Journal of Chromatography A, 1418 (2015) 1–11

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Evaluation of multistep derivatization methods for identification and quantification of oxygenated species in organic aerosol

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ARTICLE INFO

Article history:
Received 24 March 2015
Received in revised form 24 August 2015
Accepted 15 September 2015
Available online 24 September 2015

Keywords:
Multistep derivatization
Organic aerosol
Secondary organic aerosol
Oxygenated organics
GC × GC-ToF-MS

ABSTRACT

Two, 3-step methods for derivatizing mono- and multi-functional species with carbonyl (C=O), carboxylic acid (–COOH), and alcohol (–OH) moieties were compared and optimized. In Method 1, the C=O, –COOH, and –OH moieties were converted (1) to methyloximes (R–C=N–OCH_3) with *O*-methylhydroxylamine hydrochloride (MHA), (2) to methyl esters (O=C–R–OCH_3) with (trimethylsilyl)diazomethane in methanol (TMSD/MeOH), and (3) to trimethylsilyl ethers [R-$OSi(CH_3)_3$] with *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS), respectively. Steps 1 and 3 of both methods were identical; however, in Step 2 of Method 2, –COOH moieties were derivatized with 10% (v/v) boron trifluoride (BF_3) in MeOH or *n*-butanol (*n*-BuOH). The BF_3/MeOH and BF_3/*n*-BuOH were ineffective at converting species with more than 2-OH moieties. Average standard deviations for derivatization of 36 model compounds by the 3-step methods using TMSD/MeOH and BF_3/(MeOH) were 7.4 and 14.8%, respectively. Average derivatization efficiencies for Methods 1 and 2 were 88.0 and 114%, respectively. Despite the lower average derivatization efficiency of Method 1, distinct advantages included a greater certainty of derivatization yield for the entire suite of mono- and multi-functional species and fewer processing steps for sequential derivatization. Detection limits for Method 1 using GC × GC-ToF-MS were 0.3–54 pg m^{-3}. Approximately 100 oxygenated organic species were identified and quantified in aerosol filtered from 39 m^3 of air in an urban location. Levels of species were 0.013–17 ng m^{-3} and were nearly all above the Method 1 limit of detection.

1. Introduction

Aerosols play an important role in tropospheric chemistry, climate, and human and environmental health. Primary organic aerosol (POA) is directly emitted by natural and anthropogenic sources and is distributed between the fine and coarse aerosol modes [1]. Secondary organic aerosol (SOA) is the most abundant fine aerosol type in the global troposphere and is formed through a sequence of gas-phase and nucleation reactions [2]. The SOA is composed of thousands of species representing a continuum of polarity that is attributed to the types and numbers of oxygen (O)-containing functional groups [e.g., alcohol (–OH), carbonyl (C=O), carboxylic acid (–COOH)] of the SOA species. Forecasting SOA production on regional to global scales is problematic due to uncertainties in emission inventories of SOA precursors and quantification of precursor oxidation products [3]. Multidimensional gas chromatography with time-of-flight mass spectrometric detection (GC × GC-ToF-MS) has proven useful in identifying and quantifying the myriad of species that compose SOA [4–7]. However, O-containing functional groups of SOA species, particularly acidic compounds, limit transfer through GC systems due to interactions with surfaces and are poorly resolved by some GC stationary phases [8]. Chemical derivatization increases species volatility and reduces species polarity, which facilitates transfer through GC systems and improves separation, and thus, the conversion step is included in analytic methods to ease quantification of SOA [9–12].

One- and two-step derivatization methods have been used to identify and quantify O-containing aerosol species isolated in chamber experiments [9–12] and from ambient [12–17] and indoor air [18]. Derivatizing mono-functional species with –COOH [19–22], C=O [23–27], and –OH moieties [11,28–30] or the simultaneous quantitation of species with –OH and –COOH functionalities

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http://dx.doi.org/10.1016/j.chroma.2015.09.041
0021-9673/

[14,31–33] has been accomplished with one-step methods. Bi- and multi-functional compounds are typically derivatized with two-step methods [9,34–37]. Comprehensive identification and quantification of multifunctional, oxygenated organic species of aerosol has been accomplished using various derivatization methods [36,38,39]. Three-step derivatization methods, which allow unambiguous determination of –COOH and –OH species, have been reported [39–41] and applied to identify and quantify mono- and multi-functional species in aerosol [36,41–43].

The C=O functionalities are derivatized in the first step of multi-step procedures and –OH and –COOH moieties are derivatized in the second step. Carbonyls are converted to oxime derivatives with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) [24] or *O*-methylhydroxylamine hydrochloride (MHA) [38,40]. Derivatization with PFBHA requires the solvent extract of the aerosol to be reduced to dryness, which might promote losses of volatile species, and takes 24 h for the reaction to proceed to completion. However, MHA can be added to the aerosol extract and derivatization is complete in about 1 h. Carboxylic acids are typically alkylated, acylated, or silylated with methanol (MeOH) or *n*-butanol (*n*-BuOH) in the presence of a strong Lewis acid like boron trifluoride (BF_3) [16,44–47], diazomethane [40,48–50], and *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) or *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) [19]. The BF_3/butanol technique has the lowest detection limits; however, the procedure is the most time-consuming of the derivatization methods [22]. Diazomethane reacts instantaneously and forms few byproducts; however, toxicity of diazomethane is a concern [22]. (Trimethylsilyl)diazomethane (TMSD) in MeOH, which is less toxic and more stable than diazomethane, has also been used to methylate carboxylic acids [40,51,52]. Silylation of alcohols with BSTFA or MSTFA in the presence of trimethylchlorosilane (TMCS), which is an effective acid catalyst, is efficient, fast, reproducible, and quantitative [9,19,22,34,39]. However, reaction artifacts have been observed [53] and derivatives are prone to decomposition during storage of processed extracts due to infiltration of trace amounts of water [22].

