Determination of Low Molecular Weight Organic Acids in Environmental Samples by Ion Chromatography Method

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Abstract

Ion chromatography using the IonPac AS 11 HC analytical column, a 3.0 mM NaOH eluent, and a conductivity detector provides a simple, cost-effective, and fast way to quantify six organic acids, Lactate, Formate, Acetate, Propionate, Pyruvate, and Glyoxalate at concentrations as low as micrograms per liter in environmental samples. This method includes details on sensitivity, accuracy, quality control, and sampling techniques, making it suitable for analyzing cloud water, rainwater, and aerosol filters. Formate and Acetate were found to be the most abundant organic acids in all samples analyzed. Although nine anion peaks were detected, including seven organic acids, only the six specified were quantified due to challenges like coelution and volatilization challenges, which are discussed in detail in the paper.

Keywords- Ion chromatography, organic acid, cloud water, rainwater, particulate matter

Introduction

Carboxylic acids play crucial roles in industries such as food, agriculture, medicine, pharmacy, and electronics. These low molecular weight organic acids (LMWOAs), formed through the oxidation of primary alcohols and aldehydes or the hydrolysis of esters, are widely present in the environment, found in air, aerosols, solid particles, ice, snow, rain, and various water sources, making their extensive analysis important due to their impact on air quality and human health. As weak acids with low pKa values (3-9), their solubility is influenced by hydrophobic nature of carbon chains. Photochemical oxidation of volatile organic compounds significantly contributes to the formation of monocarboxylic acids like formic and acetic acids detected in various environmental matrices (Carlier et al., 1996). Dicarboxylic acids account for 0.2-16% of total carbon in aerosols, originating from both natural and anthropogenic sources. Oxalic acid is the most abundant, followed by malonic and succinic acids, which are primarily formed through photochemical reactions (Kawamura et al., 1993, 1999). Sources such as vehicular emissions, cooking and biomass burning contribute to aerosol composition, with notable seasonal variations (Kawamura et al., 1987; Rogge et al., 1993; Cong et al., 2015). Research on urban smog, laboratory experiments, and photochemical processes provides insights into diacid formation pathways from both anthropogenic and natural sources (Grosjean, 1978; Bikkina et al., 2014; Hetakeyama et al., 1981). Additionally, aqueous-phase oxidation in clouds contributes to oxalic acid and other oligomer formations (Altieri, 2008; Tan et al., 2010; Yang et al., 2008) leading to a considerable fraction of mass in secondary organic aerosol (Ervens et al., 2011). The complex interplay of chemical reactions and atmospheric conditions during long range transport influences the presence of organic acids in fine and coarse modes (Paulot et al., 2011; Carlton et al., 2009; Eugene et al., 2014; Zhao and Gao, 2008).

Determining carboxylic acids in atmospheric samples is challenging due to their trace levels, as it requires careful sampling and handling to prevent contamination as well as microbial degradation. Common analytical methods used for this purpose are spectrophotometry, enzymatic assays, capillary electrophoresis (CE), and chromatography. Gas chromatography (GC) provides efficient separation but needs complex derivatization. In contrast, CE is a faster and cost-effective alternative, while ion chromatography (IC) offers a safer option by eliminating hazardous reagents and reducing the analysis time. While conductivity detectors are most used, UV/Vis and potentiometric detectors are also explored but not as popular due to high cost. However, this detection method provides limited structural information about separated ions which can be overcome by coupling it with mass spectrometry or UV/Vis spectrophotometry. In IC for determining carboxylic acids, two main separation techniques are used: suppressed or non-suppressed IC. Several factors influence the separation process such as the stationary phase, eluent characteristics (concentration, pH, flow rate), detector type, injection volume, and sample preparation. the elution order of LMWOAs is determined by their ion charge, size, and affinity for the stationary phase. Adjustment to eluent concentration or flow rate may improve resolution but potentially extends the separation time. Hence, IC has been proved as a novel method for separating LMWOAs with improved detection methods, particularly suppressed conductivity detection, which enhances sensitivity and lowers detection limits for organic acids like formic, acetic, and oxalic acids (Mkoma et al., 2014; Hodgins et al., 2011, Berggren et al., 2010). However, IC method can't analyze the non-ionic species or molecules which don't interact with the ion exchange resin bed limiting its usage in analysis of a sample containing both ionic and non-ionic compounds.

