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Application of Factorial Designs To Study Factors Involved in the Determination of Aldehydes Present in Beer by On-Fiber Derivatization in Combination with Gas Chromatography and Mass Spectrometry

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ABSTRACT: With the aim of studying the factors involved in on-fiber derivatization of Strecker aldehydes, furfural, and (*E*)-2-nonenal with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine in beer, factorial designs were applied. The effect of the temperature, time, and NaCl addition on the analytes' derivatization/extraction efficiency was studied through a factorial 2^3 randomized-block design; all of the factors and their interactions were significant at the 95% confidence level for most of the analytes. The effect of temperature and its interactions separated the analytes in two groups. However, a single sampling condition was selected that optimized response for most aldehydes. The resulting method, combining on-fiber derivatization with gas chromatography−mass spectrometry, was validated. Limits of detections were between 0.015 and 1.60 μg/L, and relative standard deviations were between 1.1 and 12.2%. The efficacy of the internal standardization method was confirmed by recovery percentage (73−117%). The method was applied to the determination of aldehydes in fresh beer and after storage at 28 °C.

KEYWORDS: factorial design, aldehydes, beer, on-fiber derivatization, PFBHA

INTRODUCTION

A group of volatile aldehydes, some derived from the reaction of reducing sugars and amino acids and others generated from the degradation of fatty acids, may be produced during storage of beer on the shelf, thus deteriorating its flavor. Until relatively recent, Strecker aldehydes, derived from the decarboxylation and deamination of amino acids, were mainly considered as indicators of beer aging. Strecker aldehyde concentration increased significantly during beer storage, but the individual compounds rarely exceeded their flavor thresholds.[1] However, the latest studies have shown that Strecker aldehydes are important contributors to the formation of aged flavors in beers.[2−4] Saison et al.[2] demonstrated that the addition of a mixture of Strecker aldehydes to a fresh beer at concentration levels found in aged beers contributed to the formation of aged notes (caramel, burnt, bread, cardboard, and stale-sulfury), suggesting important interactions between these aldehydes for the formation of the aged flavor. Other researchers[3,4] have also found important flavor interactions between Strecker aldehydes, suggesting that they are not only indicators of beer aging but important contributors to flavor deterioration. Another relevant aldehyde is (*E*)-2-nonenal, which is produced from the oxidative degradation of fatty acids. There are differing results in the literature regarding (*E*)-2-nonenal influence on beer taste. Some authors[5] found that the concentration of this aldehyde does not increase significantly during beer aging. Most recent studies have demonstrated that (*E*)-2-nonenal is responsible for the development of cardboard flavor[2,3] and, as a consequence, does contribute to the formation of the aged flavor.[2,4] Furthermore, furfural, which is formed from the degradation of reducing sugars, has been considered to be a good indicator of beer exposure to high temperatures.[6]

The concentrations of the above group of aldehydes in beer are very low, at trace levels; thus, their determination requires decreasing the interferences caused by most volatile compounds present in the beer matrix. An appropriate method for achieving this is selective derivatization of the carbonyl moiety. Different reagents have been used to derivitize the carbonyl group; among them *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA) has been shown to be more reactive than pentafluorophenylhydrazine (PFPH), and the resulting derivatives of PFBHA derivatization are more volatile than those of PFPH.[7] The former properties explain the extensive use of PFBHA as a carbonyl derivatizing reagent in beer. Furthermore, the combined use of derivatization and solid phase microextraction (SPME) has the advantage of decreasing analysis time when compared to the many steps associated with derivatization followed by liquid−liquid extraction. Three combinations of derivatization and SPME have been reported in the literature: (1) derivatization in the sample matrix followed by SPME extraction, either by headspace or by direct immersion;[8−12] (2) simultaneous extraction and derivatization in the sample headspace;[11,13−17] and (3) derivatization in the injection port.[18]

The most convenient combination for the determination of aldehydes in beer is simultaneous extraction and derivatization (on-fiber derivatization), which is very simple and easy to apply.[13,14,17] In these works, the derivatizing reagent, PFBHA, is loaded on the fiber coating prior to analyte sampling. When the

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analyte reaches the coating, it reacts to produce the derivatives, syn and anti, directly on the fiber.

On-fiber derivatization combined with gas chromatography and mass spectrometry has provided appropriate selectivity (characteristic ions) and sensitivity (high ion abundances) in the electron impact mode as well as in the negative chemical ionization mode for the quantification of some aldehydes in beer. It has been reported that methional is one of the most difficult to detect aldehydes in beer.[14] This may be related to the analyte's intrinsic properties such as volatility, Henry's law constant, and molecular structure, which may affect its extraction and derivatization processes. Given that each analyte will show a different behavior during extraction and derivatization,[13] it is necessary to investigate the factors that affect these processes, and their overall significance, to optimize the analyte response. Most studies[8,13,14] have used a univariate approach to determine the conditions that improve the efficiency of the analyte extraction. However, univariate analysis has two main limitations.[19,20] First, it involves many experiments because each factor is optimized one at a time. Second, it does not allow the determination of whether the factors have interactions or not; this may lead to erroneous interpretations of analyte behavior and to the establishment of sampling conditions far from the optimum.

