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Analysis of the stable carbon isotope composition of formic and acetic acids

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ABSTRACT

Formic and acetic acids are ubiquitous in the environment and in many biological processes. Analysis of the stable carbon isotope composition ($\delta^{13}C$) of formic and acetic acids is important to understanding their biogeochemical cycles. However, it has been faced with poor accuracy and high detection limits due to their low carbon number, high hydrophilicity, and semi-volatility. Here we developed an analytical technique by needle trap and gas chromatography–isotope ratio mass spectrometry (GC–IRMS). The organic acids in aqueous solution were extracted using a NeedlEx needle through purge-and-trap and were analyzed by GC–IRMS for $\delta^{13}C$. The procedures incur no isotope fractionation. Defined as the point at which the mean $\delta^{13}C$ is statistically the same as the given value and the analytical error starts rising, the method's detection limits are 200 and 100 mg/L for formic and acetic acids, respectively, with an uncertainty of approximately 0.5‰ in direct extraction and analysis. They were lowered to 1 mg/L with precision of 0.9‰ after samples were subjected to preconcentration. The method was successfully applied to natural samples as diverse as precipitation, vinegars, ant plasma, and vehicle exhaust, which vary considerably in concentration and matrix of the organic acids. It is applicable to the organic acids in not only aqueous solution but also gaseous phase.

Formic and acetic acids are the simplest and most ubiquitous low-molecular-weight carboxylic acids in the environment [1], biomass [2,3], and many biological processes [4,5]. They increase free acidity of the tropospheric environment [6] and influence the terrestrial ecosystem as well as the atmospheric reactions relevant to pH [7]. They also play important roles in fermentation of organic material [8,9], rusting of metal equipment [10], and corrosion of limestone material in buildings and artistic statues [11]. These understandings were achieved primarily through concentration measurement. To elucidate their biogeochemical cycles such as source identification and apportionment, transformation, and decomposition processes, measurement of the compound-specific stable carbon isotope composition represents a more efficient way [12]. Early efforts on the analysis were performed stepwise, involving a series of manual processes from capture/separation and oxidation of the individual acid to the isotopic analysis by isotope ratio mass spectrometry (IRMS)[1] [13–16]. These methods were improved recently by continuous flow mass spectrometry LC–IRMS or GC–IRMS, which is formed by coupling liquid chromatography or gas chromatography, respectively, to IRMS through an oxidation interface. After being separated by the chromatography, the analytes are transferred successively by the carrier gas into the oxidation module to be converted to CO_2. The CO_2 of an individual analyte is delivered to the following IRMS for the isotope ratio measurement [17]. All of these operations are performed continuously and automatically. Compared with LC–IRMS, which is a newcomer to continuous flow mass spectrometry [18], GC–IRMS is more routinely employed. The latter, however, has been faced with a critical problem in introducing the analyte to the instrument in a GC-amenable way. Being semi-volatile and highly hydrophilic, formic and acetic acids are usually processed in aqueous solution irrespective of their original matrix [19]. The presence of large amounts of water prevents the sample from being injected directly into the GC, although the effort was made decades ago [20]. Solid-phase microextraction (SPME) solved the problem while keeping the contamination free of the ambient air and has been used in measurement of the stable carbon isotope composition of acetic acid and other volatile organic compounds [21–24]. However, the high hydrophilicity of the organic acids makes SPME difficult at low concentrations [25]. Furthermore, adsorption of the SPME fiber induces isotopic fractionations [21]. Last but not the least, formic and acetic acids have the minimum carbon numbers of their kind and, thus, generate the least amount of CO_2 in the oxidation. These problems impaired the methods' accuracy and precision while making the detection limit high [21,24]. Up to now, no examination has been found on the analytical precision or sensitivity for formic acid because of its extremity with these problems. Based on these understandings, we developed a new extraction technique using the dynamic SPME technology, the

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[1] *Abbreviations used:* IRMS, isotope ratio mass spectrometry; LC, liquid chromatography; GC, gas chromatography; SPME, solid-phase microextraction; VPDB, Vienna Pee Dee Belemnite; SPE, solid-phase extraction; SD, standard deviation.

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needle trap [26,27], and previously reported the optimization on the extraction temperature and pH [28] as well as the extraction efficiency [25]. Here we apply it to the measurement of the stable carbon isotope composition of formic and acetic acids by coupling with GC–IRMS.

Materials and methods

Chemicals and materials

Formic acid (>98.00% purity) was purchased from Fluka. Acetic acid (>99.99% purity) and phosphoric and sulfuric acids (99.999% purity) were purchased from Sigma–Aldrich. A batch of LC–SAX SPE tubes (500 mg/3 ml), glass vials (40 ml), and Teflon tubes (2 mm i.d.) were purchased from Supelco. Six AP-20 gas pumps were purchased from Komyo (Japan). A batch of NeedlEx needles were purchased from Shinwa (Japan). Gas bags (TMC-030) with 3 L volume capacity each were purchased from Tedlar. Vacuum fittings (SS-4-UT-1-2) were purchased from Swagelok. Helium gas (99.999% purity) and quartz tubes (20 cm long, 8 mm i.d.) were supplied by local vendors. As the kernel of this study, the NeedlEx is a needle 85 mm long with an inside diameter of 0.5 mm and an outside diameter of 0.7 mm packed inside approximately 30 mm long with polymer-based beads synthesized from divinylbenzene monomer. The copolymer of the NeedlEx is the type for extraction of fatty acid and has a total surface area of 150 m^2/g [26].