Recent studies have optimized IC methodologies for environmental applications, with the Dionex IonPac AS11-HC column widely used for effective anion separation (Vasconcellos et al., 2018). Researchers also explored hydroxide-based eluents, which improved resolution and reduced background conductivity compared to traditional bicarbonate eluents (Niu et al., 2018). This approach has been successfully applied to measure carboxylic acids in various environmental matrices, such as gas and aerosol (Mkoma et al., 2014), vapors (Hodgins et al., 2011), urban particulate matter (Tapparo et al., 2020), fog (Vasconcellos et al., 2018), rainwater (Khuntong et al., 2020; Niu et al., 2018; Jackson et al., 2006), snow (Samui et al., 2017), cloud water (Khwaja et al., 1995; Sun et al., 2016), ice (O'Após et al., 2016), surface water (Berggren et al., 2010), and soil (Marcl et al., 2017).

This study aims to develop an optimized Ion Chromatography (IC) method for the accurate measurement of six organic acids at ultra-trace levels (ppb) among a total of 9 identified anions, 7 of which are classified as organic acids.

Experimental

Sampling Method

The environmental samples analyzed in this study, along with their sampling techniques, are as follows. All bottles were thoroughly washed with deionized water prior to collection, and samples were kept frozen until analysis.

1. Cloud Water: Collected using the Mohnen Omni-directional passive cloud water collector under specific meteorological conditions: liquid water content >0.05 g m^{-3} (measured by the Gerber Particle Volume Monitor), temperature >2°C, no rain (detected by a CAPMoN sensor), and wind speed >2 m/s (measured by an RM Young anemometer). When all of these conditions are met for at least one minute, the collector rises, exposing Teflon strings to the passing air flow collecting cloud water that goes into an accumulator (Lawrence et al., 2023). Samples are collected every 12 hours in a 1-liter ISCO bottle, then transferred to a 250 ml bottle and frozen for analysis. To prevent microbial degradation, a 0.4 µm polycarbonate filter is used which gets replaced twice every week. Reanalysis of samples from 2018-2019 showed less than 10% degradation error, confirming method reliability.
2. Rainwater: Collected in 20 ml bottles every 15 minutes over an hour from Albany on June 20, 2022. Each bottle contained 40 µL of chloroform to inhibit microbial growth, and samples were frozen until analysis.
3. Aerosol (PM2.5) Filters: Collected on 47 mm PTFE filters over 24 hours using a low-volume sampler (16.7 LPM flow rate) in Pakistan (2008), Saudi Arabia (2015), and Albany (2018). Filters were weighed before and after collection and stored at 4°C. One-fourth of each filter was cut, placed in a 20 ml vial with 5 ml of DI water, shaken for 24 hours, and sonicated for 2 hours before ion chromatography analysis.

Instrumentation

For this attempt to quantify LMWOAs in trace level in environmental samples, Dionex ICS 3000 Ion Chromatography System is used, consisting of a guard column, analytical column, suppressor device, and conductivity detector. Organic acids are separated utilizing their specific affinity for the resin bed of a Dionex AS11-HC 4×250mm analytical column with an AG11-HC 4×50mm guard column and ASRS Suppressor. A sodium hydroxide eluent facilitates separation. After the separation, the organic acids pass through the suppressor, that converts them to highly conductive acid forms, while the sodium hydroxide eluent is transitioned into weakly conductive water. Conductivity measurements, compared against retention times of the standards, are used to identify separated anions. Before sample analysis, the Minimum Reportable Levels (MRL) for essential organic acids, Lactate, Acetate, Propionate, Formate, Pyruvate, and Glyoxalate are measured following with EPA guidelines, to ensure accuracy and reliability in the analytical process.

