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Method for Characterization of Low Molecular Weight Organic Acids in Atmospheric Aerosols Using Ion Chromatography Mass Spectrometry

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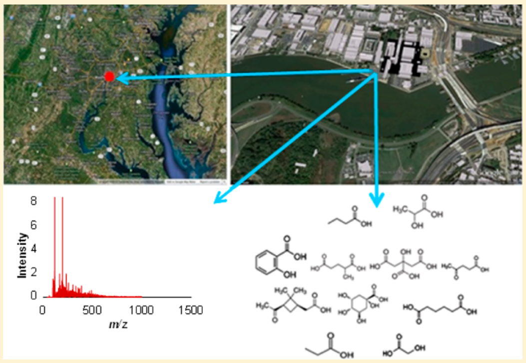
ABSTRACT: The structural composition of PM2.5 monitored in the atmosphere is usually divided by the analysis of organic carbon, black (also called elemental) carbon, and inorganic salts. The characterization of the chemical composition of aerosols represents a significant challenge to analysts, and studies are frequently limited to determination of aerosol bulk properties. To better understand the potential health effects and combined interactions of components in aerosols, a variety of measurement techniques for individual analytes in PM2.5 need to be implemented. The method developed here for the measurement of organic acids achieves class separation of aliphatic monoacids, aliphatic diacids, aromatic acids, and polyacids. The selective ion monitoring capability of a triple quadropole mass analyzer was frequently capable of overcoming instances of incomplete separations. Standard Reference Material (SRM) 1649b Urban Dust was characterized; 34 organic acids were qualitatively identified, and 6 organic acids were quantified.



Aerosols have been linked to lung cancer and cardiopulmonary morbidity and mortality.[1−3] Aerosols contribute to limiting visual range and to the Earth's radiative balance, thus affecting climate and the hydrological cycle.[4] Aerosols are identified in the U.S. Federal Clean Air act as criteria pollutant particulate matter (PM). Although the Clean Air Act designates an annual PM mass loading limit of 12 $\mu g/m^3$, substantial uncertainties remain concerning aerosol composition, mechanisms of formation, and the ability to model their fate and transport.

The composition of airborne particulate matter varies widely with respect to source, climate, and local meteorology. Routine, in situ monitoring of PM typically includes measurements of organic carbon (OC), black carbon (also called elemental carbon (EC)), and inorganic salts. Analytical techniques such as Fourier transform infrared spectroscopy (FTIR), liquid chromatography with ultraviolet detection (LCUV), or nuclear magnetic resonance (LCNMR), and aerosol mass spectrometry (AMS) measurements have independently indicated at different locations around the globe that water-soluble organic carbon (WSOC) is often largely dominated by the presence of carboxylic acids.[5−8] Instrument requirements plus sample preparation and processing pose significant challenges to both field and laboratory methods for molecular determination. Compositional analysis frequently ends with bulk property characterization.

To study the structural composition of organic aerosol, analysis is often divided into molecular classes based on polarity. Nonpolar components are often introduced by direct emission, but they can also derive from the partitioning of volatile organic compounds (VOCs) into the particle phase consisting of water-soluble and insoluble fractions.[9] Gas chromatography mass spectrometry (GCMS) methods have demonstrated reliable structural identification of water insoluble aromatic compounds and soluble hydroxylated and alkoxylated aliphatic compounds.[10−12] Water-soluble, polar organic carbon is composed primarily of secondary organic aerosol originating from biogenic and anthropogenic VOCs and is known to play a role as cloud condensation nuclei.[13,14] GC/MS has also been used for the molecular determination of organic acids, but requires derivitization.[15,16] To obviate the need for multiple preparative steps, small polar molecule analysis has largely shifted to LC electrospray ionization mass spectrometry (ESI-MS).[17−19] ESI has the advantage of being compatible with polar mobile phases, and soft ionization leaves a largely intact molecular ion as the dominant ionization product.

Numerous LC and LC/MS methods have been used for aerosol characterization in both smog chamber and field experiments. Field samples represent the end state of aerosol atmospheric processing, whereas smog chamber experiments are useful for following specific reaction pathways. Ion chromatographic (IC) methods are most often limited to measurements of inorganic ions and the simplest diacid, oxalic acid, but have also been used to suggest the presence of mono- and dicarboxylates in Brazil, Taiwan, and Germany.[20−22]

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Table 1. Precursor and Product Ion Transitions Monitored for the Organic Ions

