

We thank the reviewer for their included suggestions, questions, and points for clarification. We address the reviewer's feedback below. Our responses to the reviewers are included in italics after each reviewer comment. Additionally, the revised version of the manuscript is added.

GENERAL COMMENTS

The authors report on the development of supercritical fluid chromatography for the separation of polar products of the atmospherically important reaction between methylglyoxal and ammonium sulfate. The reaction itself has already been widely investigated. New molecular/fragment ions were found, however identification of the corresponding analytes was not in focus of the study (is also not expected due to the unit mass resolution of mass spectrometric detection). The presented SFC is an attractive and greener alternative to commonly applied LC and GC methods, but the motivation why it was developed for the analysis of the investigated reaction is not clear. Also, its better performance in comparison to conventional analytical techniques is not well justified (see below). Moreover, the use of C18 and HILIC columns seems fundamentally inappropriate; one does not expect any good results when applying nonpolar-to-polar gradient on C18 or operating HILIC without a certain amount of water.

It should be made clear, by corrections throughout the manuscript, that there is no chromatographic method that is unique and can be used for the detection of any analyte in any mixture. In this regard, it should be clearly shown at the end of the manuscript why the new chromatographic method is better performing than the conventional LC/GC separations (best by comparison of SFC, LC and GC chromatograms, a real sample analysis would be above expectations). I believe that the new identified peaks cannot be unambiguously attributed to the better separation, but may also arise from different MS detection (different instrument/ESI source, lower LOD, etc.). Please revise the manuscript addressing these issues in particular.

We agree that no chromatographic method is perfect for every analyte, and have tried to make it clearer throughout the manuscript that SFC is an alternative to LC that may be able to provide complementary information on these chemical systems, but is not necessarily the only tool that will provide useful information. We have also revised much of the text with a discussion of how it is the combination of the separation of compounds such as isomers and the use of EIC and tandem MS that make it possible to learn more about this methylglyoxal/AS system. In addition, it is likely that a lower LOD is an advantage here, but without knowing enough details about the specific ESI-MS and APCI-MS instruments used in past studies, we are not comfortable making this claim.

SPECIFIC COMMENTS

P1L3: *These methods (GC and LC) can be time-consuming and do not easily separate highly polar aqueous molecules.* -> The presented method obviously also doesn't assure separation of highly polar products (broad peak after 11 min).

We agree that our separation method does not assure separation of all compounds, but we think this strengthens our point that separation is difficult with these systems. We have revised the abstract and multiple sections throughout the text to make it clear that while complete separation does not occur with the presented chromatography, the use of EIC and MS/MS still allows for analysis of these coeluting compounds.

P2L13-17: First, use of ion-pairing reagents enables/improves separation of polar analytes on RP columns and has for instance been successfully applied to the detection of ambient organosulfates. Second, how long the method has to be is very much dependent on the complexity of the sample (simulated reactions are usually less demanding than real aerosol extracts). Thirdly, many peaks co-elute also in your case (broad peak after 11 min).

We have revised this section of the introduction to talk in general about the use of GC and LC for atmospheric system, and then we discuss more specifically the few studies that have used LC to study reactions of small carbonyls with amines or ammonium. Due to this revision of the text, we do not feel that the mention of ion pairing fits into the manuscript because, to our knowledge, ion pairing reagents are not often used for this particular system. However, we do mention that the additives we are using are ion-pairing agents. With the use of EIC and MS/MS, the complete separation of these peaks is not completely necessary to identify and study these compounds, and previous studies have not achieved complete separation, but were still able to draw many conclusions about this system. This technique is complementary to previous techniques. As we revised the text based on other referee comments, we made many changes to how we discuss this method in comparison to others, and believe we have made this point much more clearly.

“A significant challenge to the identification and quantification of atmospheric reaction 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 (Nozière et al., 2015). The two most commonly used separation techniques are gas chromatography (GC) and liquid chromatography (LC). 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 ambiguity in structural identification (Nozière et al., 2015). Reverse-phase high performance LC (HPLC) and ultra performance LC (UPLC) are also commonly coupled to mass spectrometry (MS) for separation and identification of atmospheric compounds (Lin et al., 2015; Nozière et al., 2015; Aiona et al., 2017; De Haan et al., 2018; Jayarathne et al., 2018), and several studies have used these techniques to study aldehyde – ammonium/amine reaction systems (Lin et al., 2015; Aiona et al., 2017; Kampf et al., 2016; Kampf et al., 2012). Lin et al. (2015) and Aiona et al. (2017) provided comprehensive studies of chromophores found in the methylglyoxal – ammonium sulfate system before and after photolysis using similar HPLC methods. Lin et al. (2015) found that an acetonitrile/water gradient with an SM-C18 column provided the best separation in 80 minutes at 0.2 mL min⁻¹. Kampf et al. (2012) analyzed a glyoxal – ammonium sulfate mixture with an acetonitrile/water gradient on an Atlantis T3 (C18) column in 60 minutes at 0.2 mL min⁻¹. A similar study analyzed the nitrogen-containing compounds from the reaction of small dicarbonyls and amines on HPLC and UPLC (Kampf et al., 2016). The HPLC method utilized the same Atlantis T3 column and an acetonitrile/water gradient to separate these reaction mixtures in 19 minutes at 0.5 mL min⁻¹, while the UPLC method used a Hypersil Gold C18 column with an acetonitrile/water with formic acid gradient to separate the compounds in 8.5 minutes at 0.5 mL min⁻¹ for analysis via targeted MS/MS. The use of tandem MS coupled to both chromatography systems in that study allowed for the identification of many compounds without complete separation. While GC and LC have provided many important insights into numerous atmospheric systems, there is a need for a separation method for aldehyde – amine reaction systems that does not require derivatization and can reduce the necessary separation time while still providing separation of a majority of the compounds in the mixture.”

P2L35-P3L1: not strictly true, revise

We have altered this text to be consistent with our original intended meaning, which is that modifiers and additives can expand the polarity range that can be achieved in a single chromatographic run compared to that of pure carbon dioxide mobile phase. The text now reads:

“Incorporating modifiers and additives can change the polarity of the mobile phase, thereby making it possible to separate a range of polar or nonpolar compounds and allowing SFC to be used analogously to either normal- or reverse-phase LC (Guiochon and Tarafder, 2011). As it is possible to use a mobile phase gradient that ranges from pure carbon dioxide to ~50% modifier, the mobile phase can be changed from nonpolar to relatively polar over the course of one injection onto the column. This makes SFC ideal for the separation of mixtures containing compounds with a wide range of polarities, such as the methylglyoxal – ammonium sulfate reaction system.”