PREPARATION OF STANDARDS, QCs, and SAMPLES

Reagents and Stock Solutions

1. Deionized (DI) Water: Conductivity <1 µmho/cm at 25°C.

2. Stock Eluent (2000 mg/L NaOH): Dissolve 4.70550 g of NaOH (FW = 39.997) in 1 liter of deionized water.

3. Working Eluent (3.0 mM NaOH): Transfer 76.55 mL of stock eluent to a flask and dilute to 3 L with deionized water.

4. Stock Standards (1000 mg/L): Purchased from SPEX CertiPrep (certified solutions) and utilized before expiration. Quality assurance/control samples were prepared from ACS reagent-grade materials (dried at 105°C for 30 minutes), detailed as follows:
Lactate: Dissolve 0.1258 g of Sodium Lactate (FW = 112.06) in DI water and dilute to 100 mL.
Acetate: Dissolve 0.1389 g of Sodium Acetate (FW = 82.03) DI water and dilute to 100 mL.
Propionate: Dissolve 0.1296 g of Sodium Propionate (FW = 96.07) in DI water and dilute to 100 mL.
Formate: Dissolve 0.1477 g of Sodium Formate (FW = 68.01) in DI water and dilute to 100 mL.
Pyruvate: Dissolve 0.1249 g of Sodium Pyruvate (FW = 110.04) in DI water and dilute to 100 mL.
Glyoxalate: Dissolve 0.1555 g of Sodium Glyoxalate (FW = 114.03) in DI water and dilute to 100 mL.

Calibration Standards

Mixed calibration standards at concentrations of 0.025, 0.05, 0.08, 0.1, 0.2, 0.5, 1, and 5 mg/L were prepared, establishing a linear calibration curve for each analyte with a minimum correlation coefficient (r) of 0.995. Freshly prepared QC samples were analyzed daily and after every 8-10 samples to validate the calibration curve, with control limits set at 90-110%.

Quality Assurance and Quality Control

Lab and field duplicates were analyzed with a relative percent difference (RPD) acceptance criterion of ±10%. Samples failing this criterion were reanalyzed. Matrix spike recoveries indicated sample matrix interference, requiring analyte concentrations four times the minimum detection limit (MDL) for reliable detection. For 0.05 mg/L concentration (the 2nd lowest calibration standard), seven replicates were analyzed; fortified concentration = mean ± SD. MDL was calculated as t_{n-1} = 3.143 * SD. Minimum reporting level (MRL) = MDL × 3.

ANALYTE	PEAK #	RETENTION	MDL DETERMINATION		
		TIME (min)	Measured Conc (mg/L)	MDL (mg/L)	MRL (mg/L)
Lactate	1	5.97	0.055	0.0074	0.0223
Acetate	2	6.45	0.052	0.0119	0.0357

Propionate	3	7.30	0.055	0.0132	0.0397
Formate	4	8.18	0.055	0.0041	0.0122
Pyruvate	5	10.4	0.055	0.0167	0.0320
Glyoxalate	6	15.7	0.053	0.0073	0.0220

(Table 1: Details of all measured anions)

Analytical Column	AS11 4 X 250mm HC
Guard Column	AG11 4 X 50 mm
Detector	Dionex CD25A (Conductivity)
Autosampler	Dionex AS50
Gradient Pump	Dionex GP50
Eluent	3.0 mM NaOH (isocratic)
Flow Rate	1.0 mL / min
Suppressor	Dionex Anions ASRS
Suppressor Current	50 mA

(Table 2: chromatographic conditions)

