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Original Research

Determination of Volatile Fatty Acids in Environmental Aqueous Samples

E.M. Siedlecka, J. Kumirska, T. Ossowski, P. Glamowski, M. Gołębiowski, J. Gajdus, Z. Kaczyński, P. Stepnowski*

Faculty of Chemistry, University of Gdańsk, ul. Sobieskiego 18, PL-80-952 Gdańsk

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Abstract

Volatile fatty acids (VFAs) are present in environmental waters in the range of 1 to 5,000 ppm and different methods have been reported for their determination. In this paper we have studied and compared analytical performance parameters for the distillation method followed by potentiometric titration, spectrophotometric and gas chromatographic methods. The main disadvantage of the distillation approach was quite poor absolute recovery (53-58%) from the given matrix and rather elevated limit of quantification (LOQ) at 110 mg/L. Direct potentiometric titration was characterized by acceptable accuracy (above 97%) and precision in the range 1.8%-15%. The LOQ value was 11 mg/L. The spectrophotometric method was sensitive for hydrogen carbonate alkalinity and phosphate ions; measured concentrations of acetic acid were lower than nominal. The precision and accuracy of the spectrophotometric method were in the ranges 1.3-14% and 82.1-104.2%, respectively. Limit of quantification was 28 mg/L. However, if ion exchange bed is used prior to this method the LOQ can be reduced to 5 mg/L. The GC method is characterized by quite low LOQ (5 mg/L) and seems to be the best methodology to determine low VFA concentrations in environmental waters. The precision of the method ranged from 5.7 to 14.8% and accuracy was above 92%. Additionally, this method allows for determination of individual VFAs.

Keywords: volatile fatty acids, environmental waters, spectrophotometry, gas chromatography, distillation, potentiometric titration

Introduction

Low-molecular mass carboxylic acids, (C_2-C_7 monocarboxylic aliphatic acids) are important intermediates and metabolites in biological processes. These carboxylic acids are known as volatile fatty acids (VFAs) or short-chain fatty acids (SCFAs). The presence of VFAs in a sample matrix is often indicative of bacterial activity. VFA analysis is significant in studies of health and disease in the intestinal tract [1]. In some foods VFA content is an index to quality assurance [2]. Volatile fatty acids originate from anaerobic biodegradation of organic matter. Therefore, they are widely present in activated sludge [3], waste and landfill leachates [4], and wastewater. Recently, the determination of VFAs has become of increasing interest since it has been found that they are involved in different processes, for example in biological removal of phosphorus from water [5] or nitrification- denitrification in activated sludge [6]. Carboxylic acids may also affect the storage stability of waste incineration residues by reducing pH value and increasing the mobility of heavy metals and radionuclides. In addition, VFAs constitute one of the chemical classes responsible for unpleasant

*e-mail: sox@chem.univ.gda.pl

odour generated in wastewater, together with amines and sulfur compounds.

Additionally, large quantities of potassium and sodium salts of VFAs are used as airplane, runway and apron deicers. After being used, these compounds can contribute to the increase of chemical oxygen demand, (COD) thus decreasing overall water quality. In winter, the biodegradation of used deicing solutions can be limited by microbial inhibition due to low temperatures. In these seasons, VFAs are usually accumulated in various environmental compartments and their concentrations increase in waters [7].

Volatile fatty acids exist in environmental matrices in different concentrations. The results of investigations carried out in Polish municipal wastewater plants concerning VFA levels in wastewater in 2004-05 are presented in Table 1. Additionally, the range of volatile fatty acid concentrations in surface water [8] and landfill leachates are presented [9]. It has been noticed that the range of VFA concentrations is wide; the content may vary from a few mg/l in surface water to thousands mg/L in landfill leachates.

In literature different methods for the determination of VFAs in environmental samples are reported. Traditionally, the VFA content in wastewaters and foods has been analyzed by titrimetric or gas chromatographic methods preceded by solvent extraction. The rapid colorimetric determination of organic acids are also in use [10]. More recently, ion chromatography and HPLC [11] have also been applied to VFA analysis.

