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Application of gas chromatography coupled to chemical ionisation mass spectrometry following headspace solid-phase microextraction for the determination of free volatile fatty acids in aqueous samples

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Abstract

Gas chromatography coupled to positive and negative ion chemical ionisation mass spectrometry was evaluated for the determination of free volatile fatty acids (VFAs) from aqueous samples by headspace solid-phase microextraction. Negative ion chemical ionisation in the selected ion monitoring mode using ammonia as reagent gas provided acceptable sensitivity and the highest selectivity for the determination of C_2–C_7 fatty acids using a polydimethylsiloxane–Carboxen fibre. Detection limits in the range of 150 $\mu g\ l^{-1}$ for acetic acid and from 2 to 6 $\mu g\ l^{-1}$ for the remaining carboxylic acids were achieved. The reproducibility of the method was between 9 and 16%. The developed analytical procedure was applied to the analysis of VFAs in raw sewage. The absence of interfering peaks provided a more accurate determination of acetic, propionic, butyric and isovaleric acids than a similar analytical scheme but using a flame ionisation detector.

Keywords: Water analysis; Environmental analysis; Fatty acids; Volatile organic compounds; Carboxylic acids

1. Introduction

Volatile fatty acids (VFAs) comprise a variety of low-molecular weight carboxylic acids containing up to six carbon atoms. These compounds are produced during anaerobic biodegradation of the organic matter. Therefore, they are widespread in raw sewage, activated sludge [1–3] and landfill leachates [4–6]. Monitoring of VFAs is of growing interest since they are, together with sulphur compounds and volatile amines, responsible for odour formation in wastewater treatment or during composting operations. On the other hand, they may have some profitable effects since they act as a source of carbon for microorganisms involved in the removal of phosphorus from waters [7].

Analytical schemes used in the determination of VFAs are based on liquid–liquid extraction [4,8], distillation [9] or purge and trap techniques [10] combined with gas chromatography (GC). Non-polar derivatives (i.e. pentafluorobenzylesters) [11] have been usually prepared from VFAs since they can be analysed easier than the free VFA forms. Moreover, the derivatization process allows an improvement in the detection limits in the determination of ester

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derivatives by GC coupled to electron impact mass spectrometry (EI-MS) [12–14]. On the other hand, chemical ionisation mass spectrometry (CI-MS) has not been attempted in the analysis of VFAs neither in the free nor derivatised forms.

In the present work, GC coupled to CI-MS was evaluated as detection system following headspace solid-phase microextraction (HS-SPME) of underivatised C_2–C_7 fatty acids in aqueous samples. CI-MS was selected as detection system to improve the analytical selectivity and sensitivity in comparison to flame ionisation detection (FID) or EI-MS. This gain in selectivity is particularly relevant in the determination of VFAs in complex mixtures such as wastewater. Two reagent gases (NH_3 and CH_4) were evaluated in both positive and negative ionisation modes. Other variables optimised in CI-MS were the reagent gas pressure and the ion source temperature. Finally, linearity, detection limits and reproducibility of the whole analytical procedure were calculated and the methodology was applied to the determination of VFAs in urban sewage samples.

2. Experimental

2.1. Chemicals and materials

Acetic (99.99%) and propionic (99.5%) acids were obtained from Aldrich (Milwaukee, WI, USA). Butyric (99%), valeric (99%), hexanoic (99.5%), heptanoic (99%) and 2-ethylbutyric (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 polydimethylsiloxane–Carboxen (PDMS–CAR) 75 μm fibre were obtained from Supelco (Bellefonte, PA, USA). Stock standard solutions of each analyte (1.25–10 mg ml^{-1}) were prepared in Milli-Q water. Standard mixtures for the preparation of working standard solutions were obtained diluting the individual stock solutions with Milli-Q water. All the standard solutions were stored at 4°C in the darkness.