Section 2: a summary (table) of all tested conditions is missing (best to put it in SI).

We have taken the advice of both referees and added Table S2 to the supplemental information showing the chromatography conditions.

2.3.1: four different BEH columns were used and only one is shortly named BEH. This may be misleading. I suggest changing this acronym.

We have clarified this by changing the abbreviation for all of the BEH columns to include “BEH” in the abbreviation.

P6L11 and Fig.2: Amide column does not seem any better than C18 and HILIC – improve data representation or revise the text.

We have remade the figures to make the early elution off the columns clearer and added more figures to the supplemental information. We have also updated the text slightly, and it now reads:

“It was not possible to separate the compounds of interest with the BEH C18 column, as can be seen in Figs. 2 and S1-S8, likely due to the nonpolar stationary phase. With a methanol modifier, most compounds eluted within 2 minutes even when the starting conditions contained 100% carbon dioxide. Varying degrees of separation were observed with the HILIC-based stationary phases (HILIC, BEH Amide, BEH 2-EP, and BEH). Elution times for many compounds range from <1 minute to 20 minutes, which is approximately when the mobile phase reaches its most polar condition. EICs for the first 11 minutes on each column using a methanol modifier are shown in Fig. 2, and all other chromatograms are shown in Figs. S1-S8. It was expected that the HILIC column would efficiently separate this mixture, since it is composed of bare silica and is often used in separations of similar, highly polar aqueous mixtures (Laskin et al., 2017). However, while separation on the HILIC column was improved over that of the BEH C18 column, there were still many wide, coeluting peaks between 1 and 3~minutes along with peaks that eluted much later (7 and 13 minutes), indicating that while some separation is occurring, many compounds are not well separated. BEH columns with polar functionalities such as the BEH Amide and BEH 2-EP combined with a 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. However, both exhibited similar features as the HILIC column, with several compounds eluting within 3 minutes, followed by wider peaks between 4 and 9 minutes.”

P6L13-14: how do you know how many compounds elute after 12 min? It is better to say that most compounds efficiently separate within 12 min...

We agree that this clarification will make the text more accurate, and we have changed the wording to state that:

“All columns provided better separation of the compounds that eluted within 11 minutes than 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 (see Figs. S1-S8).

P6L20-29: As already stated above, the usage of C18 and HILIC seems fundamentally inappropriate. If they were treated differently, explain in detail how.

We disagree that the BEH C18 and HILIC columns are fundamentally inappropriate for SFC separations. There is literature precedent for using both types of columns with this chromatography system. C18 columns have been used with SFC in the past to separate mixtures of imidazole derivatives, including some of the same compounds we are analyzing herein (Patel et al., 1998; Parlier et al., 1991). We found that the BEH C18 column could not separate the compounds of interest very well, but there was minimal separation. Due to the reverse-phase nature of this column, we also initially tested a polar-to-nonpolar gradient and found that there was no separation of the peaks, indicating that while this column may not work well for this system, there some separation does occur when used in a nonpolar-to-polar solvent gradient. HILIC columns have been successfully used with SFC using a variety of polar organic solvents as a modifier (methanol, acetonitrile, and isopropanol with small acids and amine additives) (Bieber et al., 2017; Dispas et al., 2012; Lesellier and West, 2015; West et al., 2012). While water is useful for these separations, separation can still be achieved with the CO₂/polar organic solvent gradients used for SFC. In fact, several of the columns used herein are HILIC columns (HILIC, BEH, BEH Amide, and BEH 2-EP) and all provide varying degrees of separation between compounds. The HILIC column used here did not provide the same chromatographic resolution as the BEH column, but was able to separate many of the compounds in the mixture.

P6L26: BEH Amide and 2-EP are not HILIC columns, but rather contain polar stationary phase. While these BEH columns do contain a polar stationary phase, they are based on HILIC technologies and are often considered to be HILIC columns by the manufacturer (Waters) and according to several published studies (Kitanovski et al., 2012; King et al., 2019). The column chemistry is slightly different from a standard HILIC column, but has many similarities, as are now discussed in the text.

“The HILIC column is a polar unbonded stationary phase and the BEH stationary phase is an ethylene bridged HILIC formulation. Both are intended to separate polar compounds. The BEH Amide and BEH 2-EP columns are modified BEH columns, with amide or 2-ethylpyridine groups bonded to the stationary phase. Both columns have previously been used for the separation of polar compounds containing amines and alcohols, functional groups found in the methylglyoxal – ammonium sulfate reaction mixture (Lesellier and West, 2015).”

P8L1: I don't understand: *elute much more cleanly from the column*

Thank you for pointing out this unclear wording. We have changed the text to explain the differences we see in the chromatogram with the ammonium formate additive. The text now reads:

“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 modifier (Fig. 3), and the compounds that elute after 11 minutes do so with better resolution and with sharper peaks than the methanol or methanol with formic acid modifiers.”

P8L6: the reaction was left for 1 month to get sufficient amounts of products for the detection, so I don't expect that a few minutes of reaction between the carbonyls and ammonium on the column can produce the measured artefacts.

We agree that the interaction of carbonyl analytes and ammonium on the column is not likely to have caused the increase in nitrogen-containing products, rather that the rapid drying of the droplets in the ESI source is where this reaction is occurring. However, we have not tested this hypothesis and therefore only know that an interaction between the species is occurring somewhere in the SFC-MS system. We have changed the wording in the manuscript to state that: “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 and do not always elute at the same times as in these systems. Thus, the increased nitrogen-containing compounds are being formed within the instrument. Ammonium is reacting with carbonyls in the sample either on the column or in the ionization source, most likely in the ionization source. Previous studies have noted increased oligomer signals as a result of ESI ionization, likely due to the rapid increase in analyte concentrations within the droplets upon drying (Hastings et al., 2005). This is likely happening here, with methylglyoxal and ammonium reacting within the droplets. This is further supported by the fact that while some earlier masses elute at similar times to the methanol system, there are nitrogen-containing compounds detected at times that do not match peaks eluted with a pure methanol modifier. It is possible that compounds eluting from the column at this time are methylglyoxal oligomers formed through aldol condensation that then react with the ammonium after elution (Krizner et al., 2009). It is also possible for analytes to react with the mobile phase during SFC analysis (Lesellier and West, 2015).”