Calibration of isotopic standards

Development and evaluation of the analytical procedures require the external isotopic standards of formic and acetic acids. These standards, however, are commercially unavailable at this time. To solve this problem, the reagents of formic and acetic acids as indicated above were calibrated with respect to their stable carbon isotope compositions. A batch of quartz tubes loaded with 2.5- to 3-g CuO wires each was decarbonized by combustion at 850 °C for 2 h in a Muffle furnace and, after cooling down, were attached to a stainless steel Ultra-Torr vacuum fitting, as shown in Fig. 1.

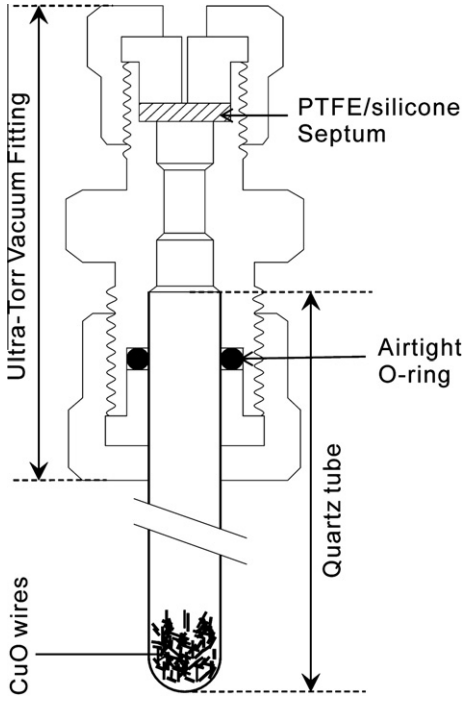

Fig.1. Apparatus for complete combustion of formic and acetic reagents.

The apparatus was attached to a vacuum system through a needle that pierces through the septum of the vacuum fitting. After the vacuum in the tube reached 8 to 10×10^{-3} mbar for 1 min, the apparatus was detached from the vacuum line and submerged in liquid nitrogen by the bottom end. Then the formic and acetic acids (~0.3 ml each) were loaded separately into the tube using syringes of 1 ml volume. After the sample was frozen to the bottom, the tube was sealed at the upper part with an oxygen acetylene cutting torch and subject to heating at 850 °C for 5 h in the Muffle furnace. The CO_2 produced by oxidation of the formic and acetic acids was cryogenically purified in a system modified after Craig [29] and analyzed in a dual-inlet way with a Finnigan MAT252 IRMS instrument for carbon isotope composition. The results were expressed as the δ notation relative to the Vienna Pee Dee Belemnite (VPDB) as follows:

$$\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000\%,$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ of the sample and standard CO_2, respectively.

Preparation of sample solution and NeedlEx

Stock solution of 1000 mg/L was prepared every week by diluting the reagent of formic and acetic acids with ultrapure water and was stored in a refrigerator at 4 °C. Aqueous samples of various concentrations were diluted daily from the stock solution.

The NeedlEx was conditioned at 200 °C prior to use in a custom-made thermostat apparatus assisted by a flush of helium gas at a rate of 7 ml/min. The conditioning lasts for 3 min on the first use, whereas it lasts for a half-minute before daily experiments.

Extraction of formic and acetic acids

The prepared aqueous solution of 20 ml was pipetted into a 40-ml glass vial with a stir bar loaded previously. Then the vial was capped tightly and injected with 500 µl of 4 mol/L phosphoric acid using a syringe.

A batch of six samples prepared in this way were each fixed on a thermostat magnetic stir plate. Each vial was installed with a preconditioned NeedlEx and a medical spinal needle through the septum of the hole cap, as shown in Fig. 2. The NeedlEx was connected to an AP-20 pump, and the spinal needle was connected to a helium gas bag through Teflon tubes. The stir plates were set at room temperature and a 2000-rpm stirring rate. After the setup, the pump was drawn to a specific volume. The negative pressure created in this way pumped the gas in the headspace of the vial out through the NeedlEx while sucking in the helium gas through the aqueous solution. The organic acids in the solution were purged by the stirred helium gas and subsequently trapped in the sorbent of the NeedlEx. Thus, this setup and these procedures created a purge-and-trap system. Normally, a 100-ml purge, which is the pump's maximum aspirating volume for one draw, takes approximately 13 min at a purge flow rate of 7.7 ml/min. Purge volumes larger than 100 ml were achieved by repeated drawings. Unless specified otherwise, the purge volume was 1000 ml.

Desorption of extracts

After the extraction, the NeedlEx was attached to a 1-ml airtight syringe that had 0.5 ml of helium gas drawn beforehand and was introduced to the GC instrument. When it remained in the injection port for 15 s, the needle was flushed with the helium gas at a rate of approximately 200 µl/s and then removed for the next extraction.

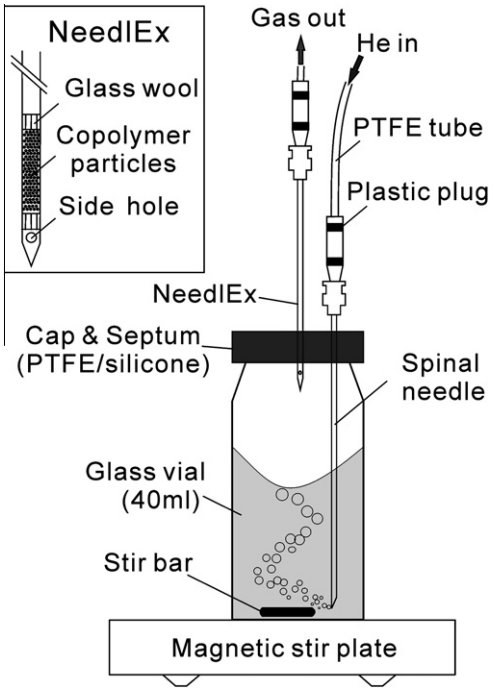

Fig.2. Setup of the purge-and-trap extraction of formic and acetic acids in aqueous solution. He, helium; PTFE, polytetrafluoroethylene (Teflon).

