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Development of a headspace solid-phase microextraction procedure for the determination of free volatile fatty acids in waste waters

M. Ábalos[a], J.M. Bayona[a,*], J. Pawliszyn[b]

[a]*Environmental Chemistry Department, IIQAB-CID-CSIC, Jordi Girona 18-26, E-08034 Barcelona, Spain*

[b]*Department of Chemistry, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada*

Abstract

An analytical procedure based on headspace solid-phase microextraction (SPME) coupled to GC–flame ionization detection/Negative Chemical Ionization Mass Spectrometry has been developed for the determination of free volatile fatty acids (C_2–C_7) in waste water samples. Five different coatings have been evaluated and polydimethylsiloxane–Carboxen was the only fiber that allows a successful extraction of the shortest chain fatty acids (acetic and propionic). Several parameters such as extraction time and temperature, desorption conditions, agitation speed and sample volume have been optimized using the polydimethylsiloxane–Carboxen fiber. The linear dynamic range was over two–four orders of magnitude, depending on the acid. Procedural detection limits were in the low to medium μg/l levels and the RSDs were between 5.6% and 13.3%. To evaluate the applicability of the developed SPME procedure on real samples, fermented urban wastewaters were analysed.

Keywords: Solid-phase microextraction; Headspace analysis; Extraction methods; Water analysis; Fatty acids

1. Introduction

Volatile fatty acids (VFAs) are low-molecular-mass organic acids with a strong hydrophilic character [1]. They originate from aerobic biodegradation of carbohydrates, proteins and fats. Therefore, they are widely present in activated sludges [1–4], waste and landfill leachates [5–8] and wastewaters. Recently, the determination of VFAs became of increasing interest since it has been found that they are involved in different processes, i.e. the biological removal of phosphorus from waters [9,10] or nitrification–denitrification in activated sludge [11,12]. Carboxylic acids may also affect the storage stability of waste incineration residues by reducing the pH value and increasing the mobility of heavy metals [13] and radionuclides [14]. In addition, VFAs constitute one of the chemical classes responsible for unpleasant odour generation in wastewaters, together with volatile amines and sulfur compounds.

Conventional analytical schemes for the determination of VFAs include liquid–liquid extraction [6,15], distillation [16] or purge-and-trap techniques [17] combined with gas chromatography (GC). However, due to the peculiar characteristics of these acids (i.e. high polarity, volatility and solubility in water), a derivatization step is currently performed to obtain non-polar derivatives that can be easier analysed. Pentafluorobenzyl esters of VFAs are usually prepared because they can be detected at high sensitivity by electron-capture detection (ECD) [8,18,19]. Alternatively, other derivatization reagents such as butyldimethylsilyl)-*N*-methyltrifluoro-

*Corresponding author. Tel.: +34-343-400-6100; fax: +34-343-204-5904.

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acetamide have been used to obtain the corresponding fluorinated amides from VFAs [20]. Some analytical procedures that involve LC determination have also been performed [5]. In the last years, capillary electrophoresis and indirect UV detection or laser-induced fluorescence have been developed for the determination of VFAs [21].

In the present work, an analytical procedure based on headspace solid-phase microextraction (SPME) has been developed for the determination of underivatised C_2–C_7 fatty acids. Application of SPME to the analysis of VFA derivatives has been previously described [22,23]. However, the lack of a commercially available fiber that allowed the extraction of the shortest chain fatty acids (acetic and propionic) with acceptable sensitivity has limited the interest of their SPME determination. In this study, a variety of fibers of different polarity (i.e. polyacrylate, PA; Carbowax–divinylbenzene, CW–DVB; polydimethylsioxane–divinylbenzene, PDMS–DVB; polydimethylsiloxane–Carboxen, PDMS–CAR; polydimethylsiloxane–Carboxen–divinylbenzene, PDMS–CAR–DVB) have been evaluated for the extraction of underivatized VFAs. Parameters such as extraction time and temperature, desorption conditions, agitation speed or sample volume have been optimised by using the PDMS–CAR fiber. Linearity, detection limits and precision of the whole analytical procedure have also been calculated. Finally, the developed procedure was applied to the determination of VFAs in urban wastewater samples. To the best of our knowledge, only one paper dealing with SPME analysis of VFAs in their free form has been published using CW–DVB [24] but no exhaustive optimisation of relevant parameters affecting to the extraction of VFAs was carried out. Moreover, the analysis of real samples was not attempted.