2.2. SPME procedure

Optimisation of the HS-SPME procedure for the determination of VFAs in water samples is described elsewhere [15]. Briefly, 10 ml of Milli-Q water or real sample were placed into a 40 ml vial together with 3.75 g of NaCl, then the vial was sealed with a septum lined with PTFE and spiked with a determined amount of the internal standard (2-ethylbutyric acid) by injection through the septum. For spiked samples, a given volume of VFA standard mixture was also added. Next, the 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. Extraction temperature was set at 25°C using a thermostated water bath.

2.3. Instrumental analysis

GC–CI-MS analyses were performed in a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA, USA) coupled to a Hewlett-Packard 5988A mass spectrometer. A tailor-made capillary column designed for free fatty acids analysis (TR-FFAP, Teknokroma, Barcelona, Spain) of 30 m× 0.25 mm I.D. coated with 0.25 μm film thickness of polyethylene glycol modified with nitroterephthalic acid was used. Helium at 1 ml min^{-1} was the carrier gas. The oven temperature was programmed from 70°C for 1 min, then to 10°C min^{-1} until 200°C holding the final temperature for 1 min. Fibre desorption was performed in a split/splitless injector at a temperature of 300°C for 5 min.

CI-MS detection was performed in the negative mode using NH_3 (>99.995%) provided by Air Liquide (Bois d'Arcy, France). The optimum pressure and ion source temperature were 1 Torr (133.3 Pa) and 210°C, respectively. In addition, CH_4 (>99.9995%) from Air Liquide was also evaluated as reagent gas in the positive mode. Optimum conditions were an ion source temperature of 150°C and 0.8 Torr (106.6 Pa) reagent gas pressure. Temperatures of the transfer line and the quadrupole analyser were set at 180°C and 100°C, respectively. In the scan mode, the acquisition range was in the 40–300 m/z range. Ions considered in the selected ion monitoring mode (SIM) are listed in the following section.

The detection limits were calculated taking into account the areas corresponding to three times the S/N ratio of a procedural blank. Data were calculated from calibration curves closer to the lowest

detectable concentration range and the obtained results were confirmed by analysing standard mixtures of these concentrations or lower.

3. Results

3.1. Selection of reagent gas and ionisation mode

In order to evaluate the VFA ionisation process in respect to different CI-MS systems, two reagent gases (i.e. NH_3 and CH_4) were investigated in both positive and negative modes.

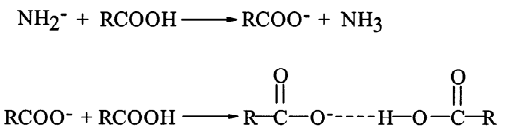
Ammonia

Determination of VFAs by CI-MS was firstly attempted in the negative ion chemical ionisation (NCI) mode using NH_3 as reagent gas. Due to the acid character of the analytes and the basic properties of the reagent gas, an acid–base reaction in the gas phase is expected giving rise to the carboxylate anion formation of the corresponding carboxylic acids, $[R–COO]^-$.

In a previous work, Grützmacher et al. [16] reported that these ions were one of the major products obtained from the reaction of a selected group of esters with the NH_2^- ion, produced by electron capture in NH_3. Mass spectra of VFAs confirmed that $[M–H]^-$ was one of the few ions obtained and no dissociative resonance capture ionisation mechanisms occur. However, a secondary ion is originated from an ion–molecule condensation reaction involving a neutral molecule of the acid M and the $[M–H]^-$ ion. A proposed mechanism involving hydrogen bonding of the ionised molecule is given in Fig. 1a as suggested elsewhere for propionic and butyric acids [17]. The relative percentage of this secondary ion in respect to the $[M–H]^-$ ion was always low, but it depends on the analyte concentration. In this regard, it increased from 0.1 to 8.6% when the concentration of VFAs in the aqueous matrix varied along two orders of magnitude. The m/z ratio of the two ions observed for every carboxylic acid is listed in Table 1.

The VFA behaviour in the NH_3 reagent gas in the positive ion mode was also evaluated. Theoretically, VFAs should not be protonated in that system since their proton affinities (PA) are below the NH_3

(a)



(b)

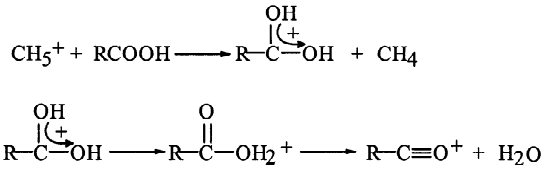


Fig. 1. Proposed mechanisms for chemical ionisation of short-chain fatty acids in: (a) NCI with NH_3, and (b) PCI with CH_4.