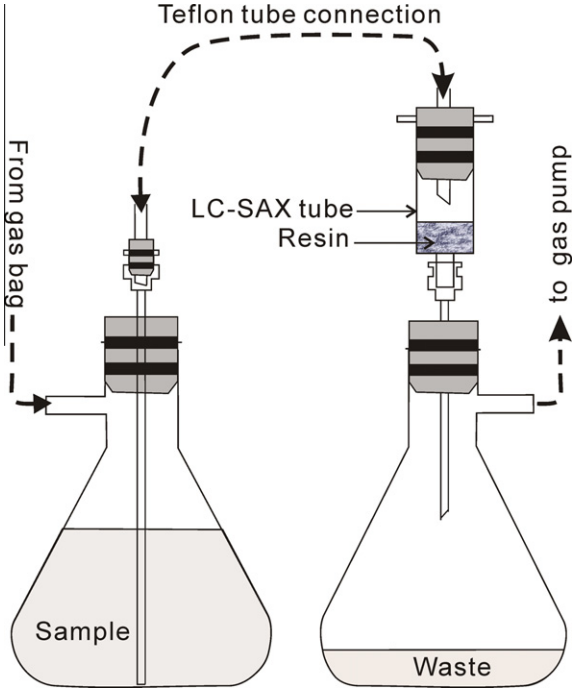

Fig.3. Assembled apparatus for preconcentration of formic and acetic acids in aqueous solution.

Preconcentration at sub-mg/L level

Measurement of $\delta^{13}C$ of the organic acids at a microgram per liter (μg/L) level or a few milligrams per liter (mg/L), referred to hereafter as sub-mg/L level, requires preconcentration before the purge-and-trap extraction. This was performed using the anion exchange solid-phase extraction (SPE) tube (Supelclean LC–SAX SPE). To prevent contamination from the ambient air, two Buchner flasks were connected by a Teflon tube through an SPE tube and a few plugs or connection adaptors, as shown in Fig. 3. Prior to the preconcentration, the SPE tube was first activated with 3 ml of methanol and then eluted successively with 3 ml of ultrapure water and 3 ml of 1% ammonia solution. Meanwhile, the sample was basified to pH 7.0 with 6 mol/L NaOH solution so as to dissociate the organic acids. After this was done, the vacuum suction was effected and the aqueous sample was forced through the SPE tube, in which the anions (including formate and acetate) were exchanged onto the resin. The volume of the preconcentrated sample was 1000 ml. To elute out the anions from the SPE tube, the sample flask was replaced by one containing 2 mol/L HCl and the waste flask was substituted by a 40-ml glass vial. The suction was introduced to the vial through a needle. Then the same operation as described above was repeated until the volume of the elution reached 20 ml.

GC analysis

A Hewlett–Packard 6890N GC instrument equipped with a Stabilwax-DA fused-silica capillary column (30 m long. 0.32 mm i.d., 0.25 μm *df*, Restek, Bellefonte, PA, USA) was used for separation of the analytes. The injection port temperature was 200 °C, and the injection mode was splitless. The oven temperature was programmed as follows: initial 65 °C, hold for 1 min, first ramp to 80 °C at 2 °C/min, then ramp to 170 °C at 15 °C/min, and then hold for 1 min. The carrier gas was helium at 2 psi pressure.

Working conditions of combustion system

The combustion system consisted of an interface, a combustor, and a Nafion instrument. The interface connected the GC instrument and the furnace, and it was set at 350 °C. The combustor containing CuO and Ag wires was set at 850 °C. The Nafion instrument was made of semi-permeable membranes and a gas drying module, and it was installed after the combustor to serve as a denuder of the water vapor produced during the combustion [17,30]. The flow rate of helium through the drying module of the Nafion instrument was 23 ml/min, and the rate of carrier gas through the combustor was 1.2 psi.

Settings of IRMS and isotopic analysis

A Micromass Isoprime IRMS instrument was connected with the combustion system through an open split interface through which the CO_2 of both samples and reference was introduced to the spectrometer. The accelerating voltage of the IRMS analysis was set at 3520 V, the magnet current at 4000 mA, the extraction voltage at 75 V, the trap current at 200 μA, the electron volts at 100 V, and the ion repeller voltage at −8 V. The carrier gas was set at 0.3 psi, and the reference CO_2 was set at 10 psi.

Ion currents generated by the ionized CO_2 in the ionization chamber of the IRMS instrument were measured continuously for m/z 44, 45, and 46 by the triple Faraday cups. The measured isotopic ratios were automatically calculated by the software in reference to the given value of the reference CO_2. The results were also expressed as the δ notation relative to VPDB.

Analysis of natural samples

The method was applied to analysis of $\delta^{13}C$ of formic and acetic acids in four types of natural samples: precipitation that fell during different months in Guiyang (the provincial capital of Guizhou in southwest China), vinegars of a few brands available in the market,

exhaust of an idle car, and body plasma of the ant *Camponotus japonicus*. These samples have a wide range of concentrations and different matrices for the organic acids.

