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Separation and detection of aqueous atmospheric aerosol mimics using supercritical fluid chromatography–mass spectrometry

Daisy N. Grace[1], Melissa B. Sebold[1,*], and Melissa M. Galloway[1]

[1]Department of Chemistry, Lafayette College, PA, USA
[*]now at: Department of Chemistry, University of Pennsylvania, Philadelphia, PA, USA

Correspondence: Melissa M. Galloway (gallowam@lafayette.edu)

Abstract. Atmospheric particles contain thousands of compounds with many different functional groups and a wide range of polarities. Typical separation methods for aqueous atmospheric systems include reverse-phase liquid chromatography or derivatization of the analytes of interest followed by gas chromatography. These methods can be time-consuming and do not easily separate highly polar aqueous molecules. This study uses supercritical fluid chromatography–mass spectrometry to separate the methylglyoxal – ammonium sulfate reaction mixture as a proxy for aqueous atmospheric aerosol mimics. Several column compositions, mobile phase modifiers, and column temperatures were examined to determine their effect on separation and the optimum conditions for separation in a minimal amount of time and sample preparation. Polar columns such as the Viridis UPC^2 ™ BEH column combined with a mobile phase gradient of carbon dioxide and methanol provided the best separation of compounds in the mixture. This separation method can be extended to analyze other aqueous atmospheric systems, including the mixtures of other aldehydes or organic acids with ammonium or small amines.

1 Introduction

Secondary organic aerosol (SOA) comprises a significant portion of the total mass of atmospheric particulate matter and has been shown to impact human health and climate (Andreae and Gelencsér, 2006; Jimenez et al., 2009; Laskin et al., 2015). The composition of SOA can vary greatly; it typically contains large fractions of organic and inorganic material and water. There are an estimated 10,000 to 100,000 compounds in the atmosphere, with many of these found within the condensed phase (Goldstein and Galbally, 2007). Traditionally, SOA formation was thought to be a result of the partitioning of low volatility gas-phase reaction products into particles, but recent studies have shown that subsequent reactions within the particle can also lead to further SOA formation (Rossignol et al., 2014; McNeill, 2015). Within the particle, functionalization and oxidation can lead to the formation of cyclic compounds and oligomers via the reaction of aldehydes and organic acids (Powelson et al., 2013; Lin et al., 2015). Current models significantly underestimate the formation of atmospheric SOA, which leads to uncertainties in predictions of its role in climate change (Heald et al., 2005; Hallquist et al., 2009; Jimenez et al., 2009; Barsanti et al., 2013;

Laskin et al., 2017). Understanding the products formed in atmospheric reactions may be an important step toward closing the gap between atmospheric measurements and models.

The aqueous aldehyde – ammonium/amine reaction mixture is one class of reaction systems that has been shown to impact aerosol growth. Light absorption of product mixtures generated from these systems often strongly resembles humic-like substances, indicating that these reactions may provide insight into reaction mechanisms occurring within atmospheric droplets (Hawkins et al., 2016; Lin et al., 2015). A significant challenge to identification and quantification of atmospheric reaction product mixtures is the separation of the compounds that compose them. This is due in part to the high degree of similarity between many of the compounds in solution (Noziére et al., 2015). The two most commonly used separation techniques are gas chromatography (GC) and liquid chromatography (LC) (Noziére et al., 2015). For many atmospheric samples, derivatization must be performed before GC analysis, which leads to increased specificity and identification. However, derivatization can also lead to side reactions and issues with structural identification (Noziére et al., 2015). Reverse-phase LC is also commonly coupled to mass spectrometry for separation and identification of atmospheric compounds (Lin et al., 2015; Noziére et al., 2015; Aiona et al., 2017; De Haan et al., 2018; Jayarathne et al., 2018). Many of these LC methods utilize HILIC or C18 columns and use methanol or acetonitrile as the organic component of the mobile phase (Lin et al., 2015; Riva et al., 2016; Aiona et al., 2017). For complete elution, these methods use mobile phase flow rates between 0.2 and 0.3 mL min^{-1} and can take as long as 80 minutes to complete (Lin et al., 2015; Riva et al., 2016; Aiona et al., 2017). Even under these conditions, many peaks coelute and must be analyzed under multiple conditions or with more complex methods (e.g., 2D-HPLC) (Lin et al., 2015). While these methods have provided many important insights into numerous atmospheric systems, there is a need for a separation method that does not require derivatization and can reduce the necessary separation time while still providing efficient separation of the compounds in the mixture.

