A Headspace Solid-Phase Microextraction Method for the Determination of Some Secondary Compounds of Brazilian Sugar Cane Spirits by Gas Chromatography

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · SEPTEMBER 2001

Impact Factor: 2.91 · DOI: 10.1021/jf000896r · Source: PubMed

5 AUTHORS, INCLUDING:

Eliana Nonato
Centro de Desenvolvimento da Tecnologia N…
4 PUBLICATIONS **64** CITATIONS
SEE PROFILE

Zenilda L Cardeal
Federal University of Minas Gerais
66 PUBLICATIONS **588** CITATIONS
SEE PROFILE

A Headspace Solid-Phase Microextraction Method for the Determination of Some Secondary Compounds of Brazilian Sugar Cane Spirits by Gas Chromatography

Eliana A. Nonato,[†] Fernando Carazza, Fernando C. Silva,[‡] Ciomara R. Carvalho,[§] and Zenilda de L. Cardeal*

Chemistry Department, ICEx, Federal University of Minas Gerais, Avenida Antonio Carlos 6627, 31270-901 Belo Horizonte, MG, Brazil

A headspace solid-phase microextraction (SPME) method was developed for the determination of secondary compounds from Brazilian sugar cane spirits, or *cachaça*, by GC-FID. An SPME holder with an 85 μm polyacrylate coating was utilized. The novel method is compared with an optimized method: liquid−liquid extraction (LLE). Both methods showed good linearity, but the repeatability for analyses done with the SPME technique (%RSD = 1.8−3.9) was better than for those done with LLE (%RSD = 10.3−11.7). The concentrations of the analytes obtained in the analysis of 12 cachaça samples with the SPME technique were higher than those obtained with LLE. In the SPME method the extraction wastes are smaller. Cachaça samples were qualitatively analyzed for GC-MS.

Keywords: *Brazilian sugar cane spirits; secondary compounds; GC-FID; SPME*

INTRODUCTION

Brazilian sugar cane spirits, commonly known as *cachaça* [kha-sha-ssa], caninha [kah-nee-ña], or pinga [peen-gah], are a product deeply linked to the history of the country. Its manufacture dates back to colonial times, and at present it is second only to beer as the most consumed alcoholic beverage in Brazil. It is estimated that daily consumption of cachaça is ∼70 million 50 mL drinking measures per day (*1*).

Growing consumption and increasing value in both national and international markets point to the need of revised methods of qualitative and quantitative analysis. There is widespread interest in establishing quality standards for the beverage, as frequently declared by academic and industrial sectors, government areas, and producer associations (*1*−*3*).

The main components of cachaça are water and ethanol. However, this beverage can be described as a complex aqueous solution, constituted by several substances other than water and ethanol, which can be grouped as higher alcohols, ethyl esters, aldehydes, ketones, and organic acids. Although representing <1% of all the compounds, these substances are largely responsible for the organoleptic properties of cachaça (*1*, *3*−*9*).

Due to their special influence on the aroma, esters play a major role in the definition of the quality of the drink. Esters originate from the reaction among acids and alcohols produced during the fermentation process, when yeast produces acyl-CoA in cells, either through the activation of fatty acid or through the oxidative decarboxylation of keto acid. The final step is the cleavage of enzymes from the complex. In the presence of alcohol, the reaction produces an ester, whereas when water is present, the result is the free fatty acid. Ethyl acetate is the main ester present in cachaça. This reaction also occurs during the aging process of the liquor but in a much slower fashion (*3*, *10*).

Higher alcohols are also responsible for the flavor of cachaça. They are composed of aliphatic and aromatic alcohols, which can originate from amino acids through transamination, decarboxylation, and reduction reactions (*10*).

The determination of secondary compounds present in alcoholic beverages is complex because components can be present in different ranges of concentrations (*11*). An efficient extraction method is still to be developed. At present, the method most frequently employed is liquid−liquid extraction (LLE). This method presents the disadvantages of being time-consuming and of using large amounts of toxic solvents in its multistep sequence. The steps can occasionally cause the loss of volatile compounds, and the method's efficiency can be further reduced by impurities in the solvents, which may significantly decrease the obtained recoveries (*12*, *13*).

Direct injection of alcoholic beverages is not recommended due to the presence of large amounts of water that result in broad peaks and disturbance of FID signal stability. This technique is favorable for secondary compounds present in higher amounts but is inappropriate for analysis of compounds present in low amounts that also are very important and responsible for flavor of alcoholic beverages. Direct injection has a further disadvantage of yielding information only on the

* Author to whom correspondence should be addressed [fax (31) 4995700; e-mail zenilda@dedalus.lcc.ufmg.br].

[†] Permanent address: Centro de Desenvolvimento da Tecnologia Nuclear − da Comissão Nacional de Energia Nuclear − CDTN/CNEN, 30123-970 Belo Horizonte-MG, Brazil.

[‡] Permanent address: Chemistry Department, Federal University of Maranhão, 65080-040 São Luis, MA, Brazil.

[§] Permanent address: Fundação Centro Tecnológico de Minas Gerais, Belo, Brazil.