(PA_{NH3}=204 kcal mol^{-1}; cal=4.184 J). However, $[M+NH_4]^+$ adducts could be formed [18,19]. In this work, the adduct formation does not seem to occur in the ionisation conditions used. Moreover, the ion source temperature was increased from 150 to 250°C in order to investigate the feasibility that a nucleophilic substitution mechanism might be favoured at high temperatures giving rise to the formation of the corresponding protonated amides, but it was not observed.

Methane

The ionisation process of carboxylic and dicarboxylic acids in CI-MS using CH_4 in positive ion chemical ionisation (PCI) has been widely studied [20–22]. For this reason, the behaviour of VFAs in the above mentioned system was evaluated as an alternative to the NH_3 negative system. The experimental mass spectra obtained agree with those found for others groups of acids reported previously [20–22], the $[M+H]^+$ was the most abundant ion followed by the H_2O elimination from that former ion (Fig. 1b). This mass spectra contrasts to the one obtained with NH_3 in the NCI, since in the PCI, the percentage of the secondary ion was significantly higher (ca. 10 and 17%), independently on the analyte concentration. The m/z ratio of these two ions observed for every acid is listed in Table 1.

The VFA mass spectra obtained in the NCI using CH_4 as a reagent gas were compared to those of the NH_3. At these conditions, CH_4 was expected to act

Table 1
Main ions obtained in the two CI-MS ionisation modes evaluated in the HS-SPME–GC–MS determination of volatile fatty acids

Volatile fatty acids	*m/z* values			
	NCI (NH_3)		PCI (CH_4)	
	$[M-H]^-$ Main ion	$[M-H+M]^-$ Secondary ion	$[M+H]^+$ Main ion	$[M+H-H_2O]^+$ Secondary ion
Acetic	59	119	61	43
Propionic	73	147	75	57
Butyric	87	175	89	71
Valeric	101	203	103	85
Hexanoic	115	231	117	99
Heptanoic	129	259	131	113
Secondary ion (%)		(0.1–8.6)[a]		13.5[b]

[a] The percentage is proportional to the VFA concentration. The range given corresponds to two orders of magnitude variation in the concentration.
[b] Mean value.

as a source of thermal electrons leading to an electron capture ionisation mechanism. Therefore, the M^- should be the most abundant ion if this mechanism. However, $[M-H]^-$ was the main ion obtained in the spectra, suggesting that the acid–base ionisation is favoured. Nevertheless, lower response was obtained with CH_4 than with NH_3 in the NCI mode reflecting the different basic strength of the reagent gases.

Accordingly, NH_3 in the NCI and CH_4 in the PCI, were selected for further optimisation of the HS-SPME GC–CI-MS as detection systems that may allow enough selectivity and sensitivity in the analysis of VFAs in real samples.

3.2. Optimisation of reagent gas ion source pressure

The reagent gas pressure was evaluated along the operational range of the CI-MS (0.5–1.4 Torr). In case of NH_3, a significant improvement in the response was found when increasing the pressure up to 1 Torr, but above this value the response dropped again (Fig. 2a). Moreover, constant fluctuations in the background level were observed at the highest ion source pressure (1.4 Torr), which could be attributed to the presence of small amounts of liquefied ammonia. On the contrary, the ionisation process of VFAs using CH_4 as reagent gas is favoured at lower pressures than with NH_3 (Fig. 2b). The maximum response was obtained in the range of 0.6–0.8 Torr. In particular, there were not significant differences between these two pressures for the ionisation of the highest-molecular weight acids (i.e. valeric, hexanoic and heptanoic acids).