The objective of the present research was to study the factors that are involved in the process of simultaneous extraction and derivatization and their effect on the extraction efficiency of a group of aldehydes, Strecker aldehydes, furfural, and (*E*)-2-nonenal, with the aim of developing a reliable and rapid method for their quantification in beer. This was accomplished by the use of two experimental designs: (a) a factorial 2^2 design to study the effect of temperature and time on PFBHA fiber loading and (b) a factorial 2^3 randomized-block design to study the effect of temperature, time, and NaCl addition on the derivatization/extraction efficiency. It was of special interest to obtain a rapid method with low detection limits for the quantification of aldehydes in fresh beer and in beers at early stages of aging at mild temperatures (28 °C), when very low concentration increments are expected to occur in relation to the fresh beer. The analytical method was validated using internal standardization, which allowed rapid and simple quantification of the aldehydes in beer. Finally, the method was applied to determine the analytes in fresh beer and after storage at 28 °C for 15 and 30 days.

MATERIALS AND METHODS

Chemicals. 2-Methylpropanal (≥97.7%) and phenylacetaldehyde (98.7%) were purchased from Chem Service (West Chester, PA). 2-Methylbutanal (95%) and 3-methylbutanal (97%) were purchased from Sigma-Aldrich (Taufkirchen, Germany). 3-Methyl-2-butenal (97%) was obtained from Sigma-Aldrich (St. Louis, MO). 2-Furaldehyde (99%), methional, and (*E*)-2-nonenal (97%) were purchased from Aldrich (Milwaukee, WI). Benzaldehyde-d_6 (98%, +0.1% hydroquinone) was purchased from Cambridge Isotope Laboratories (Andover, MA). *O*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride (98%) was obtained from Aldrich (St. Louis, MO). Sodium chloride (99.5% p.a.) was purchased from Merck (Darmstadt, Germany). Phosphoric acid (85% w/w) was purchased from Mallinckrodt Baker (Phillipsburg, NJ). Ethanol (99.9%) was obtained from Merck. Ultrapure water was taken from a Milli-Q water Millipore purification system (Billerica, MA).

Standard Solutions. Aldehyde standard solutions were prepared in ethanol 99.9% at a concentration of 1000 mg/L and stored at −20 °C. Working solutions were prepared weekly from standard solutions using ethanol 5%. Standard solutions for optimization experiments and calibration were prepared on a daily basis from working solutions in ethanol (5%) and adjusted to pH 4.5 with H_3PO_4 (0.1%). Working solutions of PFBHA were prepared in Milli-Q water and stored at 0 °C.

SPME Fiber. SPME fibers with a 65 μm polydimethylsiloxane/divinylbenzene (PDMS-DVB) coating were purchased from Supelco (Bellefonte, PA). This fiber type was selected on the basis of prior research.[14]

Beer Samples. The samples were commercial Venezuelan Pilsner beer.

Instrumentation. Gas chromatography coupled with mass spectrometry (GC-MS) analysis was performed utilizing an Agilent Technologies 7890 GC instrument (Wilmington, DE) by using a capillary HP-5MS (30 m × 0.25 mm i.d., 0.25 μm, Agilent Technologies) and a split/splitless injector held at 230 °C. Derivatized analytes desorption from the SPME fiber was performed during 2.5 min in splitless mode. The initial oven temperature was 40 °C, and the temperature was raised to 230 °C at a rate of 6 °C/min, giving a run time of 31.67 min. The carrier gas was helium at a constant flow rate of 1.3 mL/min. MS analysis was performed with an Agilent Technologies 5975C mass selective detector in the electron impact mode (70 eV), and the transfer line was kept at 280 °C. Mass range was adjusted between m/z 170 and 350, and selective ion monitoring (SIM) mode was used for aldehyde quantification.

On-Fiber Derivatization Procedure. Simultaneous extraction and derivatization comprises two steps: (1) PFBHA loading on the PDMS-DVB fiber, by exposing the coating to the headspace PFBHA solution, and (2) SPME sampling, by exposing the loaded fiber to sample headspace. Optimization of the main factors affecting both steps was carried out by means of the experimental designs described below.

Optimization of PFBHA Loading. Before the optimization of PFBHA loading, the appropriate concentration of the derivatizing reagent was determined. Different concentrations of PFBHA, ranging from 0.06 to 8 g/L, were assessed. PFBHA solutions (10 mL) were added to glass vials (20 mL) with Teflon−silicone caps and placed in a stirring hot plate (240 rpm, 40 °C). The SPME fiber (PDMS-DVB) was exposed to the headspace of the vial for 5 min and desorbed at the GC injection port. PFBHA loading effectiveness was evaluated by its chromatographic peak area. Loading factors, temperature and time, were subsequently evaluated by a factorial 2^2 experimental design, at the PFBHA concentration selected in the earlier experiment. For this experiment, loading temperature and loading time were varied at the two levels selected for the design: 30 and 40 °C and 5 and 10 min, respectively.

