

Determination of Aliphatic Acids in Natural Waters Using Distillation and Solid-Phase Extraction

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Abstract—A procedure is developed and conditions are optimized for the pretreatment of natural water using distillation with no prefiltration of acidified water in combination with preconcentration by solid-phase extraction (SPE) on a Strata-X polymeric adsorbent followed by derivatization for the determination of trace concentrations of C3–C18 aliphatic carboxylic acids by gas chromatography with mass spectrometric detection. It is shown that, at the distillation stage, 90% of free carboxylic acids of the homologous series are transferred to the distillate, which greatly facilitates the procedure of the analysis of natural water containing impurity particles and humic substances and ensures the achievement of the limit of detection for the analytes about 0.05 µg/L. It is found that, at the SPE stage, the adsorbents based on copolymers of styrene, divinylbenzene, and *N*-vinylpyrrolidone in plastic cartridges may serve as sources of additional pollution with benzoic, palmitic, and stearic acids in the eluates under investigation.

Keywords: natural water, organic acids, derivatization, solid-phase extraction on a polymer, gas chromatography with mass spectrometric detection

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Studies on the composition, distribution, and transformation of organic and inorganic compounds in natural water, ice, snow, soil, sediments, and other environmental objects are considered high-priority and contributing to the establishment of fundamental correlations between state of ecosystems, human activities, and global climate changes [1, 2]. Of particular interest is the possibility of the establishment of correlations between the qualitative and quantitative concentrations of individual classes of organic compounds, e.g., carboxylic acids in river and lake water and the disappearance of permafrost because of climate warming. As the concentration of organic substances in natural waters is low, an essential stage of the determination is preconcentration, most often by SPE [3–9]. Cartridges and discs for SPE from water samples are most often filled with C18-grafted silica and also with polymeric sorbents based on hydrophilic porous copolymers of styrene with divinylbenzene and *N*-vinylpyrrolidone. The adsorbents based on C18 silica are inferior to polymeric adsorbents in extraction efficiency to organic compounds, because they do not extract polar organic compounds (phenols and phenol carbonic acids) [3]. A comparison of various SPE adsorbents (XAD8 and C18) and versions of elution with tangential flow filtration techniques for the determination of soluble lignin was described in [5].

For chromatographic analysis, carbonic and phenol carbonic acids are modified; the choice of the

derivatization method in many respects depends on the sample type, preconcentration procedure, and method of analysis [10, 11]. Silylation, which is more complete fuller and easier with acid salts and phenolates, is used most often, which was taken into account by the authors in sample pretreatment. The most popular silylating agent is *N,N*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), which easily reacts with most of organic functional groups, ensuring high yields of trimethylsilyl derivatives of acids, phenols, and alcohols.

In most of procedures proposed to date, the initial stage of the pretreatment of water samples is purification of insoluble impurities by passing a sample through a series of filters (at final stages, with pore diameter of 0.22–0.45 µm). Humic compounds, present in the composition of natural waters, often clog fine pores of the filters, making the further filtration impossible. In addition, hardly eluted humic substances may be irreversibly adsorbed by SPE cartridges.

The aim of this study was the development of a procedure for the determination of trace concentrations of aliphatic monocarboxylic acids in natural waters for monitoring of water objects of the West Siberian region. A significant distinction of the sample pretreatment version proposed by the authors is that natural water is distilled without prefiltration and the dis-

tillate is then passed through an SPE cartridge. This ensures rather accurate determination of saturated and unsaturated aliphatic carboxylic acids with the number of carbon atoms from 3 to 18 by gas chromatography with mass spectrometric detection (GC–MS) at a level of $\mu\text{g/L}$ or lower.