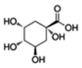
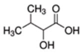
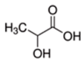
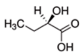
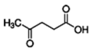
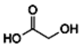
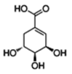
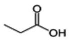
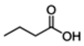
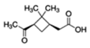
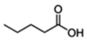
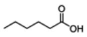
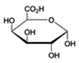
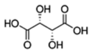
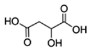
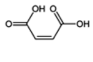
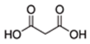
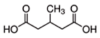
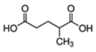
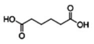
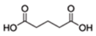
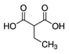
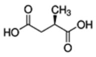
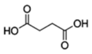
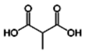
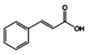
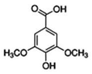
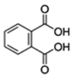
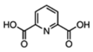
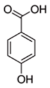
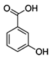
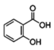
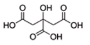
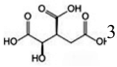
Compound	Structure	RT	Transition	Mass loss	Fragment loss	SRM comparison to standard
quinic $H_{12}C_7O_6$		3.6	191→85	106	H_2O, 2xCOO	Quantified
			191→59	132	---	Reference peak
2-OH-3-CH$_3$-butyric $H_{10}C_5O_3$		4.2	117→71	46	H_2O, CO	RT shifted left, poor peak shape, tailing
			117→45	72	COO, CO	No peak
lactic $H_6C_3O_3$ (Marine,rain forest, urban mix, Domingos et al., 2012)		4.7	89→43	46	H_2O, CO	Baseline resolved but rounded rather than pointed peak indicating interference.
			89→89	NA	NA	Low intensity
2-OH-butyric $H_8C_4O_3$		4.7	103→45		---	Baseline resolved, poor s/n, RT shifted left
			103→57		H_2O, CO	Dual unresolved peak, could be used for reference
levulinic $H_8C_5O_3$		5.0	115→97	18	H_2O	Quantifiable
			115→71	44	COO	Quantifiable
glycolic $H_4C_2O_3$ (Greenland, Jaffrezo et al., 1998)		5.2	75→47	28	CO	reference
			75→45	30	HCOH	Quantified
shikimic $H_{10}C_7O_5$		5.4	173→137	36	2xH_2O	Baseline resolved. Intensity of SRM transitions different from standards
			173→111	62	H_2O, COO	tailing
			173→93	80	2H_2O, COO	No peak
			173→73	100	---	Poor peak shape, RT shifted left
propanoic $H_6C_3O_2$ (motor exhaust, Kawamura, 1985)		5.5	73→73	NA	NA	Baseline resolved,tailing, poor peak shape due to matrix
butanoic $H_8C_4O_2$		7.8	87→87	NA	NA	Tailing. Needs both IC and MS method optimization
cis-pinonic $H_{16}C_{10}O_3$ (Eucalyptus globulus forest, kavouras, 1998)		8.2	183→57	126	---	no sensitivity
			183→139	44	COO	Poor peak shape, needs MS method optimization
pentanoic $H_{10}C_5O_2$		9.8	101→101	NA	NA	Tailing. Needs IC and MS method optimization
hexanoic $H_{12}C_6O_2$		8.8	115→115	NA	NA	Tailing. Needs IC and MS method optimization
galacturonic $H_{10}C_6O_7$		10.9	193→59	134	---	Ref peak, not baseline resolved
			193→73	120	---	Quantified.
tartaric $H_6C_4O_6$ (Amazon, Claeys et al, 2004)		18.6	149→87	62	H_2O, COO	Reference peak, baseline resolved, SRM RT shifted left
			149→73	76	---	Quantified. Best sensitivity.
malic $H_6C_4O_5$ (Amazon, Claeys et al, 2004)		18.7	133→115	18	H_2O	Quantified. SRM RT shifted left
			133→71	62	H_2O, COO	Reference peak.
maleic $H_4C_4O_4$ (Urban,Taiwan, Tsai et al., 2008)		18.7	115→115	NA	NA	Quantifiable, reference peak
			115→71	44	COO	Quantifiable, baseline resolved, SRM RT shifted left from standard.
malonic $H_4C_3O_4$ (Urban, Taiwan, Tsai et al., 2008)		18.7	103→103	NA	NA	Quantifiable. Gaussian peak shape. SRM RT shifted left from standard.
			103→59	44	COO	Reference peak.