10.1021/jf000896r CCC: $20.00
Published on Web 07/14/2001

composition of the liquid phase, leaving behind the gaseous phase of the beverage (*14*).

This paper presents an efficient method to identify and determine the amount of higher alcohols and ethyl acetate in cachaça on the basis of headspace solid-phase microextraction technique (SPME) and gas chromatography analysis with a flame ionization detector (GC-FID). The method also employs gas chromatography−mass spectrometry (GC-MS) to identify the analytes. Headspace SPME was chosen because this technique is fast, sensitive, and solvent-free (*15*−*17*). This paper also presents a comparison between headspace SPME and an optimized LLE method. Both methods have been used to analyze 12 homemade and commercial cachaça samples. Analyzed compounds are those prescribed by correlated Brazilian legislation as the main cachaça quality parameters.

MATERIALS AND METHODS

Reagents. All reagents (of at least analytical grade) were purchased from different suppliers: ethanol, ethyl acetate, isobutyl alcohol, isoamyl alcohol, sodium chloride, and sodium sulfate (Merck, Darmstadt, Germany); 1-propyl alcohol, 1-butyl alcohol (Polyscience, Niles, Illinois); pentane (Synth, Belo Horizonte, MG, Brazil); dichloromethane (QM, Belo Horizonte, MG, Brazil); and ultrapure water from a Milli-Q purification system (Millipore, Milford, MA).

Apparatus. An SPME fiber holder for manual use and fibers of polyacrylate (85 μm film thickness) were obtained from Supelco (Bellefonte, PA). All fibers were conditioned in the hot injector of the GC system according to instructions from Supelco. The analyses were performed using a Hewlett-Packard model 5890 II system equipped with an FID (Hewlett-Packard, Wilmington, DE), a split/splitless injector, and a 50 m × 0.20 mm × 0.4 μm HP-INNOWAX column (Hewlett-Packard). Temperatures were 240 and 280 °C for the injector and detector, respectively. The oven temperature program used was as follows: 40 °C hold for 5 min, raised at 5 °C/min to 100 °C, raised at 10 °C/min to 240 °C, and hold for 20 min. The carrier gas was ultrahigh-purity (UHP) hydrogen with a flow rate of 0.66 mL/min.

LLE Procedure. In a previous investigation, cachaça samples were submitted to extraction with pentane/dichloromethane 2:1 (v/v) for 3 h (*2*). In this work, an optimized LLE method was developed. The studied samples were submitted to extraction with pentane and dichloromethane mixtures in 1:2, 2:1, 1:1, 5%, and 20% proportions of pentane/dichloromethane and with pure dichloromethane. Volume proportions of cachaça and dichloromethane of 3:1, 4:1, and 5:1 and different times were also tested.

The solutions were introduced to 50 mL Pyrex vials, which were immediately sealed with Teflon septa and aluminum caps. The vials were shaken on an agitation plate and then transferred into a decantation funnel of 50 mL, for the separation phases. The addition of sodium sulfate was necessary to remove water from the organic phase. Then 1.00 μL of organic phase was injected in the GC.

For quantitative analyses a stock solution was prepared with ethyl acetate, 447.8 mg/L; 1-propyl alcohol, 652.7 mg/L; isobutyl alcohol, 396.0 mg/L; isoamyl alcohol, 1283.0 mg/L; and an internal standard stock solution of 1-butyl alcohol, 1137.9 mg/L in ethanol/water (50% *v*/*v*) stored at 3−5 °C. The utilization of the internal standard, 1-butyl alcohol, was made after the confirmation that this alcohol does not exist in the cachaça samples.

The calibration curves were prepared using aliquots of 1.00, 5.00, 10.00, 15.00, and 20.00 mL of stock solution and 3.00 mL of internal standard solution. The extractions were realized with 5.00 mL of dichloromethane. The final volume, standard solution and dichloromethane, was kept at 28.00 mL by the addition of ethanol/water (50% v/v). This procedure was necessary to keep a constant proportion of 4:1 between solution standard and dichloromethane volumes. In all solutions 3.0 g of sodium chloride was added to increase the performance of the extractions.

SPME Procedure. SPME is a process in which analytes are partitioned between a liquid or gaseous phase sample and a polymeric phase according to the partition coefficients *K*. The amount of analyte extracted under such conditions depends on the partition coefficient between the sample and the coating. For SPME sampling, there are some parameters most important to control for optimum performance: fiber polarity, fiber coating thickness, sample medium (pH and ionic strength), adsorption/absorption and desorption time, temperature extraction, and rate of sample agitation (*15*−*17*).

Therefore, an investigation was carried out to establish optimal extraction conditions for ethyl acetate and higher alcohols from cachaça samples. In this particular study, the four most important extraction parameters were varied, that is, absorption and desorption times, ionic strength by addition of sodium chloride, and extraction temperature.

The fiber selected for extraction was an 85 μm polyacrylate coating, which presented the highest yield of compounds of interest. The homemade heater unit was built using a block of aluminum covered with a heating ribbon with temperature controlled by a thermostat. The polyacrylate fiber was conditioned before initial application in the hot-port injector of the GC system by heating it at 280 °C for 3 h.