Supercritical fluid chromatography (SFC) has become popular in recent years as an alternative to GC and LC in many applications (Bernal et al., 2013; Lesellier and West, 2015; Bieber et al., 2017). SFC is often thought of as analogous to normal-phase chromatography and provides an attractive alternative to traditional LC since it is considered to be a greener technique due to the use of carbon dioxide as the main component of the mobile phase (Taylor, 2008). Overall, less solvent waste is generated than in traditional LC, and carbon dioxide is cheap and non-toxic (Patel et al., 1998). Due to the lower temperatures necessary for separation compared to GC, SFC allows for the analysis of thermally labile compounds that may not be able to be analyzed via GC (Bernal et al., 2013).

When SFC was first developed, carbon dioxide was the only component of the mobile phase (Bernal et al., 2013). However, carbon dioxide is also miscible with many polar organic solvents (e.g., methanol and acetonitrile) which can be used as mobile phase modifiers (Parlier et al., 1991). The use of modifiers can change the polarity of the mobile phase, thereby making it possible to separate polar or nonpolar compounds using SFC and allowing it to be used analogously to either normal- or reverse-phase LC (Guiochon and Tarafder, 2011). Acids, bases, or salts can also be added to the mobile phase modifier as additives to further alter the composition and polarity of the mobile phase (Lesellier and West, 2015). These additives are at much lower concentrations than the modifier itself (typically 0.05–1%), and have been shown to significantly improve both peak shape and retention of a range of compounds. The use of modifiers and additives with gradient elution allows for the separation of polar and nonpolar molecules without the need to switch solvents or columns. This makes SFC ideal for the separation of mixtures containing compounds with a wide range of polarities, such as aqueous atmospheric samples. Since SFC is often coupled to a mass spectrometer, many additives are salts that can be used as ionization agents (e.g., ammonium formate, formic acid, and tetramethylammonium hydroxide) (Cazenave-Gassiot et al., 2009; Lesellier and West, 2015). The output from an SFC column can be coupled to a variety of instruments for analysis (Bernal et al., 2013; Bieber et al., 2017). Two commonly used mass spectrometry ionization methods are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Both are soft ionization techniques that allow for identification of the molecular ion peak of a compound, which are ideal for product identification in a complex reaction system like an aqueous atmospheric reaction mimic. Many of the previously identified compounds from aldehyde – amine reaction systems have also been observed using these ionization techniques (Kampf et al., 2012; Sareen et al., 2013; Lin et al., 2015; Wong et al., 2017).

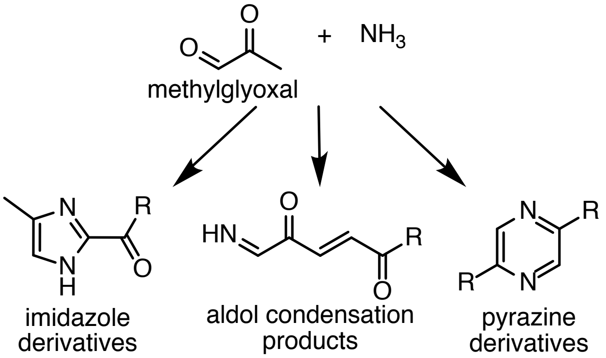

Figure 1. Methylglyoxal reacts with ammonia to form products with a variety of functional groups and polarities.

The methylglyoxal and ammonium sulfate mixture is a model system to optimize for SFC due to the fact that much is known about its chemistry and the products observed (Sareen et al., 2010; De Haan et al., 2011; Sareen et al., 2013; Lin et al., 2015; Rodriguez et al., 2017; Wong et al., 2017). This reaction provides atmospherically relevant analytes to study, as well as a system that is difficult to separate since it contains many polar oligomers and reduced nitrogen compounds (see Fig. 1) (Laskin et al., 2015; Lin et al., 2015). The ability to efficiently separate the compounds in this and similar systems may allow for identification of compounds that contribute to ambient aerosol mass. In this study, the experimental conditions for five columns, three mobile phase modifiers, and a range of temperatures are evaluated and optimized to determine appropriate SFC separation conditions for this complex mixture and others like it. An SFC method is presented that allows for the separation of this complex mixture in 30 minutes or less and with no sample preparation.