Table 1. continued

Compound	Structure	RT	Transition	Mass loss	Fragment loss	SRM comparison to standard
3-CH_3-glutaric $H_{10}C_6O_4$		20.1	145→101	44	COO	Three isomers coelute as a doublet. 3 methylglutaric precedes 2-methylglutaric and adipic acids. 2-methylglutaric and 3-methylglutaric have better method sensitivity than adipic acid. SRM RT shifted left from standard
2-CH_3-glutaric $H_{10}C_6O_4$		20.3				
adipic $H_{10}C_6O_4$ (Smog chamber, Hamilton et al 2006)		20.3	145→83	62	H_2O, COO	
glutaric $H_8C_5O_4$ (Smog chamber, Hamilton et al 2006)		20.3				Three isomers coelute as a doublet peak, glutaric and methylsuccinic acids are followed by partially resolved ethylmalonic acid. SRM RT shifted left from standard.
C_2H_5-malonic $H_8C_5O_4$		20.3	131→113 131→87	18 44	H_2O COO	
CH_3-succinic $H_8C_5O_4$		20.3				
succinic $H_6C_4O_4$ (Urban, Taiwan, Tsai et al., 2008)		20.8	117→73	44	COO	Coelute as a single peak. SRM RT shifted left from standard.
CH_3-malonic $H_6C_4O_4$		20.8	117→55	62	H_2O, COO	
cinnamic $H_9C_8O_2$ (Controlled burning, Hoffman et al., 2007)		26.1	147→77 147→103	70 44	$H_2C_3O_2$ COO	No baseline resolution due to peak fronting Signal not > s/n
syringic $H_{10}C_9O_5$		26.9	197→121 197→123	76 74	COO, OCH_3,H COO, OCH_2	Baseline resolved. Transitions observed in standards match those observed in SRM. Baseline resolved. This transition is shifted right from the same transition observed in syringic acid standard.
phthalic $H_6C_8O_4$ (Urban, Italy, Balducci et al., 2010)		28.3	165→165 165→121	na 44	na COO	Quantifiable
dipicolinic $H_5C_7O_4N$ (Aerosol chamber, Russell et al., 2004)		28.2	166→78 166→122	88 44	2xCOO COO	Peak tailing, RT shifted left Peak tailing, RT shifted left
4-OH-benzoic $H_6C_7O_3$		29.3	137→93	44	COO	These three compounds are almost baseline resolvable. There is an unidentified fourth peak that matches these transitions.
3-OH-benzoic $H_6C_7O_3$		34.0	137→41	96	---	
2-OH-benzoic $H_6C_7O_3$		37.2				
Citric $H_8C_6O_7$ (Mountain and urban, Chang et al., 2003, Po Valley Decesari et al., 2001)		35.0	191→85 191→111	106 80	2xCOO, H_2O COO, $2xH_2O$	Quantified Reference transition
Isocitric $H_8C_6O_7$		35.8	191→173	18	H_2O	Transition is specific to isocitric, but poor sensitivity in SRM matrix

Confirmation of organic acids by IC with conductivity detection (CD) can be only tentative because of the inability to determine coeluting compounds. A slightly different approach performed by Decesari et al.[23] used anion exchange chromatography to separate samples into classes of neutral compounds, combined mono- and dicarboxylates, and polycarboxylates. Structural determination was performed by NMR.[23] Procedures to elucidate the molecular composition of ambient aerosol have accounted for only 5−30% of the total aerosol mass, indicating that this effort still requires much method development.[24,25]

This manuscript reports the development of a robust method for the class separation and analysis of multiple organic acids by coupling IC with tandem mass spectrometry detection (MS/MS). Few applications of IC/MS have been reported. The utility of employing IC/MS and IC/MS/MS was demonstrated by Conboy et al.[26] for the determination of organic ions relevant to pharmaceuticals and industry. They observed an order of magnitude improvement in sensitivity over CD in the detection of quaternary and tetra alkyl ammonium and sulfonated compounds. More recently, Amorisco et al.[27] used IC/TOF/MS/MS to identify 18 unknown organic acid byproducts formed during the ozonation of wastewater. Fisseha et al.[28] reported improved signal-to-noise (S/N) ratios and the resolution of coeluting acids by IC/MS for the qualitative evaluation of OA produced in a smog chamber. Single quadropole mass analysis extracting parent ions from the total ion chromatogram (TIC) has since been used to qualitatively identify urban OA and OA products of wood burning and isoprene photoxidation[29−31] The current study reports the use of coupled IC/ESI-MS/MS for the characterization of organic acids in atmospheric aerosol. The additional features of CID and MRM offered by MS/MS instruments provide detection limits lower than those observed in total ion chromatograms and structural determination of unknowns based on fragmentation patterns. This method is used to qualitatively and quantitatively evaluate organic acids in the National Institute of Standards and Technology (NIST, formerly National Bureau of Standards) air particulate Standard Reference Material (SRM) 1649b, Urban Dust.

■ EXPERIMENTAL SECTION

Chemicals. Standard reference materials (SRM) Nitrate Anion Standard solution SRM 3185, Phosphate Anion Standard solution SRM 3186, and Chloride Anion Standard solution SRM 3182 were used in the determination of inorganic ions (Office Reference Materials, NIST, Gaithersburg, MD). Organic Acids Calibration Standard, SRM 3286, was used to quantify citric, malic, quinic, shikimic, and tartaric acids (Office Reference Materials, NIST, Gaithersburg, MD). Additional standards used for quantitation of galacturonic, glycolic, isocitric, oxalic, and sodium sulfate were purchased from Sigma-Aldrich (St. Louis, MO). The purity of these commercially obtained compounds was verified by LC/UV (210 nm) to exceed 99% (mass fraction) except for isocitric acid, whose purity was 93.5%. All other reagents used for qualitative analysis were purchased from Sigma-Aldrich, (Table 1). Deionized water, 18 MΩ cm^{-1}, delivered by a Milli-Q Advantage A10 ultrapure water purification system (MA, USA), was used for IC mobile phase and dilutions.