EXPERIMENTAL

Samples of natural water from lakes (sample 1— $63^{\circ}49'09.4''$ N, $75^{\circ}34'44.1''$ E; sample 2— $63^{\circ}46'56.6''$ N, $75^{\circ}39'13.1''$ E; and sample 3— $63^{\circ}48'29.7''$ N, $75^{\circ}33'55.0''$ E), Khanupy-Yakha (sample 4— $63^{\circ}49'37''$ N, $74^{\circ}39'41''$ E) and Kharuchei-Yakha (sample 5— $63^{\circ}51'22''$ N, $75^{\circ}08'17''$ E), rivers, and also of snow and ice ($63^{\circ}49'09.4''$ N, $75^{\circ}34'44.1''$ E) from the zone of northern taiga of the Yamalo-Nenets Autonomous Ocrug were taken by research workers of the BioClimLand Center of the National Research Tomsk State University during expeditions in 2014–2015. Some samples were preserved by acidification with hydrochloric acid to pH 2.5–3, and other samples were stored frozen and also acidified with hydrochloric acid prior to the analysis.

Reagents and materials. Standards of C3–C22 aliphatic acids, BSTFA silylating agent, and pyridine (Sigma, United States), Na_2CO_3 and concentrated HCl of reagent grade (Khimmed, Russia), and ethanol rectified product Lyuks 96% were used in the study. Distilled water with the total carbon content less than $2 \mu\text{g/L}$ purified on an Arium Pro UV (Sartorius AG, Germany) installation was used as a reference solution and for the preparation of standard acid solutions. Working solutions of acids were prepared in 96% ethanol (concentrations about 1 mg/mL); after that, working solutions were added to purified water and standard solutions with the concentration of analytes from 0.1 to $10 \mu\text{g/L}$ or higher were obtained.

For extraction from aqueous media, plastic and glass cartridges of the diameter 13 mm filled with 200 mg of a Strata-X adsorbent (Phenomenex, United States) with a particle size of $30\text{--}33 \mu\text{m}$ were used. The adsorbent was retained in the cartridge between polyethylene frits. A solution for elution from SPE cartridges was prepared by mixing a Na_2CO_3 aqueous solution with 85% ethanol.

Distillation of water samples; collection and SPE of the distillate. The setup for distillation device was assembled using standard laboratory glass with ground-glass joints: a heat-resistant round-bottom flask (KKS_h-29/32-1000/2000) was connected to a Liebig condenser with the length 250–400 mm with an adapter through a Wurtz (N1-29/32-14/23) or a Claisen (N2-29/32-14/23) nozzle. The sizes of the condenser were selected taking into account the sufficient performance of the distillation setup and the minimal internal surface on which the adsorption of

part of substances distilled with a water steam was inevitable.

Equipment with the parameters described above has performance of about 500–1000 mL of distillate per hour. The sequence of operations described below was identical for both natural water samples and standard aqueous solutions of acids necessary for quantitative analysis.

A water sample (500–1000 mL, pH 2.5–3) was distilled without prefiltration. At the final distillation stage, 100 mL of purified distilled water was added to the distillation flask, and distillation was continued until 50–70 mL of the liquid remained in the cube. After that, the cooling water flow in the cooling jacket was stopped and the collection of the hot distillate with vapors was continued. Heating of the distillation flask was stopped when the volume of the residue was no more than 10–20 mL. The distillate from the receiver was cooled to room temperature and passed through a glass cartridge connected to a vacuum station via a liquid trap. Linear distillate flow rate through the cartridge was $0.7\text{--}2.0 \text{ mm/s}$.

After passing the distillate, the cartridge was dried under the deepest vacuum for 5 min and then the adsorbed compounds were eluted with 85% ethanol (5–6 mL) containing $0.16\text{--}0.20 \text{ mg/mL}$ of Na_2CO_3 . The cartridge was filled with the eluent, allowed to stand for 3–5 min for swelling, and then elution was carried out at a rate of $0.06\text{--}0.12 \text{ mm/s}$. The eluate was collected in a glass vial of the volume 10 mL, dried in a nitrogen flow at moderate heating on a water bath (50°C) for the complete removal of alcohol and water. A $50\text{-}\mu\text{L}$ portion of BSTFA (Sigma) and $150 \mu\text{L}$ of pyridine (Sigma) were added to the vial with the dry residue, the vial was sealed, and its contents were heated for 1–3 min at 80°C and, after cooling, stirred and transferred to a microvial for GC–MS analysis. Three replicate measurements were carried out for each water sample and standard solution.