2 Materials and methods

2.1 Reagents

Methylglyoxal (40% w/w in H_2O) and ammonium sulfate were purchased from Sigma Aldrich. Food grade carbon dioxide was obtained from Airgas. Methanol (Optima^{TM} LC/MS Grade), ammonium formate (10 mM with 0.05% formic acid) in methanol (LC/MS grade), and formic acid (Optima^{TM} LC/MS Grade) were purchased from Fisher Chemical.

2.2 Methylglyoxal and ammonium sulfate mixtures

Separate standard solutions of 1 M methylglyoxal and ammonium sulfate were prepared in deionized water. Mixtures for analysis were prepared by mixing sufficient volumes of each stock solution with deionized water to make solutions containing 50 mM each of methylglyoxal and ammonium sulfate. Due to slow room temperature reaction times at these concentrations, the mixture was allowed to react for at least a month in a dark environment before analysis (Zhao et al., 2015). This ensured that the reaction had proceeded far enough to form all the previously identified major products (Amarnath et al., 1994; Bones et al., 2010; Sareen et al., 2010; De Haan et al., 2011; Lin et al., 2015; Kampf et al., 2016; Aiona et al., 2017; Hawkins et al., 2018).

2.2.1 Mass spectrometry

The methylglyoxal – ammonium sulfate mixture was separated and analyzed with a Waters ACQUITY Ultra Performance Convergence Chromatography (UPC^2) SFC system coupled to a Waters XEVO TQD triple quadrupole mass spectrometer (MS). The XEVO TQD is equipped with an ESCi ion source which allows for rapid switching between ESI and APCI modes, and all samples were analyzed via positive and negative ESI and APCI modes. The ESCi probe conditions were set as follows: desolvation temperature = 200° C, desolvation gas flow = 650 L hr^{-1}, cone flow = 1 L hr^{-1}. ESI conditions were set as follows: capillary voltage = 1.18 kV, cone voltage = 30 V. APCI conditions were: corona voltage = 1.5 kV, cone voltage = 50 V.

2.3 Supercritical fluid chromatography

Unlike an LC system, the pressure in an SFC column must be maintained throughout, so a backpressure regulator is installed after the column to ensure the mobile phase stays in a near-supercritical state throughout the entire column. In the UPC^2 system, liquid carbon dioxide is pulled into a chilled carbon dioxide pump and mixed with co-solvent before delivery to the column. A 10 μL sample loop is plumbed inline and diverts solvent flow through the loop upon sample injection. Part of the flow is sent to the backpressure regulator (set at 1500 psi), and the remaining flow is directed to the MS. In these experiments, the flow from the UPC^2 system was mixed with the output of an isocratic pump that provided 0.25 mL min^{-1} of 10 mM formic acid in methanol as makeup flow into the ionization source.

The chromatography system was optimized using a variety of columns, modifiers, and column temperatures, as described below.

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2.3.1 Columns

The columns used for analysis were chosen for their range of polarities and variety of functional groups. They are: ACQUITY UPLC® BEH Amide (Amide), CORTECS™ UPLC® HILIC (HILIC), Viridis UPC^2 ™ BEH (BEH), ACQUITY UPLC® BEH C18 (C18), and Viridis UPC^2 ™ BEH 2-Ethylpyridine (2-EP). Specific details about each column can be found in Table S1.

2.3.2 Modifiers

A binary gradient of carbon dioxide and organic modifier was used for elution of all samples. The total flow rate was held constant at 1.0 mL min^{-1}. The modifiers tested in this study were methanol, 10 mM formic acid in methanol, and 10 mM ammonium formate in methanol. Optimal mobile phase conditions varied slightly with the identity of the column, but all runs started with a low percentage of modifier (0–2%), held at this concentration for 5–8 minutes, increased linearly to 45% modifier until 20 minutes, then held at 45% modifier until 27 minutes before returning to initial conditions for the last 3 minutes of the run. The initial isocratic hold was varied slightly depending on the polarity of the column since stationary phase composition significantly changed the retention of early eluting compounds. When switching modifiers, the columns were allowed to flush with the new modifier for at least 1 hour at 1.0 mL min^{-1} to ensure there were no residual additive ions on the column (Berger and Deye, 1991).

2.3.3 Column temperature

The temperature of the columns was varied from 35–55°C to determine the effect of temperature on separation. The optimal mobile phase conditions determined in Sect. 2.3.2 were used at all temperatures.