The precipitation was characterized by high inorganic ions such as F^-, SO_4^{2-}, Cl^-, NO_3^-, Na^+, K^+, and Ca^{2+}[31] but low formic and acetic acids at a sub-mg/L level [32]. It was first submitted to the preconcentration before the purge-and-trap extraction. Based on the long-term observations of anion concentration in precipitation in Guizhou province [31,32], the highest total electronic charge of the anions did not exceed 464×10^{-3} mEq/L. The ion exchange capacity of the resin in the SPE is 0.2 mEq/g. To exchange all of the anions in a 1-L precipitation sample, five SPE tubes (500 mg/3 ml) are needed. This is because the affinity of formic and acetic acids with the resin is weaker than the inorganic anions, and the organic anions would pass through if the capacity of SPE were not provided sufficiently.

For extraction of the organic acid in the vinegars, a 0.2-ml vinegar sample was diluted to 20 ml with water in a 40-ml glass vial and then acidified and extracted as described above. For the extraction in ant plasma, 20 ants were captured in the glass vial also with 20 ml of water and 500 μl of 4 mol/L phosphoric acid. The mixture was stirred, and the organic acids were extracted.

The organic acids in the vehicle exhaust were trapped directly by drawing the exhaust air through the NeedlEx in front of the exhaust pipe. To prevent the needle from being clogged by possible carbon soot, a glass fiber filter (1.2 μm) was attached to the head of it. The volume of the air pumped through the NeedlEx was 1000 ml.

The concentrations of formic and acetic acids in the natural samples were measured separately by the ion chromatography method, as reported in Ref. [33].

Results and discussion

$\delta^{13}C$ of formic and acetic reagents

The stable carbon isotope compositions of formic and acetic acids in the reagents are listed in Table 1. The final standard deviation (SD), which is indicated by the error and may derive in any step from the sample preparation to the instrument analysis, was 0.02‰ for both acids. These errors are a magnitude smaller than the normal uncertainty of the GC–IRMS, which was specified and tested by Micromass using decane, undecane, dodecane, and methyl-deconate [34]. Complete combustion of the compound in the quartz tube leaves no room for isotope fractionation, and the dual-inlet mode of the IRMS instrument is the most accurate approach for isotopic analysis to date. Thus, the reagents calibrated would serve as a qualified isotopic standard for the following experiments.

Table 1
$\delta^{13}C$ values of formic and acetic acids in reagents.

Acid	Number	$\delta^{13}C$ (‰) ± 1σ[a]	Mean (‰) ± 1σ[b]
Formic	1	−24.88 ± 0.01	−24.87 ± 0.02
	2	−24.88 ± 0.01	
	3	−24.86 ± 0.01	
	4	−24.90 ± 0.02	
	5	−24.85 ± 0.01	
Acetic	1	−39.53 ± 0.01	−39.52 ± 0.02
	2	−39.55 ± 0.02	
	3	−39.50 ± 0.02	
	4	−39.51 ± 0.01	
	5	−39.52 ± 0.00	

[a] The error is the SD of five time measurements by IRMS on the CO_2 produced in one combustion.
[b] The error is the SD of the five replicate analyses.

Effect of acidification and basification

The purge of formic and acetic acids is a critical step in the extraction. It is facilitated by molecular form of the organic acids, whereas it is thwarted by dissociated ones in the aqueous solution according to Henry's law. Preconcentration of the acids, on the contrary, would be favored or impeded at the inverse conditions because the SPE tube works by ion exchange. Both purposes can be fulfilled through adjustment of pH because the dissociation of the acid depends strongly on pH of the solution [28] (see Fig. S-1 in the Supplementary material).

Normally, pH of the mixed aqueous solution of formic and acetic acids is approximately 3.0 to 4.0, depending on the concentration of the acids (Table 2). The addition of H_3PO_4 or H_2SO_4 would lower the pH to 2.0 to 3.0 or to 1.0 to 2.0, respectively. The effect of both inorganic acids decreases with concentration of the organic acid because of the increased buffering capacity of the latter. Despite its better effect in acidification, H_2SO_4 was not used in the following experiments due to its detriment to the needles. Instead, H_3PO_4 of 4 mol/L was employed.

The addition of 500 μl of such phosphoric acid lowers the pH of the solution to approximately 2.3, at which the molecular forms of formic and acetic acids are increased to more than 96 and 99%, respectively, of the total forms (Fig. S-1). The undissociated acetic acid is higher than the undissociated formic acid at the same pH; however, the increasing rate of the former is slower than that of the latter at the same acidification (i.e., at the same reduction of pH), suggesting that the effect of acidification on formic acid is stronger than the effect on acetic acid. These theoretical effects of acidification were verified by the signal size of the extracts measured in the IRMS, which was increased by 6% to 30% and by 4% to 14% for formic and acetic acids, respectively, after acidification (Fig. 4). The increased percentage of the signal intensity decreases with increases of concentration due to the increased buffering capacity of the solution, confirming that the acidification works better for both acids at low concentrations, as shown in Table 2. Meanwhile, the enhancement of the signal size for formic acid is 1.4 to 2.1 times higher than that for acetic acid, indicating that the acidification achieved a better effect in formic acid. The difference increases with decreasing concentrations, suggesting that acidification is particularly helpful for extraction of formic acid at low concentrations.

The effect of acidification is also proved by the analytical results for both acids in comparison with non-acidification (Table 3). The SD of six replicate extractions and analysis at acidification is smaller than that at non-acidification at all concentrations except 10 mg/L for acetic acid, in which the error with the acid-treated solution is 2.69 compared with 2.52 at non-acidification. The improvement of the uncertainty under acidification results from the increased purge-and-trap efficiency (Fig. S-1) and, thus, the enhanced signal sizes (Fig. 4). Despite the significant difference it made, the acidification does not bias the isotope composition for either acid ($P > 0.05$) (see Table S-1 in Supplementary material), suggesting that no isotopic fractionation was inflicted by the acidification.