Optimization of SPME Sampling Conditions. Three factors were selected as potentially affecting the derivatization/extraction efficiency, namely, temperature, extraction time, and NaCl addition. To study the influence of these factors and their possible interactions, a factorial 2^3 randomized-block experimental design was chosen. Response variables were the areas of the derivative aldehydes (syn and anti oximes). The experimental design was applied to both beer samples and aldehyde standard solutions (5% ethanol). For this experiment, temperature, extraction time, and NaCl addition were varied at the two levels selected for the design: 30 and 50 °C, 10 and 30 min, and 0 and 2.6 M, respectively.

Aldehyde standard solutions (2-methylpropanal, 2-methylbutanal, and 3-methylbutanal, 15 μg/L; furfural, 50 μg/L; (*E*)-2-nonenal, 0.1 μg/L; methional and phenylacetaldehyde, 20 μg/L) or beer samples (20 mL) were added to amber glass vials (40 mL) with Teflon−silicone caps, spiked with internal standard (30 μg/L, 3-methyl-2-butenal), and placed in a stirring hot plate (240 rpm). Each sample was equilibrated at the sampling temperature for 20 min and subsequently extracted according to the corresponding treatments specified in the design matrix.

Extraction Time Profiles. Extraction time profiles were studied for each of the analytes in standard solutions (ethanol 5%) as well as in beer, from 2 to 50 min, at the temperature and salt concentration selected in the experimental design. Beer samples were stored at 38 °C for 3 months before the study, to increase the aldehyde concentration

Table 1. Fragments Used for Aldehydes Quantification

PFBHA derivatized aldehyde	molecular mass (g/mol)	retention time (min)	ions *m/z*
2-methylpropanal	267	12.75	181, 195, 250
2-methylbutanal	281	14.70	181, 239
3-methylbutanal	281	15.05	181, 239
3-methyl-2-butenal (IS[a])	279	17.50	181, 264, 279
furfural	291	18.50	291, 248
methional	299	20.44	299, 252
benzaldehyde-d_6 (IS)	307	21.85	277, 307
phenylacetaldehyde	315	22.90	181, 297, 315
(*E*)-2-nonenal	335	24.40	181, 250

[a] IS, internal standard.

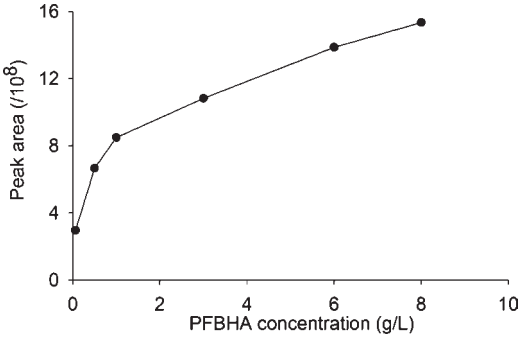

Figure 1. Effect of PFBHA concentration on its chromatographic peak area. Loading temperature, 40 °C; loading time, 5 min.

so that aldehyde spiking was not necessary before the analysis. Standard solution concentration and sample preparation were the same as in the above section. Each sample was equilibrated at the sampling temperature for 20 min and extracted at the different time points.

Method Validation. To determine the quality of the method, linearity, detection limit, repeatability, and recovery studies were performed for each analyte. Linear dynamic ranges were determined by external and internal standardization. Standard calibration solution (20 mL) was added to amber glass vials (40 mL) with Teflon−silicone caps, spiked with internal standards (30 μg/L, 3-methyl-2-butenal; and 3 μg/L, benzaldehyde-d_6) and placed in a stirring hot plate (240 rpm). Each solution was equilibrated at the sampling temperature for 20 min and subsequently extracted according to selected extraction conditions. Detection limits were determined according to IUPAC;[21] the lowest analytical signal, y_L, was given by the equation $y_L = y_b + ks_b$. In this equation, y_b is the mean of the blank measures, s_b is their standard deviation, and k is a numerical factor chosen according to the confidence level desired. In the present case, $k = 3$, corresponding to a 95% confidence level. For the detection limits estimation, 10 measures on blank solutions (5% ethanol, at pH 4.5 adjusted with 0.1% H_3PO_4, and spiked with the internal standards) were performed.

Repeatability study was performed using standard solutions at two different concentration levels with five replicates. For recovery experiments, beer samples were spiked with aldehyde standard solutions at two concentration levels.

Table 2. 2^2 Factorial Design Matrix and Response Values[a]

run	temperature	time	peak area (/10^7)
1	−1	1	201.0
2	1	−1	209.2
3	−1	−1	172.8
4	−1	1	191.5
5	1	1	217.2
6	1	1	219.6
7	1	−1	210.2
8	−1	−1	192.4
−1	30 °C	5 min	
1	40 °C	10 min	

[a] Matrix generated by Minitab statistical software, two replicates.

