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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Identification of particulate organosulfates in three megacities at the middle and lower reaches of the Yangtze River" *by* X. K. Wang et al.

X. K. Wang et al.

lin_wang@fudan.edu.cn

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RE: A point-to-point response to referee #1's comments

"Identification of Particulate Organosulfates in Three Megacities at the Middle and Lower Reaches of the Yangtze River" (acp-2015-393) by X. K. Wang , S. Rossignol , Y. Ma, L. Yao, M. Y. Wang, J. M. Chen , C. George, and L. Wang

We are grateful to referee #1 for his/her valuable technical comments, encouraging us to further develop our experimental section. A point-to-point response to this reviewer's comments, which are repeated in italic, is given below.

1. The work by Wang et al. describes molecular composition of PM2.5 samples from three megacities i.e., Wuhan (WH), Nanjing (NJ), and Shanghai (SH) determined by an UHPLC Orbitrap MS. The authors identified significant number of organosulfates and nitrooxy-organosulfates and discussed their contribution to the PM at these locations. Unfortunately the authors ignored a majority of the very important comments that were given at the initial ACPD review stage. I strongly believe that they have to be addressed before the manuscript could be published in ACP. Unfortunately I cannot support this work for publication in this current form.

Reply: We are very much surprised by this comment. In fact, during the initial review stage we had already to respond to a full, and quite extensive, review of our paper. We therefore already corrected our paper thoroughly and provided an extensive and constructive point-to-point answer to all comments we received. We therefore honestly believed, as the manuscript was accepted, that the feedbacks we provided have been appreciated. We understand now that referee 1 would like that we revise our manuscript beyond to what has already been done. Naturally, we did so and especially clarified the experimental procedures we used. We believe that our approach and data are convincing enough to warrant publication in ACP.

2. The methodology section is still confusing. Considering a very large number of detected molecules (>200), I assume the whole results and discussion section is based on the direct infusion analysis. If not, please show the LC/MS chromatogram and describe the methodology more clearly. Please add a citation for the LC/MS method.

Reply: As mentioned in the manuscript, all the analysis have been performed by LC-MS and not by direct infusion analysis. The associated methodology has been specifically developed for these analysis (therefore we cannot provide an additional reference), but we recognize that we could have still added some details. Especially, we have added some more figures in the manuscript and in the supplement showing some of the chromatograms we obtained.

We now clearly state in our revised manuscript that: “One-fourth of each filter was put into an amber vial with 6 mL of methanol (Optima® LC/MS, Fischer Scientific, UK) and shaken for 20 min on an orbital shaker set to 1000 rpm. The extract was then filtered through a glass syringe on a 0.2μm PTFE membrane (13 mm, Pall Corporation, USA). These two steps were performed twice, and the extracts of each filter were recombined and blown almost to dryness under a gentle stream of nitrogen. The extracts were then reconstituted in 1 mL of a 1:1 v/v mixture of water (Optima® LC/MS, Fischer Scientific, USA) and acetonitrile (Optima® LC/MS, Fischer Scientific, USA). For the analysis, 100 μL of the final reconstituted extract was diluted by adding 100 μL of water. 5μL of these diluted solutions (50 μL in the case of the NJSD sample) were analyzed by UHPLC (Dionex 3000, Thermo Scientific, USA) coupled to a Q-Exactive Hybrid Quadrupole-Orbitrap MS (Thermo scientific, USA). The efficiency and the repeatability on three replicates of the extraction protocol were checked using four standards: methyl sulfate, octyl sulfate, dodecyl sulfate, and camphor sulfonic acid. The results showed that their average extraction efficiencies were 71.4, 95.0, 97.7, and 94.0%, respectively (Table S2). Analytical replicates were not considered because the final sample extract volume was quite low (200 μL), and the remaining volume after the first injection was preferentially kept in case of specific analytical doubt rather than systematically injected.

Analytes were separated using a Waters Acquity HSS T3 column (1.8μm, 100$\times$2.1mm) with mobile phases consisting of (A) 0.1% formic acid in water (Optima® LC/MS, Fischer Scientific, USA) and (B) 0.1% formic acid in acetonitrile (Optima® LC/MS, Fischer Scientific, USA). The concentration of eluent B was initially kept at 1% for 2 min, then increased to 100% in 11 min, kept at 100% for 2 min, decreased to 1% in 0.1 min, and kept at 1% for 6.9 min. The Q-Exactive Hybrid Quadrupole-Orbitrap mass spectrometer was equipped with a heated electrospray ionization source. It was operated in the negative ion mode with a spray voltage of -3.0kV, a mass resolving power of 140 000 at m/z 200, and a scanning range of 50–750 m/z. The Q-Exactive mass spectrometer was externally mass calibrated daily using a 2 mM sodium acetate solution that

provides a series of negative adduct ions in the range of 50–750 m/z.

