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Application News

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Gas Chromatography

Single-Injection Analysis of Formic Acid and Free Volatile Fatty Acids Using Flame Ionization Detector with In-jet Methanizer

■ Abstract

While short chain (C2- C7) volatile fatty acids (VFAs) can usually be analyzed in free form, formic acid commonly requires derivatization to methyl formate to be detected by a Flame Ionization Detector (FID). The use of an in-jet methanizer (Jetanizer) allows simultaneous determination of formic acid along with other volatile fatty acids in free form with FID. This study demonstrates the analysis of underivatized formic acid simultaneously with VFAs, from C2 to C5, on the GC-2050 with a Jetanizer for FID detection.

■ Introduction

Analysis of low molecular carboxylic acids, or Volatile Fatty Acids (VFAs), is essential in environmental samples, especially in wastewater and sludge treatment, hydraulic fracturing operations and landfills. These chemicals are metabolites formed through the biological processes that water resource recovery facilities (formerly known as wastewater treatment plants) employ to eliminate organic matter and other contaminants. But they can also occur, under favorable conditions, in other applications that involve anaerobic biological activity, such as various fermentation processes in the food industry. VFAs can be easily determined using a gas chromatograph with flame ionization detector (GC-FID) in their free form [1], except for formic acid.

Normally, derivatization to methyl formate is required to detect formic acid by FID. Here we demonstrate that using an in-jet methanizer (Jetanizer), formic acid was successfully quantified using FID, along with other short chain volatile fatty acids, in a single injection.

■ Materials and Methods

A free fatty acids test standard (acetic, propionic, iso-butyric, butyric, iso-valeric and valeric acid, 1000 ppm each in water) was purchased from Restek (Cat #35272). Formic acid was from Chempure chemicals (Cat # CP-L9636). Standards were diluted in distilled water to specified concentrations. Table 1 summarizes the targeted compounds. Formic acid was either added to the fatty acid mix standard or run individually.

A Shimadzu GC-2050 with split/splitless injector (SPL) and flame ionization detector (FID), shown in Figure 1, plus in-jet methanizer (Jetanizer), was used for this analysis. Analysis conditions are outlined in Table 2. LabSolutions software was used for data acquisition and processing.

Table 1: Targeted compounds, retention time, calibration range and correlation coefficient.

Peak ID	Compounds	RT (min)	Conc. range (ppm)	r^2
1	Acetic acid	5.917	75-500	0.994
2	Propionic acid	6.494	75-500	0.997
3	Iso-butyric acid	6.673	75-500	0.998
4	Butyric acid	7.070	75-500	0.997
5	Iso-valeric acid	7.321	75-500	0.998
6	Valeric acid	7.737	75-500	0.997
7	Formic acid	6.369	200-1000	0.996

Table 2: Instrument Configuration and Analysis Conditions

GC system	Shimadzu Nexis GC-2050 with SPL, FID, AOC-30i autosampler
Column	SH-wax, 30 m x 0.32 mm x 1 µm, connected to short guard column
Column Temp	80 °C, 2 min – 20 °C/min – 200 °C, 3 min
Injection	0.5 µL Splitless
Carrier Gas	Helium, constant flow of 5 mL/min
FID Detector	400 °C, Air 250 mL/min, H2 32 mL/min, N2 makeup 24 mL/min

Figure 1: GC-2050 with AOC-30i autosampler.

■ Results and Discussion

Detection of Formic Acid

First, a mixture of short-chain VFAs (C1 to C5) was run on GC-FID without derivatization using regular a setup (flame ionization detector with regular jet). Previous research showed that formic acid does not have a significant response on FID. As expected, formic acid was not detected at 1000 ppm while other targeted compounds had great responses at 100 ppm (Figure 2).

Conversion of carbon-containing compounds to methane (methanization) before reaching FID is a technique that enables visualization of otherwise undetectable compounds, such as CO and CO_2[2]. It also helps to equalize response factors of organic compounds traditionally problematic for FID analysis. Therefore, a special in-jet methanizer (Jetanizer) was installed in the FID. When comparing responses of VFAs (C2-C5), it was noted that the S/N values were lower than using the normal jet (Table 3). This is because with the presence of an in-jet catalyst, column insertion depth was smaller than required for optimal FID response. However, as expected, formic acid could now be detected using the Jetanizer (Figure 3). To maximize responses of all VFAs, two changes were made from using the normal jet:

1) splitless injections were used instead of split injections;
2) total column flow rates were increased to fasten the movement of compounds through the jet.