This paper investigates advantages and disadvantages of simple procedures used for the routine analysis of volatile fatty acids in wastewater: distillation, spectrophotometric and gas chromatographic method. Our work aimed primarily to verify the repeatability, precision and limits of quantification of investigated methods and to estimate the matrix influence of the obtained results.

Materials and Methods

Chemicals and Materials

All reagents were of analytical grade and used without further purification. All reagents were purchased from PPH "POCh" SA (Gliwice, Poland), unless otherwise indicated. The inorganic salts: NaH_2PO_4 and $NaHCO_3$, were acquired from Chempur (Piekary Śląskie, Poland).

Determination of VFA by a Distilled Method

500 mL water samples containing various concentrations of acetic acid were steam-distilled in accordance to the official Polish Normalized Committee methods PN-75/C-04616 [12] and as described in *Standard Methods for Examination of Water and Wastewater (APHA, AWWA, WEF, Washington 1992)*. The content of volatile acids in distillate was determined by titration with 0.1N NaOH solution and expressed as acetic acid content. This procedure was modified in this study by replacement of the conventional titration with the potentiometric titration system. The concentration of NaOH solution was matched with acetic acid content in samples every time.

Determination of Acetic Acid by a Spectrophotometric Method

The spectrophotometric procedure is based on the well known colorimetric ferric hydroxamate method for determination of carboxylic esters known as the Montgomery method [10]. In this study the procedure was modified as follows: aqueous sample (0.5 mL) was taken into a dry test-tube, and then after the addition of 1.5 mL ethylene glycol reagent and 0.2 mL of 19.5N sulphuric acid the mixture was heated for 3 min in a boiling bath. Then, the

Table 1. The range of volatile fatty acid concentrations in wastewater and water.

Sample	population equivalent of municipal wastewater treatment plant	Range of VFA concentrations
Municipal wastewater influent to biological treatment stage [a)]	450,000	65-168 mg CH_3COOH/L
	27,000	124-150 mg CH_3COOH/L
	8,000	70-150 mgCH_3COOH/L
Municipal wastewater influent to wastewater plant [a)]	450,000	28-69 mg CH_3COOH/L
Landfills leachate [b)]		0- 19,000 mgCH_3COOH/L
Water from the airport region collected in winter [a)]		52-2,500 mg CH_3COOH/L
Surface water [c)]		0-3 mg CH_3COOH/L

a – VFAs were determined by distilled method (PN-73/C-04616). Investigations were carried out in Poland in 2004-05 by authors (data unpublished), b – [9], c- [8].

content of the test-tube was immediately cooled in cold water. After cooling, 0.5 mL of 10% hydroxylamine hydrochloride solution, 2 mL of 4.5 N NaOH solution and 10 mL of 10% ferric chloride solution were added. The absorbance was measured at 495 nm by spectrophotometer (Odyssey HACH). The standard solutions of acetic acid (1200 mg/L CH_3COOH) were diluted with deionized water to obtain appropriate concentrations in the range of 10 to 1200 mg/L. Deionized water was obtained in-house by treating tap water with a carbon filter, reversed osmosis, a mixed bed of ion exchangers and a 0.45 µm filter.

The effect of the alkalinity and phosphate ions on the spectrophotometric response for the acetic acid was also investigated.

The application of ion exchanger (Amberlite IRA 410) for VFA separation has been also investigated. The different volume of feed solution was subjected to the top of the column and flow down. VFAs were eluted using different volume of saturated solution of NaCl at the flow rate 0.4 mL/min. The analytes were collected and determined by spectrophotometric method.

Determination of VFAs by Gas Chromatographic Method

The preparation of samples for determination of VFAs by GC method was based on Manni and Caron's procedure [4]. Briefly, the samples were acidified to pH 2 using 65% of nitric acid.1 mL portions were shaken along with 1 mL of diethyl ether for approximately 10 min, and the ether phases were quantitatively transferred to 4-mL flasks, where a small amount of anhydrous sodium sulfate was added. The 500 µL portions of ether phases were transferred into new 4-mL flasks and 150 µL of diazomethane was added.