P8L16-17: same also for LC and GC

While it is true that temperature changes the mobile phase in all chromatographic systems discussed, the effects of temperature on SFC can be counterintuitive to those used to thinking in terms of the temperature effect on viscosity and therefore retention time in GC or LC. We have changed the text to make this difference clearer, and it now reads:

“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, SFC is similar to GC and LC in that retention and separation are not only controlled by the stationary and mobile phases, but also temperature and pressure (Saito, 2013). Column temperature may become an important parameter for separation of compounds and can significantly change retention. At lower temperatures, mobile phase density and solvating power increase. When this occurs, retention

times tend to shorten, which is the opposite of what would be expected for GC or LC (Saito, 2013). However, this is balanced by the effect of lower temperatures on the kinetic partitioning of analytes into the stationary phase as is typically seen in LC or GC. Due to these several convoluting factors affecting analyte-mobile phase interactions, it is useful to test the effect of temperature on the separation of these mixtures.”

P10L29: the newly identified low-intensity signals are not always separated on the column (see for instance m/z 83,87,98,139 etc.) – they probably appear because of better performing MS detection. Also, when EIC is measured, the quality of chromatographic separation often doesn't need to be supreme; selectivity is already assured with the selection of the ion.

The reviewer is correct, and there is a combination of factors that leads to the detection of these new masses. We have reworded this text to make it clearer that the separation method is not the only factor in play here and made changes throughout the document to make it clear that there are many factors that allow us to see these compounds.

“While it is not surprising to detect methylimidazole compounds within this system, the combination of tandem MS and chromatography that allows for separation of compounds with similar masses allows for the observation of these low intensity signals that have not been identified in previous studies.”

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We thank the reviewer for their included suggestions, questions, and points for clarification. We address the reviewer's feedback below. Our responses to the reviewers are included in italics after each reviewer comment. Additionally, the revised version of the manuscript is added.

-GENERAL REMARKS The authors present a supercritical fluid chromatography–mass spectrometry method for separation and detection of aqueous atmospheric aerosol mimics. In this study SFC-MS was used to study methylglyoxal and ammonium sulphate creation mixture as mimics of reaction mixtures in atmospheric droplets. ESI and APCI ionisation modes were used for the detection of various species present in the reaction mixture. five different columns were screened to optimise separation and fourteen reaction products, detected for the first time, were reported. The study address challenges like separation of compounds with different polarities and reduction of analysis time. Identification of unknown fragments/compounds can be a strength of the work presented here. The study is relevant for the scientific community however the study design is not comprehensive and several important aspects of experimental work are not completely described. I give some suggestions hereinafter.

- MAJOR COMMENTS

- Aerosols are a mixture of solid particles and liquid droplets suspended in gases (air). The terms use of terms e.g. aqueous molecules, aqueous atmospheric systems and atmospheric droplets should be explained and the terminology should be consistent throughout the text to assist readers.

We have revised the manuscript with respect to this suggestion, and have made our terminology more consistent.

- Introduction needs to be revised, ideally introduction should address 1) gaps in knowledge, 2) specific research question(s), 3) approach used to answer the research question(s) and 4) comparison with already available knowledge. In the current state, large part of introduction focuses on the theory of SFC which better fits in an SFC (P2, L28-35 and P3, L1-6 needs to be revised and should focus more on the analytes in question).

We have revised and restructured major parts of the introduction and removed some of the general SFC theory while focusing more on the atmospheric compounds in question.

– Authors compare SFC with LC and GC. With the development of UHPLC, analysis time has significantly reduced. Describing the benefits of SFC should not be stop having a nice comparison with available UHPLC methods.

While revising the introduction to address the concerns above, we have added a discussion of current UPLC methods in use for this and similar systems.

“A significant challenge to the identification and quantification of atmospheric reaction 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 (Nozière et al., 2015). The two most commonly used separation techniques are gas chromatography (GC) and liquid chromatography (LC). 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 ambiguity in structural identification (Nozière et al., 2015). Reverse-phase high performance LC (HPLC) and ultra performance LC (UPLC) are also commonly coupled to

mass spectrometry (MS) for separation and identification of atmospheric compounds (Lin et al., 2015; Nozière et al., 2015; Aiona et al., 2017; De Haan et al., 2018; Jayarathne et al., 2018), and several studies have used these techniques to study aldehyde – ammonium/amine reaction systems (Lin et al., 2015; Aiona et al., 2017; Kampf et al., 2016; Kampf et al., 2012). Lin et al. (2015) and Aiona et al. (2017) provided comprehensive studies of chromophores found in the methylglyoxal – ammonium sulfate system before and after photolysis using similar HPLC methods. Lin et al. (2015) found that an acetonitrile/water gradient with an SM-C18 column provided the best separation in 80 minutes at 0.2 mL min⁻¹. Kampf et al. (2012) analyzed a glyoxal – ammonium sulfate mixture with an acetonitrile/water gradient on an Atlantis T3 (C18) column in 60 minutes at 0.2 mL min⁻¹. A similar study analyzed the nitrogen-containing compounds from the reaction of small dicarbonyls and amines on HPLC and UPLC (Kampf et al., 2016). The HPLC method utilized the same Atlantis T3 column and an acetonitrile/water gradient to separate these reaction mixtures in 19 minutes at 0.5 mL min⁻¹, while the UPLC method used a Hypersil Gold C18 column with an acetonitrile/water with formic acid gradient to separate the compounds in 8.5 minutes at 0.5 mL min⁻¹ for analysis via targeted MS/MS. The use of tandem MS coupled to both chromatography systems in that study allowed for the identification of many compounds without complete separation. While GC and LC have provided many important insights into numerous atmospheric systems, there is a need for a separation method for aldehyde – amine reaction systems that does not require derivatization and can reduce the necessary separation time while still providing separation of a majority of the compounds in the mixture.”

- In modern SFC, there is a huge range of packed columns available today. The authors should motivate why BEH (three types), HILIC and C18 columns were used for the screening for suitable stationary phase.

We have added a paragraph explaining the choice of columns we used in this work. The paragraph now reads:

“The packed columns used for SFC separations are similar to those used for LC systems, and many UPLC columns can be used with 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 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 SFC separation (see Table S1) (Lesellier and West, 2015). The BEH C18 column was chosen as a nonpolar comparison that is similar to those used in previous studies to separate imidazole derivatives and other polar molecules with SFC (Parlier et al., 1991; Patel et al., 1998; Lesellier and West, 2015). HILIC columns are commonly used to separate atmospheric compounds with LC (Nozière et al., 2015; Laskin et al., 2017) and have previously been used for the separation of samples containing a range of polarities with an SFC system (West et al., 2012; Bieber et al., 2017). Therefore, several HILIC stationary phases were chosen for this work. The HILIC column is a polar unbonded stationary phase and the BEH stationary phase is an ethylene bridged HILIC formulation. Both are intended to separate polar compounds. The BEH Amide and BEH 2-EP columns are modified BEH columns, with amide or 2-ethylpyridine groups bonded to the stationary phase. Both columns have previously been used for the separation of polar compounds containing amines and alcohols, functional groups found in the methylglyoxal – ammonium sulfate reaction mixture (Lesellier and West, 2015).”

