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Identification and Quantification of Aldehydes in Mezcal by Solid Phase Microextraction with On-fiber Derivatization - Gas Cromatography

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Abstract. A headspace solid phase microextraction with on fiber derivatization procedure followed by gas chromatography and flame ionization detection was applied for the determination of aldehydes in mezcal. A derivatization agent o-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) was adsorbed onto a Polydimethylsiloxane/ divinyl benzene (PDMS/DVB, 65 μm) fiber and exposed to the headspace of a vial with a mezcal sample. The aldehydes selectively reacted with PFBHA, and the oximes were desorbed into a gas chromatograph injection port. Identification of the compounds was performed with standards and confirmed by mass spectrometry. The procedure was applied to the analysis of 13 mezcal samples from different agave varieties. Calibration curves for acetaldehyde and furfural showed good linearity ($r^2 > 0.99$) in the studied concentration range, 1-9 μg/mL for acetaldehyde and 0.5-1 μg/mL for furfural in the diluted mezcal samples. Good precision was obtained for acetaldehyde, CV <6.3% and furfural <10%. Based on the standard for tequila NOM-006-SCFI-2005, all mezcal samples showed concentrations of acetaldehyde within the norm, while 2 mezcals exceeded the allowed limit for furfural.
Keywords: SPME-GC-FID, SPME-fiber derivatization, aldehydes analysis, alcoholic beverages, mezcal.

Resumen. Se aplicó un método por microextracción en fase sólida (MEFS) con derivatización en fibra seguida de cromatografía de gases con detector de ionización de flama para la determinación de aldehídos en mezcal. El agente derivatizante o-(2,3,4,5,6-pentafluorobencil) hydroxylamina (PFBHA) se adsorbió previamente en la fibra de Polydimetilsiloxano/divinil benceno (PDMS/DVB, 65 μm) para MEFS y posteriormente se expuso al "headspace" de un vial que contenía la muestra de mezcal. Los aldehídos reaccionaron selectivamente con el agente derivatizante (PFBHA), y las oximas formadas fueron desorbidas en el puerto de inyección del cromatógrafo de gases. La identificación de los compuestos se realizó comparando los tiempos de retención de estándares con los obtenidos de las muestras y la confirmación de éstos se hizo utilizando espectrometría de masas. Con este procedimiento se analizaron 13 muestras de mezcal joven de diferentes variedades de agave. Las curvas de calibración para acetaldehído y furfural mostraron buena linealidad ($r^2 > 0.99$) en el rango de concentraciones estudiado, 1-9 μg/mL para acetaldehído y 0.5-1 μg/mL para furfural en la muestra de mezcal diluida. Se obtuvo buena precisión para acetaldehído, CV <6.3% y furfural <10%. De acuerdo con los límites establecidos en la norma del tequila NOM-006-SCFI-2005, todas las muestras analizadas mostraron concentraciones de acetaldehído dentro de estos límites y solo 2 muestras presentaron valores fuera de límite para furfural.
Palabras Clave: MEFS-CG-DILL, MEFS-derivatización en fibra, análisis de aldehídos, bebidas alcohólicas, mezcal.

Introduction

Mezcal is an alcoholic beverage obtained from different agave species (*A. salmiana*, *A. angustifolia, A. cupreata, A. karwinskii, A. insequidens, and A. potatorumand A. inaequidens)*, also it is a representative alcoholic beverage in the Mexican culture, with a remarkable economic and social importance, mainly in rural zones. After tequila, mezcal is the most important Mexican alcoholic beverage and its production is completely handcrafted.

This beverage is obtained by distillation of fermented juice from the agave plant. When 100% (w/v) of the sugars come from the agave juice, it is called "mezcal 100% Agave". Mezcals are beverages related to tequila (produced from A. *tequilana weber var. azul*). A full description of both processes and differences between mezcal and tequila has been previously published [1-6]. Fermentation is the most important part of the process since it is during this step that sugars are converted to ethanol and other compounds such as esters and organic acids; these compounds, along with other substances derived from the cooked agave, probably give the characteristic flavor and taste to mezcal. Although mezcal is a popular drink that is well-known in México, there are few studies on its chemical composition [7, 8].