The principal objective of the investigation is to develop a derivatization technique for SOA species with –OH, C=O, and –COOH functionalities, which will improve the quantification of SOA and the forecasting of SOA formation on regional and global scales. Here we optimize and compare two, 3-step derivatization methods developed from modifications of three techniques [39,40,44]. The first method consists of the following sequential derivatization steps: (1) conversion of carbonyls to methyloximes ($R{-}C{=}N{-}OCH_3$) with MHA, (2) reaction of carboxylic acids with TMSD in MeOH to form methyl esters ($O{=}C{-}R{-}OCH_3$), and (3) transformation of alcohols to trimethylsilyl ethers [$R{-}OSi(CH_3)_3$] with a solution containing 1% TMCS in BSTFA. Steps 1 and 3 of the second procedure are identical to first method; however, carboxylic acids are converted to methyl or butyl esters with 10% BF_3 in MeOH or *n*-BuOH in the second step. Samples were analyzed by GC × GC-ToF-MS. Derivatization yields are reported for the two methods and detection limits and mass spectral libraries for derivatives are presented for the optimized procedure. An analysis of aerosol-phase atmospheric organic matter collected at the St. Louis – Midwest Supersite (East St. Louis, MO) is presented to demonstrate application of the derivatization method to an ambient air sample [54].

2. Experimental

2.1. Chemicals

All standards were the highest purity available and with the exception of dihydroxyacetone (MP Biomedicals, Santa Ana, CA), pyruvic acid, and hydroxybenzaldehyde (Wako Chemicals, Richmond, VA) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). All solvents were GC grade (Burdick and Jackson, Muskegon, MI). Derivatizing reagents included 1% TMCS in BSTFA (v/v), 10% BF_3 in MeOH, 10% BF_3 in *n*-BuOH (v/v), 98% MHA, and TMSD (Sigma–Aldrich Chemical Co.).

2.2. Model compounds

A total of 34 compounds were selected for the method comparison (Table 1). The target analytes were grouped according to the following functionalities and bi-functionalities: –OH, –COOH, C=O and –COOH, –COOH and –OH, and C=O and –OH. The substances represent the major functionalized compound classes typically observed in OA [4].

2.3. Sample preparation

Stock standards of model compounds were grouped according to functionality and prepared in either MeOH or 1:1 (v/v) dichloromethane (DCM) in acetonitrile (ACN) at a level of 600 $\mu g\,L^{-1}$. Derivatizing reagents and stock solutions were stored at 4 °C. Modifications of two, 3-step derivatization methods were evaluated [39,40,44]. The methods differ in derivatizating reagents for carboxylic acids. Acetals and ketals, which are in equilibrium with hemi-acetals and -ketals, are formed when carbonyls react with an alcohol in the presence of an acid catalyst [44]. Thus, in the procedure using BF_3 in *n*-BuOH, carbonyls are converted to methyloximes in the first step with MHA to avoid enolation of ketones and aldehydes by BSTFA (used to derivatize alcohols in the third step), and carboxylic acids are converted to butyl esters in the second step [39]. Samples were analyzed within 24 h of derivatization.

Reactions occurring in Methods 1 and 2 are shown in Fig. 1. Carbonyls were converted to methyloximes in step 1 of Methods 1 and 2 by adding 20 μL of MHA in ACN, which is heated to 75 °C immediately before derivatization to facilitate dissolution of MHA. Eliminating water from alcohols when using BF_3/alcohol or BSTFA as derivatizing agents is necessary to avoid formation of acetals and ketals from aldehydes and ketones, respectively. The ACN was dried with activated Alumina and stored overnight over activated Molecular Sieve 3 Å. Alumina and Molecular Sieve 3 Å were activated at 360 °C for 5 h and 300–320 °C for 15 h, respectively, and used immediately or stored in a desiccator for a maximum of 3–4 months.

Carboxylic acids were converted to methyl esters in step 2 of Method 1 by ultrasonication of the working standard mixture with 1–12 μL TMSD and 1–8 μL MeOH for 20 min. In step 3 of Method 1, alcohols were converted to trimethylsilyl ethers by reacting with 100–245 μL of 1% TMCS in BSTFA at 70 °C for 60 min. In step 2 of Method 2, carboxylic acids were converted to methyl or butyl esters with 50–150 μL of BF_3/(MeOH or *n*-BuOH) at 65 °C for 20 min. Following derivatization of carboxylic acids, the acid was neutralized with 0.5 mL of a saturated aqueous solution of sodium chloride (NaCl). The aqueous solution was extracted three times with 1 mL of 1:1 (v/v) hexane (Hex):DCM and the extracts were combined. The organic extract was dried by eluting through a Pasteur pipette containing 500 mg of anhydrous sodium sulfate (Na_2SO_4) and reduced to 100 μL with a gentle stream of nitrogen (N_2). Alcohols were converted to trimethylsilyl esters in step 3 of Method 2 with 180 μL of 1% TMCS in BSTFA at 70 °C for 60 min.

2.4. Sample collection and processing

Twenty samples were collected by the high-volume sampling technique [55] over 4- or 5-h intervals during the day and in 11-h intervals overnight from 22 to 25 August 2011 at the St. Louis – Midwest Supersite (East St. Louis, MO) [54]. Briefly, aerosol-phase