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Sequential Derivatization of Polar Organic Compounds in Cloud Water Using O-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine Hydrochloride, N, O-Bis(trimethylsilyl)trifluoroacetamide, and Gas-Chromatography/Mass Spectrometry Analysis

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Sequential Derivatization of Polar Organic Compounds in Cloud Water Using *O*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine Hydrochloride, *N, O*-Bis(trimethylsilyl)trifluoroacetamide, and Gas-Chromatography/Mass Spectrometry Analysis

Jessica A. Sagona[a], James E. Dukett[b], Harmonie A. Hawley[c,d], Monica A. Mazurek[c*]

[a]Department of Environmental Sciences, Rutgers University, 14 College Farm Rd., New Brunswick, NJ 08901

[b]Adirondack Lakes Survey Corporation, 1115 State Route 86, Ray Brook, NY 12977

[c]Department of Civil and Environmental Engineering, Rutgers University, 96 Frelinghuysen Rd., Piscataway, NJ 08854

[d]Department of Civil and Environmental Engineering, University of Texas-Tyler, 3900 University Blvd., Tyler, TX 75799

*Corresponding author. Tel.: +1-848-445-2871; fax: +1-732-445-0577; email: mmazurek@rci.rutgers.edu.

Abstract

Cloud water samples from Whiteface Mountain, NY were used to develop a combined sampling and gas chromatography-mass spectrometric (GCMS) protocol for evaluating the complex mixture of highly polar organic compounds (HPOC) present in this atmospheric medium. Specific HPOC of interest were mono- and di keto-acids which are thought to originate from photochemical reactions of volatile unsaturated hydrocarbons from biogenic and manmade emissions and be a major fraction of atmospheric carbon. To measure HPOC mixtures and the individual keto-acids in cloud water, samples first must be derivatized for clean elution and measurement, and second, have low overall background of the target species as validated by GCMS analysis of field and laboratory blanks. Here, we discuss a dual derivatization method with PFBHA and BSTFA which targets only organic compounds that contain functional groups reacting with both reagents. The method also reduced potential contamination by minimizing the amount of sample processing from the field through the GCMS analysis steps. Once derivatized only gas chromatographic separation and selected ion monitoring (SIM) are needed to identify and quantify the polar organic compounds of interest. Concentrations of the detected total keto-acids in individual cloud water samples ranged from 27.8 to 329.3 ng mL^{-1} (ppb). Method detection limits for the individual HPOC ranged from 0.17 to 4.99 ng mL^{-1} and the quantification limits for the compounds ranged from 0.57 to 16.64 ng mL^{-1}. The keto-acids were compared to the total organic carbon (TOC) results for the cloud water samples with concentrations of 0.607 to 3.350 mg L^{-1} (ppm). GCMS analysis of all samples and blanks indicated good control of the entire collection and analysis steps. Selected ion monitoring by GCMS of target keto-acids was essential for screening the complex organic carbon mixtures present at low ppb levels in cloud

water. It was critical for ensuring high levels of quality assurance and quality control and for the correct identification and quantification of key marker compounds

Key Words: highly polar organic compounds; cloud water; gas chromatography/mass spectrometry; keto-acids

Highlights

1) Analysis of the total dissolved organic carbon fraction by gas chromatography/mass spectrometry with selected ion screening.
2) Highly soluble organic compounds selectively were targeted with PFBHA and BSTFA derivatizations.
3) Low molecular weight keto-mono acids and keto-diacids were common components of the total organic complex mixtures in cloud water.
4) The total keto-acids concentrations in the cloud water samples ranged from 27.8 to 329.3 ng mL^{-1} (ppb).

1. Introduction

Water-soluble organic carbon substances are of great interest due to their interaction with atmospheric water vapor and the likely presence of these species at the interfaces of condensed aqueous phases at the solid aerosol and liquid droplet surfaces. Understanding the role of water-soluble polar organic compounds in atmospheric media is important for the larger scientific questions linked to Earth's hydrologic cycle, radiative energy balance processes, weather, and climate [1, 2, 3]. Complex mixtures of organic compounds have been identified in cloud water, fog, precipitation, and particulate matter by gas chromatography-mass spectrometry (GCMS) [4], ion chromatography [5, 6] and capillary electrophoresis [7]. Field studies and laboratory experiments have shown organic compounds to act directly and indirectly with the planetary radiation balance by acting as cloud condensation nuclei [8], scattering and absorption of visible light [9], and altering the radiative properties of clouds [10, 11].

Molecular analysis of complex mixtures of organic compounds in atmospheric media often is performed via GCMS. However, concentrations of the individual compounds are in the low ng g^{-1} water range (parts-per-billion or ppb, and equivalent aqueous concentration of ng mL^{-1}) in aqueous atmospheric media [4] and at low ng m^{-3} concentrations in airborne particulate matter [12]. The organic mixtures in these atmospheric media contain hundreds of individual compounds that contain zero to several functional groups. Within an individual organic compound, one or more functional groups which contain oxygen with free non-bonded electrons (-OH alcohol, -COOH carboxylic acid, -CHO aldehyde, and –C=O carbonyl), impart high aqueous solubility to that compound due to hydrogen bonding with water molecules. We refer to compounds containing these four functional groups and soluble in water as "highly polar organic compounds", or HPOC.