A stock solution was prepared consisting of ethyl acetate, 895.5 mg/L; 1-propyl alcohol, 636.8 mg/L; isobutyl alcohol, 316.8 mg/L; 1-butyl alcohol, 325.1 mg/L; and isoamyl alcohol, 200.5 mg/L in ethanol/water (50% v/v) stored at 3−5 °C. In the optimizated SPME conditions, an aliquot of 3.75 mL of the stock solution was diluted in water in a volumetric flask of 50.00 mL, to prevent a large ethanol peak thus overlapping the analytes' peaks. An aliquot of 10.0 mL was introduced into 22 mL Pyrex vials, and the vials were immediately sealed with aluminum caps containing septa butyl faced Teflon.

For calibration curve construction a stock solution (A) was prepared consisting of ethyl acetate, 501.5 mg/L; 1-propyl alcohol, 652.7 mg/L; isobutyl alcohol, 320.0 mg/L; and isoamyl alcohol, 641.5 mg/L in ethanol/water (50% v/v); another internal standard stock solution consisted of 1-butyl alcohol (B), 1001.4 mg/L, in ethanol/water (50% v/v). The calibration curves were prepared with aliquots of 1.00, 2.00, 3.00, 4.50, and 7.00 mL of the stock solution (A) and 1.00 mL of the standard solution (B) diluted in ethanol/water (50% v/v) to a volume of 50.00 mL.

Stock solution concentrations employed for SPME analysis were different from those for LLE because these methods present distinct extraction capacities.

Mass Spectrometry Procedure. GC-MS analysis was performed using a GC Hewlett-Packard (Wilmington, DE) 5890 series II equipped with a Hewlett-Packard MSD 5951. Interface temperature was 280 °C. The same conditions were adopted for GC and GC-MS analyses.

Samples. Artisanal and commercial Brazilian sugar cane spirits were analyzed for comparison purposes. Among them were Bocaina (Minas Gerais, MG), Rainha da Lavoura (MG), Boa Vista (MG), Espírito de Minas (MG), Germana (MG), Rumo do Poeta (MG), Velho Barreiro (São Paulo, SP), Pirapora (MG), Moeda Velha (MG), Boa Vitória (MG), Barreirinha (MG), and Caninha 51 (SP).

For cachaça sample analysis by LLE an aliquot of 20.00 mL was used. This aliquot was introduced in a 50 mL Pyrex vial with the addition of 3.00 mL of the internal standard stock solution of 1-butyl alcohol (1137.9 mg/L) and 3.0 g of sodium chloride. This mixture was extracted with 5.00 mL of dichloromethane according to the same extraction procedure used for the standard solution.

For cachaça sample analysis by SPME an aliquot of 4.00 mL was used. This aliquot and 1.00 mL of the internal standard stock solution of 1-butyl alcohol (1001.4 mg/L) were diluted in water in a volumetric flask of 50.00 mL. An aliquot of 10.00 mL of this solution was used for extraction with the

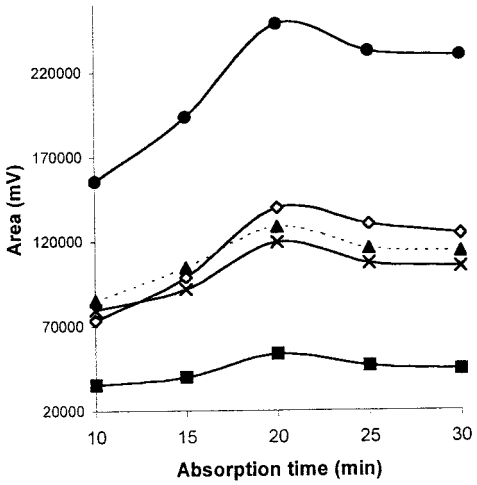


Figure 1. Effects of different absorption times on the extraction efficiencies of ethyl acetate (◇), 1-propyl alcohol (■), isobutyl alcohol (▲), 1-butyl alcohol (∗), and isoamyl alcohol (●).

Table 1. Results of LLE with Different Volume Relations between Cachaça and Dichloromethane

	relations of cachaça and dichloromethane volume areas (mV)		
compound	3:1	4:1	5:1
ethyl acetate	3847.1	3381.3	2976.0
1-propyl alcohol	52849.5	68902.6	75960.3
isobutyl alcohol	52019.5	72300.6	82438.9
isoamyl alcohol	222777.2	327710.0	388300.5

Table 2. Calibration Curve Equations, Correlation Coefficients, and Linearity Ranges for Higher Alcohols and Ethyl Acetate for LLE Analysis

compound	linearity range (mg/L)	equation	correlation coefficient (r)
ethyl acetate	15.99−319.82	$Y = 0.571X + 0.0042$	0.9999
1-propyl alcohol	23.31−466.21	$Y = 0.838X + 0.0278$	0.9996
isobutyl alcohol	14.14−282.86	$Y = 1.08X + 0.0103$	0.9999
isoamyl alcohol	45.82−916.43	$Y = 1.10X + 0.232$	1.0000

same procedure used for standard solutions. The extraction conditions used were determined by optimization analysis.