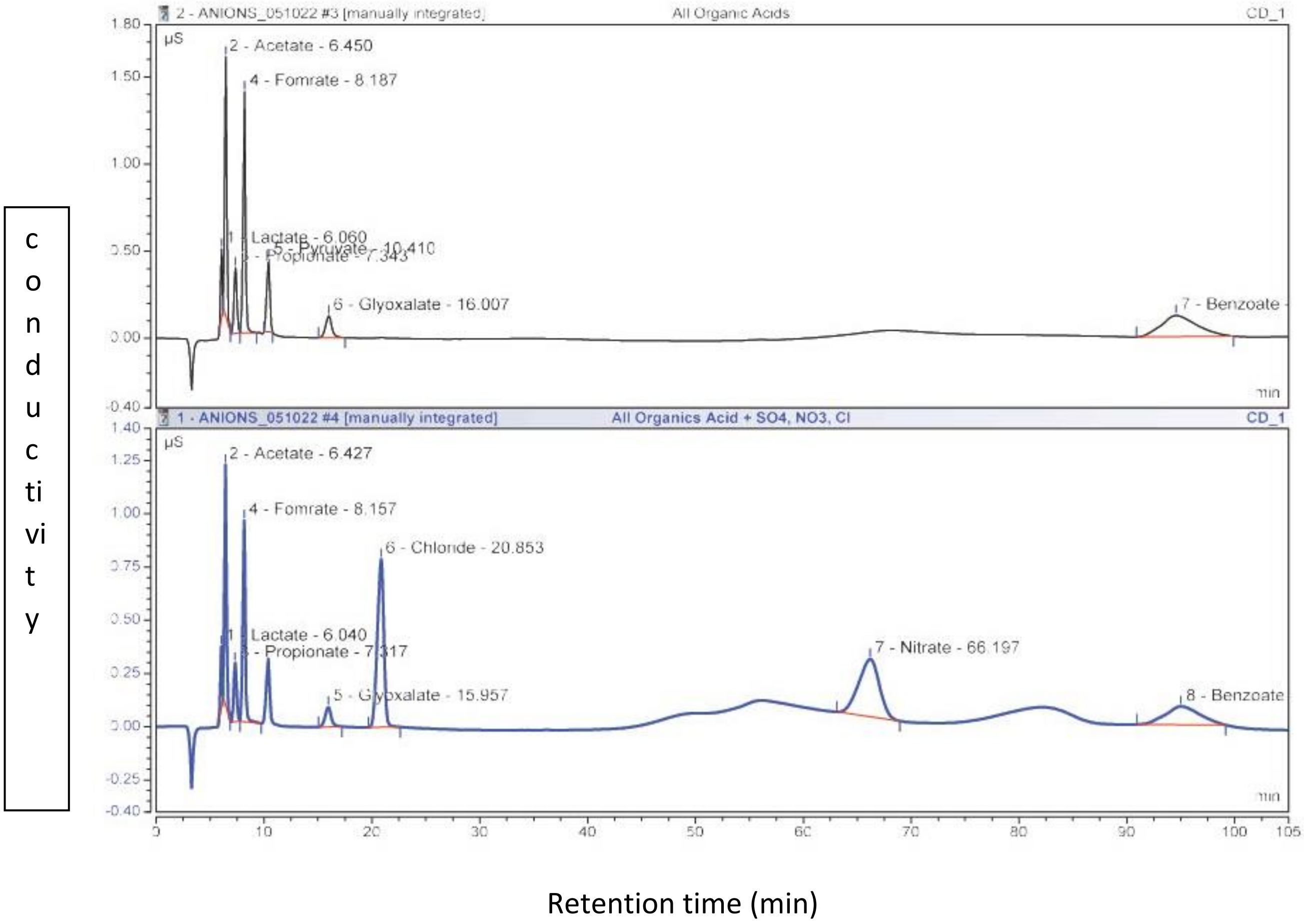
Results and Discussion

Figure 1 illustrates the elution profiles of seven organic acids and two additional anions (chloride and nitrate). All six specified organic acids were successfully eluted before the common anions, including chloride, and nitrate demonstrating the absence of interference between them. The retention times for each identified anion are detailed in Table 3. Benzoate, oxalate and sulfate were added to both mixtures as well but only benzoate eluted before the run was over i.e. 105 minutes and sulfate and oxalate did not elute in this time frame. In the calibration