A series of VFA standards for the calibration curves were prepared in the same manner as described above. Calibration curves were obtained using five aqueous solutions of acids: acetic, propionic, butyric, valeric, and caproic, in the concentration range of 5 to 1000 mg/mL.

GC analyses were performed on a GC 8000 TOP (CE Instruments) gas chromatograph equipped with a flame ionization detector and a DB-23 capillary column (30 m, 0.25 mm I.D., 0.25 µm film thickness, Alltech, Poland). The injector and detector temperatures were both 170°C. The carrier gas was argon. The analyses were performed using a temperature programme: 5 min at 30°C and a linear gradient from 30°C to 130°C at 10°C min^{-1}. In each case a 2 µL of sample was injected (a flow splitting 1:10).

Results and Discussion

Determination of VFAs by Distillation Method

The routine determination of volatile fatty acids in wastewater is preceded by quantitative isolation of VFAs from the matrix. In the distillation method, at first the wastewater sample is steam-distilled. Then, collected distillate of carboxylic acids is titrated with standard alkaline solution.

In our study validation parameters of distilled method was investigated and the obtained results are presented in Table 2.

The precision of the method was determined by calculating the relative standard deviation (RSD) while accuracy was evaluated through the comparison between the measured and nominal concentrations of acetic acid in measured samples (%). The results clearly show that mean values of measured concentrations (AVG values) were significantly lower than nominal concentrations. Therefore, the validation parameters of potentiometic titration itself were also investigated. Good results were obtained in the range from 11 to 1164 mg/L of acetic acid. The potentiometric method was characterized by acceptable accuracy (above 97%) and precision in the range 1.8%-15%, (Table 3). Limit of quantitation (LOQ) was 11 mg/L.

Secondly, the absolute recovery of acetic acid from the matrix was studied. The results given in Figure 1 indicate that the absolute recovery of acetic acid in the range from 11 to 1156 mg/L was quite poor; only 53%-58% of nominal concentration was found in a distillate.

Table 2. Assay validation results for acetic acid analysed by distillation method. Measurements of acetic acid concentrations made through automated potentiometric titration with 0.1N NaOH solution (n = 4).

Acetic acid				
Nominal concentration [mg/L]	110	123	978	1200
AVG [mg/L]	64	70	567	636
SD	1.5	1.3	14.6	31.1
Precision (RSD%)	2.3	1.8	2.6	4.9
Accuracy (%)	58.2	56.9	58.0	53

AVG- mean value of measured concentration, SD- standard deviation, RSD- relative standard deviation

Table 3. Assay validation results for acetic acid analyzed by automated potentiometric titration with 0.1N NaOH solution without distillation step (n = 4).

Acetic acid					
Nominal concentration [mg/L]	11	110	123	978	1200
AVG [mg/L]	12	111	124	972	1164
SD	1.8	2.6	2.2	25	57
Precision (RSD%)	15	2.3	1.8	2.5	4.9
Accuracy (%)	109.0	100.9	100.8	99.3	97.0

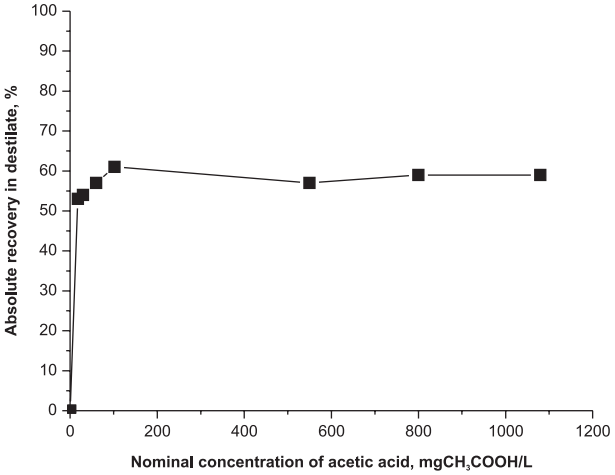

Fig. 1. Absolute recovery of acetic acid in distillate. Acetic acid concentrations measured with automated potentiometric titration with 0.1N NaOH solution.