3.3. Optimisation of ion source temperature

Three different temperatures were evaluated for both reagent gases, NH_3 and CH_4. In the case of NH_3, there were not significant differences in the response when increasing the temperature from 150 to 210°C (Fig. 3a), only for hexanoic and heptanoic acids a slight increase in the peak area was detected at the highest temperatures. For this reason, the temperature was finally set at 210°C in order to minimise the contamination of the ion source. Conversely, when CH_4 was used as reagent gas, the maximum response for all the acids was obtained at the lowest temperature evaluated (150°C) (Fig. 3b).

3.4. Linearity

The linearity of the whole analytical procedure, including the HS-SPME step, was evaluated in the two CI-MS ionisation modes optimised. Data were acquired in the SIM mode, and the $[M-H]^-$ and $[M+H]^+$ ions were chosen for quantitation in NCI with NH_3 and PCI with CH_4, respectively. 2-Ethylbutyric acid was used as internal standard.

Linear dynamic ranges together with correlation coefficients for the VFAs using CI-MS and FID are

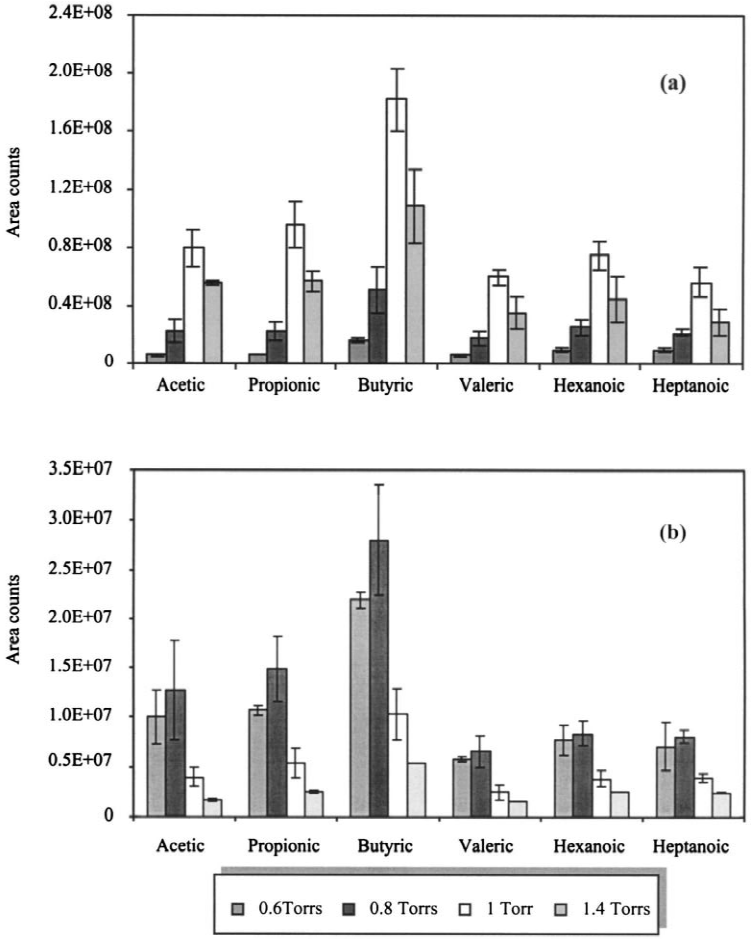


Fig. 2. Effect of the reagent gas pressure in the response in: (a) NCI-MS with NH_3, (b) PCI-MS with CH_4. Mean values and standard deviations of three replicates are displayed as vertical bars on top of each histogram.

listed in Table 2. In general, linear dynamic ranges in CI-MS were narrower than those obtained with the FID. This was particularly notorious in case of the lowest molecular weight acids (i.e. acetic, propionic and butyric). Moreover, a remarkable dependence between the correlation coefficient and the number of carbons present in the acid was found, as the hydrocarbon chain becomes longer better correlation coefficients were obtained. It could be attributable to a poorer ionisation yield for the shorter chain fatty acids. In fact, the correlation between area and concentration found for acetic, propionic and butyric acids when using PCI-MS were not acceptable to consider these calibration curves for quantitation.

