

Determination of Organic Acids in Wastewater Using Ion-Exclusion Chromatography and On-Line Carbonate Removal

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Introduction

Low-molecular mass carboxylic 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 because they can originate from anaerobic biodegradation of organic matter. Therefore, VFAs are widely present in activated sludge, landfill leachates, and wastewater.

Analysis of VFAs is significant in studies of health and disease in the intestinal tract. In some foods, VFA content is an index of quality assurance. Recently, the determination of VFAs has been of increasing interest because it has been found that they are involved in different processes of commercial interest: i.e., in biological removal of phosphorus from water or nitrification/denitrification in activated sludge. Carboxylic acids may also affect the storage stability of waste incineration residues by reducing the pH and increasing the mobility of heavy metals.¹

This study shows a method for determining eight organic acids in wastewater—acetic, formic, propionic, isobutyric, butyric, isovaleric, valeric, and caproic—using ion-exclusion chromatography and on-line carbonate removal. Normally, carbonate and inorganic anions are present in wastewater samples and interfere in organic acid analysis using ion-exchange chromatography. In this study, the interferences are eliminated using ion-exclusion chromatography. Inorganic anions are eluted in the void volume and carbonate is removed by an on-line carbonate removal device.



Equipment

Thermo Scientific™ Dionex™ chromatography system including:

- Thermo Scientific Dionex ICS-1500* system
- Thermo Scientific Dionex AS Autosampler with cooling option
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 6.80, SR10 or later

**Equivalent or improved results can be achieved using the Thermo Scientific Dionex ICS-1100 or the Thermo Scientific Dionex ICS-1600 system.*

Reagents and Standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better
- Heptafluorobutyric acid (HFBA, C₄HF₇O₂, 99.5% GC grade)
- Tetrabutylammonium hydroxide solution (TBAOH, C₁₆H₃₇NO, 40% in water)
- Formic acid (CH₂O₂, 98–100% GC grade)
- Acetic acid (CH₃COOH, 99.7% AR grade)
- Propionic acid (C₃H₆O₂, 99% GC grade)
- Isobutyric acid (C₄H₈O₂, 99.5% GC grade)
- Butyric acid (C₅H₁₀O₂, 99% GC grade)
- Isovaleric acid (C₅H₈O₂, 100% GC grade)
- Valeric acid (C₅H₉O₂, 99% GC grade)
- Caproic acid (C₆H₁₂O₂, 98% GC grade)

Preparation of Solutions and Reagents

HFBA, 0.5 mM

Add ~500 mL DI water to a 1000 mL volumetric flask, transfer 0.1 mL of concentrated HFBA to the volumetric flask, bring to volume with DI water, and mix well.

TBAOH, 5 mM

Add 897 mL DI water into a 1 L bottle, transfer 3 mL TBAOH into the bottle, and mix well.

Mixed Stock Standard Solution

Add ~50 mL DI water to a 100 mL volumetric flask and add the appropriate volume of concentrated standard solution into the volumetric flask (Table 1). Bring to volume with DI water. Perform this preparation in a fume hood.

Table 1. Stock standard concentrations and volumes used for preparation of the mixed stock standard.

Analyte (Acid)	Concentration (mg/L)	Amount of Concentrated Standard Solutions Used for 100 mL Preparation (mL)
Formic	2000	0.244
Acetic	20,000	1.947
Propionic	5000	0.502
Isobutyric	500	0.048
Butyric	200	0.019
Isovaleric	1000	0.101
Valeric	200	0.019
Caproic	2000	0.189

Calibration Standard Solutions

Dilute 0.1, 0.2, 0.3, and 0.4 mL of mixed stock standard solution in 100 mL volumetric flasks with DI water for preparing calibration standard levels 1, 2, 3, and 4, respectively. The concentrations of the calibration standards are shown in Table 2.

Table 2. Calibration standard concentrations.

Analyte (Acid)	Concentration (mg/L)			
	Level 1	Level 2	Level 3	Level 4
Formic	2.0	4.0	6.0	8.0
Acetic	20.0	40.0	60.0	80.0
Propionic	5.0	10.0	15.0	20.0
Isobutyric	0.5	1.0	1.5	2.0
Butyric	0.2	0.4	0.6	0.8
Isovaleric	1.0	2.0	3.0	4.0
Valeric	0.2	0.4	0.6	0.8
Caproic	2.0	4.0	6.0	8.0

Sample Preparation

Wastewater and Spiked Wastewater Sample Preparation

Filter the sample with a 0.45 µm cellulose acetate syringe filter before sample injection. For spiked sample preparation, spike 0.1 mL of mixed stock standard solution into 100 mL of wastewater before filtration.