The mixture of acids was run using the new setup with the Jetanizer, splitless injection and high column flow. All targeted compounds, including formic acid, were detected at 100 ppm. The use of the Jetanizer increased the responses from the compounds with the lowest molecular weight (formic and acetic acid). Excellent signal-to-noise ratios were observed for VFAs C3-C5, ranging between 1142 and 2260 (Table 3).

With current conditions, slightly more pronounced tailing of VFAs was observed than when using the normal jet (Table 3). Good separation was observed despite larger tailing factors (Figure 3). This demonstrates that the use of a Jetanizer enables the analysis of VFAs, including formic acid, in a single injection.

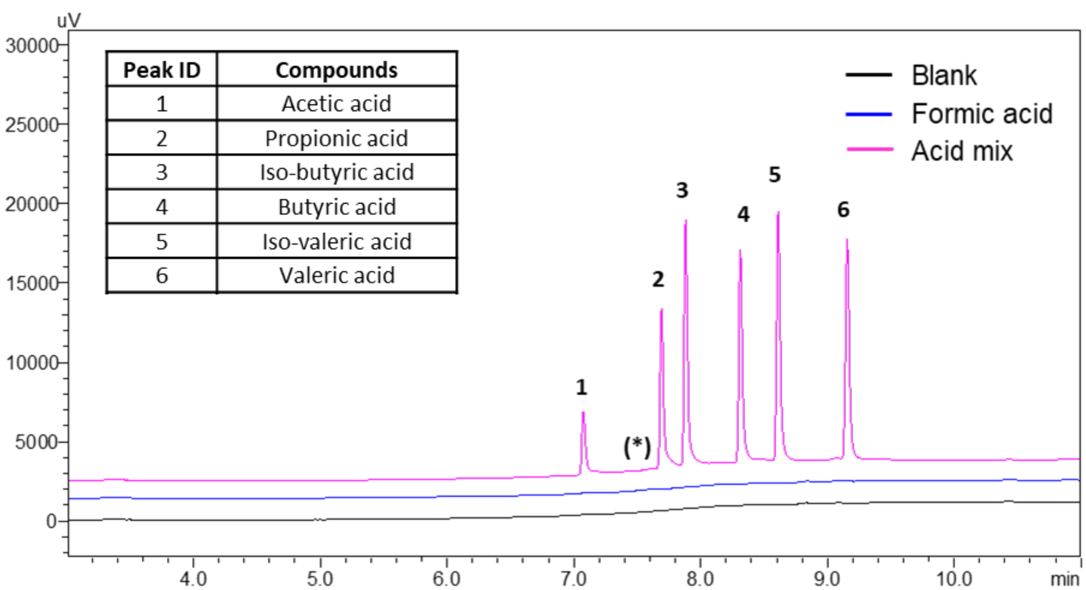

Figure 2: Chromatograms of blank (water), formic acid in 1000 ppm standard (blue) and a mix of fatty acids in 100 ppm standard (pink) using regular FID jet. (*) Expected RT for formic acid.