89 The study challenges were: to generate samples with acceptable background levels;
90 isolate the HPOC fraction; selectively derivatize the carbonyl containing compounds; generate a
91 GCMS analysis method that would separate the HPOC complex mixture; and to identify
92 individual keto compounds likely to be present in cloud water. The preparation and evaluation
93 of standard compounds would be evaluated by GCMS for characteristic retention times and mass
94 fragmentograms as the PFBHA and BSTFA derivatives. Our overall purpose was to apply new
95 molecular level analytical methods to field studies of complex HPOC mixtures present in cloud
96 water. The method also enables comparisons of the HPOC chemical composition in cloud water
97 with the inorganic and bulk carbon (total organic carbon, TOC) composition. The molecular
98 level data for the HPOC could be used further for multiyear monitoring of the dominant organic
99 species in cloud water from Whiteface Mountain, NY. Such multiyear monitoring programs are
100 necessary for understanding atmospheric emissions impacts and the processing and removal of
101 chemical species.
102 This study of HPOC targets two classes of water-soluble carbonaceous compounds: keto-
103 monoacids and keto-diacids. We performed selective chemical derivatization with 1) *O*-
104 (2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA), and 2) *N, O*-
105 Bis(trimethylsilyl)trifluoroacetamide (BSTFA). PFBHA is a derivatizing agent for non-acidic
106 carbonyl groups. For an introduction to early work with PFBHA, see Cancilla and Hee [13] and
107 references therein. PFBHA was used previously in the study of carbonyls in particulate matter
108 (e.g. Destaillats et al., [14] and Ortiz et al. [15]), but to our knowledge, PFBHA derivatization
109 has not been applied before to cloud water samples. BSTFA was used in previous studies of
110 alcohols and carboxylic acids in both particulate matter [16] and cloud water [4].

The use of PFBHA and BSTFA derivatization steps together was pioneered by Jaoui et al., [17]. A key advantage in applying both derivatizing agents to environmental samples is the expansion of identifiable HPOC to include compounds that have multiple functional groups, such as keto-monoacids and keto-diacids. These groups of compounds have significance as source markers for photochemical, biological, or combustion emission processes into the Earth's atmosphere. Dual PFBHA and BSTFA derivatization steps are compatible with analysis sequences that target only one functional group which otherwise would use only one derivatization agent (PFBHA or BSTFA) for single functional group classes such as simple carbonyls, carboxylic acids, and alcohols. In this paper we report the method development and optimization for HPOC complex mixtures in cloud water samples using both PFBHA and BSTFA. We illustrate the usefulness of this analytical approach by evaluating ten cloud water samples collected in series over a 16-hr time period from Whiteface Mountain in rural northeastern New York State. The site has been an important background continental site for cloud water monitoring and atmospheric process studies since the early 1970's [18, 19].

2. Materials and Methods

2.1 Sample Collection

Cloud water samples were collected continuously on September 22-23, 2009, from the top of Whiteface Mountain with elevation of 1483 m and geospatial coordinates of 44.36°N, 73.90°W. A full description of the sampling system was detailed by Aleksic et al., [20]. Results and discussion of the inorganic, ionic species and charge balance compositions of cloud water samples were described in Dukett et al. [21]. The cloud water collector consisted of closely spaced Teflon strings (impaction surfaces) that were exposed to the ambient air when a cloud was detected and sampling conditions [20] were met. Cloud droplets impacted the strings and

ran down into a Nalgene© collection bottle stored in a refrigerated section of the sampler system. A new collection bottle was rotated automatically into position each hour. Cloud water samples were pooled into 3-hour composites, and volume aliquots totaling 900-3000 mL were shipped overnight in an insulated cooler with blue ice solid bricks to the Rutgers University Civil & Environmental Engineering molecular analysis laboratory (Piscataway, NJ). The chilled samples were transferred immediately to a storage refrigerator (4°C) for storage until analysis. Based on the high water solubility of the HPOC target analytes (Table 1), we assumed filtration of the cloud water would not be a required step in isolating the compounds since they would be present in the aqueous phase rather than on suspended particles (if present) in the samples.

2.2 Bulk and Molecular Level Analysis by GCMS

All samples were run on a Shimadzu GCMS QP2010 instrument. The analytical column was a J&W Scientific DB-17MS with a length of 30 m, inner diameter of 0.25 mm, and a film thickness of 0.25 µm (Agilent Technologies). The temperature program consisted of a six-minute hold at 70°C, followed by a ramping rate of 5°C/minute until the temperature reached 150°C. The temperature was held for three minutes, then increased at a rate of 4°C/minute until the temperature reached 280°C, followed by a final 22-minute hold. Mostly low molecular weight solvents and reaction residues were seen for the first eight minutes of the elution profile. These components were eliminated from the chromatogram by setting the MS scanning to begin after 8.5 minutes. The MS scanned mass-to-charge (m/z) values from 45.0 to 650.0 at an interval of 0.45 seconds until 79.5 minutes.

Chromatogram peaks were integrated manually using the Shimadzu GCMS Postrun Analysis software (version 2.21). The Wiley Mass Spectral Database (version 7) was configured with the Shimadzu peak processing software and was used to verify target compounds.

Individual HPOC target compounds are given in Table 1, along with their respective GC retention times, key quantification m/z values and confirming m/z values. Standard HPOC solutions were injected and run under the same GCMS analysis conditions as the derivatized cloud water samples to determine key m/z ions and retention times. Identification of individual target compounds was accomplished using a combination of appropriate retention time, key quantification ion, and confirming ions. The mass spectrum of an individual peak was inspected manually to confirm the peak had the correct m/z diagnostic fragments, matched the Wiley MS Library search results, and did not contain a coeluting compound.

2.2.1. Relationship of TOC to HPOC Cloud water Fractions

In order to begin analysis of the HPOC fraction in cloud water, determination of the TOC in the samples was necessary. TOC was analyzed in the Adirondack Lakes Survey Corporation laboratory following EPA method 415.1 for UV/Persulfate Oxidation with Infrared Absorption using a Tekmar Dohrman Phoenix 8000 Carbon Analyzer [22]. The TOC concentration in cloud water is a rough approximation of the HPOC concentration, assuming most or all of the TOC was soluble in the cloud water. The two HPOC derivatization steps and functional groups targeted by this method development study are detailed below.

2.2.2. Carbonyl HPOC Derivatization

PFBHA has been utilized as a derivatization agent specific for carbonyl groups. The derivatization reaction of free carbonyl groups present in the TOC and HPOC subfraction is given in Figure 1. For each carbonyl the PFBHA molecule replaces the oxygen with a nitrogen functional group with a mass of 211. The resulting product is a perfluorobenzyl hydroxy oxime.