Sample Collection and Extraction. Details on the collection and handling of the first issue of SRM 1649 have been reported (NBSIR-82-2595, 1982). Briefly, the material was collected at the Navy Yard in Washington D.C. in specially designed bag houses from 1977 to 1978. SRM 1649b is a fraction of the SRM 1649 material sieved to a smaller particle size (63 μm sieve), repackaged, and reissued in 1999[32]

Masses of ∼100 mg of SRM 1649b were placed into 50 mL centrifuge tubes and recorded. Triplicate samples of SRM 1649b were analyzed. Tubes were diluted with deionized water and shaken for several hours. Prior to concentrating the extract to ∼7 mL under nitrogen, the SRM 1649b samples were filtered using a 0.2 μm Luerlock syringe (National Scientific, Rockwood, TN) and filter (Chrom Tech Inc., Apple Valley, MN). Gravimetric quantities of nitrite and fumaric acid were added as internal standards after extraction and concentration. Two milliliter aliquots of the water-soluble extract were transferred to HPLC vials for analysis.

Standard Solutions. Stock solutions for qualitative analysis were prepared gravimetrically by adding known quantities of five inorganic salts and 34 organic acids (Table 1). The mass fractions of the 34 acids were approximately 3 μg/g and quantities of the inorganic salts were 22 μg/g PO_4^{3-}, 30 μg/g Cl^-, 30 μg/g NO_3^-, and 113 μg/g SO_4^{2-}.

Calibrants for quantitative analysis were made by gravimetric dilution of the stock solutions containing the SRM acids citric, malic, quinic, shikimic, and tartaric, plus galacturonic, isocitric, glycolic, and oxalic acids. Five salts were added to the calibration solution: PO_4^{3-}, Cl^-, NO_2^-, NO_3^-, and SO_4^{2-}. The calibration solutions were targeted to bracket the SRM concentrations.

Analysis. Separations were performed on a dual system, Reagent-FreeTM ICS 3000 (Dionex at ThermoFisher, Sunnyvale, CA). The Dionex dual ICS 3000 contains two complete chromatographic systems enclosed in one instrument convenient for simultaneous anionic and cationic analysis. The system is equipped with two built in degasser pumps and two eluent generators for running ionic gradients. Continuously regenerating ion trap columns are installed between the pumps and their respective columns, removing all ionic contaminants in the eluent. After injection, compounds are separated on an ion-exchange column. Before conductivity detection (CD), the total eluent conductivity is suppressed by electrolytically removing the excess hydroxide ions across a membrane in the self-regenerating suppressor (SRS). Typically, these systems are plumbed in recycle mode, directing the CD waste effluent back to the SRS as the source of hydration and counter flow for electrolytic exchange across the membrane.

Injections were made using a 10 μL injection loop onto an IonPac AS 17-C hydroxide-selective anion-exchange column, 250 × 2 mm (Dionex, Sunnyvale, CA). The analytical column was preceded by an IonPac AG17-C guard column, 50 × 2 mm. After SRS suppression, the CD effluent was directed to the ESI chamber for tandem MS/MS analysis. Plumbed in external water mode, the SRS electrolytic counter flow was provided by a second, integral pump, at a flow of 3.33×10^{-8} m^3 s^{-1} (2 mL/min). At a flow of 0.25 mL/min, the gradient program was, in units of min/mmol/L KOH: 0/0.6, 6/0.6, 11/5.0, 18/10.0, 28/20.0, 32/32.0, 38/60.0, and 43/80.0. After each sample, 80 mmol/L KOH was run for 10 min as a post column wash and regeneration that was followed with a 7 min re-equilibration period at 0.6 mmol/L KOH.

Mass spectrometric detection was performed by interfacing the Dionex IC with an Agilent 6410 mass spectrometer (Santa Clara, CA) equipped with an ESI source and a triple quadrupole mass analyzer. Source settings were capillary voltage, 3500 V; desolvation gas temperature, 350 °C; gas flow, 1.83×10^{-4} m^3 s^{-1} (11 L/min); and nebulizer gas pressure, 2.41 × 105 Pa (35 psig). Compound identification was determined through multiple reaction monitoring (MRM) in the negative ionization mode. The MS/MS conditions for each reference standard were determined individually by selection of an appropriate precursor ion, followed by collision-induced dissociation to determine the most abundant product ions. The two most intense precursor-to-product ion transitions were selected for peak assignment. The MRM method was set so that all precursor-to-product ion transitions were scanned continuously throughout the run (Table 1).

■ RESULTS AND DISCUSSION

Optimization of Separation Conditions. The use of IC instrumentation to produce system-generated pH gradients permits programmed eluent compositions for adjusting ionic concentrations in an aqueous mobile phase. Because the competing hydroxide ions are removed from the gradient between the column and the detector by the eluent suppressor, the effluent can be introduced directly into the MS. The use of methanol, acetonitrile, and formic acid as mobile phase modifiers was evaluated by their addition to either the IC reservoir or to the effluent with splitting before introduction into the ionization chamber. Because MS signal enhancement was not observed, modifiers were not used in the final method.

