytical procedures. Three steps were followed to evaluate the suitability of the methodologies for studying beer off-flavor development:

1) Test accuracy based on linearity of standard calibration curves. An artificial beer solvent system was created, spiked with various levels of 14 known beer flavor or off-flavor chemicals (including aldehydes implicated as causing beer off-flavors), and analyzed by four PDMS methods. The linearity of plots generated from spike concentration versus peak area for each analyte was determined and used as a measure of test accuracy. The approach has been described previously (11).

2) Test precision based on standard deviations of replicate measurements. Because the SBSE method provided the best accuracy of the four tests evaluated (i.e., the highest linear least squares correlation coefficients for analyte standard calibration curves), the precision of the SBSE method was investigated. Measuring the standard deviation of replicate measurements for analytes is a common approach for determining the precision of new analytical methods. The concentrations of the 14 analytes were

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calculated in control, heat-abused, and light-abused beer samples by multiplying the peak area of the analyte determined at a specified quantification mass by the slopes of the SBSE calibration curve plots for each chemical in each of the three samples. The three beer samples were analyzed in duplicate. The standard deviations for the 14 analytes were estimated using the equation standard deviation = ($\sum d^2/2n$)$^{1/2}$, where $d$ = the positive difference between each pair of results and $n$ = the number of pairs of results.

3) Determination of chemical changes occurring in samples as a function of heat abuse and light abuse. After accuracy and precision studies showed that SBSE was an acceptable method for measuring the 14 analytes, the peak area results for more than 700 analytes that appeared in each of the SBSE chromatograms of the control, heat-abused, and light-abused samples were carefully compared. These results provided further evidence of the usefulness of SBSE GC-TOFMS as a tool for studying beer off-flavor development.

Instrumentation and Instrument Conditions

Analyses were performed on a 6890 gas chromatograph (Agilent Technologies) equipped with a CIS4 inlet and MPS2 robotic sampler with TDU option (GERSTEL) and a Pegasus gas chromatograph/time-of-flight mass spectrometer (Leco). The gas chromatograph capillary column used for all determinations was a 30-m HP-5MS (Agilent) with an internal diameter of 0.32 mm and a film thickness of 0.25 µm. Chromatographic-grade helium was used as the carrier gas, with a head pressure of 1.6 psi and a constant flow of 1.5 mL/min. The oven ramping conditions were as follows: 40°C for 1 min, then heated at a rate of 10 degrees Celsius/min to 270°C and held at 270°C for 6 min.

Extracted volatiles were thermally desorbed from the PDMS Twisters and the PDMS foam sorbents with the GERSTEL TDU desorption tube containing PDMS foam sorbent. The HSSE technique was similar to the SBSE method, except the magnetic Twister was attached to a metal wire stuck through the vial’s septum and suspended in the headspace above the sample.

With the P&T technique, the sample was placed in a purge vessel (Scientific Instruments Inc.) (Fig. 1) and purged with nitrogen at a flow rate of 30 mL/min for 20 min at room temperature into a GERSTEL TDU desorption tube containing PDMS foam sorbent. A second stream of dry nitrogen simultaneously was employed to purge the PDMS foam tube to prevent condensation of water; the flow rate of the dry nitrogen stream was 25 mL/min.

With the dynamic headspace CLS (DHSCLS) method, a 250-mL gas washing bottle was used (Fig. 2). Sample (10 mL) was placed in the bottle, and a GERSTEL TDU desorption tube with a PDMS

3. Sensory Analysis of Beer Samples

The three types of beer samples were subjected to an informal sensory evaluation by four untrained tasters. The three beer types had distinct flavor differences from each other. The control had a fresh, pleasant beer flavor, whereas the heat-abused beer had a stale, aged-beer taste and somewhat chemical-like off-flavor. The light-abused beer had a light-struck, skunky, sulfury aroma and highly objectionable taste.