RESULTS AND DISCUSSION

LLE Analysis. Dichloromethane was selected as the solvent of extraction because this introduced efficiency to the same degree when compared with the pentane/dichloromethane mixture suggested by the literature (*2*). The ionic strength increased by the addition of NaCl reduced the time of extraction from 3 h to 30 min.

The proportion selected between the cachaça and the solvent volumes was 4:1 because it is more efficient than the proportion 3:1 and presents the same proportion efficiency as 5:1 as is shown in Table 1.

For the construction of calibration curves the standard solutions were analyzed in duplicate. The linearity was evaluated by the correlation coefficient (r) of calibration curves of each analyte. Table 2 shows the concentration range, straight-line equations, and correlation coefficients.

SPME Analysis. The absorption time profile was investigated by varying the time in the range from 10 to 30 min. During this procedure the desorption time was kept at 3 min and the extraction temperature at 60 °C, and the addition of NaCl was of 2.5 g. Figure 1 shows that the equilibrium between sample and coating was obtained only after 25 min, which can be proved by the small variations in analyte areas in the absorption curves after this time. Then, the absorption time of 25 min was selected.

Table 3. Areas of Different Higher Alcohols and Ethyl Acetate on the Desorption Time of SPME Method[a]

	area mean (mV)	
compound	3 min desorption	5 min desorption
ethyl acetate	109576	95909
1-propyl alcohol	41260	35815
isobutyl alcohol	112317	96029
n-butyl alcohol	98439	89014
isoamyl alcohol	224472	205022

[a] n = three injections.

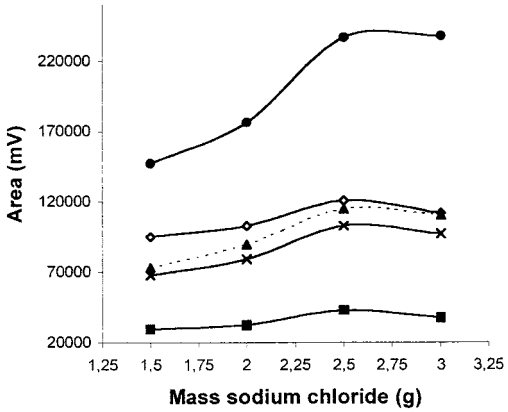


Figure 2. Effects of different salt concentrations on the extraction efficiencies of ethyl acetate (◇), 1-propyl alcohol (■), isobutyl alcohol (▲), 1-butyl alcohol (∗), and isoamyl alcohol (●).

The desorption time was studied by monitoring the peak area at 1, 3, 5, 7, and 10 min. During this procedure the absorption time was kept at 25 min and the extraction temperature at 60 °C, and the addition of NaCl was of 2.5 g. It was observed that the average analyte areas at 3 min of desorption were greater than those found using 5 min of desorption, as shown in Table 3. On the other hand, 7 and 10 min desorption times have resulted in ethanol high-intensity peaks, which superimposed adjacent peaks. The time of 1 min was not enough for total thermal desorption of compounds from fiber; the blank run done with the fiber after this time exhibited carry-over of the analytes. Then the desorption time of 3 min was selected.

The effect of ionic strength on extraction efficiency was determined by analyzing a standard solution with 1.5, 2.0, 2.5, and 3.0 g of sodium chloride. During this procedure the absorption time was kept at 25 min, the desorption time at 3 min, and the temperature extraction at 60 °C. The addition of the salt decreased the solubility of the soluble organic compounds in water, facilitating the extraction and concentration of these in the fiber coating. Figure 2 shows that analyte responses reached their maximum with the addition of 2.5 g of NaCl.

The temperature increased the concentration of the analytes in the headspace; however, a controlled temperature should be used because the concentration process of the absorption by the fiber coating is exothermic. Extraction temperatures of 50, 60, 70, and 80 °C were investigated to determine coating fiber concentration efficiency. Figure 3 shows that there is little difference in the performance of the extraction using

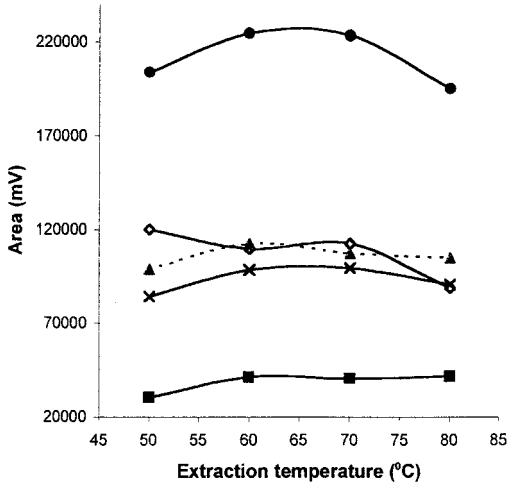


Figure 3. Effects of different extraction temperatures on the extraction efficiencies of ethyl acetate (◇), 1-propyl alcohol (■), isobutyl alcohol (▲), 1-butyl alcohol (∗), and isoamyl alcohol (●).

temperatures of 60 and 70 °C. However, a temperature of 60 °C was selected as adequate for the extraction procedure favoring the concentration exothermic process of the compounds with the fiber coating.