It must also be noticed that acetic acid, which does not form an azeotropic mixture with water, was particularly difficult to recover. Therefore, accurate analysis of acetic acid by distillation method in wastewater is mainly dependent on recovery from matrix.

Spectrophotometric Determination of VFAs

The spectrophotometric determination of VFAs is designed specifically for determining volatile fatty acids in the digester sludge. The method is based on estrification of the carboxylic acids present in sample and subsequent determination of the esters by the ferric hydroxamate reaction. All volatile fatty acids present in the sample are reported as their equivalent mg/L of acetic acid. This spectrophotometric method is characterized by a relatively simple procedure, commonly available reagents and quite short time of analysis. The validation results obtained for spectrophotometric determination of acetic acid are shown in Table 4.

The precision and accuracy of the method was in the range 1.3-14% and 82.1-104.2% for concentrations of acetic acid between 28-450 mg/L, respectively. Limit of quantification (LOQ) was 28 mg/L. A single analysis by this procedure takes 25 min., thus it is suitable for multiple determination procedure. Other advantages of this methodology are that the volume of sample is quite small (0.5 mL) and the determination is carried directly from the sample.

Table 4. Assay validation results for acetic acid analyzed by the spectrophotometric method based on the determination of carboxylic acid esters measurement (495 nm) according to the Montgomery method [10] (n = 4).

Acetic acid						
Nominal concentration [mg/L]	28	70	138	198	309	450
AVG [mg/L]	23	73	132	192	313	457
SD	3.1	3.6	3.2	2.6	6.4	43.6
Precision (RSD%)	14	4.9	1.7	1.3	2.0	9.5
Accuracy (%)	82.1	104.2	95.6	96.9	97.0	97.0

Separation of VFAs from the matrix by ion exchange bed was also investigated. The optimal conditions of ion exchanger performance for 300 mL of the feed solution were 3 mL/min. flow rate and 10 mL of eluent (saturated solution of NaCl). The ion exchanger bed used allowed us to reduce the limit of quantification to 5 mg/L of CH_3COOH.

The effect of alkalinity and phosphate ions (a common measure of the buffer capacity of environmental waters) on the spectrophotometer response for VFA determination was also investigated. The results are shown in Figure 2. It is clear that in the presence of hydrogen carbonate and phosphate ions, measured concentrations of acetic acid were lower than nominal.

Determination of VFAs by Gas Chromatography

Determination of VFAs by GC method included few important steps: acidification of sample by nitric acid, extraction of VFAs to diethyl ether phase, conversion of VFAs into methyl esters and final chromatographic analysis of obtained derivatives. For quantitative analysis the external standard methodology was used. Quantitative calibration proceeded for five acids: acetic, propionic, butyric, valeric, and caproic, in concentrations ranging from 5 to 1000 mg/mL. A series of VFA calibration standards were prepared in the same manner as for samples. A GC chromatogram of results obtained for the standard solutions of VFAs methyl esters (500 mg/mL in water) is presented in Fig. 3.