Distilled and fermented beverages organoleptic characteristics depend on different compounds that originate from raw materials or are produced during fermentation. The organoleptic compounds produced by yeast during fermentation have the greatest impact on distilled alcoholic beverage smell and taste.

Carbonyl compounds, mainly aldehydes, as well as methanol, higher alcohols, esters and organic acids are common byproducts of the fermentation process during the production of alcoholic beverages. The final concentration of these compounds depends on the raw material quality and the fermentation conditions. Carbonyl concentration is an important feature because high concentrations are responsible for the unpleasant taste and aroma of alcoholic beverages and spirits; in addition, these compounds can bind to biological cores (proteins, enzymes, DNA), resulting in toxic, mutagenic and carcinogenic effects [9].

The concentration of carbonyl compounds in alcoholic beverages can vary from several hundreds of mg/L to ng/L. Usually, gas chromatography (GC) is the technique of choice

for the determination of these volatile compounds in complex matrices; however, because of their high polarity and reactivity, a derivatization step is necessary prior to chromatographic analysis [10]. There are relatively few studies on the determination of carbonyl compounds in alcoholic beverages. Recently, aldehydes and ketones have been evaluated in spirits, vodka, beer and wine [11-13], by forming oxime derivatives with o-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) followed by Gas Chromatography-Electron Capture Detector analysis [12]. However, there are no reports on aldehydes determination in mezcal using this derivatization reaction.

The aim of this project was to develop a rapid, selective, sensitive and solvent-free method for the analysis of aldehydes in mezcal by SPME-with on Fiber Derivatization-GC.

Results and Discussion

There are several reports on the determination of low molecular mass carbonyl compounds in aqueous samples, which are based on derivatization with PFBHA before extraction coupled to GC. In some of these reports [14-16], the main factors that affect the SPME of analytes, such as fiber selection, temperature extraction, salt effect, stirring and extraction time, were optimized. On this basis only some adjustments were made to optimize experimental parameters in the present work.

The product of the reaction of PFBHA with most aldehydes is an oxime, which is formed both as the syn- and as the anti- conformer. The two conformers have different retention times, so two peaks are attributed to the oxime derivative of the same compound. Figures 1 and 2 show the chromatograms obtained from the analysis of acetaldehyde and furfural in mezcal samples, respectively; it is clear that the resolution of the conformers is good enough. Other extraneous peaks present in chromatograms were identified as PFBHA reagent by-products or SPME fiber bleed (a blank of reagents was run under the same conditions as the samples). The identification of acetaldehyde and furfural oxime derivatives in the samples was done by comparison of retention times with derivatized standards using GC-FID, and confirmed by the fragment m/z 181 characteristic of carbonyl oxime derivatives using GC-MS.

Calibration

The linearity of the calibration graphs was tested with five calibration points over the expected concentration range of aldehydes in the diluted mezcal samples. For each concentration level, three independent measurements were made. Calculated linear regression equations for calibration curves were:

$y = 181.7\ x + 36.2 (r^2 = 0.997)$, RSD ($n=3$) < 10% for acetaldehyde

$y = 15.6\ x + 8.4 (r^2 = 0.996)$, RSD ($n=3$) < 12% for furfural

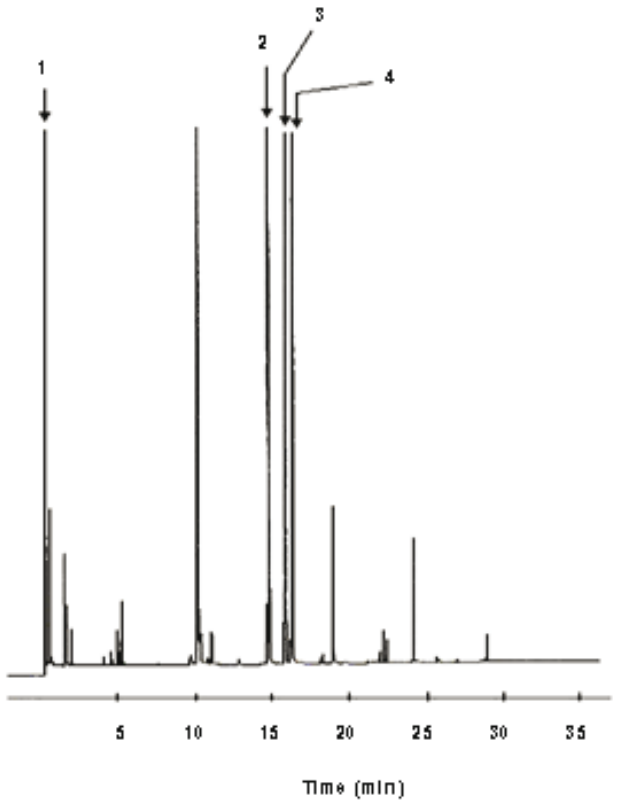

Figure 1. Gas chromatogram of a mezcal sample derivatized with PFBHA. Peaks: 1. ethanol; 2. PFBHA; 3 and 4 acetaldehyde oxime.

These results demonstrate good linearity and precision for both analytes in the studied concentration ranges. The limits of detection (LOD, s/n=3) were, 1 µg/mL and 0.02 µg/mL for acetaldehyde and furfural, respectively. Although the acetaldehyde LOD is not very low, no efforts were made to increase the method sensitivity because the concentration of this aldehyde is relatively high in this kind of alcoholic beverages as mezcal and tequila.

Mezcal analysis

Thirteen samples of mezcal (Table 1) were analyzed and the results are shown in Table 2 and Figure 3. The concentration of acetaldehyde was much higher than the concentration of furfural in all the studied mezcal samples. Because of this difference, the determination of both aldehydes was not performed simultaneously. Furfural concentration (in almost all the samples) was <10% of the acetaldehyde concentration; therefore, the extraction time for furfural was 30 min to increase the amount of recovered analyte for an accurate determination, whereas only 10 min were sufficient for acetaldehyde. SPME is not an exhaustive extraction technique, so, optimization and rigorous control of extraction time is critical for good method performance. Anyway, under the established experimental conditions, only samples 9, 11 and 12 showed furfural concentrations

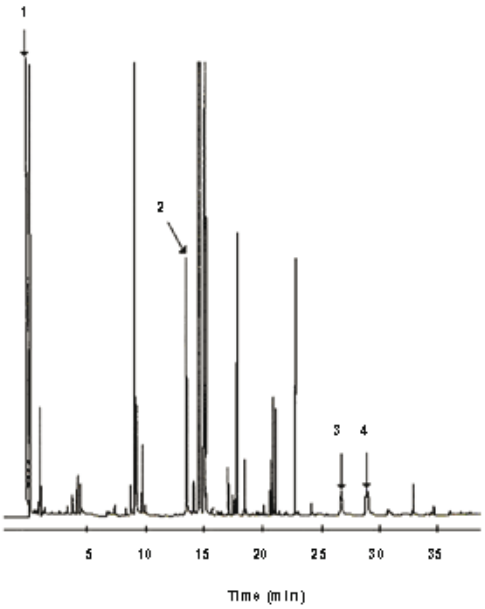

Figure 2. Gas chromatogram of a mezcal sample derivatized with PFBHA. Peaks: 1. ethanol; 2. PFBHA; 3 and 4 furfural oxime.

lower than the inferior limit of the calibration curve. Indeed, the method presented good precision for both compounds, with RSD lower than 10% in all mezcal samples.

The results of aldehydes quantification were compared with the limits set by the Mexican norm for tequila (NOM-006-SCFI-2005) [17], since the Mexican norm for mezcal does not mention a limit for these compounds (NOM-070-SCFI-1994) [18]. The limits are: 0-40 mg /100 mL of anhydrous alcohol for acetaldehyde and 0-4 mg /100 mL anhydrous alcohol for furfural. According to the determined concentrations (Table 2), acetaldehyde content in all analyzed mezcal samples was within the range established by norm, and only two samples (4 and 7) exceeded the allowed limit for furfural [17]. As expected, the range of acetaldehyde and furfural concentration in the mezcal samples was large, probably due to different agave species but also to the specific manufacturing process which is artisanal.

Conclusion

The headspace-SPME with on fiber derivatization procedure followed by GC-FID for the analysis of aldehydes in mezcal can be considered as a good alternative to the titration method proposed in the Mexican norm for tequila. The optimized method is faster, inexpensive, no solvents are consumed and sample manipulation is minimal. Determination of aldehyde concentration is very important for quality control in alcoholic beverages because these compounds can give undesirable flavors.