-Section 2.3.2, L9 (optimal mobile phase conditions varied slightly with the identity of the column.....). Why different mobile phase conditions were used to compared column efficiencies? For any comparison all the variables must be same except the one subject to comparison.

We agree that with a true comparison, only one factor should change. However, we did not intend to compare all columns with the same mobile phase composition. Instead, our intention was to compare the best chromatography we are able to achieve from each column to determine which columns might be most useful for these analyses. However, due to the nature of these systems, the optimized mobile phase conditions were very similar, as can be seen in the newly created Table S2 in the Supplemental Information. Most of the differences in gradient profiles were due to a slightly different ramping speed for the modifier.

Secondly what were the varied mobile phase compositions used for comparison? Why not to make use of supplementary information and add a figure/table to describe the actual experimental conditions?

We have taken the advice of both referees and added Table S2 to the Supplemental Information with this information.

- Section 3.2.2, L6-7: include chromatograms in supplementary information

We have added several figures (Figs. S1-9) to the supplemental information, including those showing all the chromatograms taken for the acetonitrile, methanol, and methanol with formic acid modifiers as well as a comparison between similar modifiers on each column. These show the same data, but help to show how both columns and modifiers affect separations.

- Section 3.3, L30-34: include mass spectra in supplementary information

We have added figures S12-S14 to the supplementary information showing the fragmentation data for the peaks we discuss in this section.

- ESI and APCI methods were not optimized for higher signal of the analyte, therefore, it is inappropriate to claim that APCI is not a better method based on the results. However, more information can be included from literature to motivate if APCI is a suitable ionisation source for polar compounds.

While it is time consuming to optimize either ESI or APCI conditions for each compound in such a complex mixture, optimization was performed with both ionization methods to ensure a large range of compounds were detected with each method. We have made this clear in the text:

“Data collection was performed with both ESI and APCI ionization modes for comparative purposes. Optimizing the MS method for each individual mass is time-consuming during both method development and data collection, so each ionization method was generally optimized to maximize as many signals as possible using direct infusion into the MS. 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 ionization of any compound more basic than the resulting solution, making its addition very useful for these analyses. Most compounds

were also detected in APCI mode, but at much lower intensities than in ESI mode (~20×, see Fig. S12). Therefore, ESI is the preferred mode for analysis of this aerosol mimic system, though there may be some compounds that have lower ionization efficiency with ESI and may benefit from the use of APCI in some solutions, as it has often been used for analysis of slightly less polar compounds such as polycyclic aromatic hydrocarbons, esters, and pyrazine derivatives (Walgraeve et al., 2010; Laskin et al., 2015; Nozière et al., 2015; Laskin et al., 2017; Hawkins et al., 2018). As a range of polarities are found in many atmospheric samples, 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 S3 and all extracted ion chromatograms (EICs) are shown in Fig. S14. The chromatograms presented in this study are a combination of all EIC signals in Table S3 and Fig. S14.”

MINOR COMMENTS

- Suitable keywords should be included with abstract
Keywords are not included for AMT manuscripts.

- P2, L3-4: include a reference

Thank you, references have been added to these lines. The text now reads:

“The aqueous aldehyde – ammonium/amine reaction mixture is one class of reaction systems that has been shown to impact aerosol growth (Lin et al., 2015; Hawkins et al., 2016; Aiona et al., 2017; De Haan et al., 2017).”

- Section 2.2, L10: "the mixture was allowed to react for at least a month....."; an accurate time must be included

We have included more specific information here, and the text now reads:

“Due to slow room temperature reaction times at these concentrations, the mixture was allowed to react for 6-7 weeks in a dark environment before analysis (Zhao et al., 2015).”

- P4, L22: add "that" between "to ensure" and "the mobile phase"

We have changed this wording.

- P6, L25: ".....polar molecular interactions between analytes may be driving the solution through the column", a reference must be added to support the assumption

During our revision of the discussion of stationary phases, this line was removed from the text.

- P7, L5: ".....although all temperatures and modified conditions discussed below were tested on each column with similar results"; it is insufficient to state "similar results" when there is a possibility to include chromatograms in supplementary information and generate a more quality discussion

We have added several chromatograms to the Supplemental Information to help us expand upon this discussion and referred our readers to these figures in the text for clarification.

- P7, L16: Its better to discuss the strengths/weaknesses of certain mobile phase in relation to properties of analytes rather than SFC itself.

Generally, we agree that discussing the mobile phase with respect to the analyte is more useful than blanket statements about the use of additives in a chromatography method. However, we

are trying to make the point here that ammonium formate (or small amine salts) are often used as mobile phase additives in SFC and this is why we tested ammonium formate as an additive. We then go on to describe the drawbacks of using such a modifier in a system containing carbonyl analytes. This was not immediately obvious to us, and indeed our other referee points out that "I don't expect that a few minutes of reaction between the carbonyls and ammonium on the column can produce the measured artefacts." Therefore, we feel that it is important to make the point that while ammonium formate is a common SFC additive, we must be careful of the reactions that occur within the column or ionization source, even if we do not expect them to occur.

- P9, L8-15: the text should be revised considering both mobile phase density and kinetic effects should be considered in relation to retention times

We have revised the text to discuss how solvating power changes with temperature and density, and expanded our discussion of both the theory behind the temperature effects and the results of such effects, putting more focus on the results. This is a large section of text, so is not given immediately below, but can be found in the revised manuscript.

- The language needs revision in terms of use of article "the"

As we have revised the text, we have done so with this in mind.

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

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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~~ This study introduces supercritical fluid chromatography–mass spectrometry ~~to separate as a separation method for~~ 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 UPC²™ BEH column combined with a mobile phase gradient of carbon dioxide and methanol provided the best separation of compounds in the mixture ~~. This separation and, when coupled~~ to an electrospray ionization tandem mass spectrometer, allowed for detection of several new masses in the methylglyoxal – ammonium sulfate reaction mixture as well as the possible identification of several isomers. This analysis method can be extended to ~~analyze other aqueous atmospheric systems~~ other aqueous aerosol mimics, including the mixtures of other aldehydes or organic acids with ammonium or small amines.