2. Experimental

2.1. Chemicals and materials

Acetic (99.99%) and propionic (99.5%) acids were obtained from Aldrich (Milwaukee, WI, USA). Butyric (99%), 2-ethylbutyric (99%), valeric (99%), hexanoic (99.5%) and heptanoic (99%) acids were purchased from Aldrich-Chemie (Steinheim, Germany). All standards were used as received. Analytical-grade HCl (25%) was from Merck (Darmstadt, Germany) and NaCl was from Carlo Erba (Milan, Italy).

The SPME holder and coating fibers (PA, 85 μm; PDMS–DVB, 65 μm; CW–DVB, 65 μm; PDMS–CAR–DVB, 30/50 μm and PDMS–CAR, 75 μm) were obtained from Supelco (Bellefonte, PA, USA). Stock standard solutions of each analyte (1250–10 000 μg ml^{-1}) were prepared in Milli-Q water. A mixed standard solution (250 μg ml^{-1} of C_2–C_4; 60 μg ml^{-1} C_5–C_7) for the preparation of spiked samples was obtained diluting the stocks. All the standard solutions were stored at 4°C in the darkness.

2.2. Instrumental analysis

Chromatographic analysis was carried out using a Varian 3500 GC system with flame ionization detection (FID). A tailor-made capillary column for free-fatty analysis (TR-FFAP, Teknokroma, Sant Cugat, Barcelona, Spain) of 30 m×0.25 mm I.D. coated with 0.25 μm film thickness was used. Hydrogen at 2.3 ml min^{-1} was used as carrier gas. The oven temperature was programmed from 70°C for 1 min, then 15°C min^{-1} (4°C min^{-1} in the case of real samples) until 200°C holding the final temperature for 1 min. Injector and FID temperatures were set at the highest temperature allowable for each SPME fiber and 260°C, respectively. Confirmation of analyte identity in real samples was performed by GC–MS in the negative ion chemical ionization mode (NCI) (Hewlett-Packard 5985, Palo Alto, CA, USA) using ammonia (1 Torr in the ion source; 1 Torr=133.322 Pa) as reagent gas. Chromatographic conditions were similar to those described in the GC–FID analysis but helium was used as carrier gas and column temperature was programmed at 10°C. Ion source and analyzer temperatures were held at 210°C and 110°C, respectively.

2.3. SPME procedure

Samples for method development were prepared by adding 10 or 20 ml of Milli-Q water saturated with NaCl into a 40 ml vial, sealed with a PTFE septum and then spiked with a 100 μl of the VFA standard mixture by injection through the septum.

Then, pH was adjusted to 1.5 by injecting HCl (0.2 *M*) through the septum. Extraction was performed in the headspace along 20 min under magnetic stirring (1200 rpm). Extraction temperature was set at 25°C. Mean values and standard deviations were calculated from experiments performed in triplicate. 2-Ethylbutyric acid was used as surrogate in real samples.

3. Results

Matrix pretreatment prior to headspace SPME is a key step that must be addressed to favor the volatilization from the aqueous phase to the headspace. In this regard, the pH effect and salt addition in the enhancement of the extraction of VFAs was previously evaluated by Pan et al. [22]. They found that by lowering the pH of the sample matrix, acids were mainly present in their neutral forms, which have a greater affinity for the fiber. In addition, the saturation of the aqueous phase with NaCl decreased the solubility of the neutral forms of the acids, increasing the Henry constant and consequently, the gas phase transfer. The reported optimal conditions (pH 1.5 and NaCl saturated solution) [22] were selected in this study.