Experimental

Materials

Acetaldehyde standard 99% purity (Sigma-Aldrich, USA), furfural standard 99.2% purity (Chem. Service, USA) and the derivatization reagent o-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) 98% purity (Aldrich Chem, Co.) were used. Anhydrous absolute ethyl alcohol 99% purity (JT Baker) was doubly distilled to completely eliminate all residues of carbonyl compounds. All solutions were prepared with type 1 reagent water obtained from a Nanopoure deionizer (Barnstead Thermolyne). Polydymethylsiloxane-divinylbenzene (PDMS/DVB, 65 μm) fiber and the SPME holding device were purchased from Supelco (Bellefonte, PA, USA).

Table 1. Mezcal "Joven" samples.

Mezcal	State	Agave	% Ethanol
1	Chilapa, Guerrero	*A. cupreata*	53
2	Matatlán, Oaxaca	*A. angustifolia haw*	45
3	Nombre de Dios, Durango	*A. esperrima jacobi*	50
4	Ocotlán de Morelos, Oaxaca	*A. tripón*	49
5	Ocotlán de Morelos, Oaxaca	*A. largo*	49
6	Ocotlán de Morelos, Oaxaca	Mezcla	52
7	Ocotlán de Morelos, Oaxaca	*A. rruqueño*	49
8	San Sebastian, Jalisco	*A. inaequidens*	49
9	Mihuatitlán, Oaxaca	*A. karwinskii*	39
10	Sola de Vega, Oaxaca	*A. potatorum*	49
11	Chilapa, Guerrero	*A. cupreata*	49
12	Centro, Oaxaca	*A. angustifolia haw*	42
13	Ejutla de Cresco, Oaxaca	*A. americana*	52

Table 2. Concentration of acetaldehyde and furfural in mezcal samples determined by SPME with on fiber derivatization-GC-FID, $n = 3$.

	Acetaldehyde			Furfural		
Mezcal	µg/mL	%RSD	mg/100mL*	µg/mL	%RSD	mg/100mL*
1	120.9 ± 4.6	3.8	22.8	2.7 ± 0.3	9.7	0.5
2	77.7 ± 2.2	2.8	17.3	1.7 ± 0.03	1.8	0.4
3	35.8 ± 0.5	1.4	7.1	2.3 ± 0.2	7.9	0.5
4	154.4 ± 7.4	4.8	31.5	23.5 ± 1.7	7.2	4.8
5	121.7 ± 3.3	2.7	24.8	10.3 ± 0.7	6.5	2.1
6	97.3 ± 2.7	2.8	18.7	13.8 ± 0.1	0.8	2.7
7	94.5 ± 1.98	2.1	19.3	[a]33.8 ± 3.1	9.0	6.9
8	127.5 ± 6.6	5.2	26.0	8.9 ± 0.7	9.3	1.8
9	80.8 ± 0.9	1.1	20.7	NQ	—	—
10	99.9 ± 4.1	4.2	20.4	1.9 ± 0.06	3.1	0.4
11	126.6 ± 7.2	5.7	25.8	NQ	—	—
12	128.1 ± 3.1	2.4	30.5	NQ	—	—
13	80.1 ± 5.0	6.3	15.4	19.0 ± 1.3	6.9	3.7

* Anhydrous alcohol.
[a] Quantified by extrapolation outside the range of the calibration curve.
NQ non quantified.