Copyright statement. TEXT

15 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 many of which are 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 this uptake may be reversible and 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, with the inorganic compounds present (Powelson et al., 2013; Lin et al., 2015). These reactions can change the physical and optical properties of the particle, including hygroscopicity and radiative forcing, 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 changes in the radiative forcing of SOA (Hallquist et al., 2009). In order to understand how these changes affect radiative forcing and climate, it is necessary to first identify the compounds present and the reactions that are occurring within the particle.

The aqueous aldehyde – ammonium/amine reaction mixture is one class of reaction systems that has been shown to impact aerosol growth (Lin et al., 2015; Hawkins et al., 2016; Aiona et al., 2017; De Haan et al., 2017). Light absorption of product mixtures generated from these systems often strongly resembles that of humic-like substances found in ambient SOA, indicating that these reaction systems may provide insight into reaction mechanisms occurring within atmospheric droplets (Hawkins et al., 2016; Lin et al., 2015). Methylglyoxal reacts with ammonium through imine and aldol reactions to form compounds with a variety of polarities (see Fig. 1), including cyclic imidazoles and pyrazines and acyclic aldol condensation products (Sareen et al., 2010; Lin et al., 2015; Hawkins et al., 2018). Oligomers are also formed via the addition of methylglyoxal to imidazoles or pyrazines, contributing to the complex light absorption of the system (Lin et al., 2015; Hawkins et al., 2018). Many studies have worked to understand the reactions occurring between methylglyoxal and ammonium and similar systems (e.g., aldehyde or organic acid with ammonium) to determine their significance for atmospheric radiative forcing and further reactions (Laskin et al., 2015; McNeill, 2015).

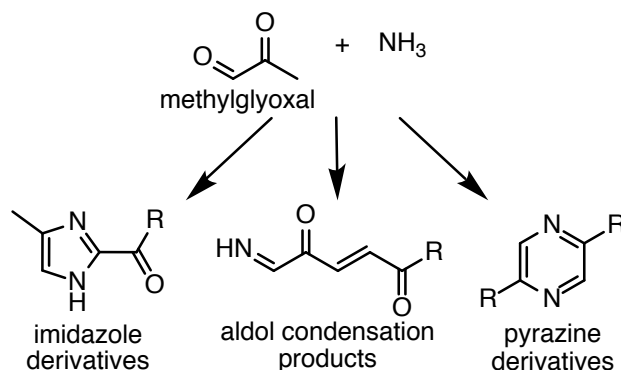


Figure 1. Methylglyoxal reacts with ammonia to form products with a variety of functional groups and polarities.

A significant challenge to the 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 (Nozière et al., 2015). The two most commonly used separation techniques are gas chromatography (GC) and liquid chromatography (LC)(~~Nozière 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 ambiguity in~~ structural identification (Nozière et al., 2015). Reverse-phase ~~LC is high performance LC (HPLC) and ultra performance LC (UPLC) are~~ also commonly coupled to mass spectrometry (MS) for separation and identification of atmospheric compounds (Lin et al., 2015; Nozière 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~~ and several studies have used these techniques to study aldehyde – ammonium/amine reaction systems (Kampf et al., 2012; Lin et al., 2015; Kampf et al., 2016; Aiona et al., 2017). Lin et al. (2015) and Aiona et al. (2017) provided comprehensive studies of chromophores found in the methylglyoxal – ammonium sulfate system before and after photolysis using similar HPLC methods. Lin et al. (2015) found that an acetonitrile/water gradient with an SM-C18 column provided the best separation in 80 minutes at 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, Kampf et al. (2012) analyzed a glyoxal – ammonium sulfate mixture with an acetonitrile/water gradient on an Atlantis T3 (C18) column in 60 minutes at 0.2 mL min⁻¹. A similar study analyzed the nitrogen-containing compounds from the reaction of small dicarbonyls and amines on HPLC and UPLC (Kampf et al., 2016). The HPLC method utilized the same Atlantis T3 column and an acetonitrile/water gradient to separate these reaction mixtures in 19 minutes at 0.5 mL min⁻¹, while the UPLC method used a Hypersil Gold C18 column with an acetonitrile/water with formic acid gradient to separate the compounds in 8.5 minutes at 0.5 mL min⁻¹ for analysis via targeted MS/MS. The use of tandem MS coupled to both chromatography systems in that study allowed for the identification of many compounds without complete separation. While GC and LC have provided many important insights into numerous atmospheric systems, there is a need for a separation method for aldehyde – amine reaction systems that does not require derivatization and can reduce the necessary separation time while still providing ~~efficient separation of~~ separation of a majority 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~~) (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~~ 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).~~

35 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 and ion-pairing reagents as additives~~ (~~Parlier et al., 1991~~) ~~. Incorporating modifiers and additives~~ can change the polarity of the mobile phase, thereby making it possible to separate a ~~range of~~ polar or nonpolar compounds ~~using SFC and allowing it and allowing SFC~~ to be used analogously to either normal-
5 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~~ ~~As it is possible to use a mobile~~
10 ~~phase gradient that ranges from pure carbon dioxide to ~50% modifier, the mobile phase can be changed from nonpolar to relatively polar over the course of one injection onto the column.~~ 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 methylglyoxal – ammonium~~
15 ~~sulfate reaction system~~. 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
20 been observed using these ionization techniques (Kampf et al., 2012; Sareen et al., 2013; Lin et al., 2015; Wong et al., 2017).
~~Methylglyoxal reacts with ammonia to form products with a variety of functional groups and polarities.~~
~~The methylglyoxal and~~ ~~The methylglyoxal –~~ 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~~ ~~observed products~~ (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
25 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~~ ~~four~~ 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~~ ~~A~~
~~method that couples SFC to ESI-MS~~ is presented that allows for the separation ~~of and identification of products within~~ this complex mixture in 30 minutes or less and with no sample preparation.

2 Materials and methods

2.1 Reagents

- 5 Methylglyoxal (40% w/w in H₂O) and ammonium sulfate were purchased from Sigma Aldrich. Food grade carbon dioxide was obtained from Airgas. Methanol (Optima™ LC/MS Grade), acetonitrile (Optima™ LC/MS Grade), ammonium formate (10 mM with 0.05% formic acid) in methanol (LC/MS grade), and formic acid (Optima™ LC/MS Grade) were purchased from Fisher Chemical.

2.2 Methylglyoxal and ammonium sulfate mixtures

- 10 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 6–7 weeks 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 (UPC²) SFC system coupled to a Waters XEVO TQD triple quadrupole mass spectrometer (~~MS~~).

- 20 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

- 25 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 that the mobile phase stays in a near-supercritical state throughout the entire column. In the UPC² 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, 30 the flow from the UPC² 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.