Figure 1A,B compares separations of a 25 compound solution achieved with IonPac AS11-HC and IonPac AS17-C columns. The mixture contained 9 monoacids, 9 diacids, 1 poly acid and 6 inorganic salts. The IonPac AS11-HC is designated a high capacity column intended for applications requiring resolution of inorganic and organic ions (IonPac AS 11-HC manual). Separations with this column have been used for aerosols collected onto filters or particle into liquid samplers (PILS) for both ground and aircraft sampling.[33,22,8,34] The IonPac AS 17-C column is designated a low-capacity column with applications for inorganic ion separations (ref IonPac AS17-C manual). Gradient conditions are indicated on the figures. Figure 1A,B demonstrates the ability to baseline-resolve inorganic ion nitrate from organic acids on the AS-17 column using mobile phase conditions suitable for organic acid class separation. Figure 1C shows a separation of the same 25 compound solution on the IonPac AS 17-C column but differs from Figure 1B by using a mobile phase program similar to that reported by Domingos et al.[20] The use of a gradient program with a higher initial KOH concentration results in better baseline separation between the aliphatic mono- and diacids, and a gradual increase in KOH concentration across the dicarboxylate region, as shown in Figure 1A,B, results in better separation of the individual diacid ions. Stronger mobile phase conditions are used after the elution of dicarboxylates to elute aromatic and trivalent ions.

The trends noted above were considered in the development of the elution conditions described in the methods section. Figure 2A shows the organic acid class separation achieved by this gradient. Organic acid standards range from 8 to15 μg/g. Aromatic acids with poor CD response, cinnamic, syringic and ferulic, are not shown in Figure 2A, but are observed to have retention similar to phthalic acid by MS. In this series, the only instance of incomplete ion class separation is the coelution of polyacids citrate and isocitrate with aromatic acids 3-hydroxybenzoic acid and ferulic acid. Figure 2B shows the separation of a standard solution containing 34 organic acids and 6 inorganic anions. The proportion of small organic acid concentrations to large inorganic anion concentrations in the standard solution captures the expectation that organic acids in ambient aerosol exist at significantly smaller concentrations than the inorganic anions. As analyte concentrations approach naturally expected abundances, the peak shape becomes more Gaussian during application of this method on a 2 mm column. The disparate conductometric response observed complicates the simultaneous analysis of organic acids and inorganic salts by CD because signal response to organic ions is frequently overwhelmed by the presence of inorganic ions. This problem is particularly significant in sampling environments heavily loaded with sulfate, as observed by Tsai et al.[21] in Taiwan.

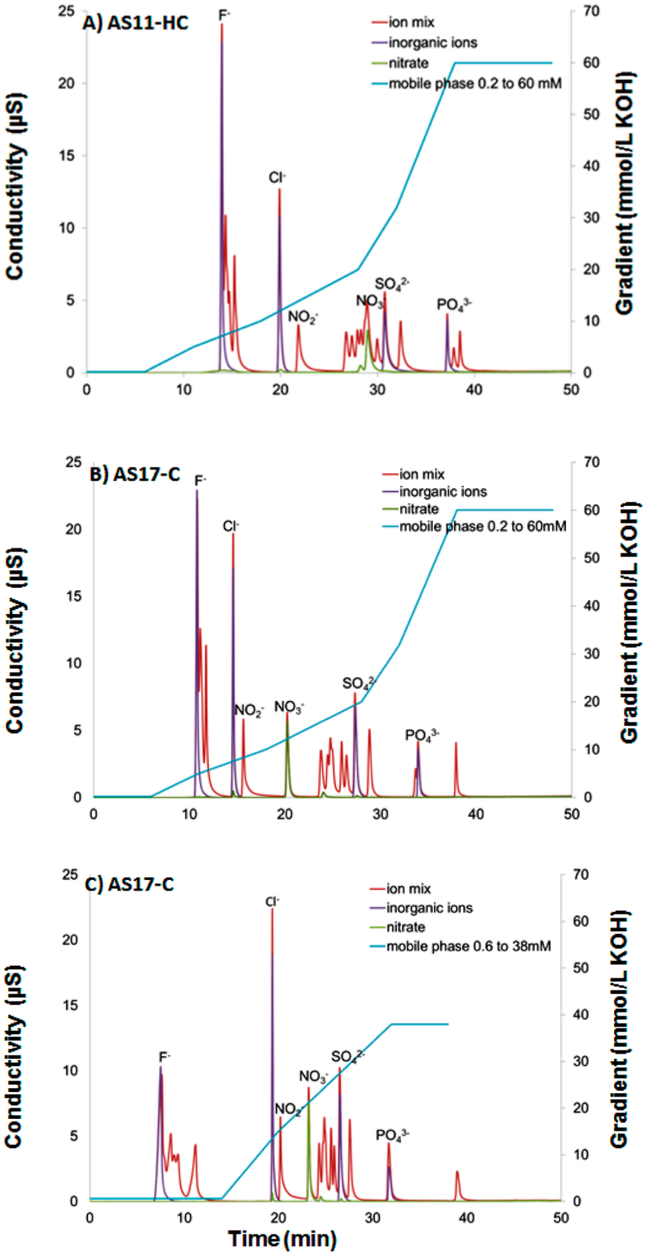


Figure 1. Chromatographic separations of 25 compounds, 9 monoacids, 9 diacids, 1 polyacids, and 5 inorganic salts on two columns and two different mobile phases. (A) Separation with IonPac AS11-HC column and 0.2−60 mmol/L KOH gradient. (B) Separation with IonPac AS17-C column and 0.2−60 mmol/L KOH gradient. (C) Separation with IonPac AS17-C column and 0.6−38 mmol/L KOH gradient.