On the other hand, headspace extraction was preferred over direct water extraction in order to prevent contact of the SPME fiber with the extreme conditions of the aqueous phase minimizing the carryover effects during the real sample analysis.

3.1. Coating selection

Five different coatings were evaluated for the VFA analysis: PA, CW–DVB, PDMS–DVB, PDMS–CAR–DVB and PDMS–CAR. At this evaluation stage, extraction time was set at 1 h in order to assure that either the equilibrium could be established or a large amount of analytes would be extracted. Generally, the highest peak area was obtained for the higher-molecular-mass acids (C_4–C_7) and all fibers gave a satisfactory peak area. Conversely, acetic and propionic acids could only be extracted with an acceptable peak area using the PDMS–CAR fiber since this coating significantly enhanced the extraction of lower-M_r analytes against the others.

A close comparison of the extraction efficiency of selected fibers (PDMS–DVB, PDMS–CAR–DVB, PDMS–CAR) with their properties was carried out in order to get further insight into the extraction mechanisms. The recently introduced dual-coated fiber (PDMS–CAR–DVB) exhibited better extraction efficiency for acetic, propionic and butyric acids compared to PDMS–DVB; whereas for valeric, hexanoic and heptanoic acids there were no significant extraction differences between these two fibers (Fig. 1). These results agreed with the hypothesis that small analytes can diffuse through the DVB layer and reach the CAR while the others remain trapped on the DVB [25]. On the other hand, PDMS–CAR was the best of the three coatings for the extraction of shorter chain fatty acids. Peak areas were from 6 to 2 times higher for C_2–C_5 acids, respectively, compared to those obtained with the PDMS–CAR–DVB fiber. For the higher homologues, the PDMS–CAR–DVB polymer showed a better performance. In this regard, the response of hexanoic acid was similar for the three coatings while for the heptanoic acid PDMS–CAR–DVB seemed to be the most appropriate (Fig. 1).

Since our target analytes were lower-molecular-mass VFAs, the PDMS–CAR fiber was chosen for further optimization of the analytical procedure.

3.2. Desorption conditions

Injector temperature and desorption time are important parameters that have to be optimized in SPME analysis in order to avoid the carryover effect. In this work, four different desorption conditions were considered: 275°C within 2 min, 275°C within 5 min, 300°C (maximum allowable temperature of the PDMS–CAR fiber) within 2 min and 300°C within 5 min. Similar peak areas were obtained along all the experiments for C_2–C_5 acids. In addition, low percentages of carryover were found for these compounds (<4%). On the contrary, temperature and time had a significant effect in controlling the desorption of the C_6 and C_7 acids. An increase of time while keeping the same temperature (275°C) improved the peak area for heptanoic acid by 52%, while increasing temperature gave rise to a lower

