

Authors' Response to Referee #1

General Comments:

The motivation and objectives of the present work are not clearly articulated by the authors. There is a lack of explanation on why the specific nine organosulfates were chosen. Were they previously identified as significant in earlier studies, thus necessitating authentic standards for enhanced quantification? Some of these synthetic OS standards have been synthesized and applied in past studies to characterize OS in ambient particulate matter. Alternatively, are they representative enough to optimize a separation method intended to improve the resolution of a broader range of ambient OSs within a matrix? The use of reversed-phase C18 column in the present work has known limitation of resolving low molecule-weight and hydrophilic compounds because of early co-elution. It would be helpful if the authors clarified the specific optimization made, with a clear identification of existing gaps, comparison with prior studies, and discussion of limitations. Moreover, the intro is overall undercited. The supposedly improved methods lack sufficient detail in relation to previous methods and related studies.

Furthermore, neither the synthetic method nor UPLC method introduced by the authors bears substantial novelty. Therefore, the expectation is that a substantial improvement was achieved compared to established methods. However, the presentation of the manuscript does not clearly demonstrate such advancements. In the results section regarding the ambient PM_{2.5} samples, only the quantification of nine OSs was presented, along with their contributions to total sulfur (~1%) and total OC (~0.1%). In my opinion, this is sufficient to substantiate the conclusion that “anthropogenic emissions and subsequent secondary processes are the main sources of OSs”. The extent of sulfur closure aligns with previous measurements by Ding et al. in Tianjin during the same sampling season, which indicated that more than 95% of organic sulfur is still unaccounted for. I wonder if authors have looked for the presence other OSs in their samples, even qualitatively? Is there any improvement in resolving additional OSs with their optimized method? Addressing these questions would greatly strengthen the author’s statement that “the optimized method has enhanced sensitivity and specificity, making it appropriate for the quantitative analysis of OSs in atmospheric PM_{2.5}”. Additionally, the authors could consider using the nine OSs as surrogate standards to quantify additional OSs resolved by UPLC-ESI-MS/MS to give

an upper/lower bound estimate. While uncertainties exist, this approach can highlight future directions.

In summary, although the topic of the manuscript falls within the scope of AMT but the significance of the method improvements and the overall presentation do not meet the journal's expectation. I recommend the authors to consider submitting to other journals, such as ACP, after improving the clarity and incorporating additional results suggested above.

Response: We thank the referee for his/her critical reading of the manuscript, appreciation of our work and constructive comments and suggestions, which helped to improve the quality of the MS. The MS is revised according to all the comments from the referee. Our point-by-point responses to all the comments are provided below. Please see the revised MS for details of the revisions.

In previous studies, organosulfates were classified into isoprene derived OSs, monoterpene derived OSs, aliphatic OSs, aromatic OSs, C₂/C₃ OSs, and organosulfates of unknown origin based on the sources of their precursors. Among them, there are more quantitative research works on the types of organosulfates derived from isoprene and monoterpenes, but there are relatively few quantitative studies on anthropogenic organosulfates (long-chain aliphatic OSs, aromatic OSs, etc.). Among the 9 organosulfates considered in this study, 5 are aromatic OSs: (phenyl sulfate (PS), benzyl sulfate (BS), 3-methylbenzyl sulfate (3MBS), phenethyl sulfate (PES) and 4-nitrophenyl sulfate (4NPS)), and the other 4 are aliphatic OSs: (methyl sulfate, ethyl sulfate, cyclohexyl sulfate, and octyl sulfate), which broaden the identification and quantification of various types of OSs in the atmosphere. The reference standards could be further used as surrogates for the same type of organosulfates to quantify more types of organosulfates in subsequent studies.

In previous studies, benzyl sulfate and octyl sulfate were directly associated with anthropogenic volatile organic compounds and primary emissions, and thus were regarded as the marker species of anthropogenic origin of OSs. Previous research reports have indicated that phenyl sulfate and benzyl sulfate may be generated through the photo-oxidation process of naphthalene and 2-methylnaphthalene (Riva et al., 2015) or by sulfate reactions with aromatic compounds such as benzoic acid and toluene under aqueous conditions (Huang et al., 2020). Octyl sulfate (OSs), as a typical representative of anthropogenic organosulfates, has been pointed out based on past laboratory experimental research results. Therefore, these two species, C₈H₁₇O₄S⁻ (referred to as

OctS) and $\text{C}_8\text{H}_{15}\text{O}_4\text{S}^-$, are very likely to originate from the photo-oxidation process of diesel vapor in the atmosphere (Blair et al., 2017). Furthermore, the source of methyl sulfate can come from both biological processes and human activities (Hettiyadura, 2018). Therefore, it is necessary for them to use real standards to enhance quantitative research.