Laboratory tests were carried out to determine the proper volume (mass based on sample TOC concentration) of PFBHA solution needed to fully derivatize the HPOC complex mixture (Table 2). A PFBHA solution of approximately 20 mg mL^{-1} was prepared by dissolving 100.1

181 mg of PFBHA.HCl (Fluka; Steinheim, Germany) in 5 mL of HPLC-grade methanol (Burdick
182 and Jackson). An aliquot of this PFBHA solution was diluted to 0.2 mg mL^{-1} for direct addition
183 to the samples. Since it was not clear how much volume of PFBHA derivatization solution
184 would be needed to fully derivatize all carbonyl groups in a cloud water sample, we evaluated
185 three identical aliquots of 20 mL from one cloud water sample. The three aliquots were
186 concentrated as described below and received 0.015, 0.15, and 1.5 grams of PFBHA per gram of
187 cloud water TOC, respectively, from the 0.2 mg mL^{-1} PFBHA solution. The mixtures were kept
188 at room temperature for 24 hours to allow for reactions to proceed at comparable ambient
189 atmospheric temperature conditions and to reduce possible thermal degradation of HPOC target
190 compounds. GCMS analysis was performed the following day. Excess PFBHA (1.5 gram trial)
191 saturated the MS detector and added significant bleed from the PFBHA throughout the analytical
192 run. The 0.015 gram trial did not saturate the detector, but the peaks of expected HPOC
193 compounds often were indistinguishable from MS total-ion-current (TIC) background noise.
194 The 0.15 gram trial appeared to be optimal, with clear, unambiguous peaks for the target HPOC
195 compounds and no saturation of the MS detector. An additional test of twice this amount of
196 PFBHA (0.3 g) on a fourth identical aliquot of cloud water did not improve peak shape or area.
197 This result indicated that 0.15 grams of PFBHA per gram of TOC was enough reagent to
198 derivatize all carbonyl groups present in the Whiteface Mountain cloud water samples, and
199 therefore, could be used in the subsequent field study program as an accurate estimator of
200 individual HPOC concentrations within the complex mixtures.
201 The next step was to apply this mass ratio of PFBHA reagent to TOC for the cloud water
202 sample set (n = 10). Initially, 20 mL of each cloud water sample were added to two 10mL glass
203 vials with Teflon-lined screw-top caps; two smaller vials were used instead of a single larger one

due to the size of the cylindrical bore spaces in the aluminum heating block. The samples were evaporated under a 2 psi stream of high purity (99.999%) nitrogen gas and the temperature was maintained at 65°C in an aluminum heating block. Once the total sample volume was 10 mL or less, the two vials' contents were combined into one 10 mL vial and the sample was concentrated further to approximately 1 mL. The concentrate was transferred to a 1.25 mL microvial (Waters with 100 µL concentration volume for GC autosampler) with a Teflon-lined silicon rubber screw-cap and was evaporated to dryness. This step removed the water, leaving the organic analytes as a residue. The required volume of PFBHA derivatization solution (0.2 mg mL^{-1} in methanol as previously described) was added to the cloud water residues in the microvials according to the sample TOC content (Table 2). 5µL of 100ppm *n*-$C_{30}D_{62}$ in methanol also was added as the injection standard for GCMS m/z ion quantification. The microvials were capped and the mixtures reacted at room temperature (25°C) for 24 hours.

BSTFA was developed as a derivatization agent specifically for hydroxyl and carboxylic acid groups. In our earlier studies of suitable silylating reagents for ppb-level atmospheric applications, we determined BSTFA was the preferred silylating agent since it had fewer starting material residues and reaction by-products that would otherwise interfere with the separation and elution of the target products as derivatives by GCMS. For each carboxylic acid group the BSTFA replaces the –OH and adds a trimethylsilyl functional group (Figure 2) with a mass of 73. PFBHA derivatization was performed first, followed by BSTFA conversion, to reduce possible hydrolysis of the BSTFA derivatives. Comparing the mass spectrometric responses of the PFBHA and the BSTFA derivatized samples, we found the GCMS source was highly sensitive to the amount of residual PFBHA present in the derivatized sample. This sensitivity to residual PFBHA reagents required close estimation of PFBHA added to a sample's TOC

concentration. However, the BSTFA reagent did not produce a similar effect with the MS detector; therefore a simple constant volume (excess) of BSTFA was added to the samples.

Once PFBHA derivatization was complete, the derivatized samples were evaporated to dryness with high purity nitrogen in the same microvial to remove the reaction solvents (methanol, water) from the first derivatization step. During this step it was essential the nitrogen gas provided positive pressure through the sample vial opening so that atmospheric moisture could not enter the vial and catalyze hydrolysis of the BSTFA derivatives, thus reversing the carboxylic acid TMS ether derivative reaction back to the free acid. Excess BSTFA reagent was added to the sample residues following the method of Hawley [23] that was adapted for low ppb levels of organic acids and alcohols in atmospheric fine particulate matter. The derivatization procedure used BSTFA with 1% trimethylchlorosilane and followed the general reaction steps given by Supelco (Bellefonte, PA). High purity anhydrous hexane (100 μL; Fluka, Steinheim, Germany) and pyridine (20 μL; Fluka, Steinheim, Germany) were added along with the BSTFA reagent (20 μL; Supelco, Bellefonte, PA) to each sample under a stream of nitrogen. The volumes of hexane, pyridine, and BSTFA added were the same for each sample regardless of TOC content. The capped reaction microvials were heated in an oven at 65°C for 35 minutes to accelerate the BSTFA reaction. Any samples that were not run immediately were stored at -4°C. All derivatized samples were analyzed by GCMS within two days to prevent hydrolysis of the BSTFA derivatives while in storage.