RESULTS AND DISCUSSION

Aldehyde Identification. Compound identification was performed by mass spectrometry and electronic impact ionization in scan mode through the injection of each aldehyde standard derivatized with PFBHA. The quantification of the different aldehydes was carried out in the selective ion monitoring mode using combinations of the most characteristic ions and/or the most abundant (see Table 1). The sum of the syn and anti oxime areas for each aldehyde was considered as a chromatographic response. Furfural, methional, and benzaldehyde-d_6 coelutions were detected when the m/z 181 ion was used; therefore, minority ions were selected to monitor the oximes of these aldehydes.

PFBHA Loading Optimization. To determine the concentration of PFBHA solution that optimizes the loading of this reagent on the PDMS-DVB fiber, the effect of increasing PFBHA concentrations on its response area after 5 min of exposure at 40 °C was studied. Figure 1 shows that a PFBHA concentration of 6 g/L allows a fiber loading close to its maximum loading capacity. This concentration was then selected for the rest of the study.

The effects of temperature and time on PFBHA loading were studied using the 2^2 factorial design matrix shown in Table 2. The response variable of this experiment, PFBHA chromatographic area, is also presented in Table 2.

The analysis of variance (ANOVA) was applied to these results, and the validity of this model assumption was confirmed. The Anderson−Darling test and the normal probability plot of the residuals confirmed the normality of the errors. Bartlett's test was applied to assess the homogeneity of variances, and the plot of residuals versus the order of data confirmed the independence of residuals. The ANOVA showed that only the temperature had a P value of <0.05, indicating that it has a significant effect on the PFBHA loading. Temperature showed a positive effect on PFBHA peak area, indicating that an increase in the loading temperature produced a greater amount of derivatizing reagent being loaded on the fiber. Because time was not found to be a significant factor, a loading time of 5 min was chosen for the experiment. Consequently, the optimized conditions for PFBHA loading on the PDMS-DVB fiber were a temperature of 40 °C and a loading time of 5 min, using a 6 g/L PFBHA solution.

SPME Sampling Optimization. To study the influence of temperature, extraction time, and NaCl addition on the areas of the derivative aldehydes, a factorial 2^3 randomized-block experimental

Table 3. 2^3 Factorial Design Matrix[a]

treatment	blocks	temperature	time	NaCl
1	1	−1	−1	−1
2	1	1	−1	1
3	1	1	1	−1
4	1	1	1	1
5	1	−1	−1	1
6	1	−1	1	−1
7	1	−1	1	1
8	1	1	−1	−1
9	2	1	1	1
10	2	1	1	−1
11	2	1	−1	−1
12	2	−1	1	−1
13	2	1	−1	1
14	2	−1	−1	1
15	2	−1	1	1
16	2	−1	−1	−1
17	3	1	−1	1
18	3	−1	−1	−1
19	3	1	1	1
20	3	−1	−1	1
21	3	1	1	−1
22	3	−1	1	−1
23	3	−1	1	1
24	3	1	−1	−1
−1		30 °C	10 min	0
1		50 °C	30 min	2.6 M

[a] Generated by Minitab statistical software.

design was applied, being repetitions of the blocks of the experiments. The number of repetitions (r) was calculated according to the equation $(T-1)(r-1) \geq 12$, where T is the number of treatments (2^3). Given that the factorial design with the three repetitions could not be performed in a single day, the complete design was executed in three blocks (one per day). This design allowed the elimination of "days" as a source of variability. Because each block consisted of a repetition of the experiment, it was possible to estimate all effects on the response variable. Table 3 shows the design matrix.

The validity of the model was confirmed by ANOVA assumptions for the responses of each oxime, as performed for PFBHA loading optimization. The results of this analysis are presented in Table 4. The experimental design was applied to beer samples as well as to aldehyde standard solutions (5% ethanol).

The design allowed assessment of main effects, interactions between them, and the block effect (day). Data processing was performed using Minitab statistical software. Results, as presented in Table 4, show that temperature, time, and NaCl addition were all significant for the vast majority of analytes; moreover, most interactions were also significant.

Temperature showed a negative effect for highly volatile aldehydes and a positive effect for the less volatile ones. This was confirmed by the time−temperature interaction results. In Figure 2a, referred to 2-methylbutanal, it can be observed that a sampling time increase causes a significant accretion of the oxime

Table 4. Main Effects, Interactions between Factors, and Block Effect for Every Analyte in Beer and Aldehyde Standard Solutions (5% Ethanol) for the Optimization of SPME Sampling[a]

		main effects			interactions				
					salt		t	T t	block
compound	matrix	T	t	salt	T	t T	salt	salt	effect
2-methylpropanal	1	−	+	+		−	+		yes
	2	−	+	+	−	−	+		yes
2-methylbutanal	1	−	+	+		−			yes
	2	−	+	+	−	−	+	−	yes
3-methylbutanal	1	+	+	+		+	+		no
	2		+	+		−	+	−	yes
3-methyl-2-butenal	1	−	+	+		−	+		yes
	2	−	+	+		−			no
furfural	1	−	+	+		−	+		yes
	2	−	+	+		−			no
methional	1	+	+	+	+	+	+	+	no
	2	+	+	+	+	+	+	+	no
phenylacetaldehyde	1	+	+	+	+	+	+	+	no
	2	+	+	+	+	+	+	+	no
(*E*)-2-nonenal	1	+	+	+	+	+	+		yes
	2	+	+	+	+	+	+		yes