standards, benzoate was not added since it’s not commonly detected in the environmental samples and fell outside the analytical scope of the study due to its delayed retention time. Oxalate and base anions such as chloride, nitrate, sulfate were measured using another analytical method (Khwaja et al., 1999) that did not account for other organic acids.

Analyte	Retention time (min)
Lactate	6.06
Acetate	6.42

Propionate	7.3
Formate	8.18
Pyruvate	10.4
Glyoxalate	15.95
Chloride	20.8
Nitrate	66.19
Benzoate	94.8

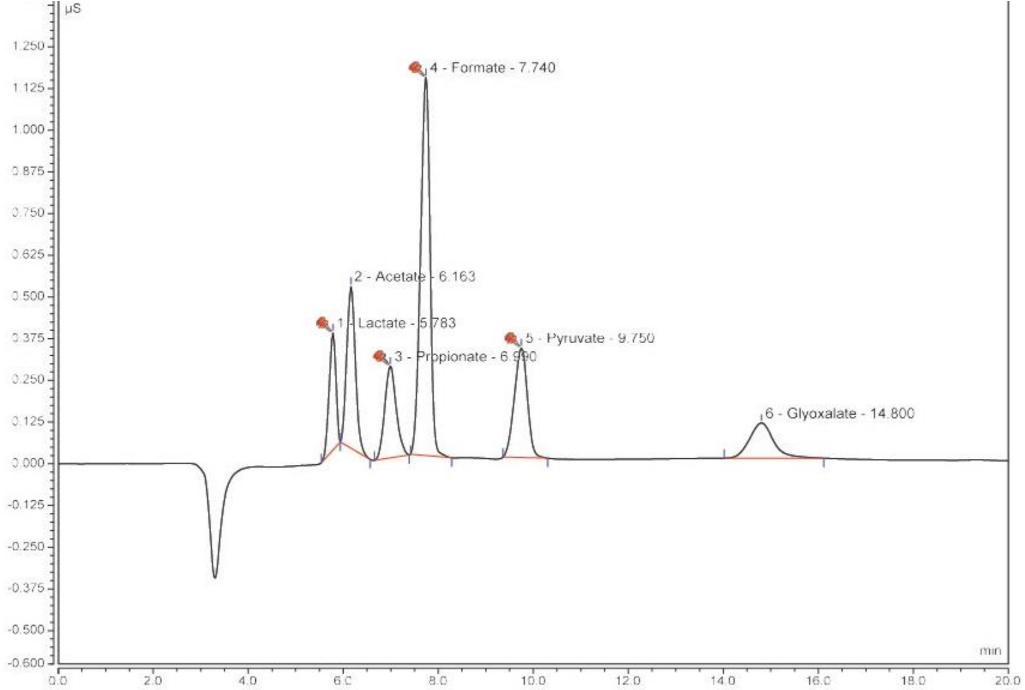
(Table 3: Retention time of all identified anions including organic acids)