2.3.1 Columns

5 The columns used for analysis were chosen for their range of polarities and variety of functional groups. They are: ACQUITY UPLC® BEH Amide ([BEH Amide](#)), CORTECS™ UPLC® HILIC (HILIC), Viridis UPC²™ BEH (BEH), ACQUITY UPLC® BEH C18 ([BEH C18](#)), and Viridis UPC²™ BEH 2-Ethylpyridine ([BEH 2-EP](#)). Specific details about each column [and chromatographic conditions](#) can be found in [TableTables S1 and S2](#).

2.3.2 Modifiers

10 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 [acetonitrile](#), 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~~ [2–5](#) minutes, increased ~~linearly~~ to 45% modifier until ~~20–15–22~~ minutes, then held at 45% modifier until [approx. 27](#) minutes before returning to initial conditions
15 for the last ~~3–4~~ 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. [Specific details can be found in Table S2](#). When switching modifiers, the columns were allowed to flush with the new modifier for at least 1 hour at 1.0 ~~mL~~ [mL](#) min⁻¹ to ensure there were no residual additive ions on the column (Berger and Deye, 1991).

2.3.3 Column temperature

20 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-with~~ both ESI and APCI ionization modes for comparative purposes. [Optimizing the MS method for each individual mass is time-consuming during both method development and data collection, so each ionization method was generally optimized to maximize as many signals as possible using direct infusion into the MS](#). 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
25 of formic acid in the makeup flow (ESI solvent) enhances ~~the~~ ionization of any compound more basic than the resulting
30

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 in ESI mode ($\sim 20\times$, see Fig. S1S12). Therefore, ESI is the preferred mode for analysis of ~~these aerosol mimics~~this aerosol mimic system, 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~~, as it has often
5 been used for analysis of slightly less polar compounds such as polycyclic aromatic hydrocarbons, esters, and pyrazine derivatives (Walgraeve et al., 2010; Laskin et al., 2015; Nozière et al., 2015; Laskin et al., 2017; Hawkins et al., 2018). As a range of polarities are found in many atmospheric samples, 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-S3 and all extracted ion chromatograms (EICs) are shown in Fig. S3S14. The chromatograms presented in this study are a combination of all ~~the~~-EIC signals
10 in Table S2-S3 and Fig. S3S14.

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~~
UPLC columns can be used ~~on~~-with an SFC system. Under the conditions presented here, nonpolar compounds should
15 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 UPC²-SFC separation (see Table S1) (Lesellier and West, 2015). The BEH 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~~
5 ~~inset of Fig. 2 for the BEH column. Most compounds elute within 12 minutes on all the columns tested, and all columns~~ that is similar to those used in previous studies to separate imidazole derivatives and other polar molecules with SFC (Parlier et al., 1991; Patel et al., 1998; Lesellier and West, 2015). HILIC columns are commonly used to separate atmospheric
compounds with LC (Nozière et al., 2015; Laskin et al., 2017) and have previously been used for the separation of samples
10 containing a range of polarities with an SFC system (West et al., 2012; Bieber et al., 2017). Therefore, several HILIC stationary
phases were chosen for this work. The HILIC column is a polar unbonded stationary phase, and the BEH stationary phase is an ethylene bridged HILIC formulation. Both are intended to separate polar compounds. The BEH Amide and BEH 2-EP columns are modified BEH columns, with amide or 2-ethylpyridine groups bonded to the stationary phase. Both columns have
previously been used for the separation of polar compounds containing amines and alcohols, functional groups found in the methylglyoxal – ammonium sulfate reaction mixture (Lesellier and West, 2015).
15 All columns provided better separation of ~~these compounds than the~~ the compounds that eluted within 11 minutes than later eluting compounds (e.g., m/z 83, 97, and 126), which either coeluted or had very wide, noisy peaks that overlapped significantly

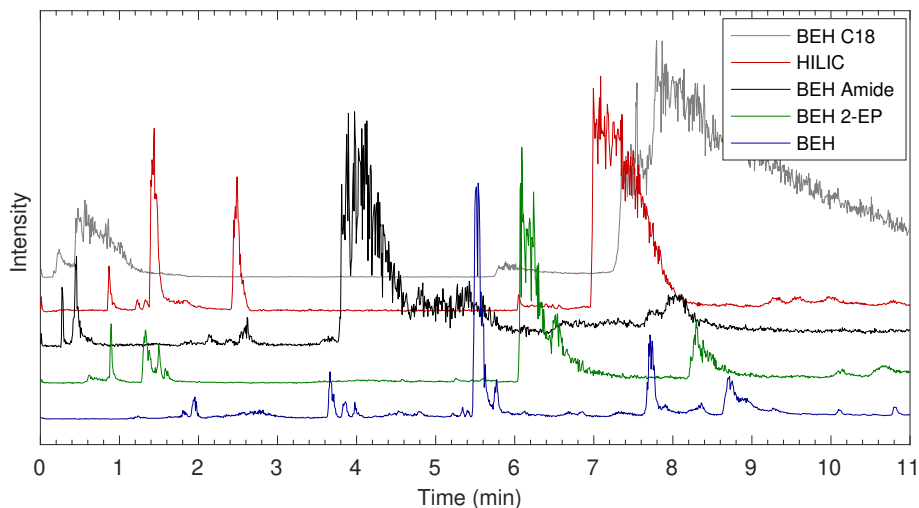


Figure 2. Comparison of EICs from five columns using a methanol modifier. The first 11 minutes are shown to make clear the differences in separation between lower intensity peaks. Full chromatograms are shown in Fig. S3 and the masses monitored are shown in Table S3. The more polar BEH columns (BEH, BEH Amide, BEH 2-EP) provide better separation, and the BEH provides the best separation of those columns tested.

depending on eluent conditions (see Figs. S1-S8). These peaks can easily be distinguished via their m/z values through the use of EIC.

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.

20 Other methylimidazole derivatives with attached methylglyoxal oligomers elute much earlier, likely since the addition of the methylglyoxal moieties decreases the polarity of the molecule-molecular polarity and, as a result, interactions with the column are decreased.

25 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.