[a] Factors: T, temperature; t, time; salt, NaCl addition. Internal standard: 3-methyl-2-butenal. Matrix: 1, aldehyde standard solution; 2, beer. Significant effects at 95% confidence: +, positive effect; −, negative effect.

response at lower temperature. A similar behavior was observed for 2-methylpropanal, 3-methyl-2-butenal, and furfural. Conversely, with phenylacetaldehyde (Figure 2b) the oxime response was enhanced significantly at higher temperature. This behavior was also observed for methional and (*E*)-2-nonenal. The effect of temperature on the oxime response was positive for 3-methylbutanal only in the standard solution, but its effect was not significant when it was in beer. This behavior was exceptional considering that this aldehyde is a volatile compound. However, the interaction plots for this analyte (Figure 4c,d) revealed an important interaction between temperature and time, which indicates that 3-methylbutanal response is also enhanced at lower temperature and longer extraction time.

Time and NaCl addition were found to have a positive effect on the oxime response for all analytes in both beer and standard solution. In the case of NaCl addition, the result indicates that an increase in the ionic strength of the matrix will increase the distribution constant of the aldehydes to the headspace; this is known as a salting-out effect. NaCl addition showed a positive interaction with sampling time, which implies that oxime response significantly increases at longer sampling time when NaCl is present in the matrix. A significant interaction between NaCl addition and temperature was also observed, which was dependent

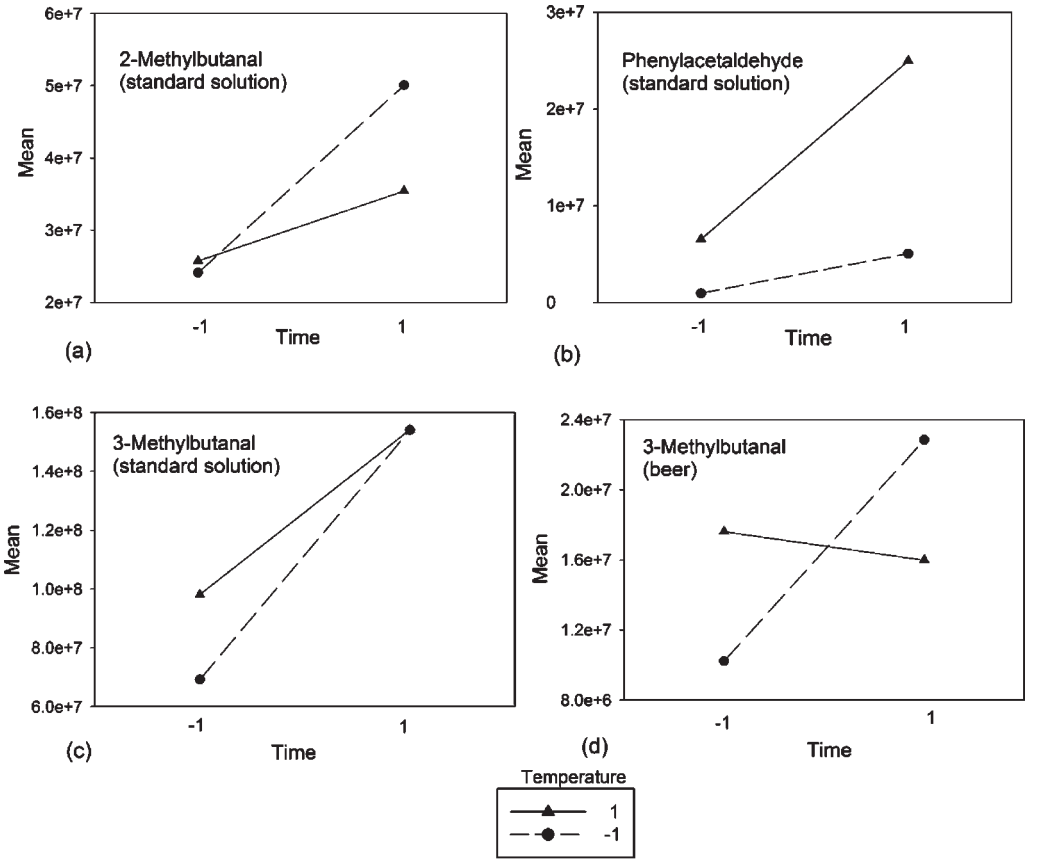

Figure 2. Time−temperature interaction plots in aldehyde standard solutions (a−c) and beer (d).

on aldehyde and/or oxime volatility. For highly volatile aldehydes, a negative interaction was observed in beer, whereas a positive interaction was obtained for the lower volatile aldehydes, both in beer and in standard solution. This behavior confirms the observed effect of the temperature on oxime response as well as the effect of the interactions between temperature and other factors.