The dissociation of both acids increases with pH and reaches nearly 100% at a pH of approximately 7.0 (Fig. S-1). The pH is approximately 4.0 in the prepared solution that was at a sub-mg/L level and not treated with phosphoric acid (Table 2), whereas it ranges from 3.0 to 6.0 in precipitation. The addition of NaOH solution can readily raise the pH to the required level.

Isotopic accuracy

Formic and acetic acids desorbed from the NeedlEx were well separated and with good peak shape at the desorption and other

Table 2
pH values measured in aqueous solution of formic and acetic acids at different concentrations and acid additions.

Concentration (mg/L)[a]	pH at different treatments[b]			
	No acidification solution	150 µl/1 mol/L H_2SO_4	150 µl/1 mol/L H_3PO_4	500 µl/4 mol/L H_3PO_4
1	4.120 ± 0.019	1.827 ± 0.021	2.517 ± 0.021	2.348 ± 0.019
10	3.975 ± 0.091	1.836 ± 0.054	2.516 ± 0.097	2.317 ± 0.026
20	3.891 ± 0.063	1.821 ± 0.078	2.511 ± 0.087	2.316 ± 0.017
50	3.621 ± 0.063	1.810 ± 0.035	2.510 ± 0.029	2.427 ± 0.171
75	3.587 ± 0.075	1.797 ± 0.109	2.497 ± 0.089	2.322 ± 0.060
100	3.496 ± 0.048	1.829 ± 0.071	2.499 ± 0.109	2.293 ± 0.016
200	3.171 ± 0.089	1.789 ± 0.085	2.490 ± 0.077	2.299 ± 0.050
300	3.006 ± 0.021	1.810 ± 0.067	2.490 ± 0.058	2.287 ± 0.087
500	2.953 ± 0.048	1.809 ± 0.017	2.489 ± 0.047	2.298 ± 0.010

[a] Both formic and acetic acids are at the same concentration.
[b] Presented as mean ± 1σ SD of three replicate measurements.

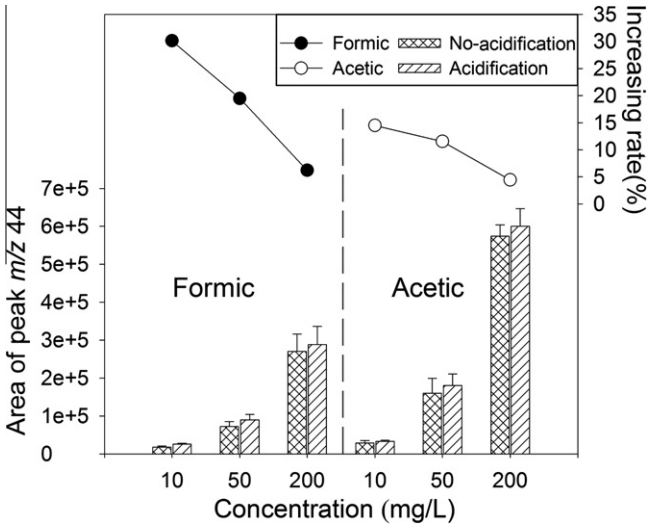

Fig.4. Effect of acidification on signal size of GC–IRMS at different concentrations.

GC conditions (Fig. 5). The retention time for formic acid is nearly 1 min longer than that for acetic acid, and the signal size for formic acid is approximately half that for acetic acid, which agrees with the difference of the carbon numbers. The separation facilitated the subsequent isotopic analysis for the individual compound. The signal size measured in the IRMS analysis for each acid decreases linearly with the concentration ($P < 0.01$). $\delta^{13}C$ of replicate analyses varies around the given value at both mg/L and sub-mg/L levels (Fig. 6). Despite the fluctuation of $\delta^{13}C$ among the replicates, especially at the low concentrations of both levels, no statistical difference to the given $\delta^{13}C$ value was found for either acid at any concentration in a Student's t test ($P > 0.05$) (Table S-2), nor did one occur between the concentrations in the analysis of variance (ANOVA) test ($P > 0.05$) (Table S-3). The excellent accuracy indicates that the extraction and analytical procedures of the method did not bias the isotope composition. Compared with the SPME fiber extraction, which was based on equilibrium partition of analytes between the solution and the fiber sorbent and led to as much as 1.5‰ isotope fractionation due to mass-dependent partition of carbon atoms [21], the extraction employed in this study purges the organic molecules dynamically and traps all of them that were drawn into the NeedlEx without mass discrimination; therefore, it causes no isotope fractionation.

Table 3
$\delta^{13}C$ values of formic and acetic acids measured at acidification and non-acidification.

Concentration (mg/L)	Formic[a]		Acetic[a]	
	Acidification	No acidification	Acidification	No acidification
10	−25.3 ± 3.5	−25.1 ± 4.0	−38.9 ± 2.7	−38.9 ± 2.5
50	−24.8 ± 1.5	−24.5 ± 2.0	−34.0 ± 0.9	−39.9 ± 1.4
200	−24.7 ± 0.5	−24.3 ± 0. 8	−39.9 ± 0.5	−40.2 ± 0.8

Note: Acidification was carried out with 500 µl of H_3PO_4 of 4 mol/L.
[a] The values listed are $\delta^{13}C$ (‰) ± 1σ SD of six replicate extractions and analyses.

Isotopic precision

Despite the good accuracy, $\delta^{13}C$ of the replicate analyses begins to fan out as the concentration and signal size decrease to certain values, as shown in Fig. 6. This suggests that the analytical precision starts deteriorating once the organic acid gets lower than a threshold concentration.