Conditions

Column:	Thermo Scientific™ Dionex™ IonPac™ ICE-AS1 Analytical, 4 × 250 mm Dionex IonPac ICE-AS1 Guard, 4 × 50 mm
Eluent:	0.5 mM HFBA
Flow Rate:	0.16 mL/min
Pressure:	750 psi
Sample Volume:	25 µL
Column Temp.:	30 °C
Sample Tray Temp.:	10 °C
Detection:	Suppressed conductivity, Thermo Scientific™ Dionex™ AMMS™ ICE 300 suppressor, 4 mm, Thermo Scientific Dionex CRD 200 Carbonate Removal Device, 2 mm, and 5 mM TBAOH regenerant delivered by pressurized bottle using N ₂ gas
Total Conductivity:	~41 µS

Results and Discussion

Separation

Inorganic anions and carbonate can interfere when determining organic acids in wastewater using anion-exchange chromatography. Inorganic anion interferences can be eliminated easily by using ion-exclusion chromatography because they are excluded from the column (i.e., not retained). Carbonate is naturally present in wastewater and can interfere with organic acid determinations, even using ion-exclusion chromatography, due to the fact that carbonate is a weak acid that is retained by the column. Hence, a Thermo Scientific Dionex CRD 200 device is used to remove carbonate in this application.

The Dionex CRD 200 device is typically placed between the suppressor and conductivity cell to convert the suppressed carbonate to carbonic acid that readily changes to carbon dioxide and is removed by the Dionex CRD 200 device. For this application, place the Dionex CRD 200 device between the column and suppressor because the eluent in this application is acidic and the carbonate in the sample is converted to carbonic acid when the sample is injected into the column. Carbonic acid is converted to carbon dioxide and removed at the column outlet by the Dionex CRD 200 device (Figure 1).

Column: Dionex IonPac ICE-AS1 Analytical, 4 × 250 mm
Dionex IonPac ICE-AS1 Guard, 4 × 50 mm
Eluent : 0.5 mM HFBA
Flow Rate: 0.16 mL/min
Pressure: ~730 psi
Sample Volume: 25 µL
Column Temperature: 30 °C
Sample Tray Temperature: 10 °C
Detection: Suppressed conductivity,
Dionex AMMS ICE 300 suppressor, 4 mm
With Dionex CRD 200 device, 2 mm and
5 mM TBAOH (regenerant)
Total Conductivity: ~41 µS
Sample: A. Spiked wastewater without Dionex CRD 200 device
B. Spiked wastewater with Dionex CRD 200 device

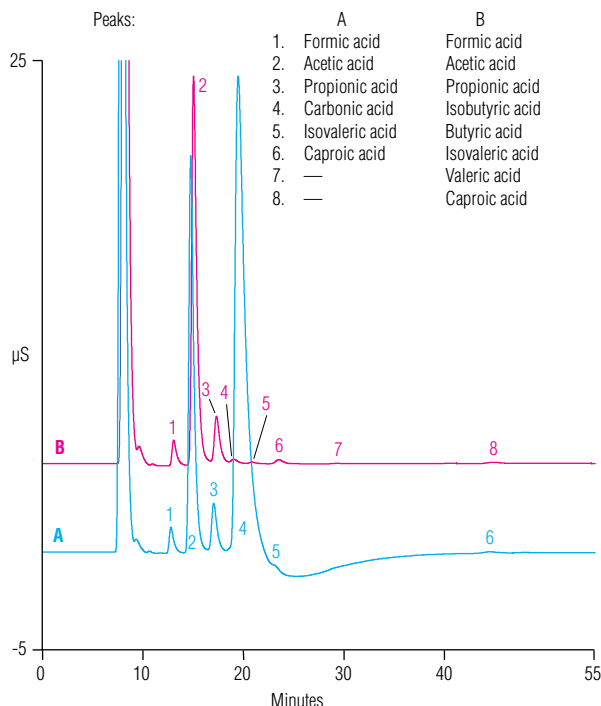


Figure 2. Overlay of chromatograms of spiked wastewater with and without a Dionex CRD 200 device installed.