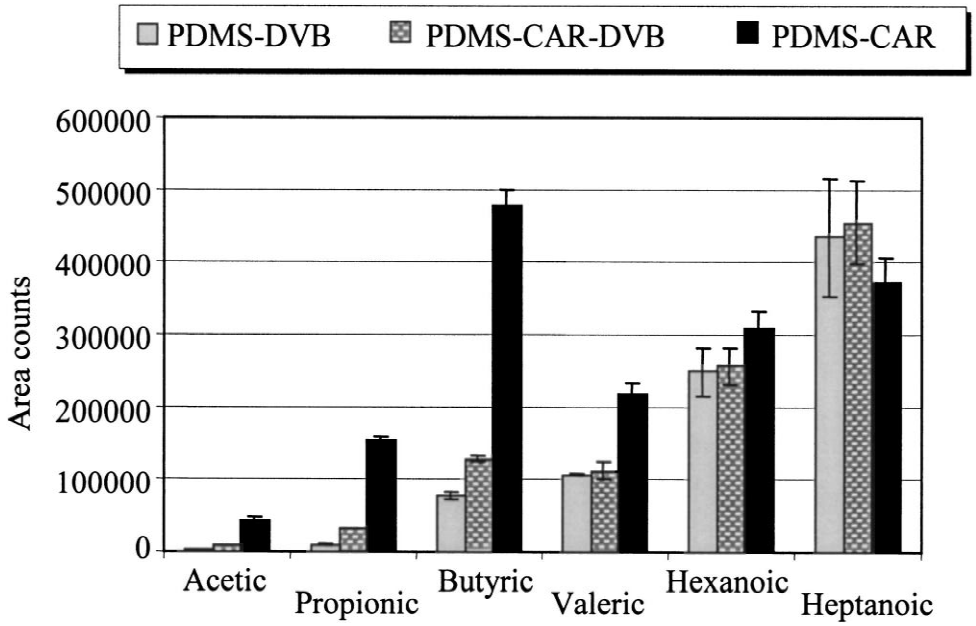

Fig. 1. Comparison of the performance of different SPME coatings. Mean values and standard deviations of three replicates are represented as vertical bars on top of each histogram.

enhancement of the peak area (30%). Nevertheless, the highest temperature had to be used in order to obtain the maximum peak area along with the minimum percentages of carryover, 8% and 12% for hexanoic and heptanoic acids, respectively.

3.3. Extraction time

Extractions were performed at 10, 20, 30, 45, 60 and 90 min. The equilibrium could not be reached for any of those analytes within this time span (Fig. 2). Conversely, most compounds showed a good linearity correlation when areas were monitored in relation to time (R^2=0.991–0.997), only heptanoic acid exhibited a lower regression coefficient (R^2= 0.98). On the other hand, it must be noticed that acetic acid not only gave the lower area response related to compound concentration but it also exhibited the lower response factor with time. Therefore, increasing the extraction time would not lead to a significant improvement of the sensitivity for this acid. Considering that chromatographic separation can be successfully completed within 10 min, the extraction time was finally set at 20 min in order to minimize analysis time without affecting the reproducibility of the whole procedure.

3.4. Extraction temperature

As has been previously discussed, 20 min extraction is a non-equilibrium situation. Therefore, the increase of temperature may have had a noticeable effect on the sensitivity enhancement since extraction kinetics is temperature dependent. Extractions were performed at 25 and 35°C. Fig. 3 shows that the peak areas obtained at 35°C were significantly higher than those achieved at 25°C (factors ranged from 1.8 to 2.6 for C_3–C_7, respectively); except for acetic acid (factor 1.1). However, the relative standard deviations at 35°C were particularly high for hexanoic and heptanoic acids, 26% and 34%, respectively. On the other hand, the PDMS–CAR fiber showed a poor performance after performing the experiments at higher temperature. An explanation to this behaviour could be that the coating might be damaged due to the presence in the headspace of a higher concentration of HCl at the higher extraction temperature.