Mass Spectral Identification of Compounds. With ESI the most common precursor ion prior to collision induced dissociation (CID) is either $[M + H]^+$ or $[M - H]^-$ resulting

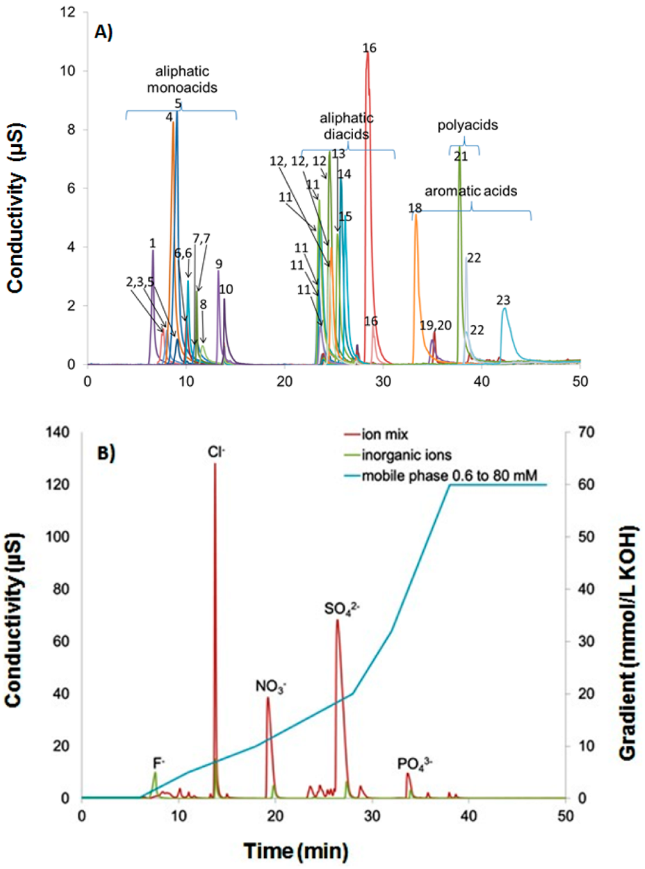


Figure 2. Retention of organic and inorganic species. (A) Separation of organic acid classes. Organic acids are identified as follows; Monoacids: (1) quinic, (2) lactic, (3) levulinic, (4) glycolic, (5) 2-hydroxybutyric, (5) propanoic, (6) butanoic, (6) dipicolinic, (6) 2-hydroxy-3-methylbutyric, (7) cis-pinonic, (7) pyruvic, (8) valeric, (9) galacturonic, (10) hexanoic. Diacids: (11) 3-methylglutaric, (11) methylsuccinic, (11) adipic, (11) 2-methylglutaric, (11) glutaric, (12) malic, (12) methylmalonic, (12) ethylmalonic, (13) malonic, (14) tartaric, (15) maleic, (16) oxalic, (16) fumaric. Aromatic acids: (18) phthalic, (19) 4-hydroxybenzoic, (20) 3,4-dihydroxybenzoic, (22) 3-hydroxybenzoic, (23) 2-hydroxybenzoic. Polyacids: (21) citric, (22) isocitric. (B) Separation of inorganic ions.

from addition or loss of a proton. Of the 51 reference standards initially studied, most exhibited $[M - H]^-$ precursor ions. Sulfate (SO_4^{2-}) and phosphate (PO_4^{3-}) formed dimerized precursors $[2M - H]^-$ in the ionization chamber and underwent transition to their molecular ions (195 → 97) during CID. PO_4^{3-} underwent an additional 195 → 177 transition corresponding to a loss of water, but SO_4^{2-} did not. The ability to distinguish PO_4^{3-} by this additional transition is important for samples that contain high levels of SO_4^{2-}, because loss of chromatographic baseline resolution may result. Nitrite (NO_2–) (MW 46) and nitrate (NO_3–) (MW 62) are chromatographically resolved, but both compounds are identified by m/z 62, suggesting possible oxidation of NO_2^- to NO_3^-. The mass spectral detection of NO_2^- at the same m/z as the NO_3^- precursor ion indicates that NO_2^- to NO_3^- oxidation occurs after leaving the column and is not a product of mobile phase interaction. Under these conditions, fluoride and chloride did not produce an MS signal and are not reported here.

During the method development of organic acids, a few instances were observed in which ESI simultaneously produced deprotonated dimers and monomers. Adjustment of the cavity accelerator and fragmentation voltages influenced the abundance of the $[M - H]^-$ versus $[2M - H]^-$ ions in standard solutions with concentrations of a few micrograms/milliliter. Dimerized product ions periodically observed in higher concentration standard solutions (low micrograms/gram) were not observed in solutions with concentrations relevant to ambient (nanograms/gram and picograms/gram) nor in the analysis of SRM 1649b. This indicates that, unlike SO_4^{2-} and PO_4^{3-}, the organic compounds are susceptible to concentration-dependent noncovalent dimerization.[35] Pan et al.[36] observed that noncovalent dimerization can be both instrument- and mode-dependent. Spray chamber conditions were always optimized to favor production of the monomer target for MRM. Concentration-dependent dimerization in the ESI chamber could become relevant to smog chamber experiments if initial reactant concentrations lead to reaction products whose concentrations significantly exceed ambient atmospheric concentrations.