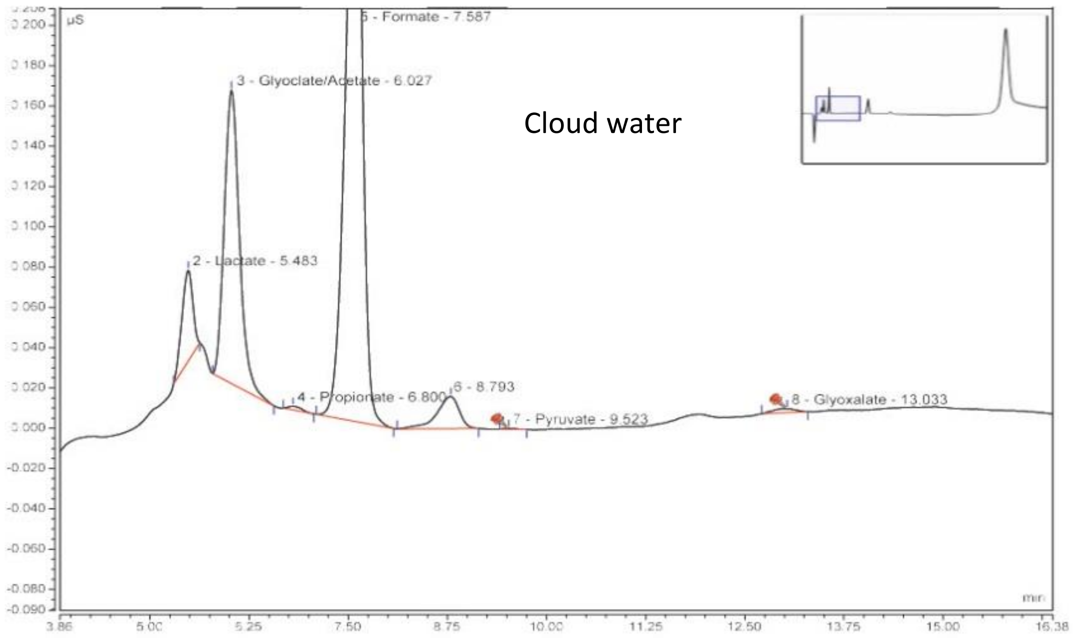


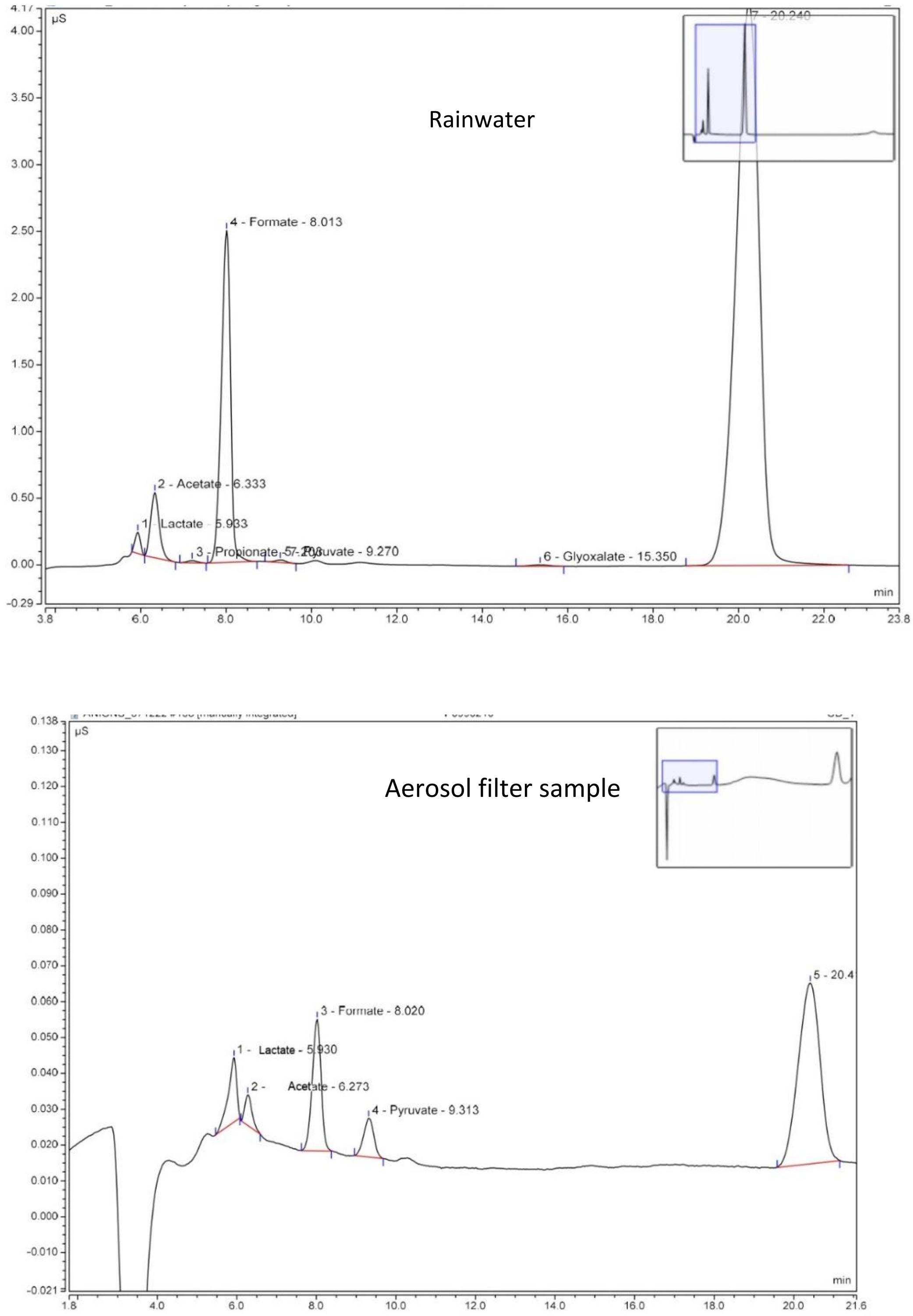
(Fig 1: Parallel comparison between the chromatograms of mixtures with and without anions)