Equipment and conditions of the determination of carboxylic acid derivatives. Analyses were carried out on a GC–mass spectrometer, consisting of an Agilent 7890 gas chromatograph and a MSD 5975C mass spectrometry detector (Agilent Technologies, United States). Organic acids were determined as trimethylsilyl derivatives thereof. A silica capillary column with an HP-5ms polyamide coating (Agilent Technologies, United States) with a grafted methylphenyl (5%) silicone phase (length 30 m, diameter 0.25 mm, and thickness of grafted phase film $0.25 \mu\text{m}$) was used for separation. Temperature programming in the analysis was as follows: 60°C for 5 min, heating to 260°C with a gradient of 10 K/min , and exposure for 35 min. Sample input mode was as follows: pulsed splitless for 15 psi, sample volume $0.5 \mu\text{L}$. Injector temperature was 280°C , and interface temperature was 290°C . Detector, electron impact ionization (70 eV), ion source temperature 230°C , and quadrupole tempera-

Table 1. Concentrations ($\mu\text{g/L}$) of acids in consecutive eluates from an SPE cartridge (Strata-X, glass, water sample 2)

Acid	1 st elution	2 nd elution	3 rd elution	4 th elution	5 th elution*	SPE** bidistillate	Rotor** bidistillate
Benzoic	7 ± 2	5 ± 1	4 ± 1	2.6 ± 0.6	7 ± 2	4 ± 1	0.2 ± 0.1
C12:0	0.6 ± 0.2	—	—	—	—	—	—
C14:0	0.10 ± 0.05	—	—	—	—	—	—
C15:0	0.6 ± 0.2	—	—	—	—	—	—
C16:0	2.0 ± 0.4	0.25 ± 0.05	0.18 ± 0.05	0.12 ± 0.05	0.08 ± 0.05	0.60 ± 0.05	0.02 ± 0.05
C18:0	1.8 ± 0.4	0.03 ± 0.05	0.01 ± 0.05	0.01 ± 0.05	—	0.50 ± 0.05	0.01 ± 0.05
Total	12 ± 3	6 ± 2	4 ± 1	2.8 ± 0.8	7 ± 2	5 ± 1	0.2 ± 0.1

* After standing for 4 h.

** Analysis of twice-distilled water with preconcentration by SPE and on a rotary evaporator.

ture 150°C . Detector mode, scan 50–500 a. m. u. Qualitative identification and quantitative determination were performed by characteristic mass numbers of ions, and calibration was performed by standard aqueous solutions of acids.

RESULTS AND DISCUSSION

In the selection of the optimal conditions of SPE on polymeric adsorbents, we studied the degree of desorption of preconcentrated impurities by successive elutions. To do this, after each elution the concentration cartridge was dried under vacuum for 3–5 min, then the adsorbent was compacted and a repeated elution was carried out (Table 1). The results obtained indicate that, in repeated extractions from Strata-X cartridges, benzoic acid was present in all eluates; its possible source was polymeric adsorbent, which decomposed under the influence of atmospheric oxygen. The concentration of benzoic acid gradually decreased in successive elutions and then increased again after a short storage of the cartridge. We should also note the constant presence of traces of C16 and C18 carboxylic acids in the eluates, the source of which found in additional studies was shown to be the material of the plastic body of SPE cartridges, in which they were present, probably, because of the production technology of the cartridges (the contact with grease substances at stamping or casting of the bodies). Particularly significant amounts of C16 and C18 acids were eluted from unused SPE cartridges with a plastic body. In carrying out repeated elutions, the amount of artifact C16 and C18 acids decreased, but still continued to introduce errors into the results of analyses of real samples. Because of this, the Strata-X adsorbent packed into glass cartridges should be used for the quantitative determination of the mentioned acids at a level of $10 \mu\text{g/L}$ or lower. After the transfer of the adsorbent to a glass body, it may be washed from traces of C16 and C18 acids; however, the determination of benzoic acid remained impossible. A comparison of the results of GC–MS analyses of twice-distilled water

with preconcentration by SPE and distillation in a rotary evaporator confirms that the source of benzoic acid is the adsorbent material for SPE (Table 2). However, polymeric adsorbents for SPE are irreplaceable in the preconcentration of polar compounds and are more suitable for multiple use as opposed to silica-based adsorbents. The latter adsorbents are unstable in aqueous solutions and solutions with extremely high and low pH values and poorly reproduce the results in repeated use.