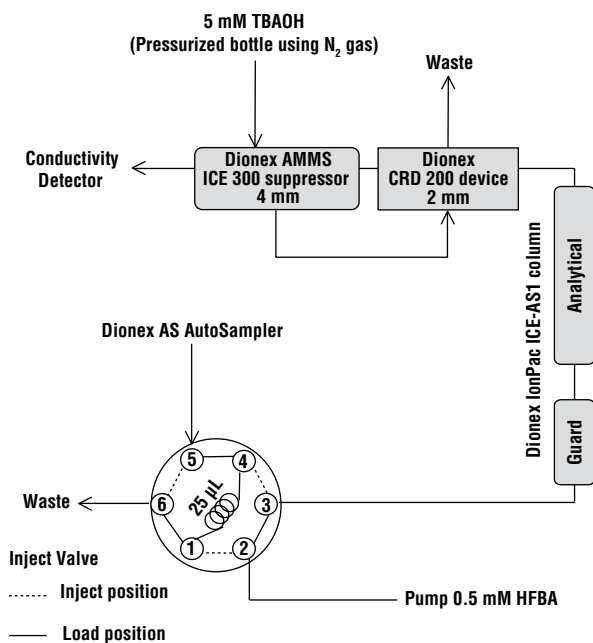


Figure 1. System configuration.

To minimize the extra column volume that can cause peak broadening, use a 2 mm Dionex CRD 200 device. Excessive backpressure can damage the Dionex CRD 200 device and cause it to leak. This application runs at a low flow rate, so system backpressure will not affect the performance of the Dionex CRD 200 device. Figure 2 shows chromatograms of a spiked wastewater sample with and without a Dionex CRD 200 device installed in the IC system. Note that with the Dionex CRD 200 device installed and carbonate removed, two additional organic acids are identified. Figure 3 shows a chromatogram of a DI water blank.

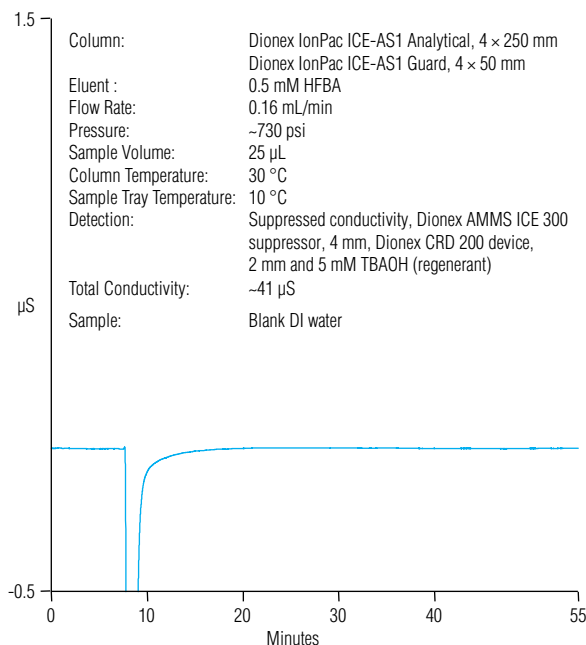


Figure 3. Chromatogram of a DI water blank.

Method Calibration

Calibrate the method before sample analysis using four mixed standards with different concentrations. To achieve an accurate measurement using this method, prepare the calibration standard concentration in a similar concentration ratio to each analyte in the wastewater sample. Chromatograms of the calibration standards are shown in Figure 4. Calibration results for each analyte are shown in Table 3.

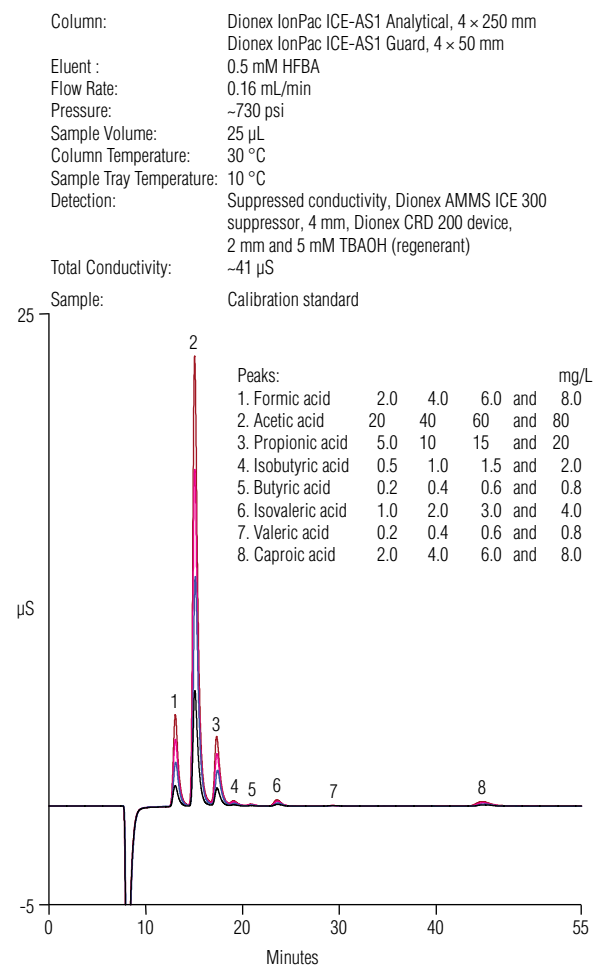


Figure 4. Overlay of chromatograms of the calibration standard solutions.