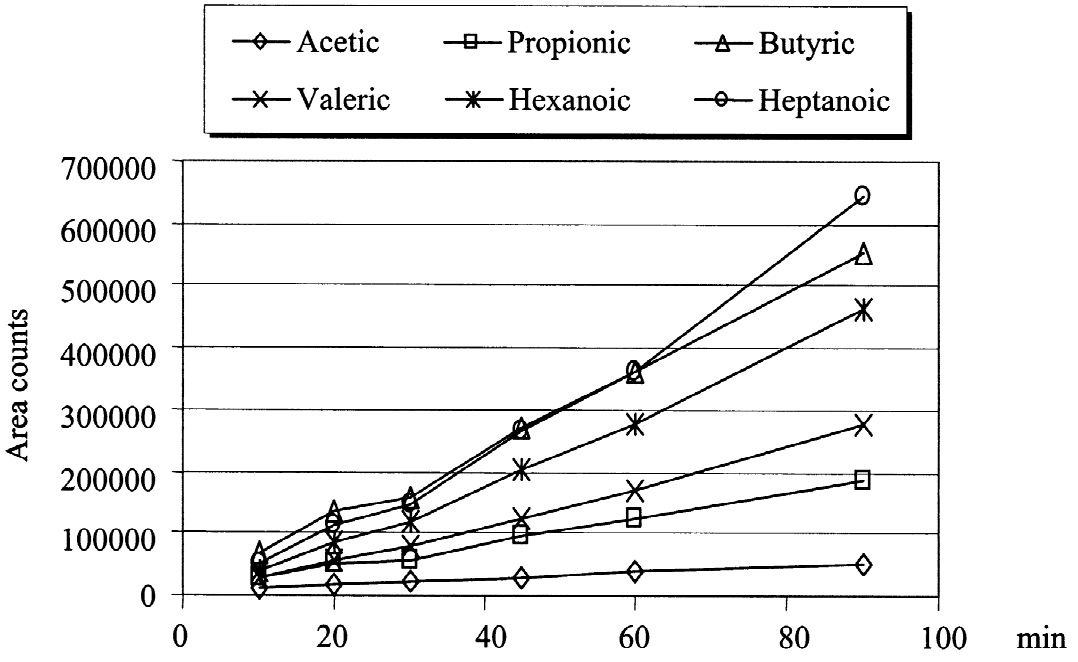

Fig. 2. Extraction time profiles of C_2–C_7 fatty acids using a PDMS–CAR fiber.

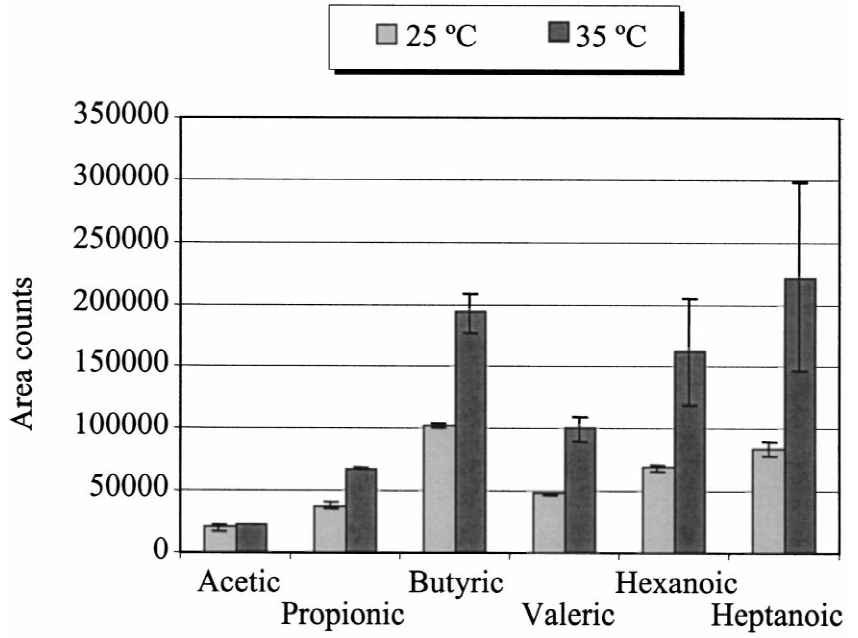

Fig. 3. Effect of extraction temperature on the SPME of C_2–C_7 fatty acids using a PDMS–CAR fiber. Mean values and standard deviations of three replicates are represented as vertical bars on top of each histogram.

In view of these fiber problems and taking into account that the response of acetic acid could not be improved by increasing the extraction temperature, 25°C was finally chosen for further analyses. In fact, if the extraction rate has to be increased in order to obtain a satisfactory sensitivity in lightly polluted real samples, a longer extraction time should be considered instead of increasing the temperature.

3.5. Sample agitation

Theoretically, extraction is always favored if the sample is perfectly agitated [26]. In this study, results obtained when extraction was carried out without agitation of the aqueous phase were compared to those achieved at 600 rpm and 1200 rpm magnetic stirring. As it was expected, peak area increased with the stirring speed. Nevertheless, it must be noticed that the areas obtained when no agitation was performed were relatively high (45–67% of the total peak area at 1200 rpm). This fact could be explained by the volatility of the C_2–C_7 fatty acids, which allow a large amount of the analytes in the headspace even when no agitation is used.