3 Results and discussion

3.1 Mass spectrometry

Data collection was performed in both ESI and APCI ionization modes for comparative purposes. The mobile phase in SFC separations is acidic (Lesellier and West, 2015), which helps to protonate analyte molecules during the ESI ionization process. Therefore, it is not surprising that the overwhelming majority of these compounds are detected in positive ESI mode since they contain alcohols and nitrogen-containing functional groups that are easily protonated. The presence of formic acid in the makeup flow (ESI solvent) enhances the ionization of any compound more basic than the resulting solution, making its addition very useful for these analyses. Most of the compounds were also detected in APCI mode, but at much lower intensities than ESI mode ($\sim 20\times$, see Fig. S1). Therefore, ESI is the preferred mode for analysis of these aerosol mimics, though there may be some compounds that have lower ionization efficiency with ESI and may benefit from the use of APCI in some solutions (Hawkins et al., 2018). The ability to switch back and forth between modes in the same separation is useful for such analyses.

All masses observed in this study are given in Table S2 and all extracted ion chromatograms (EICs) are shown in Fig. S3. The chromatograms presented in this study are a combination of all the EIC signals in Table S2 and Fig. S3.

3.2 Chromatographic conditions

3.2.1 Columns

The packed columns used for SFC separations are similar to those used for LC systems, and many Ultra Performance LC columns can be used on an SFC system. Under the conditions presented here, nonpolar compounds should elute earlier than polar compounds on a reverse-phase column since the polarity of the mobile phase increases over the course of the separation. As some of the compounds in this mixture are highly polar, most of the columns that were chosen for this work are intended to separate polar compounds in the slightly acidic environment (pH 4–5) present during UPC^2 separation (see Table S1) (Lesellier and West, 2015). The C18 column was chosen as a nonpolar comparison. Figure 2 shows a comparison of EICs from all columns tested in this work, using methanol as the modifier. The Amide, 2-EP, and BEH columns all provided separation of the reaction products in the system. Many of the low intensity peaks are difficult to see when compared to the most intense peaks in the chromatogram but can be viewed within the inset of Fig. 2 for the BEH column. Most compounds elute within 12 minutes on all the columns tested, and all columns provided better separation of these compounds than the later eluting compounds (e.g., *m/z* 83, 97, and 126), which either coeluted or had very wide, noisy peaks that overlapped significantly depending on eluent conditions. Two of these late eluting compounds have been identified as small methylimidazole derivatives (*m/z* 83 and 97), and it is likely that they interact very strongly with the columns and require a highly polar mobile phase for complete elution. Other methylimidazole derivatives with attached methylglyoxal oligomers elute much earlier, likely since the addition of the methylglyoxal moieties decreases the polarity of the molecule and, as a result, interactions with the column are decreased.

Efficient separation on the C18 and HILIC columns was not possible under the conditions tested, as most compounds eluted within 2 minutes even when the starting conditions contained 100% carbon dioxide. It is likely that the C18 column is not polar enough to retain many of these compounds. It was expected that the HILIC column would efficiently separate this mixture, since it is composed of bare silica and is often used in LC separations of similar, highly polar aqueous mixtures (Laskin et al., 2017). However, separation on the HILIC column was similar to that of the C18 column, indicating that this separation is affected by more than just partitioning to the polar stationary phase and that polar molecular interactions between analytes may be driving the solution through the column. The HILIC columns with polar functionalities such as the Amide and 2-EP combined with the polar methanol modifier provided improved separation, as the methylglyoxal – ammonium sulfate mixture produces many polar compounds that contain nitrogen- and oxygen-containing functional groups that have heightened interactions with the nitrogen-containing stationary phase (amide or 2-ethylpyridine) in these columns.

Under these conditions, the best separation was achieved with the BEH column. The bridged ethylene groups on the BEH column slightly reduce the polarity of the stationary phase as compared to the open silanol sites on the HILIC column while still providing a polar stationary phase and separation of highly polar compounds. In addition to slight improvements in resolution while using the BEH column over the 2-EP column, the less intense peaks that eluted before 11 minutes also had higher