30 Efficient separation on the C18 and HILIC columns-It was not possible under the conditions tested, as to separate the compounds of interest with the BEH C18 column, as can be seen in Figs. 2 and S1-S8, likely due to the nonpolar stationary phase. With a methanol modifier, 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. Varying degrees of separation were observed with the HILIC-based stationary phases (HILIC, BEH Amide, BEH 2-EP, and BEH). Elution times for many compounds range from <1 minute to 20 minutes, which is approximately when the mobile phase reaches its most polar condition. EICs for the first 11 minutes on each column using a methanol modifier are shown in Fig. 2, and all other chromatograms are shown in Figs. S1-S8. 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, ~~while~~ separation on the HILIC column was ~~similar to improved over~~ that of the BEH 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 there were still many wide, coeluting peaks between 1 and 3 minutes along with peaks that eluted much later (7 and 13 minutes), indicating that while some separation~~
5 ~~is occurring, many compounds are not well separated.~~ BEH columns with polar functionalities such as the ~~Amide and BEH~~ ~~Amide and BEH~~ 2-EP combined with ~~the a~~ 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~~ However, both exhibited similar features as the HILIC column, with several compounds eluting
10 ~~within 3 minutes, followed by wider peaks between 4 and 9 minutes. With all modifiers tested, the~~ best separation was achieved with the BEH column, ~~with elution spread out until approx. 11 minutes using the methanol-based modifiers.~~ 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~~ other HILIC columns, the less intense
15 peaks that eluted before 11 minutes also had higher intensities than with the other columns. Therefore, further analysis will focus on the BEH column, although ~~all the~~ temperature and modifier conditions discussed below were tested on each column with ~~similar results.~~ ~~consistent results, as can be seen in Figs. S1-S11 in the Supplemental Information.~~

3.2.2 Modifiers

~~Several~~ Four modifiers were tested to determine suitable mobile phase conditions for separation on each column (see Fig. 3
20 for an example ~~and Figs. S1-S8 for all chromatograms~~). 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~~ ~~compounds were still eluting from the column after~~
~~27 minutes, when the mobile phase was switched back to initial conditions (see Fig. S1). Preliminary testing showed that these~~
~~compounds are not finished eluting even if the modifier is held at 45% for an additional 15 minutes, indicating that acetonitrile~~
5 ~~is not polar enough to elute all compounds present~~ 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
10 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~~ ~~could explain~~ the improved separation ~~due to of~~ 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.

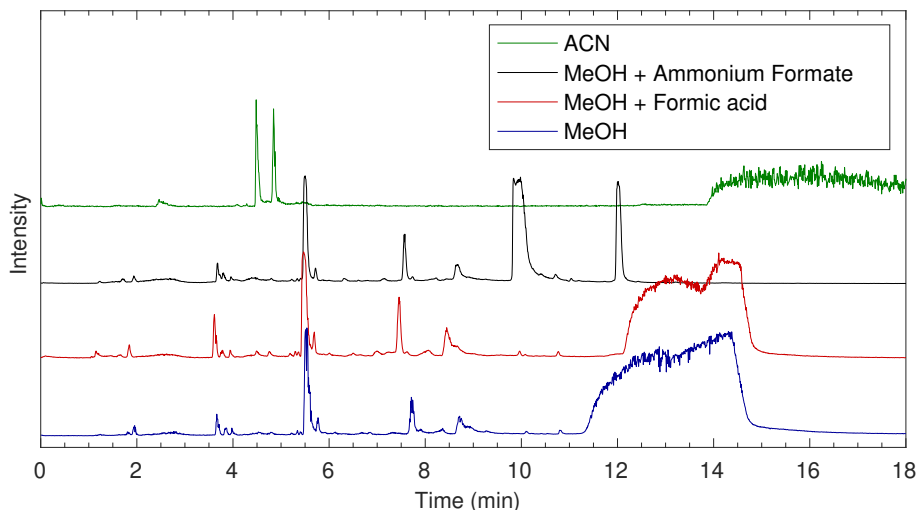


Figure 3. Comparison of EICs showing chromatography from methanol-based modifiers on the BEH column. The masses monitored are shown in Table S2 S3. Mobile phase modifiers can affect separation on the column as well as chemistry occurring in the ionization source.

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. 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). 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 modifier (Fig. 3), and the compounds that elute after 11 minutes also elute much more cleanly from the column do so with better resolution and with sharper peaks than the methanol or methanol with formic acid modifiers. 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 and do not always elute at the same times as in these systems. Thus, the increased nitrogen-containing compounds are being formed within the instrument. The ammonium is likely reacting with the Ammonium is reacting with carbonyls in the sample either on the column or in the ionization source during rapid drying of the sample., most likely in the ionization source. Previous studies have noted increased oligomer signals as a result of ESI ionization, likely due to the rapid increase in analyte concentrations within the droplets upon drying (Hastings et al., 2005). This is likely happening here, with methylglyoxal and ammonium reacting within the droplets. This is further supported by the fact that while some earlier masses elute at similar times to the methanol system, there are nitrogen-containing compounds detected at times that do not match peaks eluted with a pure methanol modifier. It is possible that compounds eluting from the column at this time are methylglyoxal oligomers formed through aldol condensation that then react with the ammonium after elution (Krizner et al., 2009). It is also possible for analytes to react with the mobile phase during SFC analysis (Lesellier and West, 2015). 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. Since ammonium formate additives in this system lead to falsely enhanced signals of nitrogen containing compounds, no further analysis was carried out in this work, and ammonium formate modifiers are not included in Figs. S1-S7.

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, and the optimal modifier will depend on the compound mixture in question. As there is little difference between separations with methanol and methanol with formic acid on the BEH column in this system, further analysis in this work uses pure methanol as the mobile phase modifier.

10 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,~~ SFC is similar to GC and LC in that 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~~ Column temperature may become an important parameter for separation of compounds and can significantly change retention. At lower temperatures, ~~the mobile phase density is higher and~~ and solvating power increase. When this occurs, retention times tend to shorten, which is the opposite of what would be expected for GC or LC (Saito, 2013). However, this is balanced by the effect of lower temperatures on the kinetic partitioning of analytes into the stationary phase as is typically seen in LC or GC. ~~Therefore, it can be~~ Due to these several convoluting factors affecting analyte-mobile phase interactions, it is 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) (Guiochon and Tarafder, 2011; Saito, 2013). 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 ~~—(see Figs. S9-S11 for a comparison).~~ Retention time typically ~~increased~~ changed slightly with an increase in temperature, but this small change did not necessitate a change in mobile phase conditions for optimal separations. No trend in retention time was seen with some modifier-column combinations, such as methanol-HILIC or methanol-BEH 2EP, while others showed obvious differences, such as acetonitrile-BEH, formic acid-BEH Amide, and methanol-BEH. Beyond retention times, peak shapes can also be affected by column temperature, as can be seen on the BEH column with an acetonitrile modifier. The peak at 6 minutes at 35°C elutes as a narrower peak in 4.8 minutes at 55°C (see Fig. S9). On the BEH column with the methanol modifier, separation improves at 55°C as compared to 35°C or 45°C (see Fig. 4). ~~The increased temperature decreases the~~