3.6. Sample volume

Sample volume and the ratio between the headspace and the aqueous phase were optimized in the final step of method development. Thus, 10 ml of a spiked sample was placed in vials of different volume (i.e. 15 ml and 40 ml), in order to assess the effect of vial shape and volume on the extraction efficiency. Higher areas where obtained when extraction was performed in the largest vial, the area increase was particularly important in the case of the highest molecular weight acids (hexanoic and heptanoic). On the other hand, responses of valeric, hexanoic and heptanoic acids were significantly decreased when the sample volume was increased from 10 to 20 ml in a 40 ml vial, whereas for acetic, propionic and butyric acids similar areas were obtained (Fig. 4). Therefore, the analyte transfer into the gas phase was slow when the headspace/aqueous

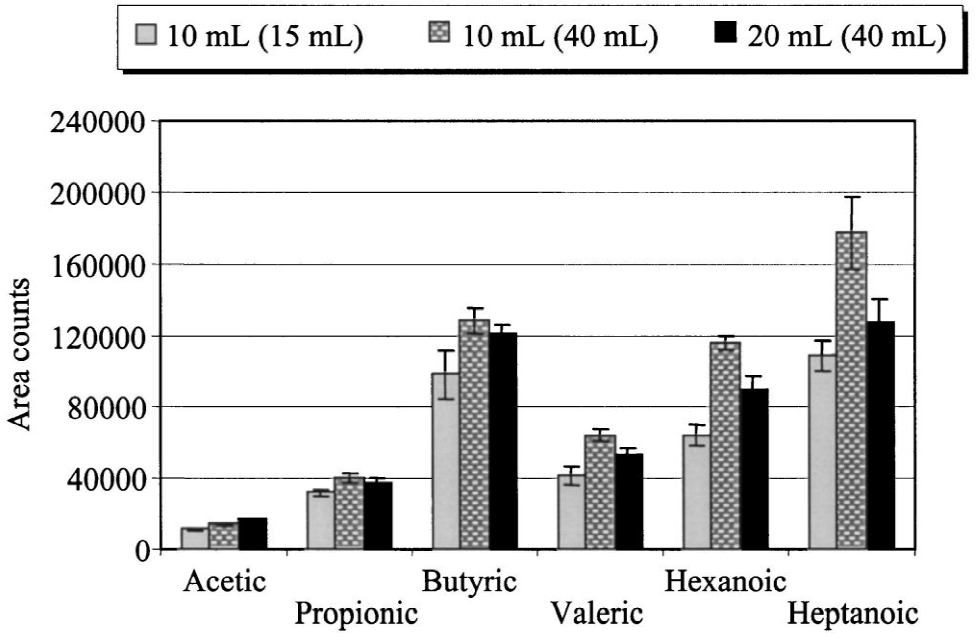

Fig. 4. Effect of sample volume and ratio between headspace/aqueous phase on the SPME of C_2–C_7 fatty acids using a PDMS–CAR fiber. Aqueous phase volume together with vial volume (in parenthesis) are indicated. Mean values and standard deviations of three replicates are represented as vertical bars on top of each histogram.

phase ratio was reduced according to theoretical calculations [27].

3.7. Linearity

The linearity of the SPME–GC–FID procedure was evaluated over a range of four orders of magnitude taking into consideration the concentrations in the aqueous phase. Peak area of the different compounds increased with the alkyl chain length. Therefore, concentrations of C_5–C_7 acids were always set at five times lower than those considered for C_2–C_4 acids in the same standard solution. The linear dynamic ranges together with the response factors for each of the analytes are given in Table 1. In general, the acids with a relatively low response factor (C_3–C_4) have shown good linearity along the whole evaluated range. For valeric and hexanoic acids two different slopes of the calibration curve were found depending on the concentration range considered. Acetic and heptanoic acids exhibited the narrower linear dynamic ranges (two orders of magnitude). Acetic acid linearity was limited at the lowest concentrations due to an exceptionally high detection limit.