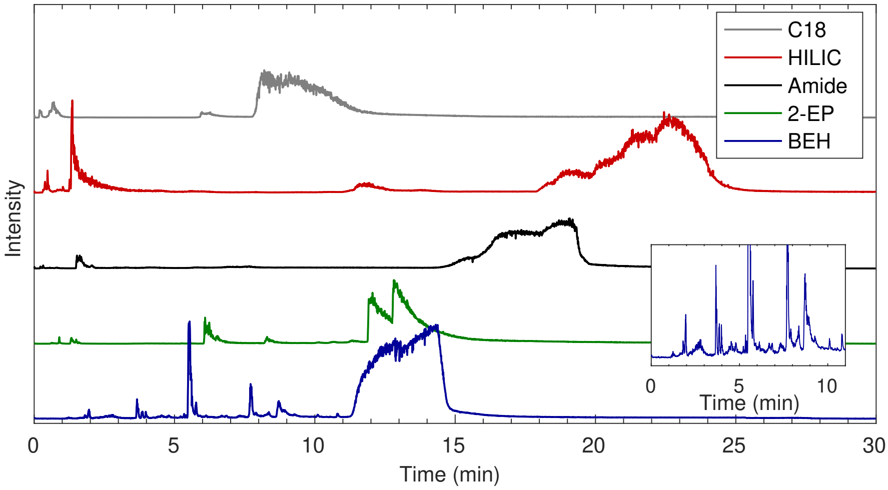

Figure 2. Comparison of EICs from five columns using a methanol modifier. The masses monitored are shown in Table S2. More functionalized polar columns (Amide, BEH, 2-EP) provide better separation. The inset shows the first 11 minutes of elution from the BEH column to show the clean separations between many of the lower intensity masses.

intensities than with the other columns. Therefore, further analysis will focus on the BEH column, although all temperature and modifier conditions discussed below were tested on each column with similar results.

3.2.2 Modifiers

Several modifiers were tested to determine suitable mobile phase conditions for separation on each column (see Fig. 3 for an example). Common SFC modifiers include small alcohols (e.g., methanol and ethanol) and acetonitrile. Acetonitrile has a lower polarity index than methanol and was initially tested. In a carbon dioxide/acetonitrile gradient, the most polar compounds did not elute from any of the columns tested within 40 minutes. This is likely because a higher polarity solvent is needed to elute some of the more polar compounds from the column. There was also no improvement in separation of the earlier eluting compounds when using acetonitrile, and in most cases, separation efficiency decreased. These observations, combined with the fact that significant precipitate forms when the reaction mixture is diluted in acetonitrile in the bulk phase, led to the decision to exclusively use methanol-based mobile phases in this work. Most of the compounds within these mixtures are soluble in methanol, and methanol provided improved separation over acetonitrile. Methanol is the most polar of the commonly used SFC modifiers, which likely explains the improved separation due to the high polarity of some of the products in the methylglyoxal – ammonium sulfate reaction mixture. This study then focused on determining which mobile phase additive provided the best separation for the methylglyoxal – ammonium sulfate system.

Ammonium formate is a common mobile phase additive for SFC and is an ideal additive for use in mass spectrometry because it promotes ionization in the electrospray ionization source. When using 10 mM ammonium formate in methanol as the mobile phase modifier, separation of compounds that elute in less than 11 minutes is similar to that of a pure methanol

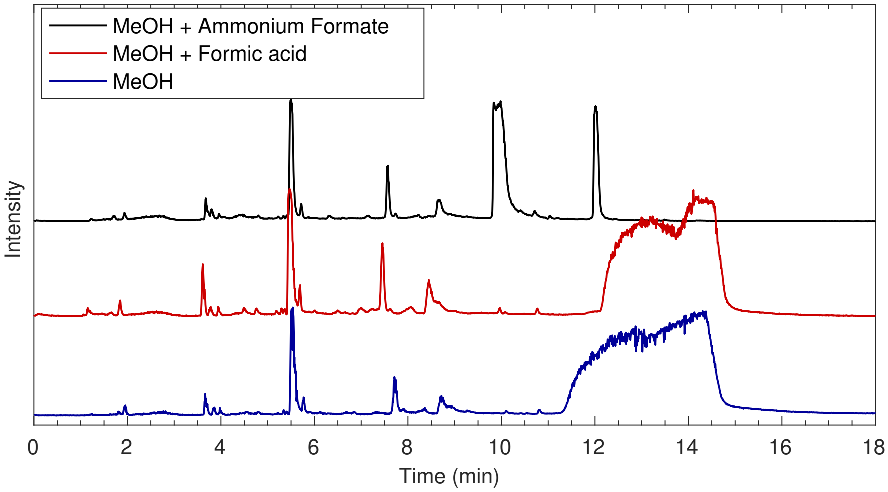

Figure 3. Comparison of EICs showing chromatography from methanol-based modifiers on the BEH column. The masses monitored are shown in Table S2. Mobile phase modifiers can affect separation on the column as well as chemistry occurring in the ionization source.