The applied design revealed that temperature and its interactions with other factors allowed separating the analytes into two groups according to the significance, positive or negative, regardless of the matrix, beer or standard solutions. The first group, with negative significance, comprises the higher volatile aldehydes: 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, 3-methyl-2-butenal, and furfural. The vapor pressures of these aldehydes are between 1 and 2 orders of magnitude greater than those of aldehydes from group 2.[22] The same tendency would be expected to occur in the corresponding PFBHA derivatives. The former would explain, for aldehydes in group 1, the decrease of oxime responses at higher temperature, probably as a result of oxime desorption from fiber. The second group, with positive significance of temperature and its interactions, comprises the lower volatile aldehydes: methional, phenylacetaldehyde, and (*E*)-2-nonenal. For this group, with low vapor pressures, an increase of temperature seems to favor the distribution equilibrium of the aldehydes toward the headspace with no effect on oxime desorption from fiber, thus obtaining an increase in oxime response.

Statistical analyses demonstrated that, for most analytes, the effect of the day was significant (Table 4, block effect), indicating the relevance of the application of the randomized-block design. Additionally, the selection of the appropriate number of repetitions assured the precision of the study of the factors and their interactions in the process of aldehyde simultaneous extraction and derivatization.

Even though the study suggests that aldehydes in beer shall be determined at two different sampling temperatures, according to their vapor pressure, this would significantly increase analysis time and reagent consumption. Consequently, the use of a single condition for all aldehydes is proposed, performing the extraction and derivatization at 30 °C during 30 min, in the presence of NaCl. These conditions optimize the response of the higher volatile aldehydes, including the internal standard, but reduce the extraction efficiency of phenylacetaldehyde, methional, and (*E*)-2-nonenal.

Extraction Time Profiles. Extraction time profiles allowed the formation rate of the different derivatives, in beer and standard solutions, to be examined under the sampling conditions established in the previous section. It was of special interest to determine whether the formation of the oximes reaches a plateau during the sampling time. Figure 3 shows the extraction time profile for the different analytes. It can be seen that no plateau was attained for any of the analytes during the 50 min of analysis. For 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, and furfural in beer, the formation rate was faster during the first 10 min and progressively slowed through 40 min. In standard solution, the formation rate for these analytes was slower than in beer. A different behavior was observed for phenylacetaldehyde, methional, and (*E*)-2-nonenal; in beer and in standard solution derivatives the formation rate was almost constant throughout the 60 min of sampling time.

When the formation rates of the different groups of analytes are compared, it is observed that oximes of highly volatile