Sample Preparation Methods for SBSE, HSSE, P&T, and Dynamic Headspace CLS

In all cases, 10 mL of beer or standard was extracted, and all extractions were conducted at room temperature. For SBSE, a Twister stir bar was placed in the beer or standard in a 20-mL gas chromatograph vial, sealed, and stirred for 2 hr at 900 rpm. After extraction, the Twister was rinsed in distilled water for 3 sec and patted dry with a clean lintless towel, as recommended by GERSTEL operating instructions for Twister. The HSSE technique was similar to the SBSE method, except the magnetic Twister was attached to a metal wire stuck through the vial’s septum and suspended in the headspace above the sample.

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Time-Course Studies and Preparation of Standard Solutions

The first step in developing the analytical methods was to study the recovery behavior of a variety of flavor compounds known to occur in beer as a function of extraction time. An artificial beer solvent system was prepared by adjusting a 5% ethanol/water solution to pH 4.5 with 0.1% phosphoric acid as previously described (16). Spiking this solvent with flavor standards was used to prepare time-course samples, as well as standard stock and working standard calibration samples. The 14 analytes used in the standards are listed in Table I. The stock solution was used as an internal standard at a level of 55 ppb.

Time-course studies were conducted with a mid-range working standard solution. These studies revealed that 2-hr extraction times for SBSE and HSSE were sufficient, with only slight increases in trapped volatiles occurring after 2 hr. For P&T with the PDMS foam trap, 20 min of extraction at room temperature provided good recovery of volatiles under the conditions used. Time-course DHSCLS results were unexpected. Maximum extraction time was 10 min. Sampling times greater than 10 min resulted in a reduced analyte signal. Because the amount of volatiles extracted at 5 and 10 min were essentially the same, 5-min extraction times were employed for DHSCLS.

A stock solution of 14 analytes was prepared in ethanol. This stock solution was added to the artificial beer solvent system at six concentration levels to prepare working standards. The six working standards were analyzed by each of the four sample preparation methods. Linear least squares correlation coefficients were determined and used as a measure of test accuracy for each of the sample preparation methods (Table I).

Working standard calibration samples were prepared by diluting the stock standard solution to give analyte concentrations of 0, 5, 10, 25, 100, and 200 ppb for all standards except isoamyl acetate (also known as 1-butanol, 3-methyl-, acetate) and phenyl ethyl alcohol. For these two standards, working standard concentrations of 0, 50, 100, 250, 1,000, and 2,000 ppb were prepared. The linear least squares correlation coefficients for the working standard curves are shown in Table I. Also, quantitation of these 14 analytes in the control, heat-abused, and light-abused samples was made to allow estimation of test precision (as measured by the standard deviation of replicate determinations).

RESULTS AND DISCUSSION

Total ion chromatograms (TIC) for the control beer sample analyzed by SBSE and HSSE (which used Twisters) are shown in Figure 3. Although more than 700 analytes were detected in each beer sample analyzed, only the largest peaks are identified in Figure 3. TIC results for P&T and DHSCLS (which used PDMS foam trapping) are shown in Figure 4. Chromatograms for HSSE, P&T, and DHSCLS were most similar.

Although all PDMS extraction methods worked well, based on the average linear least squares correlation coefficients (Table I) SBSE provided the most accurate quantitation and was capable of detecting the most odor-active compounds. SBSE was the only method that could detect all 14 standard analytes quantitatively. HSSE calibration curves for furfural were nonlinear. Methional was not detected by P&T and HSSE in any of the standards. Phenyl ethyl alcohol calibration curves were nonlinear with CLS, P&T, and HSSE. Standard deviations of replicate analysis were less than 10% for all analytes analyzed by SBSE, except for phenyl ethyl alcohol (PEA) (Table II). The poor precision for PEA may be due to the fact that it was present in all beer samples at levels outside the range of the PEA standard calibration curve.

Most of the peaks appearing after 1,200 sec in the DHSCLS chromatogram in Figure 4 were polysiloxane background peaks contributed by the PDMS foam. Many of these same PDMS foam background peaks appear in the P&T chromatogram in Figure 4, but they were present in smaller amounts. These late-eluting polysiloxane background peaks tended to be greatly reduced in the SBSE and HSSE chromatograms in Figure 3. Both SBSE and HSSE used PDMS Twister stir bars instead of the PDMS foam as the absorbent. There was much more PDMS absorbent associated with the PDMS foam compared with the Twister stir bar; therefore, the higher polysiloxane background levels for P&T and DHSCLS were expected.