In the direct purge-and-trap extraction, the SD of six replicate analyses for formic acid is quite stable at approximately 0.5‰ at or above a concentration of 200 mg/L and increases only slightly to 0.7‰ as the concentration is reduced to 100 mg/L. It rises dramatically as the concentration is lowered further. Similarly, the SD of acetic acid stabilizes at approximately 0.5‰ at a concentration of 100 mg/L or above, increases slowly to 0.9‰ at 50 mg/L, and then soars as the concentration decreases further. As a result, the change of the uncertainty with concentration abides by the power function ($P < 0.01$) for both acids (Fig. 7).

The preconcentration condensed the analytes by 50 times, raising a concentration of µg/L to a corresponding one at mg/L (Table S-4). Due primarily to this effect, the threshold concentration was lowered considerably. The SD of six replicate analyses stabilizes at 0.9‰ at or above a concentration of 1000 µg/L for both acids and begins to increase as the concentration is lowered further (Fig. 7).

Besides the effect of condensation, the preconcentration also increased the effect of ionic strength by the addition of Na^+ and OH^- in the basification of the samples, which in turn increased the extraction efficiency of the organic compounds [35,36] and, thus, contributed to the decrease of the threshold concentration. After being subjected to the preconcentration, the thresholds for formic and acetic acids are 4 times (as 50 vs. 200 mg/L) and 2 times (as 50 vs. 100 mg/L), respectively, lower than in the direct extraction (Table S-4). The ionic strength plays a stronger role in the case of formic acid compared with acetic acid.

Despite the substantial decrease of the threshold concentration by preconcentration, analytical uncertainty still changes with concentration by power functions ($P < 0.01$) (Fig. 7). The error above the threshold concentration is generally compatible with that at the corresponding concentration in the direct extraction.

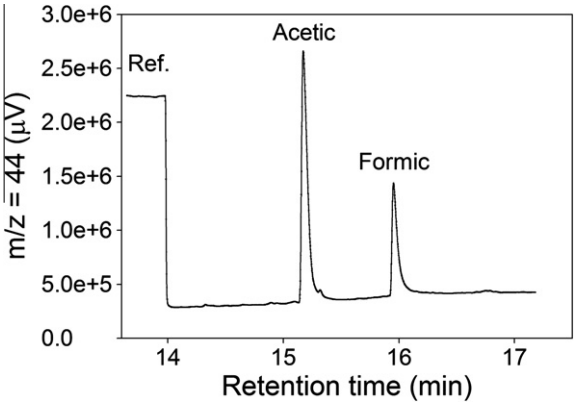

Fig.5. Chromatogram for formic and acetic acids separated at the desorption and other GC conditions following extraction at 500 mg/L. Ref., reference.

However, it becomes increasingly lower as the concentration drops below the threshold point (Fig. 7 and Table S-4), suggesting that the effect of the ionic strength gets stronger at low concentrations.

Method range

The analytical error for formic acid is always larger than that for acetic at low concentrations (Fig. 7). This is a result of the different sensitivity of the method, or detection limit, to the individual acid, which is defined as the minimum concentration of the acids for accurate and precise measurement of the isotope composition. The method is more sensitive to acetic acid in that its molecular carbon number, and thus the amount of CO_2 produced in its oxidation, is twice that of formic acid. This is a common phenomenon in isotopic analysis of organic compounds with different carbon numbers [37]. Due to the extremely low carbon number(s), formic and acetic acids (especially the former) are notoriously poor in sensitivity.

The critical point determining a method's sensitivity for a specific compound lies in where to settle the minimum concentration for the accurate and precise isotope measurement. Dias and Freeman [21] reported the threshold as the concentration that generates 50 pmol of CO_2 in the ion source of GC–IRMS, whereas Jochmann and coworkers [38] recently determined the minimum concentration as the point where the SD of triplicate measurements does not exceed ±0.5‰ and the running average of the mean