3.5. Detection limits

Detection limits of the VFAs obtained with CI-MS and FID are reported in Table 3. In nearly all cases, the detection limits with CI-MS were lower than those obtained with FID. Particularly, in the case of NCI the detection limits of propionic, valeric and heptanoic acids were clearly below to those reported using FID.

3.6. Precision

The reproducibility of the method was determined for both, NCI and PCI modes. In general, the relative

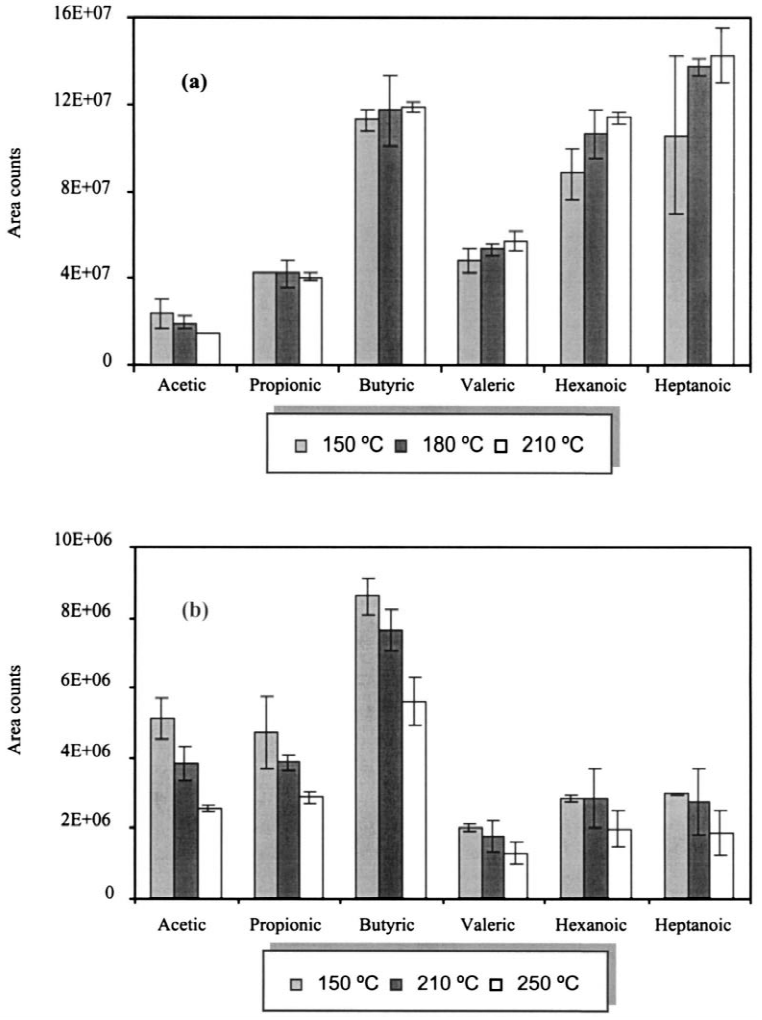


Fig. 3. Effect of the ion source temperature in the response in: (a) NCI-MS with NH_3, (b) PCI-MS with CH_4. Mean values and standard deviations of three replicates are plotted as vertical bars on top of each histogram.

Table 2
Comparison of the linear dynamic range and linear correlation coefficient (values in parentheses) obtained with FID and the two CI-MS methods evaluated

VFAs	Linear range ($\mu g\ L^{-1}$), (n=4–13)[a]		
	FID	NCI-MS (NH_3)	PCI-MS (CH_4)
Acetic	1095–45350 (0.989)	1058–2995 (0.988)	250–1084 (0.984)
Propionic	55–46450 (0.994)	514–2603 (0.989)	58–1903 (0.956)
Butyric	5–36240 (0.996)	526–2665 (0.987)	48–2697 (0.955)
Valeric	70–6345 (0.995)	10–820 (0.990)	20–836 (0.995)
Hexanoic	20–5785 (0.994)	11–907 (0.996)	16–635 (0.995)
Heptanoic	70–6700 (0.984)	10–5759 (0.999)	20–516 (0.991)

[a] A interval of 13 concentrations was evaluated in all the cases. However, the number of points defining the linear range varies depending on the width of this range.