3.8. Precision

The reproducibility of the method was determined by performing the extraction of seven water samples spiked at the same concentration (1000 μg l^{-1} C_2–C_4; 300 μg l^{-1} C_5–C_7). The relative standard deviations are shown in Table 1. They are comparable to those usually obtained in others SPME procedures developed for volatile organic compounds [28–30].

Table 2
Concentration of VFAS in fermented raw sewage

Compound	Concentration (μg l^{-1})	
	Sample 1	Sample 2
Acetic acid	10 390	19 614
Propionic acid	7812	2849
Butyric acid	1338	911
Valeric acid	n.d.[a]	n.d.
Hexanoic acid	45	n.q.[b]
Heptanoic acid	n.d.	n.d.

[a] n.d. not detected.
[b] n.q. not quantifiable.

3.9. Limits of detection

Detection (LODS) and quantitation (LOQs) limits given in Table 1 were calculated from the calibration curves by considering the peak area corresponding to three and ten times the signal-to-noise ratio, respectively, of a procedural blank. Standards with concentrations close to the determined detection limits were also analysed in order to confirm them. It is remarkable that the great ability of the PDMS–CAR fiber to extract volatile compounds gives rise to a significant background level that precludes the possibility to obtain lower detection limits. This problem was especially noticeable in the case of acetic acid, which was solved by improving the selectivity in the detection technique.

3.10. Analysis of real samples

The developed procedure was applied to the

Table 1
Figures of merit of headspace SPME–GC–FID in the determination of VFAs

Compound	Linear dynamic range (μg l^{-1})	Response factor	LOD (μg l^{-1})	LOQ (μg l^{-1})	RSD (%) (n=7)
Acetic acid	1095–45 350	4.88	675	6340	11.2
Propionic acid	55–46 450	19.96	54	224	5.6
Butyric acid	5–36 240	67.4	6	55	11.5
Valeric acid	70–6345	174	46	153	8.2
	6345–10 660	99.4			
Hexanoic acid	10–5785	375	19	64	10.4
	5785–9720	120			
Heptanoic acid	70–6700	490	38	127	13.3