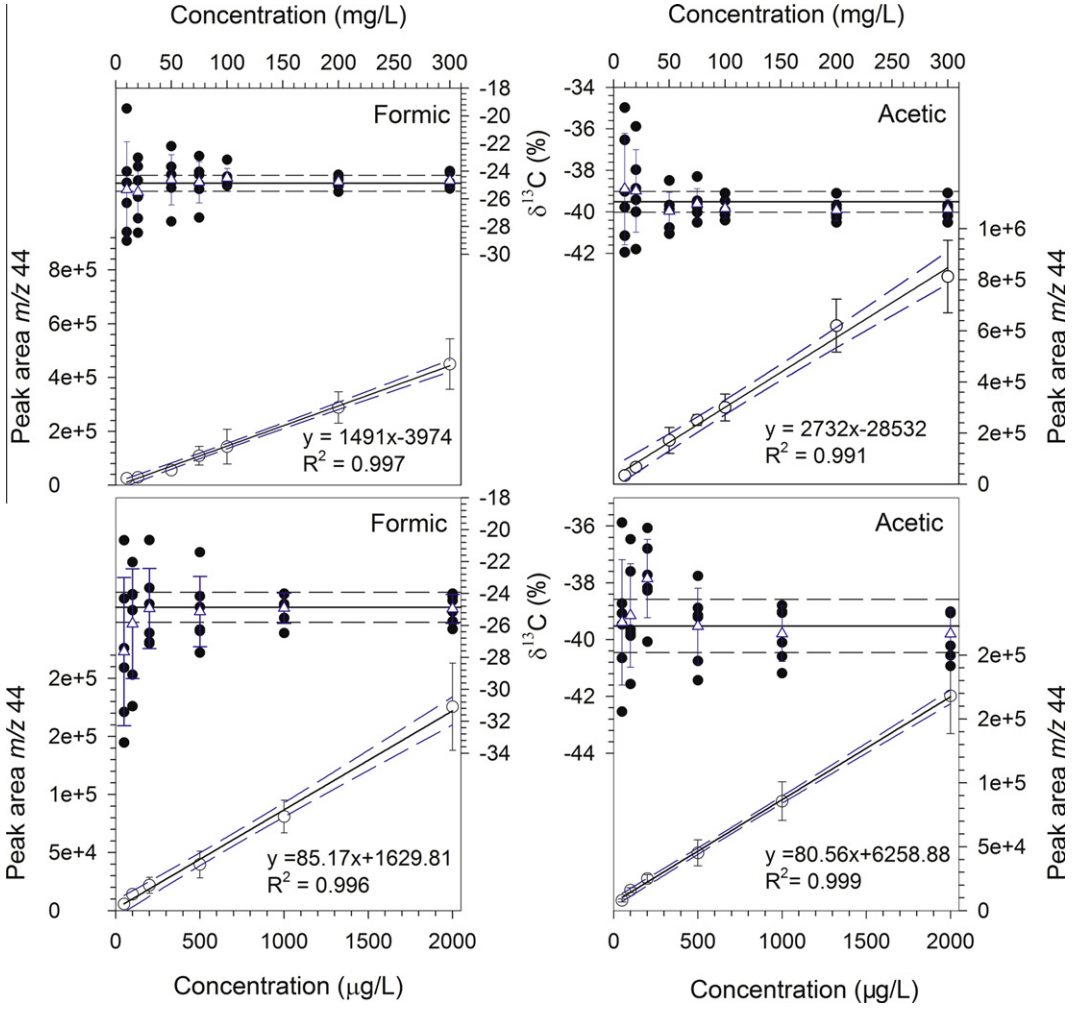

Fig.6. $\delta^{13}C$ and signal size of GC–IRMS for formic and acetic acids at both mg/L and μg/L levels. For the isotopic composition, the filled circles indicate $\delta^{13}C$ of each replicate analysis, and each triangle indicates the average $\delta^{13}C$ of all six replicates at a specific concentration. The solid horizontal lines mark the given $\delta^{13}C$ of the individual acid. The dashed horizontal lines indicate the average SD of the given $\delta^{3}C$ plus the SD in the GC–IRMS analyses above 100 mg/L and 1000 μg/L, respectively. The accumulated errors are ±0.58‰ and ±0.50‰ for formic and acetic acids, respectively, at the mg/L level, whereas the accumulated error is ±0.94‰ for both acids at the μg/L concentration. For the signal size, the circles are the mean signal sizes of the six replicate analyses, the solid lines are the linear regression ($P < 0.05$), and the dashed lines bracket the 95% confidence band. All error bars are 2σ SD.

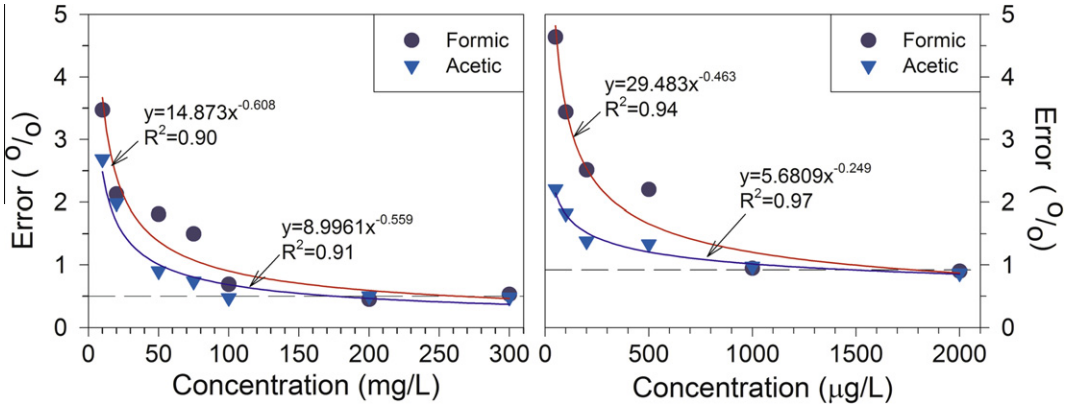

Fig.7. Change of the analytical uncertainty with concentration of the organic acids at both mg/L and sub-mg/L levels. The errors are 1σ SD of six replicate analyses. The dashed lines mark 0.49‰ and 0.92‰ for mg/L and sub-mg/L, respectively.

$\delta^{13}C$ values is located within the ±0.5‰ interval. Because the SD of replicate analyses is influenced by, among other factors, molecular carbon number of the compound as demonstrated by the difference between formic and acetic acids in this study, confining the precision to ±0.5‰ is not suitable for formic and acetic acids due to their extremely low carbon numbers, although ±0.5‰ is typical in GC–IRMS analysis for organic compounds with high carbon numbers [39]. For formic and acetic acids higher than a certain concentration, the SD of replicate analyses stabilized at approximately 0.5‰ and 0.9‰ in cases of direct extraction and preconcentration, respectively. With the concentration decreasing below the threshold, the analytical error increases exponentially (Fig. 7). Based on these facts, we propose the minimum concentration as the point at which the mean isotope composition of replicate analyses is statistically no different from the given value and the analytical error begins rising. According to the experimental results, these points are located at 200 and 100 mg/L for formic and acetic acids, respectively, in the direct extraction and at 1 mg/L for both acids after preconcentration. Compared with the SPME fiber extraction that was also coupled with GC–IRMS, which achieved the detection limit at 41.4 mg/L for acetic acid [21], the method reported in this study improved the sensitivity by more than 40 times. Direct injection and LC–IRMS analysis of formic and acetic acids that were mixed with methanol and ethanol in water solution reduced the detection limit to 0.4 mM/L of carbon, which corresponds to 18.4 and 12 mg/L for formic and acetic acids, respectively [40]. They are still 10 to 20 times higher than the limits achieved in this study.