modifier (Fig. 3), and the compounds that elute after 11 minutes also elute much more cleanly from the column. However, the addition of ammonium in the mobile phase or makeup flow leads to artificially high signals from nitrogen-containing compounds, even in samples that contain no nitrogen (e.g., aqueous methylglyoxal). These compounds are also seen in samples containing ammonium sulfate, but their signals are enhanced with additional ammonium added into the system via the mobile phase or makeup flow. The increased nitrogen-containing compounds are being formed within the instrument. The ammonium is likely reacting with the carbonyls in the sample either on the column or in the ionization source during rapid drying of the sample. Therefore, the chosen additive must be one that does not react with the analytes of interest within the instrument, and additives containing ammonium are not suitable for systems containing carbonyl compounds.

Formic acid is another common SFC mobile phase modifier that promotes ionization in the ESI source and does not contain the ammonium that can react with the analytes in the mixture. The use of pure methanol or 10 mM formic acid in methanol as the mobile phase resulted in very similar separations. Therefore, either of these modifiers could be used for separation of these compounds. As there is little difference between separations with methanol and methanol with formic acid, further analysis in this work uses pure methanol as the mobile phase modifier.

3.2.3 Column temperature

The solvating power of a super- or sub-critical fluid depends on the density of the fluid, which is affected by the temperature and pressure of the system. Therefore, in SFC, retention and separation are not only controlled by the stationary and mobile phases, but also the temperature and pressure (Saito, 2013). The temperature of the column may become an important parameter for separation of compounds and can significantly change retention. At lower temperatures, the mobile phase density is higher and retention times tend to shorten. However, this is balanced by the effect of lower temperatures on the partitioning of analytes

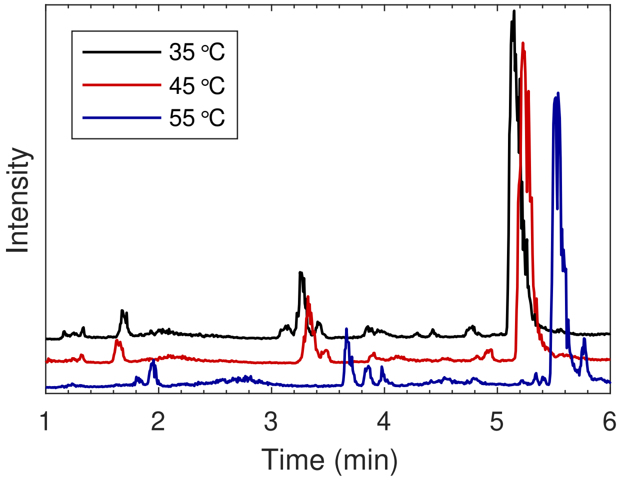

Figure 4. Comparison of EICs showing separation at various temperatures on the BEH column with MeOH as the modifier. Separation is improved at higher temperatures. On all columns, most peaks elute slightly earlier at lower temperatures and are somewhat sharper.

into the stationary phase as is typically seen in LC or GC. Therefore, it can be useful to test the effect of temperature on the separation of these mixtures. In this work, column temperature was varied from 35°C to 55°C to test the working range of the SFC columns. The critical temperature of carbon dioxide is 30.1°C, so pure carbon dioxide is supercritical under all of these temperature conditions, but the addition of a modifier or additive to the mobile phase raises the critical temperature of the system (Guiochon and Tarafder, 2011). It is very likely that the mobile phase is subcritical for these lower temperature analyses, but Guiochon and Tarafder (2011) showed that it does not matter whether the mobile phase is supercritical or subcritical, as long as the retention factors fall within a useful range and analyte does not precipitate on the column during analysis.

Changing the temperature of the column did not consistently affect separation of the analytes between columns. Retention time typically increased slightly with an increase in temperature, but this small change did not necessitate a change in mobile phase conditions for optimal separations. On the BEH column, separation improves at 55°C as compared to 35°C or 45°C (Fig. 4). The increased temperature decreases the density of the mobile phase, which changes its interaction with some analytes. While not all analytes are significantly affected by this difference, some retention times are impacted, as can be seen in the small peaks that elute near 3.5 minutes and the small peak that is present at 5.8 minutes at 55°C but is not separated from the larger nearby peak at lower temperatures. Overall, higher temperatures lead to improved separation with the BEH column. For other columns, temperature did not have as large of an impact on separation, but slight improvements in peak shape were observed as the temperature decreased. Thus, the effects of changing column temperature on the separation efficiency of a system depend strongly on the column, and it is likely that they also depend on the analyte and mobile phase. Therefore, temperature is a variable that must be tested for each individual system to determine how separation will be affected.