Changes in Flavor-Significant Chemicals in Heat-Abused and Light-Abused Beer and How They Relate to Perceived Off-flavors

After the linear least square correlation coefficients for the standard curves showed that the SBSE method was the most accurate of the PDMS extraction techniques and replicate analyses of beer samples showed that the SBSE method demonstrated acceptable precision, SBSE chromatograms of control, heat-abused, and light-abused beer samples were inspected carefully to see what potential malodorous chemicals were generated by heat abuse and light abuse. Several interesting flavor chemicals were created or increased in concentration in the beer sample stored at 30°C for 12 weeks compared with the beer control sample (Fig. 5; Tables II and III), including furfural; furfuryl ethyl ether; furfuryl hydroxymethyl ketone; 2,4-dodecadienal, (E,E); benzeneacetic acid ethyl ester; β-damascenone; 3-pyridinecarboxylic acid ethyl ester; octanal; and nonanal. The increases in β-damascenone and furfuryl ethyl ether...
may be most significant to off-flavor development considering their extremely low flavor threshold levels (Table III). Furyl hydroxy-methyl ketone also may be an important off-flavor contributor; however, the flavor threshold value for this compound in beer is unknown. All four PDMS extraction techniques demonstrated increases in \( \beta \)-damascenone for the heat-abused beer compared with the control but slight decreases in \( \beta \)-damascenone in light-abused beer compared with the control (Fig. 6). Figure 6 also is useful for

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**Fig. 3.** Total ion chromatogram plots for the control beer sample analyzed by stir bar sorptive extraction (SBSE) and headspace sorptive extraction (HSSE) with Twister. PDMS = polydimethylsiloxane; R.T. = peak retention time.

**Fig. 4.** Total ion chromatogram plots for the control beer sample analyzed by purge and trap (P&T) and dynamic headspace closed-loop stripping (DHSCLS) with polydimethylsiloxane (PDMS) foam traps. R.T. = peak retention time.
comparing the sensitivity or response of each test method for β-damascenone. SBSE was the most sensitive test for β-damascenone, and HSSE was the least sensitive test. In general, similar trends in test sensitivity were observed for other flavor compounds.

At least two mechanisms have been proposed for the formation of β-damascenone in beer. Acid hydrolysis of precursors might explain the increase in β-damascenone during aging. β-Damascenone can be generated by acid-catalyzed conversion of polyols derived from enzymatic transformation of the carotenoid neoxanthin (4,9). Therefore, the pH of the beer may have a strong effect on the amount of β-damascenone that forms in beer during aging. β-Damascenone precursors also have been linked to sugars and could arise during aging through the chemical hydrolysis of glycosides.

With the observed increases in β-damascenone shown in the beer aged at 30°C for 12 weeks compared with the control beer, it is important to emphasize the extremely low odor threshold of this.

Fig. 5. Chemicals generated in heat-abused beer after aging at 30°C for 12 weeks as analyzed using stir bar sorptive extraction with gas chromatography/time-of-flight mass spectrometry. Quantitation mass is shown in parentheses after each chemical name on the y-axis.

Fig. 6. Changes in levels of β-damascenone in control, heat-abused, and light-abused beers analyzed by stir bar sorptive extraction (SBSE), headspace sorptive extraction (HSSE), purge and trap (P&T), and dynamic headspace closed-loop stripping (DHSCLS) methods.