The obtained chromatograms were analyzed with Progenesis QI software (V1.0, Waters Corporation) by assuming that the extracted ions in the range of 50–750 m/z [M-H] were formed from loss of a proton from the analytes. In contrast to direct infusion, the LC separation provides meaningful help in distinguishing quasi-molecular ions and potential in-source formed adducts for the same chromatographic retention time. A molecular formula calculator was then used to mathematically assign all possible formulas for an extracted quasi-molecular ion with a mass tolerance of $\pm$2 ppm (Page 6-8, Line 152-192).”

3. It is not clear whether the mass spectra were blank corrected. If yes, please describe how. What was the signal to noise threshold for keeping the formulae for further evaluation? Orbitrap MS is known to result in the formation of shoulder ions, which significantly increase a number of identified molecules. Were the shoulder ions removed from the mass spectra? Were the analytical replicates considered? Were the C, N, P and S isotopes considered for the correct molecular formulae assignment?

Reply: The way in which the LC/MS dataset was processed is described in section “2.3 Data processing”. The peak search was performed manually. For all the detected m/z, the exact mass was extracted to obtain the extracted ion chromatogram. The occurrence of shoulder peaks eluting at an identical retention time was checked to identify potential “shoulder ions”, that could come from H2O loss for example, and to determine if the detected m/z corresponds effectively to a [M-H]- quasi-molecular ion or not. Chromatographic peak areas were then determined from the extracted quasi-molecular ion chromatograms only. The analytical replicates were not considered but one can assume an uncertainty corresponding to the whole analytical process, including the extraction step, which is now given in the supporting information. Molecular formula assignment was performed including the following elements: C, H, N, S and O. Isotopes are a classical way to confirm molecular formula assignments when the resolution of the instrument is not sufficient. Here, in most cases, the isotopic peak is

not detected due to the low intensity of the related quasi-molecular ion. Nevertheless, we are confident in our assignments as, within an error of 2 ppm, only one molecular formula is generally chemically relevant.

We now clearly state in our revised manuscript that: “These molecular formula can be expressed as CcHhOoNnSs, where c is the number of carbon atoms in the range of 1–40, h is the number of hydrogen atoms in the range of 2–80, o is the number of oxygen atoms in the range of 0–40, n is the number of nitrogen atoms in the range of 0–3, and s is the number of sulfur atoms in the range of 0–2. Formulas were further constrained by setting H/C, O/C, N/C, S/C, and double bond equivalent to carbon number ratios (DBE/C) in the ranges of 0.3–3.0, 0–3, 0–0.5, 0–0.2, and 0–1, respectively. This was done to ensure that the retrieved molecular formula do exist in nature (Fuller et al., 2012; Lin et al., 2012a; Lin et al., 2012b).

The number of ions with more than one reasonable formula within 2 ppm mass tolerance accounted only for 1.5% of the total number of tentatively determined ions, and the formulas with the best accuracy are listed in Table S3. The peak intensities of isotopically substituted ions were constrained by their low abundance and were hence not systematically checked. Compounds that satisfy these criteria and present a number of oxygen atoms greater than or equal to 4s+3n (4s+3n$\leq$o) were tentatively regarded as OSs or nitrooxy-OSs. However, other S- and N-containing compounds, such as sulfonates or compounds bearing nitro groups, may also be involved (e.g., Riva et al., 2015b; El Haddad et al., 2013) (Page 8, Line 192-209).”

“Blank filters were processed and analyzed in an identical way, and blank correction was made as follows. The presence of targeted quasi-molecular ions in the blanks was systematically verified, and if a chromatographic peak was indeed detected, then it was retained (i.e., considered as real) only if the sample-to-blank ratio of the peak area was greater than 10, with the blank value being subtracted prior to further processing (Page 9, Line 225-230).”

“The efficiency and the repeatability on three replicates of the extraction protocol were checked using four standards: methyl sulfate, octyl sulfate, dodecyl sulfate, and camphor sulfonic acid. The results showed that their average extraction efficiencies were 71.4, 95.0, 97.7, and 94.0%, respectively (Table S2). Analytical replicates were not considered because the final sample extract volume was quite low (200 μL), and the remaining volume after the first injection was preferentially kept in case of specific analytical doubt rather than systematically injected. (Page 7, Line 163-170).”

4. What was the mass scan range of the Orbitrap analysis?

Reply: The mass scan range of the orbitrap analysis was m/z 50 to 750. We have stated that “It was operated in the negative ion mode with a spray voltage of -3.0kV, a mass resolving power of 140 000 at m/z 200, and a scanning range of 50–750 m/z” (Page 7, Line 178-180).