Table 3
Comparison of the limits of detection (LODs) obtained with HS-SPME using different detection systems

Volatile fatty acids	LOD ($\mu g\ L^{-1}$)		
	FID	NCI-MS (NH_3)	PCI-MS (CH_4)
Acetic	675	150	115
Propionic	54	5	25
Butyric	6	2	26
Valeric	46	2	11
Hexanoic	19	6	13
Heptanoic	38	5	10

standard deviations obtained from the analysis of seven standard mixtures with the same concentration were in the range of 9–16%. They were comparable to those previously obtained using FID [15]. However, unusually high values were obtained for acetic and propionic acids when using PCI (37 and 18%, respectively). These variations could be related with the non-linear behaviour observed for these compounds (see Section 3.4).

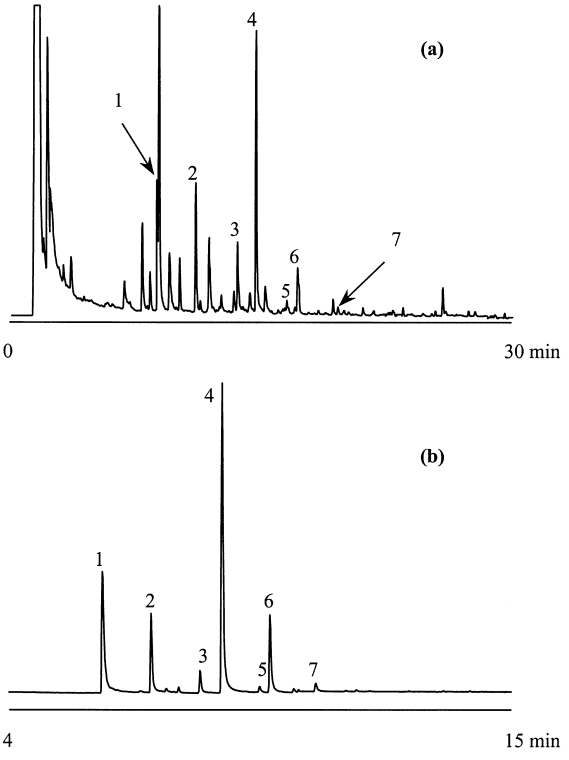


Fig. 4. HS-SPME–GC chromatograms of a fermented urban sewage using: (a) FID, ramp: 4°C/min; (b) NCI-MS in the SIM mode with NH_3 as reagent gas, ramp: 10°C/min. Peak identification: (1) acetic acid; (2) propionic acid; (3) butyric acid; (4) isovaleric acid; (5) valeric acid; (6) 2-ethylbutyric acid (I.S.); (7) hexanoic acid.

3.7. Application to real samples

Taking into account the results reported in the previous sections, GC–MS in the NCI mode using NH_3 was selected as the most convenient detection system following HS-SPME of VFAs in water. As mentioned above, lower detection limits were obtained in respect to FID, besides a great improvement in the selectivity (analyte/interference ratio $>10^4$) was observed in the analysis of real samples. Fig. 4 shows a comparison between the FID chromatogram and the selected ion monitoring chromatogram obtained in GC–NCI-MS of a fermented urban sewage. The absence of interfering peaks in the GC–NCI-MS chromatogram allows an accurate quantification of all the VFAs of interest.

4. Conclusions

An analytical method for the determination of free VFAs based on HS-SPME–GC–MS in the NCI mode, using NH_3 as reagent gas, has been developed for the first time. This method improves both detection limits and selectivity in respect to FID and GC–MS in the EI mode because the ionisation mechanism is based on proton abstraction from the carboxylic group by the ammonia reagent gas. The improvement in the selectivity is particularly important when analysing complex samples because interferences from other compounds adsorbed on the SPME fibre are not detected in the NCI-MS selected ion monitoring mode. Therefore, HS-SPME combined to GC–NCI-MS has been shown to be a reliable, low-time consuming and cost-effective method for the determination of VFAs in water, even if complex samples (i.e. raw sewage) are considered.

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