### TABLE II
Concentrations of 14 Flavor Chemicals in Control, Heat-Abused, and Light-Abused Beers

<table>
<thead>
<tr>
<th>Name</th>
<th>R.T. (sec)</th>
<th>Quant. Mass (ppb)</th>
<th>Control</th>
<th>Heat Abused</th>
<th>Light Abused</th>
<th>Average Std. Dev. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentanal</td>
<td>150</td>
<td>58</td>
<td>&lt;2.5</td>
<td>&lt;2.5</td>
<td>&lt;2.5</td>
<td>...</td>
</tr>
<tr>
<td>Hexanal</td>
<td>215</td>
<td>56</td>
<td>&lt;2.5</td>
<td>&lt;2.5</td>
<td>&lt;2.5</td>
<td>...</td>
</tr>
<tr>
<td>Furfural</td>
<td>242</td>
<td>96</td>
<td>89</td>
<td>1,007</td>
<td>369</td>
<td>8.3</td>
</tr>
<tr>
<td>1-Butanol, 3-methyl-, acetate</td>
<td>272</td>
<td>43</td>
<td>535</td>
<td>608</td>
<td>569</td>
<td>5.4</td>
</tr>
<tr>
<td>Methional</td>
<td>298</td>
<td>48</td>
<td>&lt;2.5</td>
<td>&lt;2.5</td>
<td>&lt;2.5</td>
<td>...</td>
</tr>
<tr>
<td>Hexanoic acid, ethyl ester</td>
<td>378</td>
<td>88</td>
<td>66.8</td>
<td>74.1</td>
<td>68.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Octanal</td>
<td>382</td>
<td>57</td>
<td>3.17</td>
<td>6.25</td>
<td>3.04</td>
<td>3.6</td>
</tr>
<tr>
<td>Benzenacetaldehyde</td>
<td>419</td>
<td>91</td>
<td>2.33</td>
<td>2.54</td>
<td>3.80</td>
<td>4.9</td>
</tr>
<tr>
<td>Nonanal</td>
<td>472</td>
<td>57</td>
<td>2.59</td>
<td>5.18</td>
<td>3.10</td>
<td>6.5</td>
</tr>
<tr>
<td>Phenyl ethyl alcohol</td>
<td>487</td>
<td>91</td>
<td>5,491</td>
<td>5,917</td>
<td>6,268</td>
<td>11.0</td>
</tr>
<tr>
<td>2-Nonenal, (E)-</td>
<td>523</td>
<td>83</td>
<td>0.43</td>
<td>0.39</td>
<td>0.37</td>
<td>4.9</td>
</tr>
<tr>
<td>Ethyl octanoate</td>
<td>555</td>
<td>70</td>
<td>169.0</td>
<td>162.2</td>
<td>141.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Decanal</td>
<td>566</td>
<td>112</td>
<td>0.71</td>
<td>0.88</td>
<td>0.81</td>
<td>3.9</td>
</tr>
<tr>
<td>Ethyl decanoate</td>
<td>720</td>
<td>88</td>
<td>42.3</td>
<td>37.1</td>
<td>18.8</td>
<td>4.2</td>
</tr>
</tbody>
</table>

a Heat-abused beer = control beer abused by heat (12 weeks of storage at 30°C), and light-abused beer = control beer abused by exposure to sunlight for 8 hr (analytical method was stir bar sorptive extraction with gas chromatography/time-of-flight mass spectrometry).
b Peak retention time.
c Ion mass used for peak integration.
d Standard deviation = (Σd²/2n)¹/².
compound and its likely strong impact on aging off-flavors. For example, based on olfactometry studies, Gus et al. (7) reported a rhubarb, red fruit, strawberry odor for β-damascenone and determined that it was among the most intense odor-active compounds in aged beer, with approximately the same odor impact as 3-methyl-2-buten-1-thiol and significantly more odor impact than t-2-nonenal. They concluded that 3-pyridin-carboxylic acid ethyl ester probably should not be considered a flavor active in aged beer based on olfactometry studies. In this study, extraction of flavor volatiles from beer was performed on 50 mL of beer with XAD-2 resin, followed by washing of the resin with water, elution of extracted flavor compounds with ethyl ether, drying with anhydrous sodium sulfate, and, finally, concentration to 0.5 mL.

Another chemical that increased significantly in heat-abused beers was furfuryl ethyl ether. The impact of this chemical on beer off-flavor development may be significant. For example, Vanderhaegen et al. (14) attributed the development of a solvent-like stale flavor of aged beer to furfuryl ethyl ether. The rate of production of this important flavor compound is proportional to the concentration of furfuryl alcohol in beer. According to this research team, the precursors of furfuryl ethyl ether are ethanol and furfuryl alcohol, which mainly derive from reactions between amino acids and sugars in boiling wort. Because beer is slightly acidic, furfuryl alcohol can acquire an extra proton and react with ethanol to form an intermediate compound that dehydrates and splits off another proton to become furfuryl ethyl ether. The researchers discovered that boiling the wort at higher temperatures for longer times produced more furfuryl alcohol. The chemical or solvent flavor from furfuryl ethyl ether has a flavor threshold in beer of approximately 6 ppb.