5. Orbitrap is known to have mass dependant ion transmission. Therefore, by selecting either low or high mass range one can miss out high or low molecular weight compounds. The mass error of 2 ppm for formulae assignments is rather high, especially considering that the majority of the assigned OSs have MW >200 (see Figure 2). Kind and Fiehn (2007) demonstrated that even at 1 ppm error a very large number of chemically realistic formulae is possible in this mass range.

Reply: 2 ppm was the mass error achieved in this study. As previously discussed at the initial review stage, similar values are reported for a consequent number of recent studies also dealing with chemical analysis of aerosol samples. The rules used here to constrain molecular formula assignments within these 2 ppm (Page 8, Line 190-192), or very closed ones, are also quite common (Fuller et al., 2012; Lin et al., 2012a; Lin et al., 2012b; Tao et al., 2014). Clearly, the accuracy of 2 ppm corresponds already to high resolution analysis on complex dilute samples only available in very low amounts. The study of Kind and Fiehn (2007) is of high interest, and very helpful to constrain the possible identification of chemical formula. Especially, they considered simulated MS

spectra at ± 3 ppm mass accuracy and ± 5% isotope ratio measurement errors. In any case, we do agree that all molecular formula are only tentatively assigned and not positively identified. We have added more information about other possible formula within 2 ppm for the major ions in the footnote of Tables S3, as it had been done by Lin et al. (2012b) for example in their supplement. Also, we now state in our manuscript (Page 8, Line 201-203) that "The number of ions with more than one reasonable formula within 2 ppm mass tolerance accounted only for 1.5% of the total number of tentatively determined ions, and the formulas with the best accuracy are listed in Table S3".

6. Please mention reproducibility of the ion appearance in the mass spectra for the ions with low intensity in the replicates.

Reply: No replicate was performed. Second injections of a larger volume of sample were performed - for which the peak shapes were degraded - allowing to confirm the presence the ions but not to give reproducibility. In any case, even the "low" intensity ions present signal to noise ratios much greater than 10 or, for a very large majority, were not present in the blank at all.

7. Please also clarify whether the mass spectra (Figure 2) was obtained by integration of chromatographic area of the LC chromatogram or from a direct infusion analysis.

Reply: The intensity of individual mass spectra in Figure 2 was obtained by integration of chromatographic peaks. We stated in our manuscript (Page 8, Line 210-212) that "In this study, the abundance of an OS refers to the area of its chromatographic peak, and the number of isomers for an OS is based on the number of chromatographic peaks observed for given m/z values". Please also refer to our reply to the 2nd comment.

8. It is important that all extracts have comparable OC or PM load, overwise the comparison of molecular composition in the samples from different sampling locations is highly speculative as such differences could be attributed to the analytical artefacts (e.g., ion suppression which is known to be an issue in the ESI direct infusion analysis). Please justify it.

Reply: OC data are unfortunately unavailable and PM data are only available for Shanghai (with relatively comparable values, see Table S1). We do agree that these values would have been particularly interesting. Nevertheless, the samples were not analyzed here by ESI direct infusion. LC separation allowed to minimize artifacts due to the matrix, diluting the low amount of sample injected (5μL) in the LC solvent flow (300 μL min-1) and resolving most the matrix components (these latter being previously solvent extracted and filtered).

Reference: Fuller, S. J., Zhao, Y. J., Cliff, S. S., Wexler, A. S., and Kalberer, M.: Direct Surface Analysis of Time-Resolved Aerosol Impactor Samples with UltrahighResolution Mass Spectrometry, Anal. Chem., 84, 9858-9864, doi: 10.1021/ac3020615. Kind, T., and Fiehn, O.: Seven Golden Rules for heuristic filtering of molecular formulas obtained by accurate mass spectrometry, Bmc Bioinformatics, 8, doi:10.1186/1471-2105-8-105, 2007. Lin, P., Rincon, A. G., Kalberer, M., and Yu, J. Z.: Elemental composition of HULIS in the Pearl River Delta Region, China: results inferred from positive and negative electrospray high resolution mass spectrometric data, Environmental science technology, 46, 7454-7462, doi: 10.1021/es300285d, 2012a. Lin, P., Yu, J. Z., Engling, G., and Kalberer, M.: Organosulfates in humic-like substance fraction isolated from aerosols at seven locations in East Asia: a study by ultra-highresolution mass spectrometry, Environmental science technology, 46, 13118-13127, doi: 10.1021/es303570v, 2012b. Tao, S., Lu, X., Levac, N., Bateman, A. P., Nguyen, T. B., Bones, D. L., Nizkorodov, S. A., Laskin, J., Laskin, A., and Yang, X.: Molecular Characterization of Organosulfates in Organic Aerosols from Shanghai and Los Angeles Urban Areas by NanosprayDesorption Electrospray Ionization High-Resolution Mass Spectrometry, Environmental science technology, 48, 10993-11001, doi: 10.1021/es5024674, 2014.

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