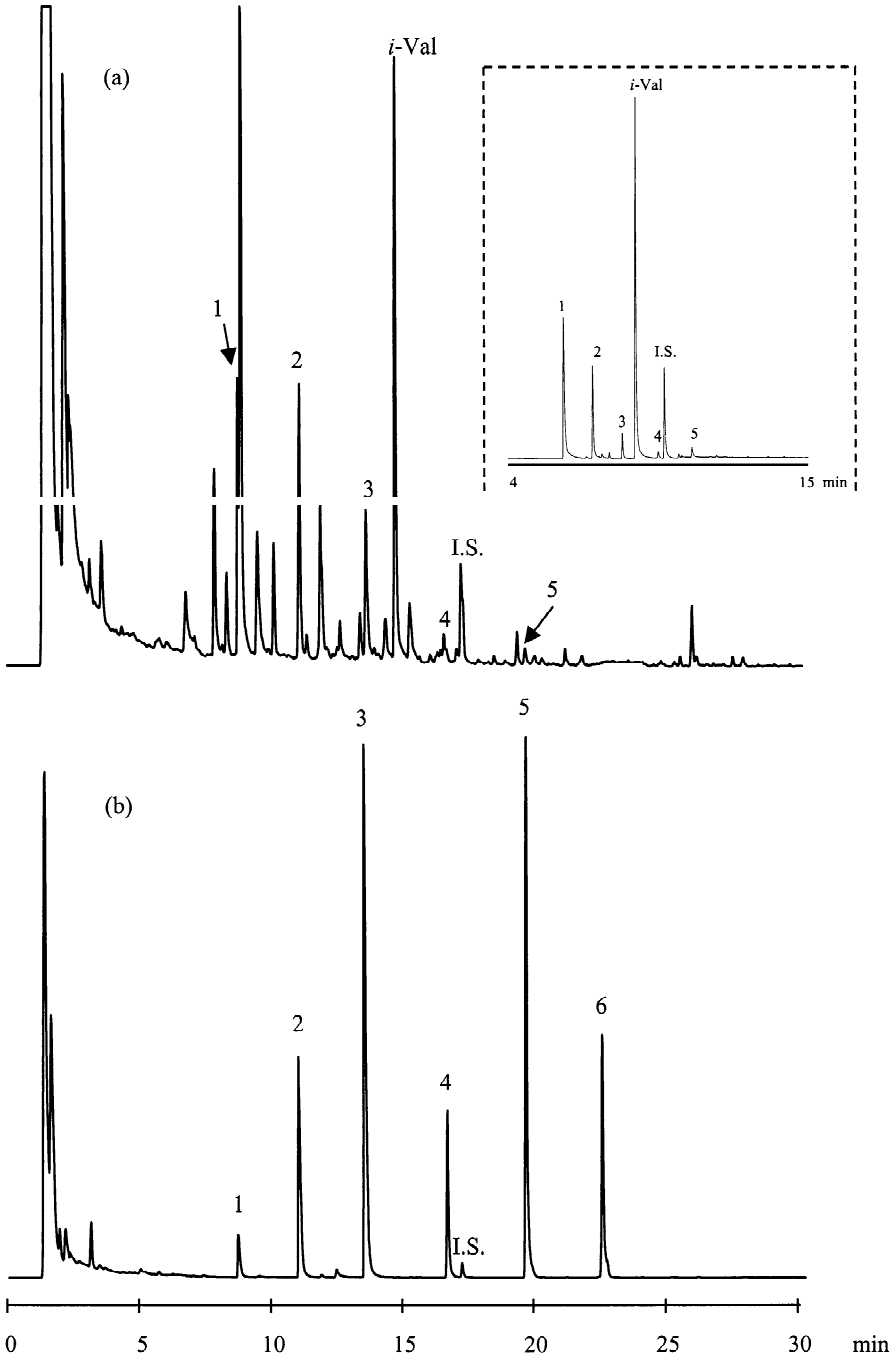

Fig. 5. Headspace-SPME–GC–FID chromatograms of: (a) Fermented urban water sample; (b) Standard solution containing C_2–C_7 fatty acids. The insert included in the chromatogram (a) corresponds to a GC–NCI-MS corresponding to the same sample. Peak identification: (1) acetic acid; (2) propionic acid; (3) butyric acid; (4) valeric acid; isovaleric acid (*i*-Val); (5) hexanoic acid and (6) heptanoic acid. IS: internal standard (2-ethylbutyric acid).

analysis of urban waste waters that were submitted to a fermentation process in order to increase its VFA content. The concentration of VFAs found in these samples are listed in Table 2. The chromatogram corresponding to one of the samples is shown in Fig. 5, together with the FID chromatogram of a C_2–C_7 standard solution is included. The presence of VFAs in the samples was confirmed by GC–NCI-MS. It was demonstrated that despite the presence of additional peaks not related to VFAs, that were coextracted with the fiber, most of the VFAs were well separated and determined using the non-selective GC–FID. However, acetic acid presented interference from the matrix that precluded its determination either by GC–FID or GC–EI-MS since both analytes exhibited similar diagnostic ions. Its determination was only possible by GC–NCI-MS because its higher selectivity of acidic analytes using ammonia as reagent gas [31].

4. Conclusions

A simple, fast and solvent-free method for the analysis of VFAs in moderately to highly polluted waters has been developed. The SPME procedure is based on the use of a PDMS–CAR fiber combined with GC–FID that allows high sensitivity and precision. Parameters affecting the method performance have been optimized. Thus, extraction was finally carried out within 20 min at 25°C, maintaining a vigorous agitation of the aqueous phase, then desorption in the GC injector was kept for 5 min at 300°C. The optimized SPME procedure has been shown to be reliable for fast monitoring of digested urban waste waters. Further research is in progress to expand its application to a wide range of aqueous matrices.

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