2.2.3. Efficiency and Stability of Derivatization Reactions

To test the methods yields, lab standards of keto-monoacids and keto-diacids were prepared and derivatized with only BSTFA, only PFBHA, and both derivatizing agents. PFBHA derivatization resulted in a pair of isomers; thus, each target compound could have as many as five different derivatives along with the underivatized form. As an example of isomer formation

from the different combinations of PFBHA and BSTFA additions, Table 3 summarizes the five products for *cis*-pinonic acid. If up to five products formed for each of the other keto-monoacids (3 compounds) and keto-diacids (5 compounds) given in Table 1, then extensive follow-up confirmation studies were needed to understand the possible products formed for each HPOC target and to ensure accurate detection, identification and quantification by GCMS. Separate standard solutions were prepared for all nine keto-acids. Each was derivatized according to the protocol given for *cis*-pinonic acid. Full derivatization of the target HPOC was monitored in separate test runs by GCMS with selected ion monitoring (SIM) of characteristic m/z fragments. Chromatographic retention times and characteristic m/z mass fragments were determined for each HPOC derivative formed for a given compound. Eventually, the order and amounts of PFBHA and BSTFA reagents added to the Whiteface Mountain cloud water samples were determined from the above studies with the HPOC standard solutions, GCMS analysis, and the TOC concentrations present in each sample.

The HPOC cloud water concentrations we report in Section 3 (Table 5) are based on the sum of isomers formed from a parent compound. All identifications were confirmed based on the known isomers formed and retention times for each HPOC. Each cloud water sample was screened by SIM for all of the fully- and partially-derivatized compounds of all nine parent HPOC. Only the two full derivatives (PFBHA-a with BSTFA and PFBHA-b with BSTFA) of keto-monoacids and keto-diacids were seen in the cloud water samples for eight of the HPOC. Any partial derivatives involving only one of the derivatizing agents were below the detection limits. These derivatization results for the cloud water samples indicated the masses of derivatizing agents were sufficient for 100% reaction conversion and that the order of reaction produced consistent fully derivatized products as a single compound or as only two isomers.

Some keto-monoacids did not react with both derivatizing agents. Glyoxylic acid was not derivatized easily by BSTFA alone in previous work [23]. However, in this study we applied the above protocol and found glyoxylic acid reacted with PFBHA, but did not produce quantifiable results when reacted with BSTFA. Pyruvic acid was the only HPOC parent compound that did not form PFBHA or BSTFA derivatives that could be identified and measured by this method. Derivatized pyruvic acid was not consistently identified even in laboratory standards prepared at high concentrations. No PFBHA or BSTFA derivatives for this compound were seen in the cloud water samples. It is possible pyruvic acid simply was not present in measurable quantities in the cloud water, or it does not form stable derivatives with these reagents. If pyruvic acid is a desired target compound in future work, other derivatization methods would need to be explored.

We studied the stability of PFBHA and BSTFA derivatives while stored in a -4°C freezer until GCMS analysis was completed. Twelve replicates of 20 mL each were taken from one cloud water sample (collected at 9pm on 9/22/09). Each replicate was concentrated to dryness as described above, and all were derivatized at the same time. One derivatized sample was analyzed by GCMS immediately to create a benchmark response for the m/z quantification ion. The other replicates were stored at -4°C and were run on the GCMS at a rate of one per week for 11 weeks. The same procedure was followed with 12 × 20 mL aliquots of the field blank. Results for the cloud water and field blank samples indicated the PFBHA derivatives were stable for about a week, and the BSTFA derivatives were stable for approximately four weeks without noticeable differences in the responses of the m/z quantification ions and overall chromatogram characteristics.

2.2.4. Individual HPOC Analysis

Target HPOC compounds (Table 1) in the cloud water samples were compared with the retention times and mass spectra of authentic laboratory standards and the Wiley NIST database. Five-point calibration experiments were performed on the GCMS instrument for quantification of each compound as the fully derivatized PFBHA and PSTFA derivative and for evaluation and verification of instrument performance with time. Keto-monoacids were run at concentrations ranging from 0.57 to 20 ppm (ng μL^{-1}) and keto-diacids were run at concentrations ranging from 2 to 20 ppm. The ratio of the m/z quantification ion area of the target compound to that of the internal standard, *n*-$C_{30}D_{62}$ (m/z 66) was recorded for each run to determine that compound's relative response factor (RRF) and *y*-intercept. A linear model of the GCMS data was used to estimate the numerator of *y*. The form of the regression line was $y = mx + b$, where,

y = [concentration target (ng mL^{-1})]/[concentration internal standard (ng mL^{-1})];

m = Relative response factor generated from the 5-point calibration curve;

x = [area count integration m/z target]/[area count m/z 66 for internal standard]; and

b = *y*-intercept.

Each compound's RRF and *y*-intercept were used to convert the m/z peak areas to the total mass of the compound per aliquot in the reaction vial, and then to mass concentrations (ng mL^{-1}) in the cloud water.

Table 1 shows the main mass fragments that were used to identify and measure the HPOC compounds. Generally the most important m/z ion was that of the fully derivatized compound minus one, with other secondary identifying, confirming ions. Keto-monoacids often were characterized by a mass fragment that was 17 less than the molecular weight, which suggests the loss of -OH from the hydroxyl PFBHA oxime group.

2.2.5. Limits of Detection and Quantification for HPOC

Limits of detection (LOD) were identified using the level 5 concentration of the nine-component keto-acid standard. Approximately 107 ng of each compound was injected. The injection standard was *n*-$C_{30}D_{62}$. LODs were calculated individually for each HPOC target compound by integrating ten background peaks (5 before, 5 after) occurring near the retention time of that compound and using the quantification ion m/z for every compound. The ten background peak areas of a m/z value were averaged, and the LOD was established as three times higher than that average [24]. Correspondingly, the limit of quantification (LOQ) was established as ten times the same average area of the background peaks. LOD and LOQ values for each of the target compounds are listed in Table 4.

A field blank (sampler rinsate consisting of high purity water) was collected to assess the level of contamination, if any, from the collection, transport, and storage methods. The field blank represented the cumulative HPOC background composition for the entire sampling and analysis steps. None of the target analytes were found above the detection limit in the field blank.