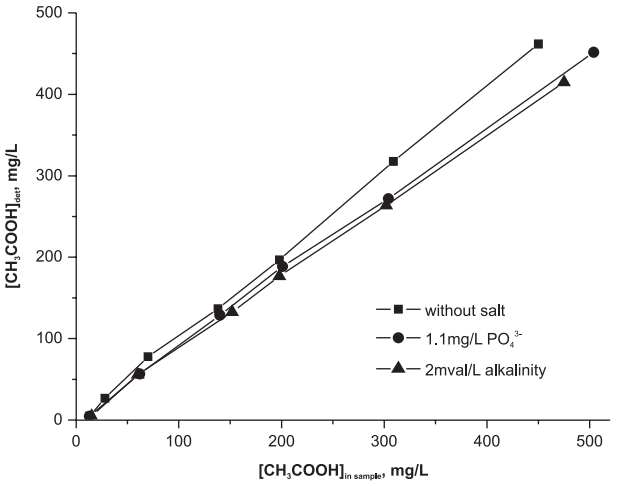

Fig. 2. Influence of hydrocarbonate (2 mval/L) and phosphate ions (1.1 mg/L PO_4^{3-}) on the determination of acetic acid with the spectrophotometric method based on the determination of carboxylic acid esters measurement (495 nm) according to the Montgomery method [10]

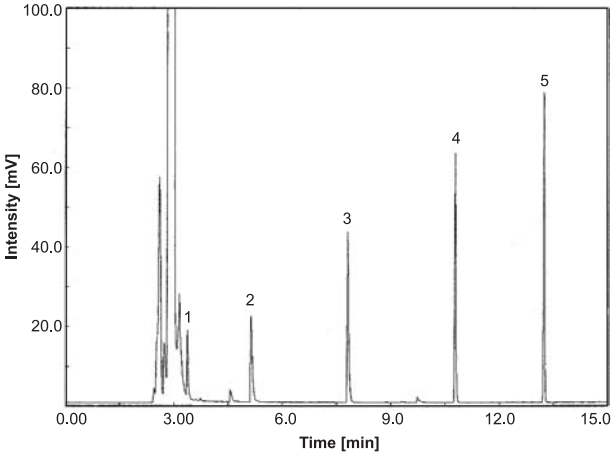

Fig. 3. GC chromatogram of methyl esters obtained from standard solutions of five acids (each 500 mg/mL): acetic, propionic, butyric, valeric, and caproic acids. The preparation of samples for there determination by GC method was based on Manni and Caron's procedure [4]. Chromatographic conditions as follows: DB-23 capillary column (30 m, 0.25 mm I.D., 0.25 μm film thickness, Alltech, Poland). The injector and detector temperatures at 170(C. The carrier gas was argon. The temperature programme: 5 min at 30°C and a linear gradient from 30°C to 130°C at 10°C min^{-1}. In each case a 2 μL of sample was injected (a flow splitting 1:10).

The calibration curve of the most important VFA – acetic acid – was linear over the range from 5 to 1000 mg/L with the square correlation coefficient of 0.9994, which confirmed a good fit to the regression lines (Fig. 4).

Precision and accuracy of the method was determined by analysis of replicates (n = 6) of samples containing known concentrations of acetic acid. RSDs ranged from 5.7 to 14.8% and accuracy was above 92% (Table 5). These results demonstrated acceptable accuracy and precision of the presented method. The limit of quantification

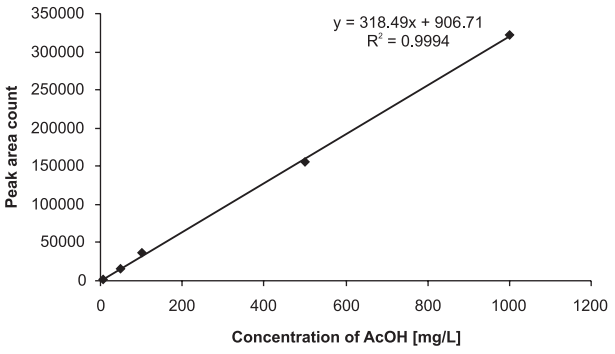

Fig. 4. Curve calibration of acetic acid obtained by GC method, where: y – peak-area of methyl derivative of acetic acid; x – the concentration of acetic acid in water. DB-23 capillary column (30 m, 0.25 mm I.D., 0.25 μm film thickness, Alltech, Poland). The injector and detector temperatures at 170(C. The carrier gas was argon. The temperature programme: 5 min at 30°C and a linear gradient from 30°C to 130°C at 10°C min^{-1}. In each case a 2 μL of sample was injected (a flow splitting 1:10).