Figure 2 shows chromatograms of a calibration standard with a concentration of 1 mg/L, followed by representative samples from various sample sets, including cloud water, rainwater, and aerosol filters with the measured organic acids. Chloride and nitrate ions were not measured although the peaks were identified in some samples.

Calibration standard (1mg/L)




Cloud water



(Fig 2: A calibration standard and 4 sample chromatograms of different types of environmental samples)

The table presents measured concentrations of six organic acids in various environmental samples. Formate (not calibrated), acetate and lactate were prevalent across all sample types.

Propionate was undetectable in all samples except rainwater. Pyruvate was detected in cloudwater and aerosol filters. Glyoxalate was not quantifiable in aerosol filters. In some instances, peaks were present in chromatograms but too noisy, thus classified as below detection level (BDL).

Sample type	Lactate (mg/L)	Acetate (mg/L)	Propionate (mg/L)	Formate (mg/L)	Pyruvate (mg/L)	Glyoxalate (mg/L)
Cloud water	.052	1.175	BDL	1.811	BDL	.052
Cloud water	.06	.984	BDL	.919	BDL	.041
Cloud water	.087	.40	BDL	.529	.035	.105
Rainwater	.040	1.072	.056	2.2	.054	.117
Rainwater	.039	1.177	.053	2.193	.055	.109
Aerosol filter	.044	1.017	BDL	.60	0.072	BDL
Aerosol filter	.073	1.027	BDL	.40	0.061	BDL
Aerosol filter	.06	.53	BDL	.277	BDL	BDL

(Table 4: Measured concentrations of organic acids in different types of environmental samples)

Conclusion

This improved technique serves as a powerful tool across various scientific fields. It allows the separation and measurement of six organic acids simultaneously, without interference from each other and even from common inorganic anions such as sulfates, nitrates, and chlorides, all within a short analysis time. This efficiency and reliability make it an excellent option for a wide range of analyses, making it valuable in industries like pharmaceuticals, petrochemicals, semiconductors, food, beverages, explosives, and cosmetics.

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