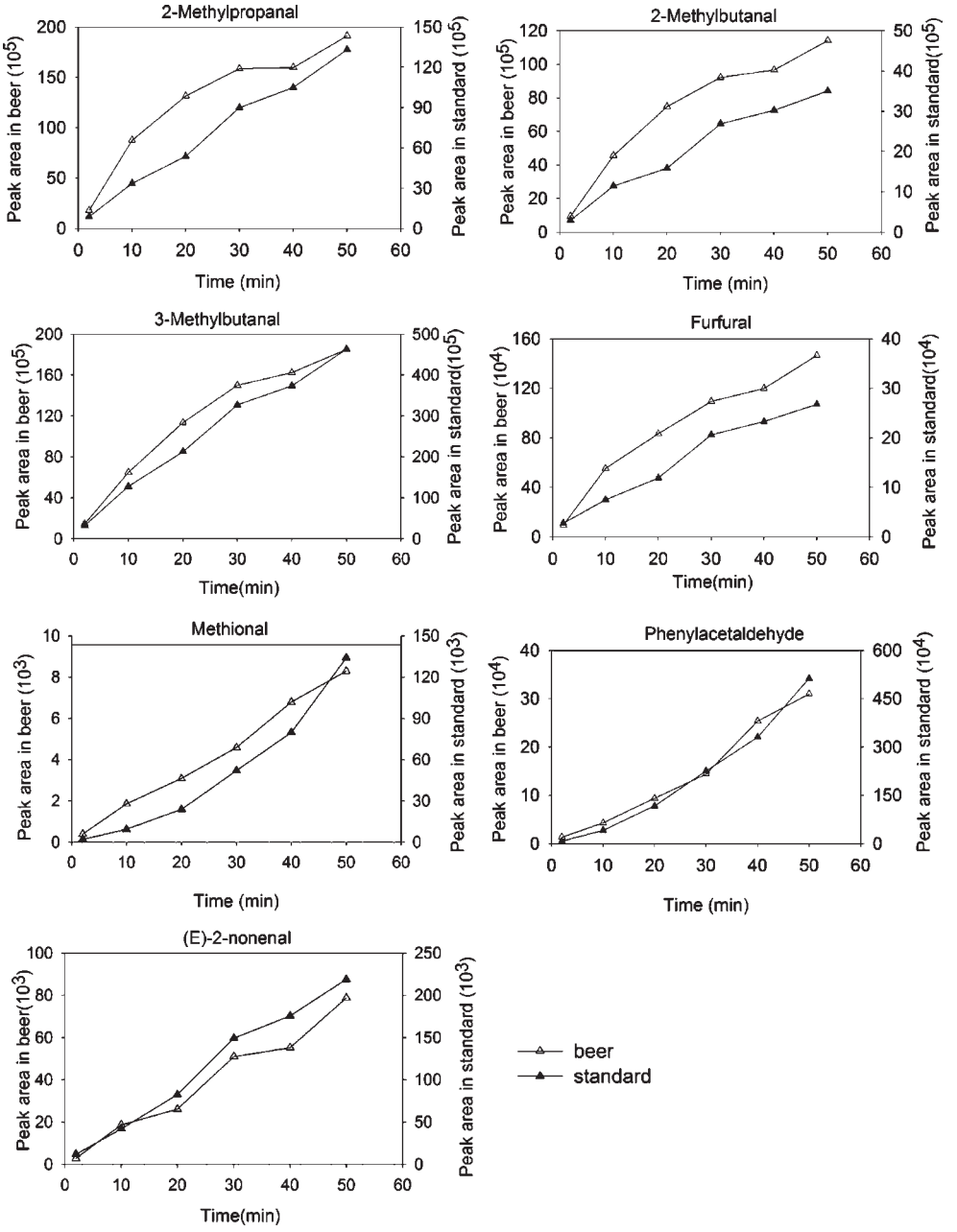

Figure 3. Extraction time profiles for the oximes of aldehydes in beer and standard solutions. Extraction temperature: 30 °C, 3 g (2.6 M) NaCl.

aldehydes are formed at a higher rate than those of methional and phenylacetaldehyde, in both beer and standard solution. The same behavior was observed for these analytes in a previous study.[13] This behavior can be explained by the differences in aldehyde affinity for the aqueous solution. It is expected that methional and phenylacetaldehyde have a greater affinity for the aqueous solution, given their lower Henry's law constant. In the case of the higher volatile aldehydes, they distribute rapidly from the aqueous solution to the headspace during the first minutes of extraction. Then the oxime formation rate decreases, probably as a result of the reduction of aldehyde concentration in the headspace because of their reaction with PFBHA.

The extraction time profiles showed that a plateau condition was not observed for any of the analytes during the 50 min of sampling. This indicates that a precise control of the extraction time is critical for the quantification because small variations in it may lead to significant variations in oxime response areas. In the case of highly volatile aldehydes this factor is less critical given that their formation rate tends to be slower at the selected extraction time (30 min).

Method Validation. Method validation was performed under the sampling conditions established above. Table 5 shows quality parameters using internal standardization method. The linear dynamic range was determined for all analytes except for methional, phenylacetaldehyde, and (*E*)-2-nonenal, the upper limits of which were established at lower values than the actual linear dynamic range. However, dynamic ranges were broad enough to include aldehyde concentrations found in any beer.

The use of 3-methyl-2-butenal as internal standard allowed better correlation coefficients to be obtained for all aldehydes

Table 5. Linearity, Detection Limit, Quantification Limit, and Repeatability of the Optimized Method Using 3-Methyl-2-butenal as Internal Standard

	linearity				repeatability RSD (%) (n = 5)	
aldehyde	range (μg/L)	R^2	LOD (μg/L)	LOQ (μg/L)	low level	high level
2-methylpropanal	0.4−75	0.9978	0.11	0.40	2.7 (0.3)[a]	1.1 (75)
2-methylbutanal	0.1−50	0.9987	0.03	0.11	3.9 (0.1)	3.6 (50)
3-methylbutanal	0.3−50	0.9972	0.09	0.30	3.0 (0.5)	7.4 (50)
furfural	5−300	0.9993	1.60	5.20	5.4 (5)	9.1 (300)
methional	1−20	0.9941	0.30	1.00	7.6 (3)	8.0 (20)
phenylacetaldehyde	0.6−20	0.9975	0.23	0.76	12.2 (0.8)	6.3 (20)
		0.9982[b]	0.17[b]	0.55[b]	9.5 (0.8)[b]	7.8 (20)[b]
(E)-2-nonenal	0.05−0.25	0.9967	0.015	0.05	5.6 (0.04)	8.9 (0.25)

[a] The values in parentheses indicate the actual concentration (μg/L) of the aldehyde at each level. [b] Values corrected with benzaldehyde-d_6 as internal standard.

compared with the values obtained without this correction (data not shown). Additionally, the correction of the internal standard compensated for the loss of linearity observed for 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal. The use of an aromatic internal standard (benzaldehyde-d_6) was also tested for the correction of phenylacetaldehyde variations during extraction and derivatization steps. It can be observed in Table 5 that the use of benzaldehyde-d_6 also showed good linearity. No optimization experiments were performed for the use of this internal standard because it was available only at the final stage of the research.

In Table 5, the figures of merit for each aldehyde can be observed. Low detection and quantification limits were obtained for all aldehydes, considering the concentration of these analytes found in fresh beer. Twenty-eight percent lower limits were obtained for phenylacetaldehyde using benzaldehyde-d_6 as internal standard. Detection and quantification limits obtained in the present work are comparable to those reported in the literature.[13−16]

An estimation of the precision of the method is given by repeatability, which was determined at two concentration levels as shown in Table 5. Good relative standard deviation (RSD) values were obtained for all analytes, ranging from 1.1 to 12.2%, which indicate a good precision when compared with similar SPME methods.[10,13,14] The highest RSD value (12.2%) was obtained for the lower concentration level of phenylacetaldehyde when 3-methyl-2-butenal was used as internal standard. The use of benzaldehyde-d_6 improves this RSD value to 9.5%.