For the construction of calibration curves the standard solutions were analyzed in duplicate in the conditions optimized by the SPME method. The concentration range, straight-line equations, and correlation coefficients are shown in Table 4.

Analysis of Real Samples. Twelve samples of homemade and industrialized types of cachaça were analyzed in duplicate. Ethyl acetate, 1-propyl alcohol, isobutyl alcohol, and isoamyl alcohol concentrations were determined.

Figure 4 illustrates a comparison of the chromatograms made by LLE and SPME procedures. The presence of a larger number of compounds with intense responses was observed in the chromatograms made by SPME procedures, indicating a better extraction performance with this technique. This can be attributed to the greater affinity of these compounds with fiber polyacrylate coating. It does not happen with LLE, in which dichloromethane did not show such efficiency for the extraction of the same compound. SPME introduced additional advantages of requiring a smaller sample

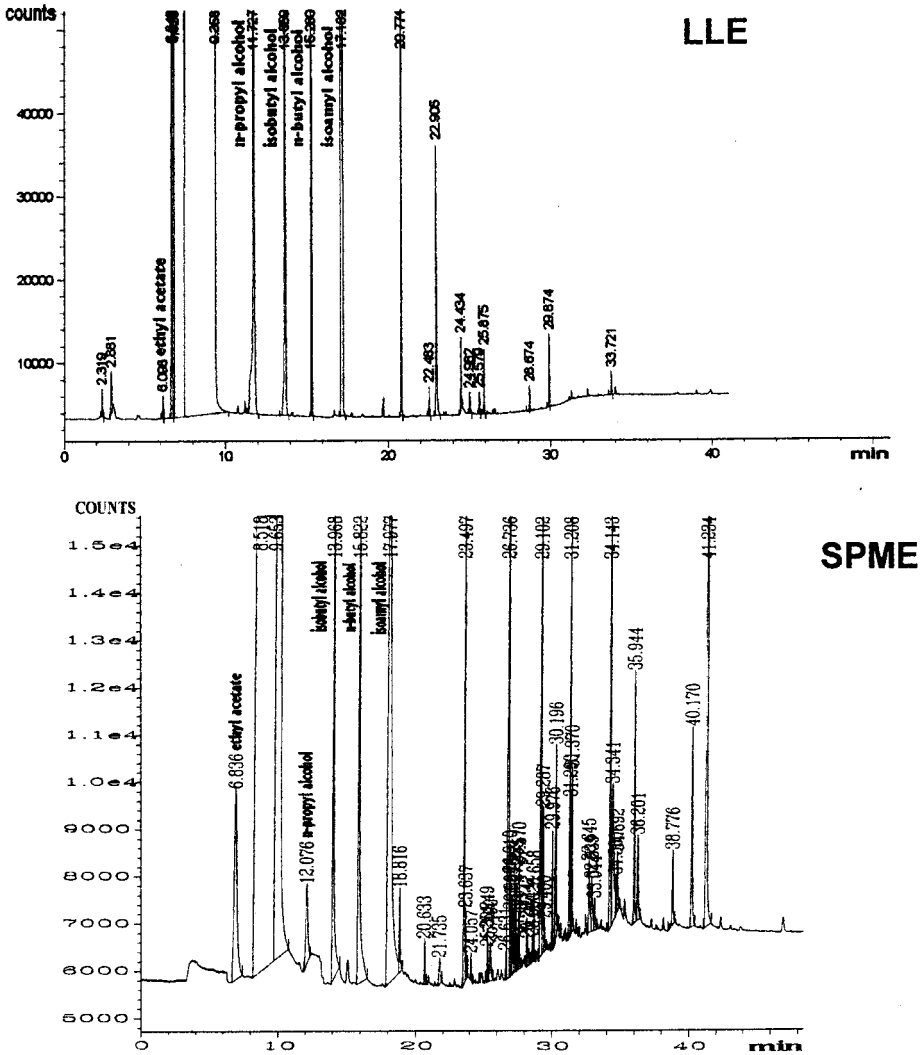
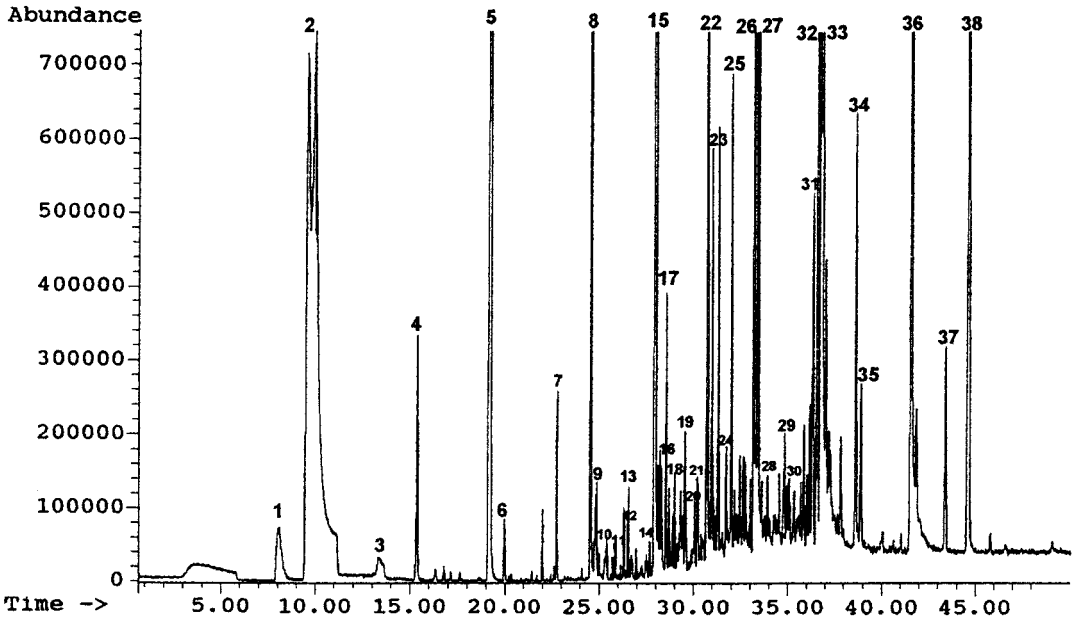