In our studies, the chemical that increased most significantly in the heat-abused beer compared with the control beer was furfuryl hydroxymethyl ketone (Fig. 5). This significant increase in furfuryl hydroxymethyl ketone in heat-abused beer is noteworthy. This compound has been identified as a major flavor contributor to strawberry jam (but not fresh strawberries) (2). We were unable to find any literature reports of this compound detected in beer. Further investigations, such as olfactometry and model system studies, should be conducted to evaluate the contribution of this compound to the staling flavor of beer.

Increases in aldehyde levels were relatively insignificant to off-flavor development considering the relatively high flavor threshold levels of most of the alkanals. t-2-Nonenal, which has been implicated as a major off-flavor contributor in aged beer and has a low flavor threshold in beer of 0.11 ppb (16), was found at relatively constant levels in control, heat-abused, and light-abused beers. Clearly, as the results shown in Table III and Figures 5–7 demonstrate, there is more to staling off-flavor development in beer than can be explained simply on the basis of increases in the concentrations of t-2-nonenal and other aldehydes.

The most significant changes in the light-exposed beer samples versus the control were the formation of dimethyl disulfide and dimethyl trisulfide in the light-exposed beer (Fig. 7). Benzenacetaldehyde concentration increased significantly in the light-exposed sample. 3-Methyl-2-buten-1-thiol (MBT), a compound widely known to be a major contributor to the skunky odor of light-damaged beer, was not detected in the light-exposed sample. It likely was present in the light-abused sample at levels too low to be detected by the analytical methods used but high enough to be perceived by smell. SBSE combined with a detector more sensitive for sulfur compounds (pulsed flame photometric detector) previously was reported as detecting MBT in beer (6). Another approach for improving the detection limit of MBT would be to extract 200 mL or more of beer sample, as previously reported (12).

Importance of GC-TOFMS Peak Deconvolution Capabilities

Accurate detection and quantitation of several key flavor compounds could not have been accomplished without the peak de-
convolution capabilities of the Pegasus gas chromatograph/time-of-flight mass spectrometer (Leco). More than 700 chemicals were detected in each beer sample tested. Peak deconvolution allowed for the detection and accurate quantitation of coeluting chemicals (Figs. 8–11). Without peak deconvolution, many of the important odor-active chemicals, which often occur at trace levels, undoubtedly would have been missed. The importance of peak deconvolution for β-damascenone, furyl hydroxylmethyl ketone, furfuryl ethyl ether, and t-2-nonenal are illustrated in Figures 8–11, respectively.

Fig. 8. Importance of peak deconvolution for detection and measurement of β-damascenone in heat-abused beer after aging at 30°C for 12 weeks and analysis using stir bar sorptive extraction with gas chromatography/time-of-flight mass spectrometry. R.T. = peak retention time.

Fig. 9. Importance of peak deconvolution for detection and measurement of furyl hydroxymethyl ketone in heat-abused beer after aging at 30°C for 12 weeks and analysis using stir bar sorptive extraction with gas chromatography/time-of-flight mass spectrometry. R.T. = peak retention time.
Fig. 10. Importance of peak deconvolution for detection and measurement of furfuryl ethyl ether in heat-abused beer after aging at 30°C for 12 weeks and analysis using stir bar sorptive extraction with gas chromatography/time-of-flight mass spectrometry. R.T. = peak retention time.

Fig. 11. Peak deconvolution for (Z)-2-nonenal showing caliper (nondeconvoluted), true, and library mass spectra for the control beer sample. SBSE = stir bar sorptive extraction with gas chromatography/time-of-flight mass spectrometry.
The nondeconvoluted and deconvoluted chromatograms for β-damascenone coeluting with ethyl 9-decanoate are shown in Figure 8. Each vertical line in the chromatogram denotes detection of a unique chemical peak by the ChromaTOF software. The TIC (Fig. 8A) shows what appears to be a single chemical eluting at a peak retention time of 711 sec. An expanded TIC view of the peak is shown in Figure 8B; note, it appears to be one chemical (i.e., one chromatographic peak). After deconvolution, however, the two components that comprise the peak are clearly evident (Fig. 8C).