A list of acids selected for qualitative and quantitative evaluation and relevant information is provided in Table 1. The CID trends observed in this study can be summarized as the following:

(1) Nonbranched, straight chain aliphatic monoacids do not fragment under the specified ionization conditions. These species were monitored by targeting the precursor ion and maintaining the collision energy at zero.
(2) Branched aliphatic monoacids and branched and straight chain aliphatic diacids typically produced product ions corresponding to losses of COO (m/z 44), H_2O (m/z 18), and CO (m/z 28) or combinations of losses CO and H_2O (m/z 46) and COO and H_2O (m/z 62).
(3) Cyclic compounds always produced at least two transitions, resulting from loss of COO and loss of a second, unspecified fragment. Frequently, aromatic rings remained intact.

An understanding of fragmentation patterns is useful for obtaining structural information on unknown compounds. In this work, the above trends were used in conjunction with acid class retention for structural determination of WSOC in SRM 1649b.

SRM 1649b. The atmospheric PM constituting SRM 1649b was collected over a 12 month time frame at the Navy Yard in Washington D.C. The composition of this time-integrated sample typifies the analytical challenges associated with the chemical analysis of atmospheric particulate matter.[37] As reported for water-soluble extract collected around the globe, a full spectrum scan (m/z 40–1000) of SRM 1649b extract indicates that the water-soluble contents are composed primarily of low-molecular-weight ions with the intensity of abundances tapering off around m/z 400.[5,7,33]

Qualitative Analysis. A multistep approach was applied for the qualitative identification of organic acids in SRM 1649b WSOC extract. SRM 1649b was screened for alkanoic acids previously reported to have been observed in atmospheric particulate matter (see Table 1). Ions that did not match the initial list of standards were tentatively identified on the basis of precursor ion m/z and retention data. Verification was made through comparison to reference standards. For example, in Figure 3A, m/z 191 was tentatively identified as citric acid. The presence of such polyacids in aerosol was reported by Decesari et al.[6] and Chang et al.[23] As shown in Table 1, MS/MS of citric

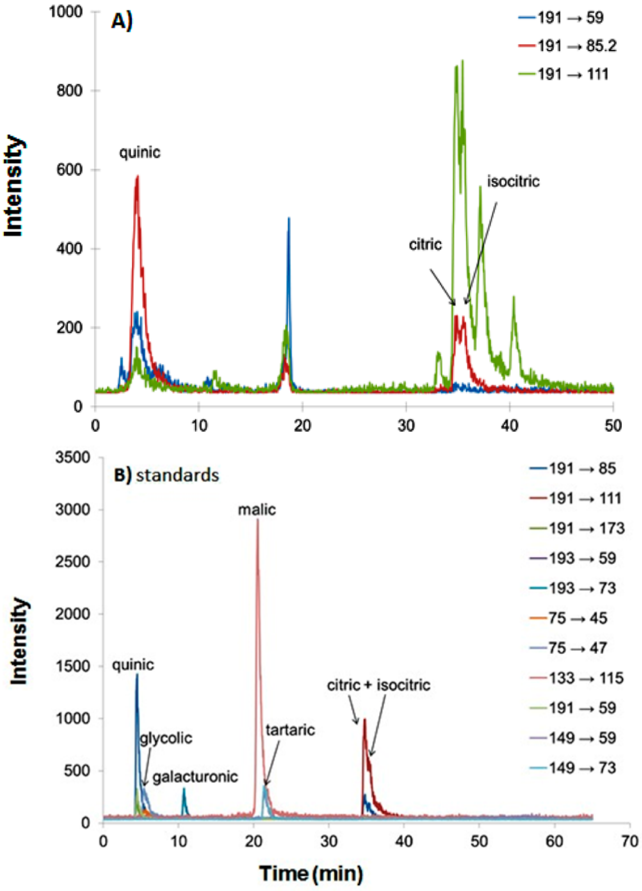


Figure 3. Separation of an extract of (A) SRM 1649b and (B) organic acid reference standards by IC/MS/MS.

acid produces product ions corresponding to losses of COO and H_2O. The doublet peak shape with the same m/z precursor and product ions suggested that an isomer of citric acid was also present, possibly isocitric acid. An isocitric acid standard was obtained and confirmed to be present in SRM 1649b. Another constituent with precursor ion m/z 191 was observed in the monoacid region of the separation. The molecular weight was inconsistent with water-soluble nonbranched alkanoic acids and tentatively attributed to either a linear branched or cycloalkanoic acid. The presence of quinic acid (a cycloalkanoic acid, MW of 192, present in the leaves of many plants) was confirmed on the basis of reference standards.