Figure 4. Chromatograms of cachaça E extracted by LLE obtained by GC-FID using dichloromethane in proportion of sample/solvent of 4:1 and addition of 3.0 g of sodium chloride and of cachaça E extracted by SPME obtained by GC-FID using a 85 μm polyacrylate fiber, absorption time at 25 min, desorption time at 3 min, extraction temperature of 60 °C, and addition of 2.5 g of sodium chloride. An HP-INNOWAX column 50 m × 0.20 mm × 0.4 μm was used, injector temperature of 240 °C and detector temperature of 280 °C. The temperature program was as follows: 40 °C (5 min), raised at 5 °C/min to 100 °C and at 10 °C/min to 240 °C (20 min).



1 – ethyl acetate
2 – ethyl alcohol
3 – 1-propyl alcohol
4 – isobutyl alcohol
5 – isoamyl alcohol
6 – ethyl hexanoate
7 – ethyl 2-hydroxy-propanoate
8 – ethyl octanoate
9 – acetic acid
10 – 2-ethyl-1-hexyl alcohol
11 – hexylmethylcarbinol
12 – ethyl 2-hydroxy-4-methylpentanoate
13 – caprylic alcohol
14– 4-methyl-1-(1-methylethyl) 3-cyclohexen-1-ol
15 – ethyl decanoate
16 – β-farnesene
17 – diethyl butanoate
18 – α,α,4-trimethyl-3-cyclohexene-1-methanol
19 – 1-decyl alcohol
20 – β-citronellol
21 – cyclodecene
22 – ethyl dodecanoate
23 – 3-methyl-butyl decanoate
24 –2,6-bis-(1,1-dimethylethyl)-4-methylphenol
25 – phenyl-ethanol
26 – nerolidol
27 – octanoic acid
28 – 2,6-dimethylnaphthalene
29 – ethyl pentadecanoate
30 – cyclododecane
31 – α-bisabolol
32 – ethyl hexadecanoate
33 – decanoic acid
34 – cis-farnesol
35 – cyclotetradecane
36 – dodecanoic acid
37 – ethyl linoleate
38 – bis-2-methyl propylate 1,2-benzenedicarboxyilate

Figure 5. Total ion chromatogram of cachaça B extrated by SPME.

Table 4. Calibration Curve Equations, Correlation Coefficients, and Linearity Ranges for Higher Alcohols and Ethyl Acetate for SPME Analysis

compound	linearity range (mg/L)	equation	correlation coefficient (r)
ethyl acetate	10.03−70.21	$Y = 0.496X + 0.0198$	0.9981
1-propyl alcohol	13.05−91.38	$Y = 0.218X + 0.0402$	0.9966
isobutyl alcohol	6.40−44.80	$Y = 1.05X + 0.0149$	0.9998
isoamyl alcohol	12.83−89.81	$Y = 3.58X + 0.263$	0.9988

volume in the extraction and not requiring an organic solvent.

Figure 5 shows a GC-MS analysis of cachaça B extracted by SPME. Extracted compounds, organic acids, esters, and cyclic and aromatic hydrocarbons, were identified by means of the Wiley library and also on the basis of a study of fragmentation pathways for the formation of the compounds.