Furfuryl hydroxymethyl ketone and ethyl heptanoate in the TIC chromatogram region between 463 and 471 sec are shown in Figure 9A. The deconvoluted peaks for this region of the chromatogram are shown in Figure 9B. Distinct Gaussian-shaped peaks are apparent for furfuryl hydroxymethyl ketone (467 sec) and ethyl heptanoate (469.3 sec), which can now be accurately integrated after peak deconvolution.

The detection and accurate integration of furfuryl ethyl ether, a potentially very significant off-flavor chemical, was particularly challenging in this sample. A cluster of small, poorly resolved trace peaks eluted in the TIC region of the chromatogram between 280 and 310 sec (Fig. 10A). An expanded TIC view of this region is shown in Figure 10B. After deconvolution (Fig. 10C), it became apparent that the peak occurring at 294 sec in Figure 10B actually comprises four components, one of which is furfuryl ethyl ether.

The benefits of the Pegasus gas chromatograph/time-of-flight mass spectrometer and peak deconvolution are further illustrated in Figure 11. The automatic peak find algorithm of the Pegasus ChromaTOF software was able to detect four closely eluting peaks: 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (peak no. 304), octamethyl-cyclotetrasiloxane (peak no. 305), ethyl octanoate (peak no. 306), and (Z)-2-nonenal (peak no. 307) (Fig. 11A). The peak apexes of ethyl octanoate and (Z)-2-nonenal nearly exactly coeluted (i.e., they were less than 500 ms apart). Spectral deconvolution of (Z)-2-nonenal from coeluting ethyl octanoate is illustrated in Figure 11B. The polysiloxane peak (peak no. 305) was a background peak contributed by the PDMS (note in the caliper nondeconvoluted) mass spectrum the 73, 88, and 101 ions contributed by ethyl octanoate and the 144 ion contributed by the pyranone (peak no. 304)). (Z)-2-Nonenal could be an important contributor to beer off-flavor. Accurate quantitation would be difficult for this chemical in this complex sample matrix without the software’s peak find and deconvolution capabilities.

As these examples illustrate, the problem of peak coelution is common in chromatograms of complex samples like beer. An excellent detailed explanation of peak deconvolution has been described previously (8).

CONCLUSIONS

After linear least squares correlation coefficients for the standard calibration curves of 14 flavor-contributing analytes found in beer showed that SBSE was the most accurate of the PDMS extraction methods investigated and precision studies showed that SBSE demonstrated acceptable precision for replicate analyses, SBSE chromatograms of control, heat-abused, and light-abused beers were compared to see what malodorous chemicals were generated by heat- and light-abuse conditions. This comparative study showed that increases in furfuryl compounds produced in Maillard reactions and β-damascenone may be more important than aldehyde formation in influencing off-flavors in aged beer. Additional sensory studies involving spiking of fresh-tasting control beer with β-damascenone, furfuryl ethyl ether, furyl hydroxymethyl ketone, and other compounds shown in Figure 5 should be conducted to determine the contribution of these chemicals to staling off-flavors in beer.

The application of TOFMS detection and peak deconvolution was critical for measuring low ppb levels of low-threshold off-flavor chemicals. As analytical flavor chemists apply increasingly sophisticated techniques that extract more analytes and higher concentrations of analytes, the problem of GC-MS peak coelution increases. The significant advantage of peak deconvolution was clearly illustrated for key off-flavor chemicals in beer.

The partnering of PDMS extraction techniques (particularly SBSE) with GC-TOFMS provides a new tool for detecting ultratrace levels of significant flavor-contributing chemicals in beer. Combining this new tool with olfactometry experiments, model system sensory studies, chemometrics, and other techniques available to beer researchers should provide the industry with a better understanding of the chemicals that most influence staling off-flavors in beer, how they form, and how they may be reduced.

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LITERATURE CITED