Table 5. Assay validation results for acetic acid analysed by GC method. DB-23 capillary column (30 m, 0.25 mm I.D., 0.25 μm film thickness, Alltech, Poland). The injector and detector temperatures at 170°C. The carrier gas was argon. The temperature programme: 5 min at 30 ºC and a linear gradient from 30 ºC to 130 ºC at 10 ºC min^{-1}. In each case a 2 μL of sample was injected (a flow splitting 1:10).

Acetic acid					
Nominal concentration [mg/L]	5	50	100	500	1000
AVG [mg/L]	4.6	49.2	111.5	484.6	1000.7
SD	0.68	4.82	8.03	32.95	57.04
Precision (RSD%)	14.8	9.8	7.2	6.8	5.7
Accuracy (%)	92	98.5	111.4	96.9	100.7

for acetic acid was 5 mg/mL and it was the lowest standard concentration in the calibration curve.

Developed GC method could be a reliable alternative to other analytical procedures for the analysis of VFAs. A significantly lower limit of quantification was achieved. The sample preparation procedure is rather simple and demands only 2 mL of the aqueous sample. In addition, this method allows for the measurement of individual VFA concentration. In the case of the presence of additional volatile contaminations in the analyzed water sample, selectivity problems with determination of acetic acid can appear. The main disadvantage of this method is necessity to prepare a derivative's agent, diazomethane, which is toxic and non-stable, on the other hand allowing an increase analytical sensitivity.

Application of Examined Methods to Environmental Samples

The studied methods of VFA determination were used for the measurement of VFA concentrations in exemplary surface- and wastewater samples. Both type of samples were collected from a Polish airport facility in winter 2006. Each winter, sodium and potassium acetates are used there as airplane runway and apron deicers. In this season, low temperatures usually inhibit biodegradation, therefore VFAs may be present not only in surface- but also in wastewaters.

In the case of GC method, the concentrations of VFAs were calculated as a sum of concentrations of acetic, propionic, butyric, valeric and caproic acids The results are presented in Table 6.

The agreement between investigated methods is not sufficient. Results obtained by distillation method were lower than those obtained by spectrophotometric procedure. This is primarily due to a very low absolute recovery of acetic acid by steam distillation. Differences in results are also due to the presence of inorganic ions (HCO_3^-, PO_4^{3-}) originated from the matrix. The GC method seems to be the most reliable between all studied methods determining low VFA concentrations.

Table 6. VFAs determined in environmental samples by distilled, spectrophotometric and GC methods.

Sample	Concentration of VFAs expressed as mg/L of CH_3COOH		
	Distillation method	Spectrophotometric method	GC method
Wastewater	84	128	-
	124	146	-
	n.d.	98	120
Surface water from the airport	1630	1920	-
	410	560	-
	n.d.	n.d.	27
	n.d.	n.d.	22
	n.d.	n.d.	15
	n.d.	n.d.	18
	n.d.	n.d.	13
	n.d.	n.d.	22

n.d. not detected (below LOQ) – not analyzed by this method

Conclusions

In conclusion, we have examined in detail the most popular analytical procedures applied for determination of VFAs in environmental waters. It was found that the distillation method commonly used in routine tests suffers from relatively high limit of quantification and very low precision. These parameters are far more improved if the spectrophotometric method is used. Moreover, relatively short time of analysis allows to propose this method for high throughput measurements, usually demanded in environmental analysis. Additionally, a pre-separation step onto ion exchanger bed significantly reduces the limit of quantification. Determination of VFAs by gas chromatographic method is characterized by satisfactory precision and good accuracy. The limit of quantification is sufficient for typical environmental concentrations The sample preparation procedure is rather simple and demands small volume of the aqueous sample. Moreover, this is the only method allowing measurement of individual VFA concentrations. Comparison of practical applicability onto exemplary surface- and wastewater samples shows that GC methods seem to be the most reliable between all studied methods for determining low VFA concentrations.

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