The repeatability of the LLE and SPME techniques was evaluated by making seven extractions on concentrations relative to the calibration curve average point. Table 5 shows the results obtained for the repeatability. It can be noted that the relative standard deviations

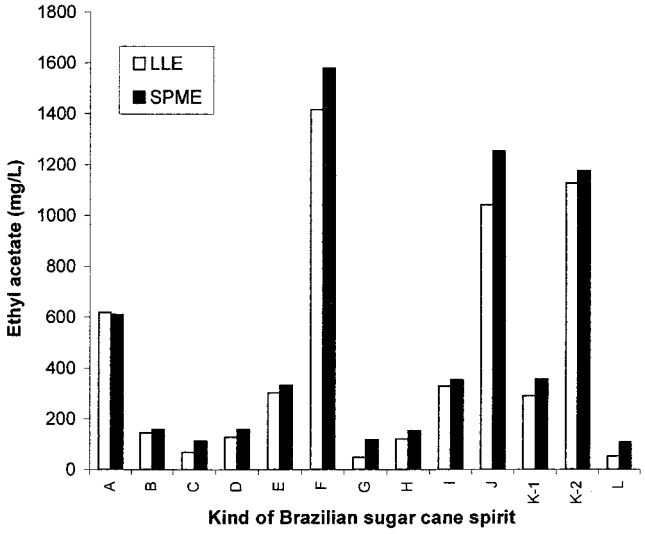


Figure 6. Comparisons of the SPME and LLE techniques in the quantification of ethyl acetate in the cachaças.

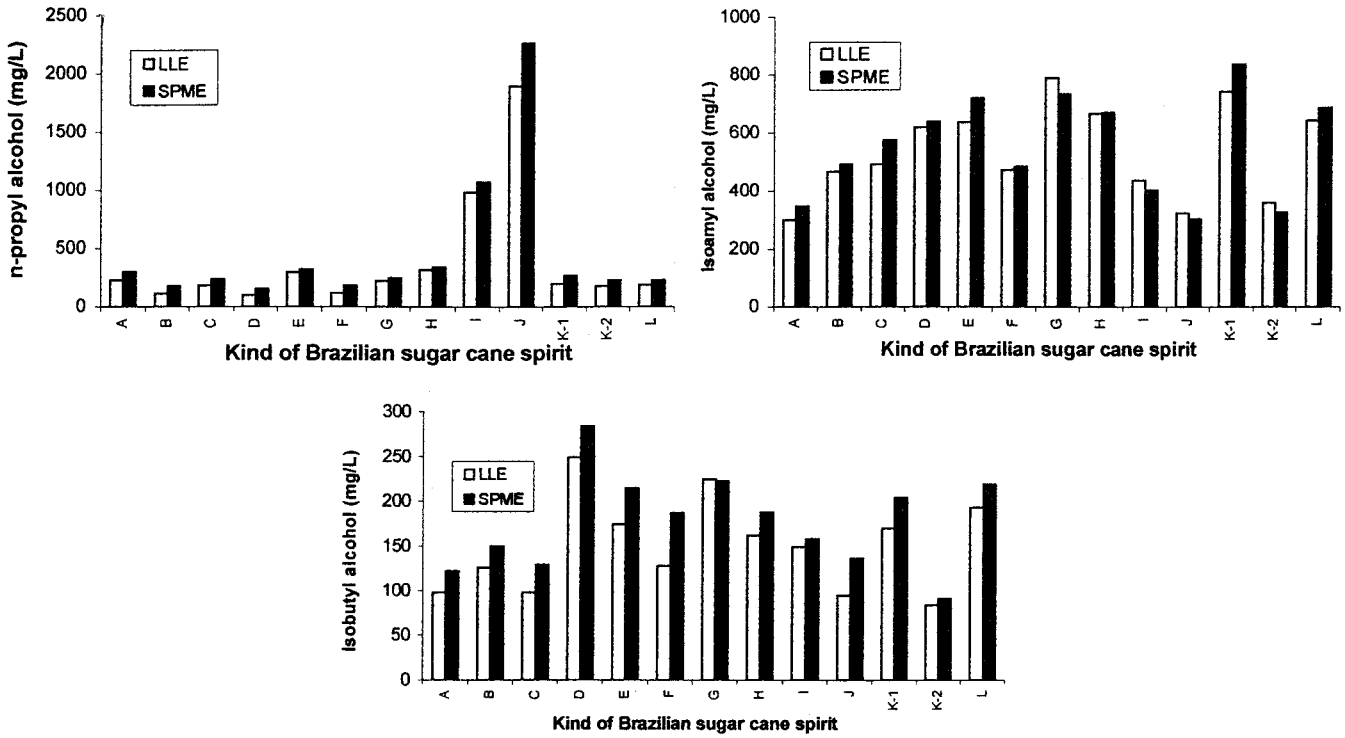


Figure 7. Comparisons of the SPME and LLE techniques in the analysis of 1-propyl, isobutyl, and isoamyl alcohols in different cachaças.

Table 5. Comparative Study of the Repeatability of LLE and SPME, Using Relative Standard Deviations of the Seven Extractions on Concentrations Relative to the Calibration Curve Average Point

	repeatability % RSD ($n = 7$)	
compound	SPME	LLE
ethyl acetate	1.8	11.0
1-propyl alcohol	3.1	11.7
isobutyl alcohol	3.9	10.5
isoamyl alcohol	3.9	10.3

for the analysis by SPME (RSD = 1.8−3.9%) were smaller than obtained those for the analysis by LLE (RSD = 10.3−11.7%). Therefore, it can be said that the SPME method enabled a minor variation in the concentration measures. This improvement was achieved because this method reduced the manipulations, which allowed for fewer lost analytes in the extraction.

