Atmospheric
Measurement
Techniques
Open Access

Discussions

EGU

**AMTD**

Interactive
comment

# *Interactive comment on* "Separation and detection of aqueous atmospheric aerosol mimics using supercritical fluid chromatography–mass spectrometry" *by* D. N. Grace et al.

**D. N. Grace et al.**

gallowam@lafayette.edu

Received and published: 30 May 2019

*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.

[Printer-friendly version]

[Discussion paper]

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

Interactive comment

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 (Noziére 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 (Noziére 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; Noziére 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 (Noziére 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 sep-*

*arate 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 modi-*

*fiers 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 ($\sim 20\times$, 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; Noziére 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 manucripts.*

- 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 then 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.*

REFERENCES:

Aiona, P. K., Lee, H. J., Leslie, R., Lin, P., Laskin, A., Laskin, J., and Nizkorodov, S. A.: Photochemistry of products of the aqueous reaction of methylglyoxal with ammonium sulfate, ACS Earth Space Chem, 1, 522–532, https://doi.org/10.1021/acsearthspacechem.7b00075, 2017.

Bieber, S., Greco, G., Grosse, S., and Letzel, T.: RPLC-HILIC and SFC with mass spectrometry: Polarity-extended organic molecule screening in environmental (water) samples, Anal Chem, 89, 7907–7914, https://doi.org/10.1021/acs.analchem.7b00859, 2017.

De Haan, D. O., Hawkins, L. N., Welsh, H. G., Pednekar, R., Casar, J. R., Pennington, E. A., de Loera, A., Jimenez, N. G., Symons, M. A., Zauscher, M., Pajunoja, A., Caponi, L., Cazaunau, M., Formenti, P., Gratien, A., Pangui, E., and Doussin, J.-F.: Brown carbon production in ammonium- or amine-containing aerosol particles by reactive uptake of methylglyoxal and photolytic cloud cycling, Environ Sci Technol, 51, 7458–7466, https://doi.org/10.1021/acs.est.7b00159, 2017.

De Haan, D. O., Tapavicza, E., Riva, M., Cui, T., Surratt, J. D., Smith, A. C., Jordan, M.-C., Nilakantan, S., Almodovar, M., Stewart, T. N., de Loera, A.,

De Haan, A. C., Cazaunau, M., Gratien, A., Pangui, E., and Doussin, J.-F.: Nitrogen-containing, light-absorbing oligomers produced in aerosol particles exposed to methylglyoxal, photolysis, and cloud cycling, Environ Sci Technol, 52, 4061–4071, https://doi.org/10.1021/acs.est.7b06105, 2018.

Hawkins, L. N., Lemire, A. N., Galloway, M. M., Corrigan, A. L., Turley, J. J., Espelien, B. M., and De Haan, D. O.: Maillard chemistry in clouds and aqueous aerosol as a source of atmospheric humic-like substances, Environ Sci Technol, 20, 7443–7452, https://doi.org/10.1021/acs.est.6b00909, 2016.

Hawkins, L. N., Welsh, H. G., and Alexander, M. V.: Evidence for pyrazine-based chromophores in cloud water mimics containing methylglyoxal and ammonium sulfate, Atmos Chem Phys, 18, 12 413–12 431, https://doi.org/10.5194/acp-18-12413-2018, 2018.

Jayarathne, T., Stockwell, C. E., Bhave, P. V., Praveen, P. S., Rathnayake, C. M., Islam, M. R., Panday, A. K., Adhikari, S., Maharjan, R., Goetz, J. D., DeCarlo, P. F., Saikawa, E., Yokelson, R. J., and Stone, E. A.: Nepal Ambient Monitoring and Source Testing Experiment (NAMaSTE): Emissions of particulate matter from wood- and dung-fueled cooking fires, garbage and crop residue burning, brick kilns, and other sources, Atmos Chem Phys, 18, 2259–2286, https://doi.org/10.5194/acp-18-2259-2018, 2018.