3.3 Comparison to LC

In order to ensure that this system performs as well as comparable chromatography – mass spectrometry coupled systems, the masses detected in this system were compared to those found in the literature for the methylglyoxal – ammonium sulfate system (Amarnath et al., 1994; Bones et al., 2010; Sareen et al., 2010; De Haan et al., 2011; Lin et al., 2015; Kampf et al., 2016; Aiona et al., 2017; Hawkins et al., 2018). Each of the masses in Table S2 were monitored. While the retention times of compounds cannot be directly compared from column to column in the SFC system or between SFC and LC in order to confirm structures, the observed masses can still be compared. All the previously published masses shown in Table S2 were observed as well as some that have not been seen in the literature. Figure 5 shows EICs from selected *m/z* values. Many of the observed masses elute in multiple peaks, as reported by Lin et al. (2015). There are also several coeluting peaks, but EICs are useful to determine the elution time of an individual mass.

The chromatograms depicted in green correspond to masses that have been shown to be important chromophores in previous work, and those in black have not yet been published for this system (Lin et al., 2015; Hawkins et al., 2018). Peak intensities for many of these new masses are comparable to those that have already been detected. Many of these peaks are also very low in intensity compared to *m/z* 83, 97, and 125 (imidazole derivatives), probably due to the variation in ionization efficiency of the ESI source for different functional groups present on the molecule and concentrations of products. However, many peaks are apparent when viewed as individual EICs and it becomes possible to detect multiple within the reaction mixture. Therefore, the intensities in Fig. 5 have been normalized to see the separation between compounds. The highest intensity in each chromatogram is given in the right-hand column.

Through the use of tandem MS, it is possible to determine similarities between the observed molecules. Common building blocks for the methylglyoxal – ammonium sulfate reaction system include methylimidazoles. These compounds fragment to several common masses, including *m/z* 69, 83, and 97 (Kampf et al., 2016). The use of tandem MS helps to confirm the presence of methylimidazole in previously published masses such as *m/z* 125, 196, 197, 232, and 269. All of these masses contained *m/z* 83 as a fragment, indicating that these methylimidazole derivatives fragment to *m/z* 83 more readily than *m/z* 69 or 97. Therefore, *m/z* 83 was used as an identifying fragment mass for compounds that contain methylimidazole. Several of the previously undetermined compounds in this system fragment to *m/z* 83, including *m/z* 139, 169, 190, and 253 (see Table S2). These compounds are likely to contain a substituted methylimidazole group. This conclusion is further supported by the fact that these compounds all elute within the range of retention times of previously identified methylimidazole compounds, as can be seen in Fig. 5. While it is not surprising to detect methylimidazole compounds within this system, SFC can be used to separate these low intensity signals that have not been identified in previous studies using LC for separation.

Tandem MS also shows that many of the masses that elute in multiple peaks may in fact be very similar compounds since the fragmentation patterns for multiple peaks are similar or the same. Many of the higher mass compounds with multiple retention times may be isomers with slight differences in structure due to oligomerization reactions occurring on different carbons within each molecule. This is true for masses such as *m/z* 126. Both of the compounds that lead to the highest intensity peaks in this EIC fragment to *m/z* 42, 55, 70, 80, 98, and 108. Similarly, there are two major peaks in the EIC for *m/z* 165 (6.1 and 6.7