Figures 6 and 7 show the quantitative results of a comparison between extractions using both techniques with ethyl acetate and higher alcohol. It can be observed that the concentrations introduced variations, and the analyte concentrations were larger in the analyses done with the SPME technique.

It can be concluded that the determination of secondary compounds present in cachaça can be made with GC-FID using extraction techniques LLE and SPME. The SPME method showed better repeatability in analyses than LLE, as well as fewer stages in the analytes' extraction. With the LLE method it is necessary to be careful in extraction to avoid solvent losses, and when Na_2SO_4 is added for water elimination, in some cases filtration is necessary before injection in the GC system. LLE introduces disadvantages of producing residue of toxic organic solvents and demanding a careful extraction process to minimize solvent losses by evaporation.

Therefore, due to its practical advantages and the fact that its reliability is linear and repeatable, the SPME method should be the technique of choice to assay ethyl acetate and higher alcohols from Brazilian sugar cane spirits by GC.

LITERATURE CITED

(1) Nonato, E. A. Identificação e quantificação de compostos secundários em aguardentes de cana por CG e CG/EM. Departamento de Química, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, 1999; 114 pp.

(2) Bezerra, C. W. B. Caracterização Química da Aguardente de Cana-de-açúcar: Determinação de álcoois, ésteres e dos íons Li^{+1}, Ca^{+2}, Mg^{+2}, Cu^{+2} e Hg^{+2}. Instituto de Química de São Carlos, São Carlos, Brazil, 1995; 53 pp.

(3) Vargas, E. A. Qualidade da Aguardente de Cana Produzida, Engarrafada e/ou Comercializada em Minas Gerais. Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, 1995; 88 pp.

(4) Kahn, J. H.; Blessinger, E. T. Collaborative study of the quantitative gas−liquid chromatographic determination of fusel oil and other components in whisky. *J. Assoc. Off. Anal.Chem.* **1972**, *55*, 549−556.

(5) Martin, G. E.; Burggraff, J. M.; Randolph, H. D.; Buscemi, P. C. Gas−liquid chromatographic determination of congeners in alcoholic products with confirmation by gas chromatography/mass spectrometry. *J. Assoc. Off. Anal.Chem.* **1981**, *64*, 186−190.

(6) Lehtonen, M. Gas−liquid chromatographic determination of volatile phenols in matured distilled alcoholic beverages. *J. Assoc. Off. Anal.Chem.* **1983**, *66*, 62−70.

(7) Wilson, L. A.; Ding, J. H.; Woods, A.E. Gas chromatographic determination and pattern recognition analysis of methanol and fusel oil concentrations in whiskeys. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 248−257.

(8) Nascimento, R. F.; Marques, J. C.; Neto, B. S. L.; Keukeleire, D.; Franco, D., Qualitative and quantitative high-performance liquid chromatographic analysis of aldehydes in Brasilian sugar cane spirits and other distilled alcoholic beverages. *J. Chromatogr. A* **1997**, *782*, 13−23.

(9) Nascimento, R. F.; Cardoso, D. R.; Neto, B. S. L.; Franco, D. Determination of acids in Brazilian sugar cane spririts and other alcoholic beverages by HRGC-SPE. *Chromatographia* **1998**, *48*, 757−757.
(10) Nykánen, L. Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages, *Am. J. Enol. Vitic.* **1986**, *37*, 84−96.
(11) Ferreira, V.; Sharman, M.; Cacho, J. F.; Dennis, J. New and efficient microextration/solid-phase extraction method for the gas chromatographic analysis of wine volatiles. *J. Chromatogr. A* **1996**, *731*, 247−259.
(12) Villén, J.; Señoráns, F. J.; Reglero, G.; Herraiz, M. Analysis of wine aroma by direct injection in gas chromatography without previous extraction. *J. Agric. Food Chem.* **1995**, *43*, 717−722.
(13) Eisert, R.; Levsen, K. Solid-phase microextraction coupled to gas chromatography: a new method for the analysis of organics in water. *J. Chromatogr. A* **1996**, *733*, 143−157.
(14) Etievant, P.; Maarse, H.; Van den Berg F., Wine analysis: study and comparison of techniques developed for the study of volatile constituents. *Chromatographia* **1986**, *21*, 379−386.
(15) Zhang, Z.; Yang, M. J.; Pawliszyn, J. Headspace solid-phase microextraction. *Anal. Chem.* **1993**, *65*, 1843−1852.
(16) Zhang, Z.; Yang, M. J.; Pawliszyn, J. Solid phase microextraction integrates sampling, extraction, concentraction and sample introduction into a single step. *Anal. Chem.* **1994**, *66*, 844−853.
(17) Pawliszyn, J. *Solid-Phase Microextraction: Theory and Practice*; Wiley-VCH: Ontario, Canada, 1997.

Received for review July 18, 2000. Revised manuscript received May 17, 2001. Accepted May 21, 2001.

JF000896R