This process was used to confirm the presence of levulinic, shikimik, 2-hydroxybutyric, 2-hydroxy-3-methylbutyric, and galacturonic monoacids. The presence of alkanoic monoacids butanoic, pentanoic, and hexanoic acids was also evaluated because shorter straight-chained monoacids have been reported.[38] The presence of these monoacids is tentatively identified in the SRM but incompletely resolved by this method. In the aromatic region, this process was used to identify syringic plus the three hydroxyacid isomers. Isomeric dicarboxylates were, at best, partially resolved by IC and not resolved by CID. In these cases, only the total area under the respective m/z can be estimated. These initial screening methods should be followed up with more precise quantification methods. Decomposition or hydrolysis of condensed phase species in water extracts may affect the organic anion composition, and the stability of these complex samples warrant additional study.

Quantitative Results. Six water-soluble species were quantified by MS/MS (Figure 3 and are listed in Table 2) using SRM-based calibrants. Requisites for quantification are (1) baseline resolution and (2) CID transition intensity ratio match with reference standards. Quantitative evaluation was limited to acids referenced against an SRM or a purity verified standard.

Shifts in solute retention were observed for extracts of SRM 1649b compared with calibration solutions. These retention shifts were caused by high concentrations of sulfate and nitrate in SRM 1649b; shifts of several minutes were observed in the diacid region. The source of the retention shift was verified by comparing organic acid stock solutions, spiked with different levels of organic species and inorganic species, with WSOC SRM 1649b extract. As ratios of the inorganic to organic species in the stock solution approached ratios representative of SRM 1649b, the diacid retention times of the standard and the SRM 1649b samples approached one another. Shifts in retention did not significantly affect quantitation of the organic acids.

Choosing appropriate internal standards with complex matrix samples is challenging. Several internal standards representative of the constituents in each molecular class are needed. In this work, two species that achieved baseline resolution in standard solutions (nitrite and fumaric acid) and whose presence was not observed in 1649b did not result in baseline resolution when added to SRM 1649b. Quantitation was based on an external calibration approach using SRM-based calibrants.

The acid mass fraction in SRM 1649b ranges from 0.143 to 20.28 μg/g (see Table 2). R^2 values for all calibration curves were >0.99. Single factor analysis of variance was run to evaluate the intraday precision and the interday precision. The intraday precision represents variation of the mean as a result of the measurement method and interday precision represents variation of the mean resulting from the extraction procedure. A dash for the interday precision indicates that the variation

Table 2. Measurements of 6 m/z's in SRM 1649b

acid	acid av (μg/g)	σ	95% confidence interval	LOD	LOQ	intraday precision	interday precision	calibration range (μg/g)
quinic 191 → 85	4.163	0.012	0.032	0.0002	0.0005	0.213	–	12–485
glycolic 75 → 47	20.28	0.26	0.709	0.023	0.078	1.365	0.839	19–795
galacturonic 193 → 73	3.823	0.007	0.019	0.0006	0.0021	0.239	–	2–97
tartaric 149 → 73	0.143	0.063	0.175	0.003	0.009	0.035	0.228	12–507
malic 133 → 115	3.641	0.086	0.238	0.003	0.009	0.155	0.306	12–496
citric + isocitric 191 → 111	7.219	0.031	0.085	0.002	0.007	0.544	–	17–695

The average is calculated as the averaged acid value of each extract measured over 5 days. The uncertainty of the mean is the standard deviation of the averaged values. LOD is 3 times the blank signal divided by the slope of the calibration curve (LOD = 3 × Sbl/m). LOQ = 10 × Sbl/m.

measured between extractions was greater than day to day method variation. Limits of detection and limits of quantitation, calculated as 3× and 10× the standard deviation of the blank and divided by the slope of the calibration curve, were in the high picograms/gram to low nanograms/gram range by this method. Individual compound signal-to-noise ratios could be improved by running single ion monitoring instead of simultaneously scanning all MRMs throughout the entire run.

■ CONCLUSIONS

This study reports the application of directly coupled IC/MS/MS to the molecular characterization of organic acids in atmospheric PM. The combination of ion chromatography and mass spectrometry significantly increases the number of ions for which a single IC procedure can be optimized due to the increased selectivity of the approach.

The application of tandem IC/MS/MS resulted in the identification of nine additional compounds present in SRM 1649b that were not initially selected for screening against known standards. Both the IonPac AS 17-C low-capacity column and the IonPac AS11 HC columns achieve organic acid class separation. Baseline resolution between nitrate and organic diacids was achieved with an IonPac AS 17 column. Baseline resolution of individual acids with their acid class was achieved through selective detection provided by MRM.

The molecular speciation of aerosol composition is important for understanding mechanistic pathways and ultimately for apportioning aerosol sources. Atmospheric aerosols are complex matrixes with many sources that lead to multiple products. Only through a combination of methods can the link between sources and products be fully discovered and properly integrated into models. Further development of IC/MS/MS based methods is planned for application to the quantification of WSOC components in particulate matter.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available.

The authors declare no competing financial interest.

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