2.2.6. Precision, Reproducibility, Accuracy of Method

Cloud water samples are unique in time and space, have limited storage lifetimes. As demonstrated in this study (Table 5), samples had organic carbon concentrations in the range of 600 to 3350 ng mL^{-1} with individual HPOC concentrations in the low parts-per-billion (ng mL^{-1}). Given the low ppb concentrations, it was difficult to design meaningful evaluations of repeatability and reproducibility that would not be heavily influenced simply from the ppb concentrations of the target analytes in this environmental matrix.

We performed preliminary recovery estimates on two aliquots of the same cloud water sample to test the precision of the derivatization reactions. Perdeuterated succinic acid and *n*-

343 $C_{30}D_{62}$ (GCMS injection standard) were added to the paired aliquots. The precision of the results
344 was within 20%. Further refinement of the dual PFBHA and BSTFA derivatization steps is
345 recommended for monitoring precision of the conversions by creating multiple paired aliquots
346 from a single cloud water sample from the field.

347 We also conducted repeatability tests on the GCMS (22 injections over 72 hours) using
348 derivatized standards (PFBHA+ perdeuteratedbenzophenone). Good reproducibility ($R^2 = 0.97$)
349 was demonstrated from multiple injections on the GCMS (22 injections over 72 hours). This test
350 indicated a stable, reproducible analysis for the PFBHA derivatives by GCMS. The BSTFA
351 reproducibility was not evaluated by repeated injections of a sample because of the rapid
352 hydrolysis of the derivative once the vial septum was pierced with the first GCMS injection.

353 The scheduling of the preparing the derivatized cloud water samples followed by rapid
354 GCMS analysis was critical to the overall stability of the time series of samples collected in the
355 field. Generally, the preparation of derivatives and the GCMS analysis for a cloud water sample
356 series occurred over a 2-3 day time period. This allowed consistent responses of the GCMS
357 instrument. The GCMS instrument itself was verified and monitored routinely by 5-level
358 standard calibration experiments.

359 There are no certified standards for cloud water TOC, nor for the individual HPOC of
360 atmospheric significance we targeted in the overall method. Consequently, there was no
361 rigorous way to validate the method with a known quantifiable standard for the keto-acid species
362 we evaluated. Also, due to the short recommended storage times for aqueous environmental
363 samples, it is not likely cloud water or precipitation samples at appropriate atmospheric pH
364 values would be available from a certified source for evaluations of accuracy and precision at
365 low ng mL^{-1} concentrations. Given the above discussion the method, nevertheless, offers

significant improvements to current analytical protocols for the quantification of single HPOC in cloud water media. It compares samples processed with the same collection, storage, derivatization, and GCMS analysis steps.

3. Results

3.1. HPOC Complex Mixtures in Cloud Water

The gas chromatographic separation of the derivatized HPOC cloud water samples demonstrated the complexity of the soluble carbon mixtures. Figure 3a shows the total ion current (TIC) plot of the HPOC-dual-derivatized complex mixtures for the field and analytical blank, and Figure 3b and Figure 3c show the corresponding TIC plots for two cloud water samples. All three figures are plotted at the same *y*-scale to allow for direct comparison. Of interest is the relatively straight baseline of the TIC mass detector response for the field and analytical blank (Figure 3a) throughout the entire run. Only a few early eluting peaks were present (10-25 minutes retention time) with TIC relative intensities of ~ 0.3 x 10^6, and one large peak at 51.5 minutes with TIC intensity of ~ 1.5 x 10^6. The peak at 51.5 minutes was identified as a phthalate compound (1,2-dicarboxylic acid mono(2-ethylhexyl) ester) and is a common plasticizer. This contaminant was traced to the derivatization step involving the BSTFA reagent and we surmise this chemical was the source. The field blank showed no detectable concentrations of any keto-monoacids or keto-diacids by applying SIM analysis (see section 2.2.5). In contrast, the TICs corresponding to the cloud water samples (Figure 3b and Figure 3c) show complex HPOC chemical compositions spanning retention times between 11 and 55 minutes. The phthalate contaminant at ~51.5 minutes is common to both TIC plots. Chromatographic separation with the GC analytical column and the temperature ramping program allows a detailed qualitative picture of the approximate number and molecular weight,

reflected by relative retention times corresponding to the mixture profile over time. Qualitatively, all ten of the Whiteface Mountain cloud water samples showed roughly the same sample complex mixture profiles of derivatized HPOC as seen in Figure 3b and 3c. Most of the compounds comprising the complex chemical mixture eluted between 10 and 40 minutes.

3.2. Individual HPOC in Cloud water

Single compound HPOC concentrations (ng mL^{-1}) are listed in Table 5 for the ten sequential Whiteface Mountain, NY, cloud water samples. The values are reported as the underivatized parent HPOC compound, taking into account the sum of the isomer masses. Also, given for each sample are the TOC concentrations and the summed concentrations of the detected keto monoacids and keto diacids. There was no clear pattern seen for the ratio of the total concentration of HPOC present to the TOC concentration.

Three HPOC, *cis*-pinonic acid, β-keto adipic acid and γ-keto pimelic acid, generally were below detection limits for the cloud samples. Glyoxylic acid, 4-oxopentanoic acid, and 5-oxohexanoic acid each were found on the order of 1 to 100 ng mL^{-1}. Glyoxylic acid often dominated the total keto monoacids present. Among the keto diacids, α-keto succinic acid and α-keto adipic acid showed similar concentration levels, while α-keto glutaric acid was present at lower concentrations that were roughly the same throughout the sampling periods. Combined concentrations of α-keto succinic and α-keto adipic acids accounted for the vast proportion of keto diacids seen in the cloud water.

The protocol provides a stable method for tracking an atmospheric molecular marker within a cloud water time series. Figure 4 shows an example of three superimposed SIM traces (m/z = 266) of the two fully-derivatized isomers of 4-oxopentanoic acid present in three sequential samples (3-hr integrated) collected on 9/22/09, starting at 6:00 am, 9:00 am, and 3:00 pm. The chemical compositions of the dominant HPOC species were similar for all three

samples. Overall, the same compounds in addition to the 4-oxopentanoic isomers were present at the same levels for all samples. This is a new level of understanding of the TOC and its individual components present in cloud water.