The performed investigations of distillation efficiency and the evaluation of losses of organic substance carried out on standard solutions of acids (Table 2) showed that the losses vary from 4 to 13% for basic carboxylic acids. Part of compounds is adsorbed on the internal surface of the Liebig condenser used for vapor condensation. At the end of each distillation, the condenser should be steamed with disconnection of the circulation of cooling water in its jacket and collection of hot distillate and vapors thereof. This measure ensures the reduction of the error of determinations.

Additionally we estimated the probability of the hydrolysis of triglycerides in distillation in an acidic medium (pH ~ 2.5) with a possible formation of free carboxylic acids. In the analysis of samples of twice-distilled water with additions of lipids (triglycerides of vegetable oil in the concentration $100 \mu\text{g/L}$), no effect of lipids on the determination of carboxylic acids at the distillation stage was found.

Table 3 presents data on the concentrations of carboxylic acids in samples of natural water from the West Siberian region. The results obtained suggest that the most common acids in all studied samples are long-chain fatty acids bearing 16 and 18 carbon atoms in a molecule; their concentrations vary from 17 to 63% of the total amount of aliphatic acids.

In the determination of carboxylic acids in natural water samples, preservation, preventing the activity of microorganisms, is of great importance. As an example, Table 3 presents data on concentrations of acids in water sample stored in a refrigerator without preserva-

Table 2. Influence of distillation on the determination of organic acids in standard solutions

Sample pretreatment conditions	Standard sample	Concentration of acids, $\mu\text{g/L}$				
		C5:0	C13:0	C15:0	C16:0	C18:0
Without distillation	1	8.4	2.5	5.8	3.4	3.1
	2	8.3	2.5	5.8	3.5	3.3
	3	9.3	3.0	7.4	4.5	3.8
	μ	8.7	2.7	6.3	3.8	3.4
	s_x	0.55	0.29	0.92	0.61	0.36
	$\Delta(0.95)$	0.62	0.33	1.05	0.69	0.41
	$\delta_x, \%$	7	12	16	18	12
With distillation	1	8.4	2.6	5.8	3.5	3.2
	2	8.3	3.1	7.2	3.8	3.4
	3	8.0	2.3	5.4	3.0	2.7
	μ	8.2	2.7	6.1	3.4	3.1
	s_x	0.21	0.40	0.95	0.40	0.36
	$\Delta(0.95)$	0.24	0.46	1.07	0.46	0.41
	$\delta_x, \%$	3	17	17	13	13
Losses due to distillation, %		5	7	13	12	10

Designations: μ is average value; s_x is standard deviation; $\Delta(0.95; s_x; n)$ is confidence interval; δ_x is relative error, %.

Table 3. Concentrations ($\mu\text{g/L}$) of organic acids in natural water samples

Acid	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 2*
Lactic	3.1 ± 0.7	0.7 ± 0.1	2.3 ± 0.6	2.5 ± 0.6	2.8 ± 0.6	—
C5:0	—	—	—	—	—	1.0 ± 0.2
C6:0	—	—	—	—	—	2.3 ± 0.6
3-Hydroxybutanoic	—	—	—	—	—	0.6 ± 0.1
C7:0	—	—	—	—	—	0.5 ± 0.1
C8:0	—	—	—	—	—	2.1 ± 0.4
C10:0	—	—	—	—	—	5 ± 1
C11:0	—	—	—	—	—	1.4 ± 0.3
C12:0	0.6 ± 0.1	0.10 ± 0.05	0.10 ± 0.05	0.10 ± 0.05	0.20 ± 0.05	13 ± 2
C13:0	—	—	—	—	—	4 ± 1
C14:0	1.1 ± 0.2	0.20 ± 0.05	0.40 ± 0.05	0.6 ± 0.1	0.8 ± 0.2	184 ± 20
3-Hydroxytetradecanoic	1.7 ± 0.3	—	—	—	—	—
C15:0	0.6 ± 0.1	6 ± 1	1.3 ± 0.2	1.3 ± 0.2	2.3 ± 0.4	15 ± 3
C16:1	—	—	0.9 ± 0.2	0.9 ± 0.2	1.6 ± 0.3	—
C16:0	2.0 ± 0.3	4.6 ± 0.6	0.9 ± 0.2	1.4 ± 0.3	1.3 ± 0.3	257 ± 26
C17:0	—	—	—	—	—	15 ± 2
C18:1	0.4 ± 0.1	—	1.3 ± 0.2	0.7 ± 0.2	2.5 ± 0.4	17 ± 4
C18:0	1.8 ± 0.3	9 ± 1	0.6 ± 0.1	1.0 ± 0.2	1.1 ± 0.2	127 ± 16
C19:0	—	—	—	—	—	4 ± 1
Total concentration of glycerol and C14–C18 carboxylic acid monoethers	—	—	—	—	—	212 ± 25
Total concentration	18 ± 4	21 ± 4	21 ± 5	17 ± 4	33 ± 8	872 ± 106