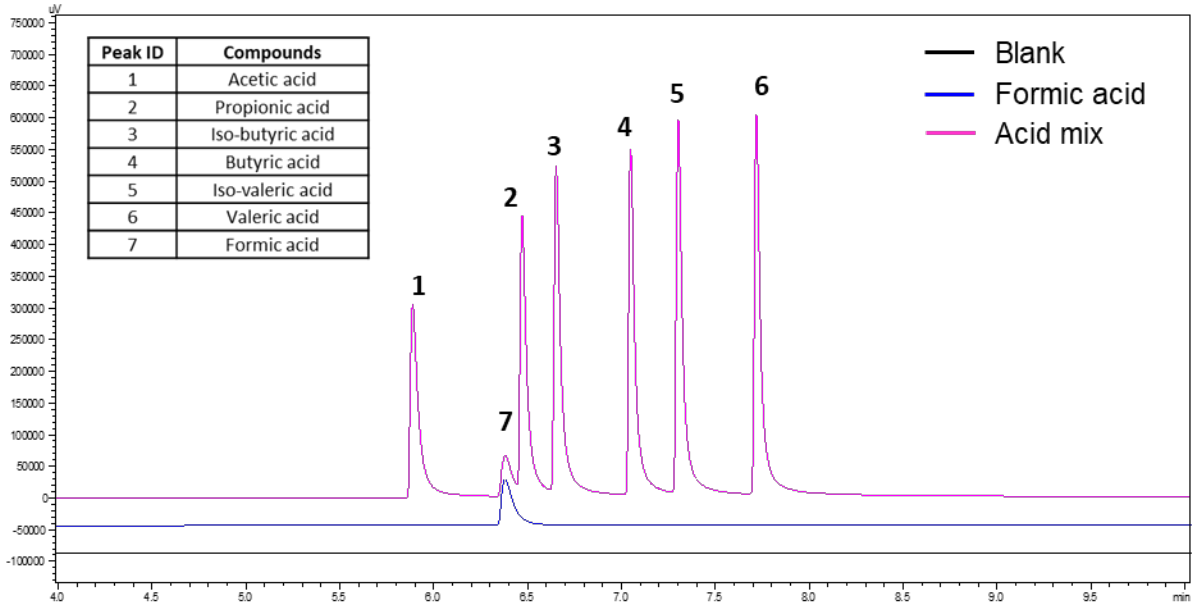

Figure 3: Chromatograms of blank (water), formic acid only (blue) and a mix of fatty acids (pink) using Jetanizer. All VFAs are 500 ppm each.

Table 3: Comparison of peak shape and sensitivity of VFAs using normal jet vs Jetanizer, split ratio of 10 unless otherwise noted. Concentration of each acid is 100 ppm.

		Tailing Factor			S/N (100 ppm)		
Peak ID	**compounds**	**Normal Jet split**	**Jetanizer split**	**Jetanizer splitless**	**Normal Jet split**	**Jetanizer split**	**Jetanizer splitless**
1	Acetic acid	2.82	2.92	4.62	220	30.4	481
2	Propionic acid	2.32	--	--	684	73.0	1142
3	Iso-butyric acid	1.58	--	--	1183	133	2074
4	Butyric acid	1.81	--	--	968	82.6	1678
5	Iso-valeric acid	1.44	--	--	1175	108	2260
6	Valeric acid	1.40	4.83	5.05	939	68.9	1884
7	Formic acid	*n.d.*	1.80	2.76	*n.d.*	3.16	89.2

n.d. peak not detected
-- baseline resolution not achieved; tailing factors cannot be calculated.

Calibration Curves

A five-point calibration curve for each VFA targeted was analyzed, from 75 to 500 ppm, except for formic acid (four-point calibration curve from 200 to 1000 ppm). The curves were fitted to linear regression and all compounds showed $r^2 > 0.994$. Individual results are included in Table 1.

■ Conclusions

Rapid analysis of VFAs is critical for ensuring proper performance of biological processes in environmental and food industries. Without an in-jet methanizer, formic acid cannot be detected on a GC-FID together with other short-chain VFAs, and laboratories need to rely on sample derivatization or the use of alternative detectors, such as a Dielectric-Barrier Discharge Ionization Detector, or BID.

In this study, underivatized formic acid was analyzed simultaneously with VFAs, from C2 to C5, on the GC-2050 with an in-jet methanizer (Jetanizer) for flame ionization detection. Calibration was linear from 75 ppm to 500 ppm for all target compounds, except formic acid (calibration 200-1000 ppm). This method overcomes the drawbacks from sample derivatization (time consuming and prone to error) and provides a good alternative for the analysis of formic acid and other VFAs using a common instrument in environmental labs.

■ References

1. Henderson, M.H.; Steedman, T.A. Analysis of C2–C6 monocarboxylic acids in aqueous solution using gas chromatography. Journal of chromatography A, 244(1982), 337–346.
2. Analysis of and Characterization of ARC's Injet Methanizer for Permanent Gases, Carbon Dioxide, and Light Hydrocarbons, Shimadzu Scientific Instruments Application News No. GC-2103.

■ Consumables

Part Number	Description	Unit	Instrument
227-35511-01	Xtra life inlet septa	Pk of 25	GC-2050
227-35007-01	Split liner with wool	Pk of 5	
220-94673-30	Jetanizer (in-jet methanizer)	each	
221-74469-00	Syringe, 10µL, fixed needle, Teflon tip	each	AOC-30i
221-75897-30	SH-wax, 30 m x 0.32 mm x 1 µm	each	Column
227-36305-01	Column, GC, SH-I Guard, 5m x 0.32mm	each	

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SHIMADZU Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS
7102 Riverwood Drive, Columbia, MD 21046, USA
Phone: 800-477-1227/410-381-1227, Fax: 410-381-1222
URL: www.ssi.shimadzu.com