4. Conclusions

Selected ion monitoring as part of GCMS analysis is an essential technique for screening complex organic environmental mixtures present at low ppb levels. It is critical for ensuring high levels of quality assurance and quality control and for the correct identification and quantification of key marker compounds. For ultratrace analysis, such as cloud water time-series samples, the SIM technique enables detection and quantification of individual compounds without sample cleanup and pre-separation steps. This reduces both loss of the target analytes and possible addition of contaminants that could interfere with their identification and measurement.

An important outcome of combining PFBHA and BSTFA derivatization steps for cloud water HPOC analysis is the expanded range of individual compounds that can be detected and measured by chromatographic separation and mass detection in a single sample run. The chromatographic analysis separated the reaction mixtures from the starting reagents and the reaction by-products from the derivatized target keto-acids. Once separated using chromatography and SIM, the PFBHA and BSTFA keto-acid derivatives were identified and quantified. The cloud water concentrations of individual HPOC ranged from 0.6 to 138.1 ng mL^{-1}. Concentrations of the sum of all detected keto-acids in a single cloud water sample ranged from 27.8 to 329.3 ng mL^{-1}. Method detection limits for the individual HPOC ranged

435 from 0.17 to 4.99 ng mL^{-1} and the quantification limits for the compounds ranged from 0.57 to
436 16.64 ng mL^{-1}.

437 The sequence of the reactions selectively targeted, first, the carbonyl compounds
438 (PFBHA derivatives only) and then second, the carboxylic acid and aromatic hydroxyl
439 compounds (BSTFA derivatives). This sequence allows greater flexibility in "screening" an
440 ultratrace-level organic sample for target compounds of environmental significance. This is
441 particularly true for process level studies evaluating amounts of target marker compounds that
442 can help identify HPOC produced by atmospheric photochemical reactions, biogenic sources or
443 human activities.

444 We have demonstrated the sequence of PFBHA followed by BSTFA derivatization steps
445 prior to GCMS analysis to be effective in chemically selecting keto-monoacids and keto-diacids
446 in cloud water samples. The method generates stable derivatives for GCMS analysis and can be
447 applied to unfiltered cloud water samples.

448 This derivatization method allows for analysis of an extended range of multi-functional
449 compounds without compromising analysis of compounds containing just one functional group.
450 The method is simple and does not require solid-phase extraction or other analytical steps that
451 might result in loss of target compounds or addition of contaminants. The method also is
452 versatile and can be applied potentially to other environmental matrices containing water-soluble
453 organic matter. Establishing a mass ratio of 0.15 grams of PFBHA per gram of organic carbon
454 in the sample is a useful mass estimating parameter for extending this trace-level molecular
455 technique to water and airborne particle samples where highly oxygenated organic compounds
456 are expected to be present. Additional studies in our lab have shown this ratio can be applied to
457 urban and rural atmospheric fine particulate matter samples. The ambient fine particle samples

458 from urban airsheds contain typically 1 to 3 orders of magnitude higher amounts of total organic
459 carbon per sample than present in the summer 2009 Whiteface Mountain cloud water samples.
460
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468
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Figure Captions

Figure 1. Reaction of a generic ketone with PFBHA derivatization reagent not in series with BSTFA derivatization. The ketone functional group is replaced with a O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine group.

Figure 2. Reaction of a generic carboxylic acid only using BSTFA as derivatization reagent. Treatment with BSTFA converts the -OH functional group to a trimethylsilyl ether.

Figure 3. Total Ion Current (TIC) chromatograms from of (a) the sampler rinsate (field blank undergoing entire laboratory analysis procedures) and (b, c) 3-hr integrated cloud water samples collected at Whiteface Mountain, NY on 9/22/09 beginning at 7:30am EDT (b) and later at 7:30pm EDT (c). The x-axis is minutes (retention time), and the y-axis is TIC intensity. The TIC shows the complexity of the dissolved organic carbon HPOC in the two cloud water samples (b,c). In contrast, the TIC for the sampler rinsate blank shows few, low-intensity peaks. This GCMS analysis confirmed a high level of quality control for the cumulative chemical background from sampling, processing and analysis. Relatively little background organic chemical species contributed to the two cloud water samples as indicated by clean baselines in the TIC plots.

Figure 4. Superimposed selected ion current (mass/charge, m/z = 266) chromatograms for three Whiteface Mountain cloud water samples (9/22/09, 3-hr integrated samples starting at 6:00 am (black), 9:00 am (pink), and 3:00 pm (blue)). The chemical compositions of the dominant HPOC species are similar for all three samples. Two isomers (“a” and “b”) are shown for the 4-oxopentanoic acid PFBHA+BSTFA derivative product. The isomers were resolved by GCMS

523 analysis as separate peaks with different elution times. Peak areas (m/z = 266) from isomers were
524 summed for the total mass of derivatized 4-oxopentaoic acid.

525
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Figure 1.

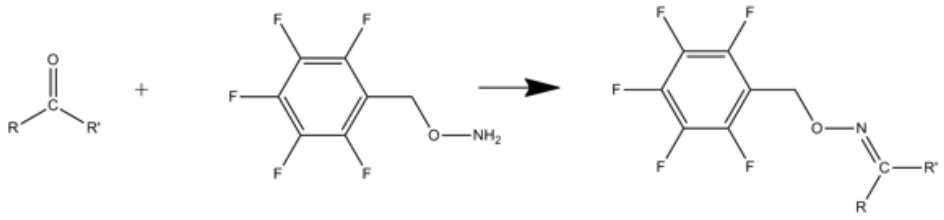


Figure 2.