* Water sample after prolonged storage without conservation.

tion at 4–6°C for 3 months (the results of analysis of fresh water sample 2 may be used for comparison). Within the mentioned storage period, the total concentration of acids and their derivatives in water increased from 22 to 872 µg/L, probably, because of bacterial activity. The source of additional acids could be bacteria in themselves and also plant residues decomposed by them. Esters of glycerol and unsaturated C14–C18 carboxylic acids, which may be degradation products of structural lipids of microorganisms under the influence of enzymes, also appear in water. In the studied water sample, we also found significant concentrations of free saturated and unsaturated carboxylic acids with odd numbers of carbon atoms from 5 to 19.

Thus, the proposed procedure of sample pretreatment of river and lake natural waters ensures the correct determination of aliphatic carboxylic acids at a concentration level in water 1 µg/L or lower. The total concentration of aliphatic acids in the studied samples of river and lake waters from Western Siberia was from 17 to 33 µg/L with a predominance of long-chain (C16–C18) fatty acids, which may be used for the monitoring of water objects of the zone of northern taiga of the Yamalo-Nenets Autonomous Okrug for revealing season and global climate processes.

REFERENCES

1. Tate, R.L., *Soil Organic Matter: Biological and Ecological Effects*, New York: Wiley, 1987.
2. Zsolnay, A., *Geoderma*, 2003, vol. 113, p. 187.
3. Dittmar, T., Koch, B., Hertkorn, N., and Kattner, G., *Limnol. Oceanogr. Methods*, 2008, vol. 6, p. 230.
4. Kim, S., Simpson, A.J., Kujawinski, E.B., Freitas, M.A., and Hatcher, P.G., *Org. Geochem.*, 2008, vol. 34, p. 1325.
5. Spencer, R.G., Aiken, G.R., Dyda, R.Y., Butler, K.D., Bergamaschi, B.A., and Hernes, P.J., *Org. Geochem.*, 2010, vol. 41, p. 445.
6. Nazarkina, S.G., Bulanova, A.V., and Larionov, O.G., *J. Anal. Chem.*, 2001, vol. 56, no. 4, p. 349.
7. Filippov, O.A., Tikhomirova, T.I., Shapovalova, E.N., Tsizin, G.I., Shpigun, O.A., and Zolotov, Yu.A., *J. Anal. Chem.*, 2003, vol. 58, no. 7, p. 625.
8. Zolotova, M.Yu. and Rozhmanova, N.B., Abstracts of Papers, *Mezhdunarodnaya konferentsiya po ekstraktzii* (Int. Conf. on Extraction), Voronezh, 1992, p. 139.
9. Smirnov, R.S., Rodin, I.A., Smolenkov, A.D., and Shpigun, O.A., *J. Anal. Chem.*, 2010, vol. 84, no. 12, p. 1266.
10. Smolenkov, A.D., Chernobrovkina, A.V., Smirnov, R.S., Chernobrovkin, M.G., and Shpigun, O.A., *Int. J. Environ. Anal. Chem.*, 2013, vol. 93, no. 12, p. 1286.
11. Sobolevsky, T.G., Alexander, I.R., Miller, B., Oriedo, V., Chernetsova, E.S., and Revelsky, I.A., *J. Sep. Sci.*, 2003, vol. 26, p. 1474.

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