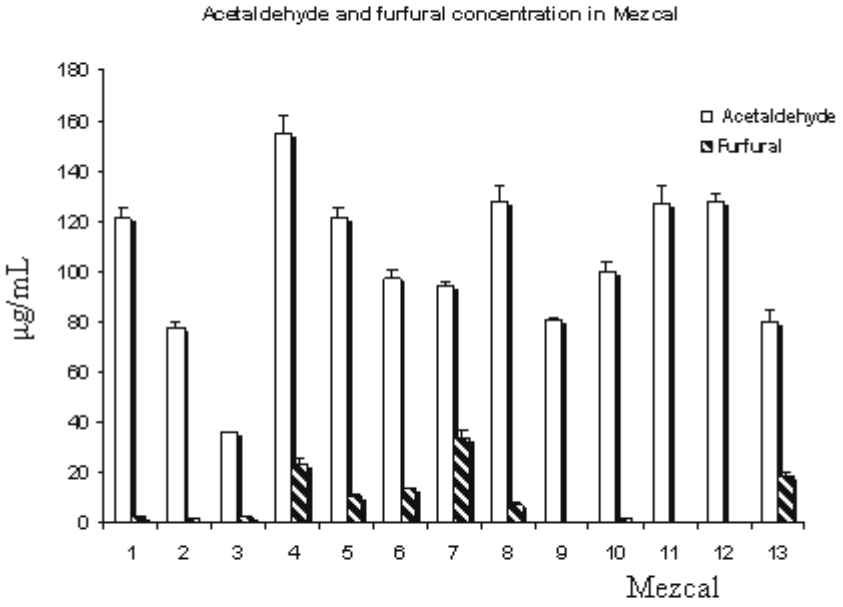

Figure 3. Comparison of acetaldehyde and furfural content (µg/mL) in the analyzed mezcal samples.

Samples

Thirteen samples of Mezcal "Joven" 100% Agave from different agave species were analyzed. All the samples were obtained from local producers from the states of Oaxaca, Guerrero and Durango. The characteristics of the samples are shown in Table 1.

GC Analysis

Analyses were performed on a Hewlett Packard 5880A Gas Chromatograph equipped with flame ionization detector and split/splitles injector. Capillary column ZB-5 (5% phenyl 95% dimethylpolysiloxane) 30m x 0.25mm id x 0.25µm film thickness (Zebron, Phenomenex, USA) was used. Injector tempera-

ture was 260 °C in split mode (13:1); detector temperature was 250 °C. Temperature program was: initial temperature 50 °C for 1 min, increasing 3 °C/min up to 160 °C, then increasing 5 °C/min to 220 °C, and holding for 5 min. Carrier gas was hydrogen (2 mL/min).

Extraction and derivatization of aldehydes

The PDMS/DVB fiber was conditioned prior to the first use by heating at 270 °C for 30 min in a GC inlet. The conditioned fiber was coated with PFBHA by exposing it to the headspace of 1.7 mg/mL aqueous PFBHA hydrochloride solution. Previously, 2 mL of PFBHA solution was equilibrated to 25 °C for 5 min in a 4 mL glass vial sealed with Teflon and a perforated cap septum, stirring at 1200 rpm [16]. The fiber was then exposed to the headspace of the solution for 20 min to obtain an acceptable fiber loading. Finally, the coated fiber was exposed to the headspace of 2 mL of standard solution (acetaldehyde or furfural) or mezcal sample in a 4 mL vial held at 25 °C and stirring at 1200 rpm during 10 min for acetaldehyde and 30 min for formaldehyde extraction. After extraction of the oxime derivative the fiber was desorbed in the GC injector (260 °C) during 5 min.

Sample preparation

Depending on the alcohol content (Table 1), each mezcal sample was diluted with deionized water (free of acethaldehyde) to obtain a concentration of 2% ethanol in order to avoid the damage of the fiber. A 2-mL volume of this diluted mezcal sample was analyzed according to the above described procedure. Three replicates of each sample were analyzed.

Calibration curves

Quantification of acetaldehyde and furfural was performed using calibration curves. A stock solution of acetaldehyde (100 μg/mL) in aldehyde-free deionized water was prepared; from this stock, five working standards containing 2% ethanol and acetaldehyde concentrations in the range 1-9 μg/mL were prepared. This procedure was carried out between 0 and -4 °C due to the high volatility of acetaldehyde.

From the furfural stock solution (10 μg/mL in aldehyde-free deionized water), five working standards containing 2% ethanol and furfural concentrations in the range 0.05-1.0 μg/mL were prepared. All standards were derivatized and analyzed by headspace-SPME-GC as described previously.

Quantification of acetaldehyde and furfural in mezcal samples

The reaction of PFBHA with the carbonyl group produced two oxime isomers; therefore, the sum of peak areas of the two oxime derivatives was obtained for the quantification of acetaldehyde and furfural. This value was substituted in the calibration curve (acetaldehyde or furfural). The concentration of acetaldehyde and furfural in the samples was reported as mg/100 mL anhydrous alcohol.

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