Kampf, C. J., Jakob, R., and Hoffmann, T.: Identification and characterization of aging products in the glyoxal/ammonium sulfate system – implications for light-absorbing material in atmospheric aerosols, Atmos Chem Phys, 12, 6323–6333, https://doi.org/10.5194/acp-12-6323-2012, 2012.

Kampf, C. J., Filippi, A., Zuth, C., Hoffmann, T., and Opatz, T.: Secondary brown carbon formation via the dicarbonyl imine pathway: Nitrogen heterocycle formation and synergistic effects, Phys Chem Chem Phys, 18, 18 353–18 364, https://doi.org/10.1039/C6CP03029G, 2016.

Laskin, A., Laskin, J., and Nizkorodov, S. A.: Chemistry of atmospheric brown carbon, Chem Rev, 115, 4335–4382, https://doi.org/10.1021/cr5006167, 2015.

Laskin, J., Laskin, A., and Nizkorodov, S. A.: Mass spectrometry analysis in atmospheric chemistry, Anal Chem, 90, 166–189, https://doi.org/10.1021/acs.analchem.7b04249, 2017.

Lesellier, E. and West, C.: The many faces of packed column supercritical fluid chromatography – A critical review, J Chromatogr A, 1382, 2–46, https://doi.org/10.1016/j.chroma.2014.12.083, 2015.

Lin, P., Laskin, J., Nizkorodov, S. A., and Laskin, A.: Revealing brown carbon chromophores produced in reactions of methylglyoxal with ammonium sulfate, Environ Sci Technol, 49, 14 257–14 266, https://doi.org/10.1021/acs.est.5b03608, 2015.

Noziére, B., Kalberer, M., Claeys, M., Allan, J., D'Anna, B., Decesari, S., Finessi, E., Glasius, M., Grgíc, I., Hamilton, J. F., Hoffmann, T., Iinuma, Y., Jaoui, M., Kahnt, A., Kampf, C. J., Kourtchev, I., Maenhaut, W., Marsden, N., Saarikoski, S., Schnelle-Kreis, J., Surratt, J. D., Szidat, S., Szmigielski, R., and Wisthaler, A.: The molecular identification of organic compounds in the atmosphere: State of the art and challenges, Chem Rev, 115, 3919–3983, https://doi.org/10.1021/cr5003485, 2015.

Parlier, D., Thiébaut, D., Caude, M., and Rosset, R.: Supercritical fluid chromatography of imidazole derivatives, Chromatographia, 31, 293–296, https://doi.org/10.1007/BF02275753, 1991.

Patel, Y., Dhorda, U. J., Sundaresan, M., and Bhagwat, A. M.: Separation and estimation of five imidazoles by packed column supercritical fluid chromatography, Anal Chim Acta, 362, 271–277, https://doi.org/10.1016/S0003-2670(98)00006-3, 1998.

Walgraeve, C., Demeestere, K., Dewulf, J., Zimmermann, R., and Van Langenhove, H.: Oxygenated polycyclic aromatic hydrocarbons in atmospheric particulate matter: Molecular characterization and occurrence, Atmos Environ, 44, 1831–1846,

https://doi.org/10.1016/j.atmosenv.2009.12.004, 2010.

West, C., Khater, S., and Lesellier, E.: Characterization and use of hydrophilic interaction liquid chromatography type stationary phases in supercritical fluid chromatography, J Chromatogr A, 1250, 182–195, https://doi.org/10.1016/j.chroma.2012.05.008, 2012.

Zhao, R., Lee, A. K. Y., Huang, L., Li, X., Yang, F., and Abbatt, J. P. D.: Photochemical processing of aqueous atmospheric brown carbon, Atmos Chem Phys, 15, 6087–6100, https://doi.org/10.5194/acp-15-6087-2015, 2015.

Please also note the supplement to this comment:
https://www.atmos-meas-tech-discuss.net/amt-2019-42/amt-2019-42-AC1-supplement.pdf