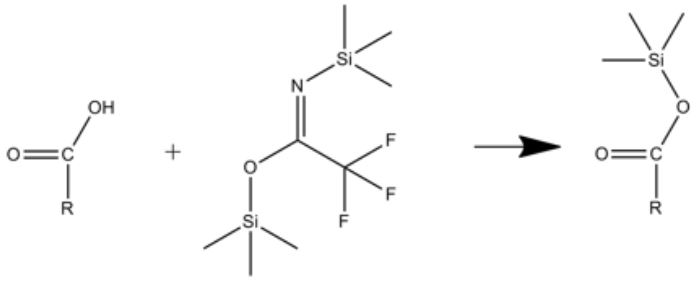
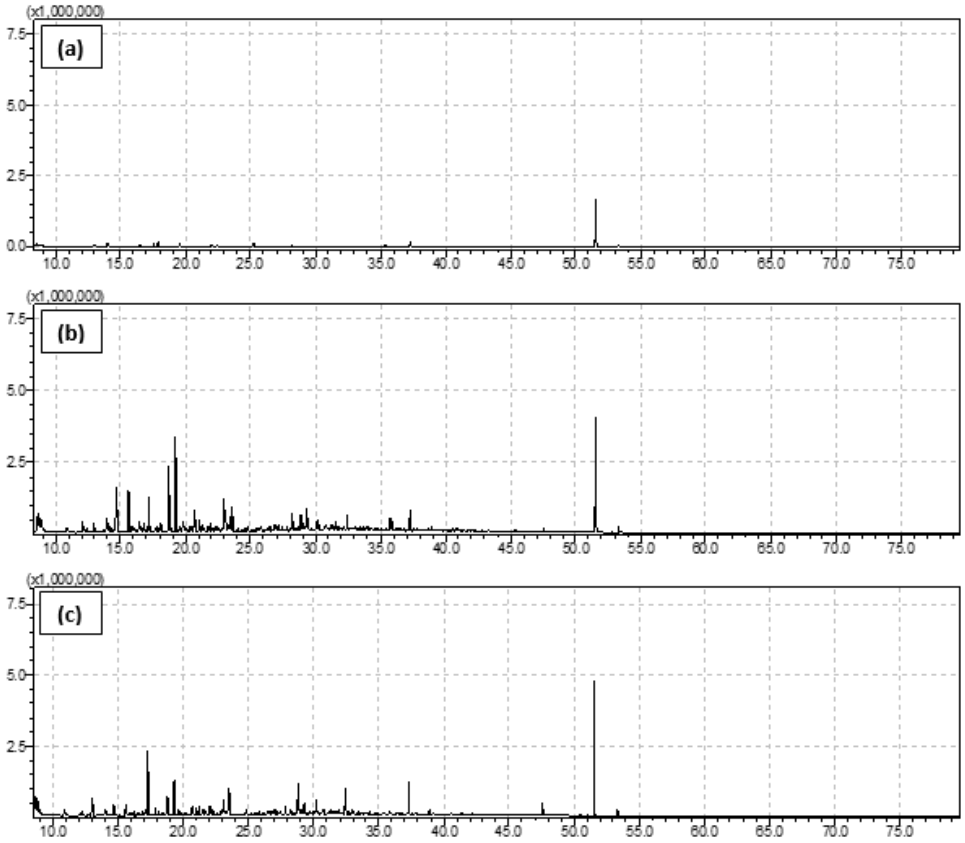


Figure 3.


(x1,000,000)
(a)
(b)
(c)
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5.0
2.5
0.0
10.0
15.0
20.0
25.0
30.0
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40.0
45.0
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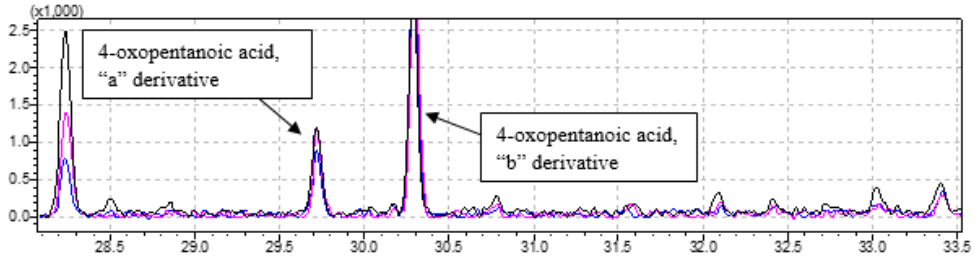
55.0
60.0
65.0
70.0
75.0

Figure 4.


(x1,000)
2.5
2.0
1.5
1.0
0.5
0.0
4-oxopentanoic acid,
"a" derivative
4-oxopentanoic acid,
"b" derivative
28.5
29.0
29.5
30.0
30.5
31.0
31.5
32.0
32.5
33.0
33.5

546 **Table 1.** Targeted HPOC compounds derivatized by PFBHA and BSTFA. The quantification
547 (quant) ion and confirming ions were the same for the "a" and "b" isomers of each compound.
548 [*Though glyoxylic acid and α-keto succinic acid each should theoretically form two isomers,
549 only one was found in all samples and lab standards.]

550

Compound Name	CAS Number	Ret. Time [minutes]	Quant Ion	Confirming Ions	Area Ratios of a:b Isomers
Keto-monoacids					
4-oxo pentanoic acid (a)	123-76-2	29.704	266	383, 202, 293	0.14 ± 0.015
4-oxo pentanoic acid (b)		30.307			
5-oxohexanoic acid (a)	3128-06-1	32.585	200	397, 266	0.25 ± 0.019
5-oxohexanoic acid (b)		33.078			
glyoxylic acid	298-12-4	23.02	326	--	* (not detected)
cis-pinonic acid (a)	61826-55-9	39.189	266	270, 320, 212	19 ± 0.67
cis-pinonic acid (b)		39.86			
Keto-diacids					
α-keto succinic acid	328-42-7	23.598	340	181	* (not detected)
α-keto glutaric acid (a)	328-50-7	36.31	352	470, 485, 288, 352	6.0 ± 1.6
α-keto glutaric acid (b)		37.24			
α-keto adipic acid (a)	3184-35-8	38.857	302	484, 258	0.14± 0.13
α-keto adipic acid (b)		39.337			
β-keto adipic acid (a)	689-31-6	39.28	409	484, 292	0.18 ± 0.022
β-keto adipic acid (b)		39.513			
γ-keto pimelic acid	502-50-1	41.995	498	306, 242, 423, 396	None; symmetrical molecule

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Table 2. Volume of PFBHA solution added to cloud water samples, based on TOC content determined by independent analysis of a sample aliquot.