Table 3. Calibration standard concentrations and calibration results.

Acid	Concentration (mg/L)				Calibration Result			
	Level 1	Level 2	Level 3	Level 4	Points	r ²	Offset	Slope
Formic	2.0	4.0	6.0	8.0	4	0.9998	-0.0352	0.3476
Acetic	20.0	40.0	60.0	80.0	4	0.9998	0.2322	0.1648
Propionic	5.0	10.0	15.0	20.0	4	0.9998	0.0362	0.1018
Isobutyric	0.5	1.0	1.5	2.0	4	0.9997	0.0018	0.0470
Butyric	0.2	0.4	0.6	0.8	4	0.9997	0.0017	0.0607
Isovaleric	1.0	2.0	3.0	4.0	4	0.9999	0.0043	0.0692
Valeric	0.2	0.4	0.6	0.8	4	0.9984	-0.0005	0.0519
Caproic	2.0	4.0	6.0	8.0	4	0.9993	-0.0017	0.0461

Table 4. Amount of organic acids in wastewater sample.

Injection No.	Amount (mg/L)							
	Formic	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Caproic
1	—	50.1	9.32	1.59	0.66	1.56	0.39	—
2	—	50.0	9.32	1.57	0.64	1.55	0.38	—
3	—	49.8	9.28	1.58	0.65	1.55	0.37	—
4	—	49.8	9.27	1.59	0.64	1.56	0.34	—
5	—	49.7	9.27	1.60	0.62	1.55	0.34	—
Average	—	49.9	9.29	1.59	0.64	1.55	0.36	—
RSD	—	0.28	0.25	0.78	1.86	0.30	5.95	—

Table 5. Amount of organic acids in spiked wastewater sample and recovery results.

Injection No.	Amount (mg/L)							
	Formic	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Caproic
	(Spiked 2 mg/L)	(Spiked 20 mg/L)	(Spiked 5 mg/L)	(Spiked 0.5 mg/L)	(Spiked 0.2 mg/L)	(Spiked 1 mg/L)	(Spiked 0.2 mg/L)	(Spiked 2 mg/L)
1	2.08	68.5	13.4	2.11	0.81	2.60	0.56	2.02
2	2.07	68.6	13.4	2.12	0.89	2.59	0.51	1.92
3	2.07	68.5	13.4	2.13	0.79	2.58	0.55	1.97
4	2.06	68.3	13.4	2.15	0.78	2.59	0.52	1.96
5	2.07	68.2	13.4	2.12	0.77	2.57	0.53	1.93
Average	2.07	68.4	13.4	2.13	0.79	2.59	0.53	1.96
RSD	0.26	0.23	0.17	0.57	1.61	0.37	3.97	1.99
Recovery (%)	104	92.5	82.2	108	75.0	104	85.0	98.0

Sample Analysis

The wastewater sample was collected from a domestic wastewater source. The sample and spiked sample were prepared immediately after the sample arrived at the lab, then placed in the sample tray set at 10 °C. Each sample was injected five times. The RSD range of the concentrations of the organic acids was 0.25–5.95% for the sample and 0.17–3.97% for the spiked sample. The results of sample and spiked sample analysis are shown in Tables 4 and 5, respectively. The average concentrations of each analyte obtained from sample and spiked sample analysis were used for recovery calculations to determine the method accuracy. Recoveries ranged from 75.0 to 108% (Table 5), demonstrating method accuracy. Chromatograms are shown in Figure 5.

Conclusion

This application demonstrates the determination of organic acids in a wastewater sample using ion-exclusion chromatography and on-line carbonate removal. Ion-exclusion chromatography excludes the inorganic anions in the sample and the Dionex CRD 200 device removes carbonate on line. The results show that this combination allows the accurate determination of organic acids in a wastewater sample.

Reference

1. Siedlecka, E.M.; Kumirska, J.; Ossowski, T.; Glamowski, P.; Gołbiowski, M.; Gajdus, J.; Kaczyński, Z.; Stepnowski, P. Determination of Volatile Fatty Acids in Environmental Aqueous Samples. *Polish J. of Environ. Stud.* Vol. 17, (No. 3) 2008, 351–356.

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