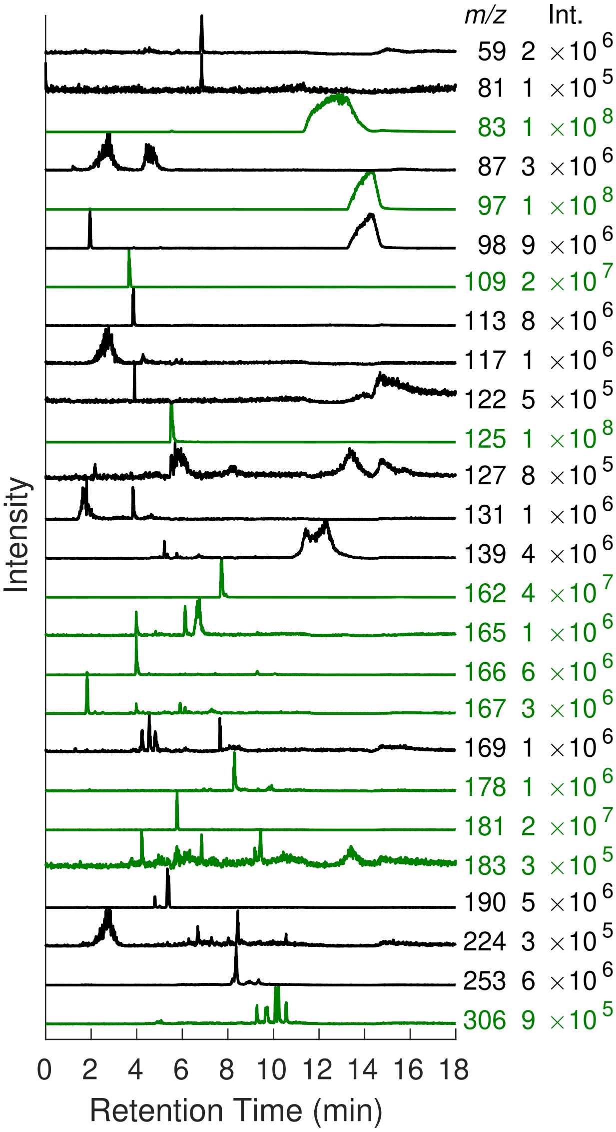

Figure 5. EICs of selected *m/z* values seen under the conditions described above. The intensity of the largest peak is given to the right of each trace. All chromatograms have been normalized to their maximum value for ease of viewing due to the very intense signals given by imidazole derivatives. The traces shown in green correspond to masses that have been identified in previous work. Those those in black have not yet been published for this system (Lin et al., 2015; Hawkins et al., 2018).

minutes, see Fig. 5), and both fragment to *m/z* 43, 123, and 147. Since these compounds elute at slightly different times, it is likely that they are isomers that reacted slightly differently as they oligomerized but have the same general structure. In general, this seems to hold true in the higher mass compounds, suggesting that differences in structure do not start to appear until higher order oligomerization has occurred (i.e., more methylglyoxal units have been added).

4 Conclusions

The use of SFC is ideal for separation of compounds with a wide range of polarities and is thus an excellent choice for separating aqueous atmospheric aerosol mimic solutions containing compounds with a variety of functional groups. There are many options for columns and mobile phase modifiers that can be used to fine-tune the separation of multiple compounds of interest. For systems containing such a large range of polarities, a polar or polar functionalized column with a highly polar mobile phase such as pure methanol or methanol with an acidic additive is ideal for this separation. ESI is also an attractive and commonly used ionization source for mass spectrometry and allows for the detection of the compounds separated via SFC. This includes the previously published products of this system and several new masses.

The total run time for the separations described herein is 30 minutes, including time for the column to reequilibrate to initial conditions, which is significantly shorter than many current LC methods for methylglyoxal – ammonium sulfate and similar systems (Lin et al., 2015; Riva et al., 2016; Aiona et al., 2017). This is due in part to the fact that initial mobile phase conditions are highly nonpolar (∼99% carbon dioxide), allowing for the more nonpolar compounds to elute early. The strongly polar mobile phase at the end of the gradient provides efficient separation on the column that may not be possible with traditional LC. It is also not necessary to moderate the flow rate to 0.2–0.3 mL min^{-1} with this system in order to achieve complete separation, and separation can be achieved with flow rates of 1.0 mL min^{-1} or more, contributing to faster total chromatography times with maximum separation.

Many other small aldehydes and organic acids are found within atmospheric droplets that react with ammonium and other amines to form similar product mixtures to that studied here. These have been explored by several groups (Heald et al., 2005; Hallquist et al., 2009; Jimenez et al., 2009; Laskin et al., 2017; Lin et al., 2015; Noziére et al., 2015; De Haan et al., 2018; Hawkins et al., 2018), but more studies are necessary to accurately determine the reactions that occur and products formed within the atmospheric aqueous phase. SFC is a useful tool for the separation of these reaction mixtures and can be utilized to provide more information about the reaction products formed.

Author contributions. MMG designed the experiments and DNG, MBS, and MMG carried them out. MMG prepared the manuscript with contributions from all co-authors.

Competing interests. The authors declare that they have no conflict of interest.

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Data availability. Chromatograms are publicly available as text files at http://sites.lafayette.edu/gallowam/publications/ (last access: 7 February 2019).

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