TOC ($mg\ L^{-1}$)	**Amount of 0.2 $mg\ mL^{-1}$ PFBHA soln. added (µL)**
≤ 3.4	50
3.4 to 6.8	100
6.8 to 13.6	200
13.6 to 27.2	400
27.2 to 54.4	800

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Table 3. Partial derivative product possibilities for *cis*-pinonic acid and scheme for MS detection.

Derivative	Retention time (minutes)	Quant ion (m/z)	Other m/z values
BSTFA derivative, no PFBHA *(COOH only product)*	24.865	171	83, 75
PFBHA (a) derivative, no BSTFA *(C=O only product, "a"isomer)*	37.315	266	362
PFBHA (b) derivative, no BSTFA *(C=O only product, "b"isomer)*	38.05	266	362
PFBHA (a) + BSTFA derivative *(C=O & COOH products, "a"isomer)*	39.189	266	270, 320, 212
PFBHA (b) + BSTFA derivative *(C=O & COOH products, "b"isomer)*	39.86	266	270, 320, 212

565 **Table 4.** Limits of detection (LOD) and limits of quantification (LOQ) for cloud water HPOC

566 species, in ng mL^{-1}.

HPOC Target Species[1]	Quant ion (m/z)	Average background peak area counts (quant m/z)[2]	Target peak m/z area counts in standard (sum of isomers)	HPOC injected concentration (ng μL^{-1})[3]	LOD (ng in an injection)[4]	LOD (ng mL^{-1})[5]	LOQ (ng in an injection)[6]	LOQ (ng mL^{-1})[7]
Oxomonoacids								
4-oxopentanoic acid	266	137.9	702558	107.1	0.063	0.442	0.210	1.472
5-oxohexanoic acid	200	130.8	1714955	107.1	0.025	0.172	0.082	0.572
glyoxylic acid	326	170.1	319868	107.1	0.171	1.197	0.570	3.988
cis-pinonic acid	266	137.9	845004	107.1	0.052	0.367	0.175	1.224
Oxodiacids								
α-keto succinic acid	340	162.8	211558	107.1	0.247	1.731	0.824	5.771
α-keto glutaric acid	352	226	319190	107.1	0.228	1.593	0.759	5.310
α-keto adipic acid	302	278.8	421224	107.1	0.213	1.489	0.709	4.964
β-keto adipic acid	409	212.3	95661	107.1	0.713	4.993	2.378	16.645
γ-keto pimelic acid	498	188.2	139535	107.1	0.434	3.035	1.445	10.116

Notes:

[1] Level 5 standard calibration run 03/24/11. Total injection volume of standard was 1.0 μL.

[2] Determined from the areas of measureable background peaks (10 total) occurring at retention times less than and greater than the quant ion RT

[3] Calculated as (150 μL of standard used *100 ng μL^{-1} standard)/140 μL final volume after BSTFA derivatization

[4] LOD mass calculated as [(ng of target HPOC/μL)*(3)*(average area counts background target quant ion)/(target quant ion in standard)]

[5] LOD concentration in cloud water (CW) calculated as [LOD mass (ng target HPOC)/Initial volume CW sample (mL)]

[6] LOQ mass calculated as [(ng of target HPOC/μL)*(10)*(average area counts background target quant ion)/(target quant ion in standard)]

[7] LOQ concentration in cloud water (CW) calculated as [LOQ mass (ng target HPOC)/Initial volume CW sample (mL)]

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572 **Table 5.** HPOC concentrations (ng mL^{-1}) in cloud water time series collected over 9/22-
573 23/2009 from Whiteface Mountain, NY. Sample collection times were local time (duration was
574 3-hr per collection). Compounds below the detection limit (LOD, Table 4) are indicated by a
575 dashed line. TOC cloud water concentrations are in units of [ng mL^{-1}] and were determined by
576 independent TOC analysis [22].

	9/22/2009							9/23/2009		
	4:30 AM	7:30 AM	10:30 AM	1:30 PM	4:30 PM	7:30 PM	10:30 PM	1:30 AM	4:30 AM	7:30 AM
Keto-monoacids										
4-oxopentanoic acid	7.3	11.6	34.0	14.4	12.0	19.7	15.5	13.3	13.3	15.9
5-oxohexanoic acid	--	--	2.8	1.0	0.9	2.9	1.4	1.3	0.6	1.8
glyoxylic acid	138.1	93.6	125.9	2.7	50.8	17.5	74.8	82.5	53.2	96.9
cis-pinonic acid	--	--	--	--	--	--	--	--	--	--
SUM	**145.4**	**105.2**	**162.7**	**18.1**	**63.7**	**40.0**	**91.7**	**97.1**	**67.1**	**114.6**
Keto-diacids										
α-keto succinic acid	56.5	59.1	71.2	9.7	23.7	64.5	40.0	52.2	32.0	78.6
α-keto glutaric acid	19.7	19.1	19.7	--	18.3	18.8	19.4	19.8	17.9	18.8
α-keto adipic acid	76.6	58.1	75.6	--	40.8	25.9	55.4	51.2	33.8	60.7
β-keto adipic acid	--	--	--	--	--	--	--	--	--	--
γ-keto pimelic acid	--	--	--	--	--	--	--	--	--	--
SUM	**152.8**	**136.3**	**166.6**	**9.7**	**82.8**	**109.2**	**114.8**	**123.2**	**83.7**	**158.2**
TOC	3350	1861	1121	607	1266	970	929	979	863	961

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