Results of natural samples

The analytical results of the natural samples are listed in Table 4. The concentration of formic and acetic acids in the precipitation was at a sub-mg/L level. After being subjected to preconcentration, the uncertainty for formic acid in precipitation is better than that in the artificial samples because the high concentration of inorganic ions in the precipitation increased the effect of ionic strength. These results provided the proof of concept of the method in analyzing the precipitation samples at low concentrations. $\delta^{13}C$ of acetic acid is 1.32‰ more depleted in ^{13}C compared with that of formic acid, probably indicative of the different sources or atmospheric reactions in the troposphere.

The vinegars are extraordinarily high in acetic acid. After being diluted by 100 times, the concentration still ranges between 360 and 510 mg/L. These are the samples of the highest acetic acid in this study. As expected, the $\delta^{13}C$ was determined with exceptionally high precisions, ranging between 0.11‰ and 0.32‰. Compared with the best precision of ±0.4‰ by SPME coupled with GC–IRMS analysis [23], this study provided a more precise approach for verification of vinegar authenticity.

Ants are a known source of formic acid to ambient air. Formic acid in the body plasma of *C. japonicus*, however, is much less depleted in ^{13}C than in the precipitation ($P < 0.05$), confirming that ants do not contribute significantly to the budget of formic acid in the troposphere [41].

Vehicle emission is believed to be an important source of formic and acetic acids in the troposphere [41]. Despite being in a gaseous phase in exhaust, organic acids can be extracted in the same way as in aqueous solution because the NeedlEx works by trapping the analytes in the gas phase. The subsequent analysis of $\delta^{13}C$ should also be legitimate because the calibration of $^{13}C/^{12}C$ in the IRMS analysis is based on the CO_2 reference rather than that derived from the acids in the solution. In fact, gaseous samples make the purge operation redundant and, thus, simplifies the method. The SDs are 1.25‰ and 0.85‰ for formic and acetic acids, respectively. The absolute amounts of formic and acetic acids trapped in the NeedlEx are 0.356 and 0.589 μg, respectively, estimated by the concentration in the vehicle exhaust. If the same amount is trapped by the NeedlEx from the aqueous solution in the direct extraction, the concentrations of formic and acetic acids should be 58 and 66 mg/L, respectively, in the solution, as estimated by Henry's law [25]. Calculated by the fitting curves in Fig. 5, the SDs are 1.26‰ and 0.86‰ for formic and acetic acids, respectively, which are nearly the same as actually analyzed in the gaseous phase. This confirms that the method measures $\delta^{13}C$ of the organic acids in the air as well as it does in the aqueous solution.

The natural samples analyzed above have a variety of matrices for the organic acids and cover a wide range of concentrations, yet they were analyzed with compatible precisions as the artificial samples. These results proved the robustness of the method in analyzing natural samples from wide ranges.

Conclusions

Formic and acetic acids are usually at a few mg/L or sub-mg/L concentrations in the environment and in some biological materials. Lowering the method's detection limit is critical for analyzing isotopic composition of the acids in these samples. Defined as the point at which the mean $\delta^{13}C$ is statistically no different from the given value and the analytical error begins rising, the detection limits were achieved at 200 and 100 mg/L for formic and acetic acids, respectively, with both errors being approximately 0.5‰ in the direct extraction and analysis. Aided by preconcentration, the limit was lowered to 1 mg/L with an error of approximately 0.9‰

Table 4
$\delta^{13}C$ values of formic and acetic acids measured in natural samples.

Sample		Concentration[a]		$\delta^{13}C$ (‰) ± 1σ (n)	
		Formic	Acetic	Formic	Acetic
Rain	8 October 2008	1122	838	−29.1 ± 0.5 (6)	−30.4 ± 0.4 (6)
Vinegar	Guiyang brand	–	51	–	−27.8 ± 0.1 (3)
	Shanxi brand	–	39	–	−19.7 ± 0.3 (3)
	Fushan brand	–	36	–	−29.8 ± 0.3 (3)
Ant (*C. japonicus*) extract		–	–	−22.4 ± 0.4 (3)	–
Vehicle exhaust		356	589	−23.3 ± 1.3 (3)	−24.6 ± 0.9 (3)

[a] The units of concentration are μg/L, g/L, and μg/m^3 for the samples of precipitation, vinegar, and vehicle exhaust, respectively.

for both acids. Although the precision is higher than 0.5‰ due primarily to the extremely low carbon numbers of the organic acids, the method considerably expanded the range of samples eligible for isotope analysis. It is cheap and practicable in laboratories with access to GC–IRMS and also can be easily performed automatically in laboratories with commercialized purge-and-trap systems. The use of NeedlEx in purge-and-trap extraction avoided isotope fractionation while solving the problem of analyte introduction from aqueous sample to GC–IRMS; thus, it made important progress in the accurate measurement of the isotope composition of organic acids. Although the method focused on formic and acetic acids, which are the simplest organic compounds and, thus, the most difficult ones in isotopic measurement, it is also suitable for other volatile carboxylic acids of higher molecular weights. The method is designed for isotope measurement of formic and acetic acids in aqueous solution and is also applicable to the acids in the gaseous phase.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ab.2013.01.029.

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