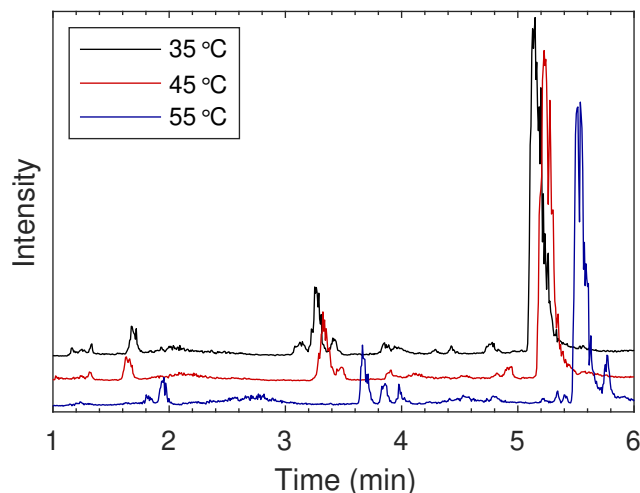


Figure 4. Comparison of EICs showing separation at various temperatures on the BEH column with MeOH as the modifier. Separation is improved at higher temperatures. On all columns, most peaks elute slightly earlier at lower temperatures and are somewhat sharper (see Figs. S9-S11).

15 ~~density of the mobile phase, which changes its interaction with some analytes. While~~ With this system, retention times tended to increase with increased temperatures, which was generally the opposite of what was observed for the other columns. As the solvating power of the mobile phase increases, interactions between the mobile phase, stationary phase, and analyte begin to change. Interestingly, as can be seen in Fig. 4, 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 affected to the same degree by this change in

20 mobile phase. The retention time of the larger peak (mostly comprised of m/z 125) at 5.2 minutes at 35 °C is not significantly affected as temperature is increased, but the fact that the small peak that is present (m/z 181) at 5.8 minutes separates from this larger peak at 55 °C but is not separated from the larger nearby peak at lower temperatures indicates that this compound was far more affected by the change in temperature than the compound or compounds that comprise the larger peak. Other compounds that are affected by this change in temperature elute near 3.5 minutes and have better resolution at 55 °C. Overall,

25 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. temperature decreased (see Figs. S9-S11). 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 mobile phase, and analyte. Therefore, temperature is a variable that must be tested for each individual system to determine how separation will be affected.

30 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-S3~~ 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-S3~~ 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.~~

~~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).~~

~~The chromatograms individual masses, especially in the case of coeluting peaks, and allow for further analysis of that mass (Lin et al., 2015). 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 compounds 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 (see Fig. S17), 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 ~~S2S3~~ and Fig. S17). 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 the combination of tandem MS and chromatography that allows for separation of compounds with similar masses allows for the observation of~~ these low intensity signals that have not been identified in previous studies ~~using LC for separation.~~

Tandem MS also ~~shows that many of the~~ indicates that many 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~~ identical. Many of the higher mass

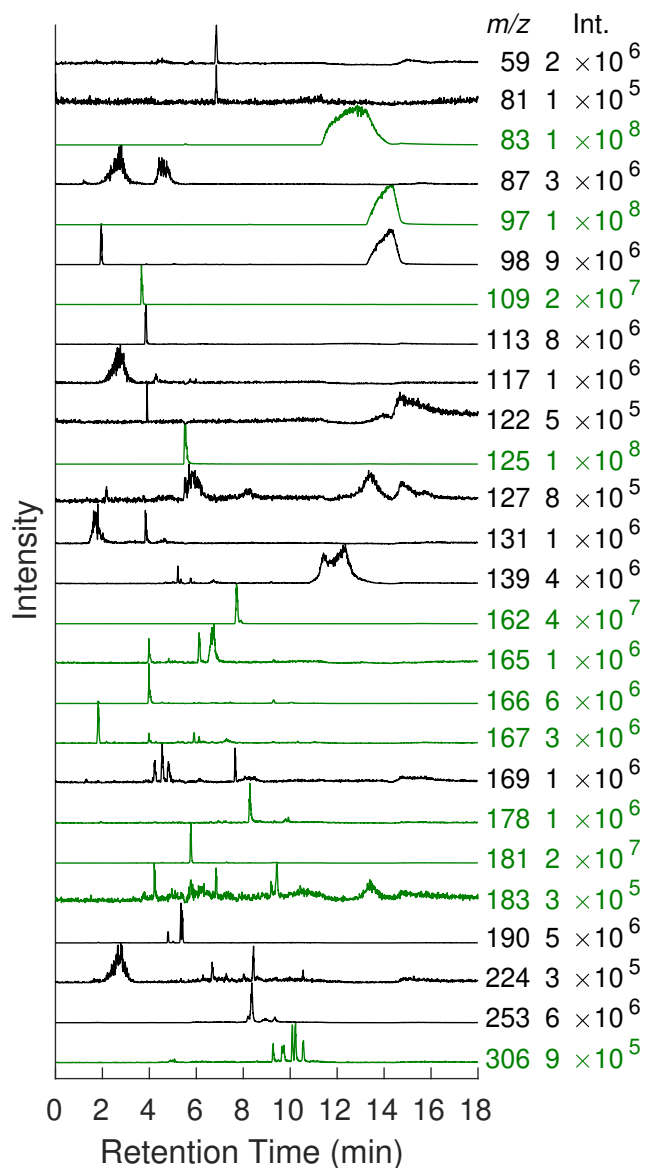


Figure 5. EICs of selected m/z values seen under the conditions described above. The intensity of the largest peak is given to the right of each trace. All chromatograms have been normalized to their maximum value for ease of viewing due to the very intense signals given by imidazole derivatives. Traces 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).

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~~-108 (see Fig. S15). Similarly, there are

two major peaks in the EIC for m/z 165 (6.1 and 6.7 minutes, see Fig. 5), and both fragment to m/z 43 (very low intensity), 123, and ~~147-147~~ (see Fig. S16). 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~~ alternative 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~~ The ability to monitor various masses via EIC or tandem MS along with a separation technique that can separate many of the less intense peaks in the chromatogram leads to the observation of previously published products of this system and several new masses, including the detection of likely isomers.

~~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~~ aqueous SOA 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; Nozière 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 the products formed within the atmospheric aqueous phase. SFC is a useful tool for the separation of these reaction mixtures and can ~~be utilized to~~ add to current analysis techniques 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.

20 *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: 31 May 25 2019).

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