Atmos. Chem. Phys. Discuss., 15, C7790–C7797, 2015
www.atmos-chem-phys-discuss.net/15/C7790/2015/

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.

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Received and published: 9 October 2015

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 is given below.

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(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 be done. Naturally, we will do so and especially clarify the experimental procedures we used. We believe that our approach and data are convincing enough to warrant publication in ACP.

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(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 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 still add some details. Especially, we will add some more figures (Figure S1) showing some of the chromatograms we obtained.

We will 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®

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LC/MS, Fisher Scientific, U.K.) and shaken for 20 min on an orbital shaker set at 1000 rpm. The extract was then filtered through a glass syringe onto a 0.2 μm PTFE membrane (13 mm, Pall Corporation, USA). These two steps were performed twice and the extracts of each filter were recombined and blown to almost dryness under a gentle stream of nitrogen. The extracts were reconstituted in 1 mL of a 1:1 v/v mixture of water (Optima® LC/MS, Fisher Scientific, USA) and acetonitrile (Optima® LC/MS, Fisher Scientific, USA). Blank filters were processed and analyzed in an identical way, and blank correction has been made accordingly. For the analysis, 100 μL of the final reconstituted extract was diluted by a factor 2 adding 100 μL of water. 5μL of these diluted solution were analyzed by an ultra-high performance liquid chromatograph (UHPLC, Dionex 3000, Thermo Scientific, USA) coupled to a Q-Exactive Hybride Quadrupole-Orbitrap MS (Thermo scientific, USA) in the mass range of m/z 50-750. The efficiency and the repeatability on three replicates of the extraction protocol were checked on four standards, methyl sulfate, octyl sulfate, dodecyl sulfate and camphor sulfonic acid. Protocol and results are given in the Supplement (Table S1).

Analytes were separated using a Waters Acquity HSS T3 column (1.8μm, 100$\times$2.1mm) with mobile phases consisting of (A) 0.1

The obtained chromatograms were analyzed with a Progenesis QI software (V1.0, Waters Corporation) by assuming that the extracted ions in the m/z range of 50-750 were [M-H]- formed from loss of a proton from the analytes. The LC separation allowed identifying this pseudo-molecular ion and potential in-source formed adducts for a same chromatographic peak. A molecular formula calculator was used to assign all mathematically possible formulas for the extracted ions with a mass tolerance of $\pm$2 ppm.

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(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 sig-

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nificantly 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 research 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, that are now given in the supporting information Molecular formulae assignment was performed including the following elements: C, H, N, S and O. Isotopes are a classical way to confirm molecular formulae 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 will now clearly state in our revised manuscript that

- The obtained molecular formula can be expressed as CcHhOoNnSs, where c is the number of carbon atoms confined in the range of 1-40, h is the number of hydrogen atoms confined in the range of 2-80, o is the number of oxygen atoms confined in the range of 0-40, n is the number of nitrogen atoms confined in the range of 0-3, and s is the number of sulfur atoms confined in the range of 0-2. Formulas were further constrained by setting H/C, O/C, N/C, S/C, and DBE (Double Bond Equivalent)/C ratios in the range of 0.3-3.0, 0-3, 0-0.5, 0-0.2, and 0-1, respectively, to assure that the obtained compound exists in nature (Fuller et al., 2012; Wozniak et al., 2008). The

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number of ions that possess more than one reasonable formula within 2 ppm mass tolerance accounted for 1.5

- The presence of the identified quasi-molecular ions in the blanks was systematically checked. If a chromatographic peak is detected in the blank, the compound is retained only if the sample to blank ratio of the area is above 10, and the blank area is subtracted to the sample area. This sample to blank ratio can be considered as a first signal to noise ratio. A second one can be defined from the intensity of random peaks in a mass spectrum (< 1×104 arbitrary unit). In that case, the signal to noise ratio chosen for compound detection is 3, at the maximum of the chromatographic peak. The compound is involved in intensity comparison between samples only if this last ratio reaches a value of 10.

- Analytical replicates were not considered as the final sample extract volume was quite low (200 μL) and as the remaining volume after the first injection was preferentially kept in case of specific analytical doubt rather than systematically injected. An estimation of the method repeatability is provided in the supplement from three standards.

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(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 "5μL of these diluted solution (50 μL in the case of the NJ daytime sample) were analyzed by an ultra-high performance liquid chromatograph (UHPLC, Dionex 3000, Thermo Scientific, USA) coupled to a Q-Exactive Hybride Quadrupole-Orbitrap MS (Thermo scientific, USA) in the mass range of m/z 50-750."

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(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, espe-

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cially 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 formulae assignments within these 2 ppm (Page 7, Line 188-189), 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 correspond 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 $\pm$ 3 ppm mass accuracy $\pm$ 5In any case, we do agree that all molecular formulae are only tentatively assigned and not positively identified. We will add more information about other possible formulae within 2 ppm for the major ions in the footnote of Tables S3-S10, as it had been done by Lin et al. (2012b) for example in their supplement. Also, we will state in our manuscript (Page 7, Line 197-199) that "The number of ions that possess more than one reasonable formulas within 2 ppm mass tolerance accounted for 1.5

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(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.

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(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 7, Line 203-205) that "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 the chromatographic peaks with the same m/z values (Figure S1)". Please also refer to our reply to the 2nd comment from referee 1.

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(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 are only available for Shanghai (with relatively comparable values, see Table S2). 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).

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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 Nanospray-Desorption Electrospray Ionization High-Resolution Mass Spectrometry, Environmental science technology, 48, 10993-11001, doi: 10.1021/es5024674, 2014.

Interactive comment on Atmos. Chem. Phys. Discuss., 15, 21415, 2015.