To evaluate the interference of beer matrix components in the analytical determination, the analyte recovery was determined at two aldehyde concentration levels,[23] and the results are shown in Table 6. Recovery values are reported using two calibration methods, internal and external standardization, to evaluate the effectiveness of internal standards. In Table 6, it can be noted that the use of internal standards significantly improves analyte recovery. Recovery percentage obtained by external calibration is in the range from 24 to 210%, whereas internal calibration recovery ranges between 73 and 117%. Recovery values below 85% were obtained only for 2-methylpropanal, 3-methylbutanal, phenylacetaldehyde (corrected with benzaldehyde-d_6), and (E)-2-nonenal at the lower concentration level. It is important to mention that phenylacetaldehyde determination can be performed using any of the two internal standards studied, 3-methyl-2-butenal or benzaldehyde-d_6. 3-Methyl-2-butenal showed to be a good internal standard for both aliphatic and aromatic aldehydes. Its effectiveness may be associated with the selected extraction conditions, which seem to be favorable for this compound.

Table 6. Recovery Results of Spiking Aldehydes in Beer Samples Using 3-Methyl-2-butenal as Internal Standard

		recovery (%)	
aldehyde	standardization method	low level	high level
2-methylpropanal	internal	79 (5)[a]	104 (20)
	external	24 (5)	57 (20)
2-methylbutanal	internal	88 (5)	85 (10)
	external	88 (5)	74 (10)
3-methylbutanal	internal	78 (5)	80 (20)
	external	89 (5)	72 (20)
furfural	internal	97 (50)	102 (100)
	external	92 (50)	97 (100)
methional	internal	87 (6)	90 (15)
	external	77 (6)	210 (15)
phenylacetaldehyde	internal	80 (5)[b]	90 (15)[b]
		87 (5)	100 (15)
	external	80 (5)	75 (15)
(E)-2-nonenal	internal	73 (0.05)	117 (0.15)
	external	74 (0.05)	99 (0.15)

[a] Values in parentheses indicate the spike concentration (μg/L). [b] Values corrected with benzaldehyde-d_6 as internal standard.

Quantification of Aldehydes in Beer. The analytical method was applied to determine the concentration of aldehydes in fresh beer and after storage at 28 °C for 15 and 30 days. Concentration

Table 7. Aldehyde Concentration in Fresh Beer and in Beer after Storage at 28 °C for 15 and 30 Days

	aldehyde concentration[a] (μg/L)		
compound	fresh beer	beer 15 days at 28 °C	beer 30 days at 28 °C
2-methylpropanal	1.90 ± 0.12	6.2 ± 0.4	7.7 ± 0.2
2-methylbutanal	0.58 ± 0.03	1.09 ± 0.05	1.16 ± 0.02
3-methylbutanal	0.58 ± 0.04	1.52 ± 0.05	1.88 ± 0.07
furfural	25.7 ± 1.5	28.9 ± 0.9	59.3 ± 4.2
methional	nq	1.0 ± 0.1	1.69 ± 0.04
phenylacetaldehyde	1.3 ± 0.3	1.45 ± 0.05	1.7 ± 0.1
(*E*)-2-nonenal	0.04 ± 0.01	0.0647 ± 0.0003	0.078 ± 0.002

[a] Concentrations are reported as means of triplicate experiments ± one standard deviation. nq, not quantifiable.

values are presented in Table 7. It was confirmed that the quantification limits for all aldehydes were satisfactory for the determination of these analytes at the low concentrations found in fresh beer, except for methional, the concentration of which was slightly below the quantification limit. The method can be applied for the study of the changes in aldehyde concentrations during the beer aging process.

We have demonstrated in this work that the application of factorial designs allowed, in a simple way, the study of factors involved in simultaneous extraction and derivatization and the selection of conditions for the determination of Strecker aldehydes, furfural, and (*E*)-2-nonenal in beer. Under the selected conditions, quality parameters of the internal standardization method were shown to be satisfactory for the determination of all analytes in beer, comparable to those obtained by the application of the standard addition method.[13,14] The application of the internal standardization method to the quantification of aldehydes in beer was consistent with the finding that the behavior of the analytes during the extraction/derivatization was similar in beer samples and in ethanolic standard solutions. The study of principal factors and their interactions was shown to be useful for comparing the behavior of the analytes in different matrices and could help in the selection of the most convenient standardization method.

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ABBREVIATIONS USED

PFBHA, *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine; PFPH, pentafluorophenylhydrazine; SPME, solid phase microextraction; PDMS-DVB, polydimethylsiloxane/divinylbenzene; SIM, selective ion monitoring; GC-MS, gas chromatography−mass spectrometry; ANOVA, analysis of variance; RSD, relative standard deviation.

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