From the perspective of chromatographic principles, reversed-phase C18 columns are indeed more suitable for the separation of compounds ranging from moderately polar to non-polar. For short-chain aliphatic OSs such as methyl sulfate and ethyl sulfate, their retention on the C18 column is very weak, almost eluting along with the solvent front edge, and thus failing to achieve the separation effect at all. But interestingly, substances like octyl sulfate with long alkyl chains or benzyl sulfate containing aromatic rings may be appropriately retained on the C18 column. Moreover, in the study of Hettiyadura et al. (2015), ethylene bridged hybrid (BEH) amide columns were used for hydrophilic interaction liquid chromatography (HILIC) separation (Hettiyadura et al., 2015). Based on the articles of Hettiyadura et al. (2015) and Huang et al. (2018), a comparative experiment was conducted using a BEH chromatographic column in this study. BEH amide column and HSS T3 column were selected to compare the retention efficiency of organic sulfate esters. Among them, the mobile phase A of BEH amide column was composed of acetonitrile and ammonium acetate buffer solution in ultrapure water (95:5, V/V) (10mM, pH = 9). Mobile phase B (aqueous phase) is composed of an ammonium acetate buffer solution in ultrapure water (pH = 9). For the BEH chromatographic column, we compared two elution gradients. Method 1: The mobile phase A is maintained at 100% for 2 minutes, then drops to 85% within 2 to 4 minutes and remains at this level for 7 minutes. Subsequently, it returns to 100% within 11 to 11.5 minutes and remains at this level for 2.5 minutes. Method 2: The mobile phase A is maintained at 98% for 2 minutes, then drops to 60% within 3 minutes and is maintained for 2 minutes. Subsequently, it returns to 98% within 7 to 7.5 minutes and is maintained for 4.5 minutes. In contrast, the separation effect of each organic sulfate ester in Method 2 is more significant, but their retention times are all within 2 minutes, and there is a co-elution effect. Compared with the HSS T3 chromatographic column in Section 3.1, although the peak intensity of the BEH amide column has increased because the alkaline environment can better adsorb the anions of organosulfates, the HSS T3 column has the longer retention time, better separation effect, and there are no significant interference peaks. The retention effect of the HSS T3 column is weak on strongly polar organosulfates (methyl sulfates, ethyl sulfates). However, compared with the BEH amide column, it has a better ability to separate other organosulfates, a longer retention time and

higher resolution. Therefore, the HSS T3 column is selected for further optimization and environmental sample analysis. The content of this part has been added to Section 3.2. Moreover, by comparing the limit of detection and limit of quantification in this quantitative methods in other articles, this study has better accuracy in the detection and quantification of methyl sulfate, ethyl sulfate and benzyl sulfate, indicating that the quantitative detection method of this study can be widely applied in quantification analysis of the OSs. Please see the lines 240-270.

In this study, the authors only focused on the establishment of quantitative detection methods for organosulfates with standard substances and their quantitative applications in environmental (aerosol) samples. They did not search for other organosulfates with similar structures through the existing standards for organosulfates. The comments raised by the reviewers are highly operational and forward-looking. In future research, we can utilize these synthesized as well as the other available authentic standards to use as surrogates to identify the other organosulfates with similar structures in aerosols.

We included most of these points in the revised MS, and strongly believe that the revised MS certainly meet the standards of AMT for final publication in the AMT.

Detailed Comments:

1. Lines 129-145: The NMR information, if presented as is, can go to the SI. key information should be summarized and phrased in sentences (like lines 146-148) in the main text to improve readability.

Response: Following the reviewer's suggestion, we have moved the text in lines 129-145 (NMR details) to the SI, and added the summarized sentences in the revised MS (please see lines 164-167).

2. Lines 159-160: Does the 2mL contain 20% methanol by volume? The remainder is 5 $\mu\text{g L}^{-1}$ CA solution? Is the CA solution aqueous? Please clarify.

Response: CA refers to a camphorsulfonic acid solution, which is water-soluble and has a concentration of 5 $\mu\text{g L}^{-1}$. Given that the mobile phase selected is a methanol solution containing 0.1% formic acid, to mitigate solvent effects, comparative evaluations were conducted using 50% methanol, 20% methanol, and 10% methanol solutions. Upon assessing the peak shapes of the standard substances under these conditions, it was observed that the 20% methanol solution yielded the most favorable peak shape. Consequently, 20% methanol was chosen as the solvent for the CA solution.

We have revised the "The extracts were then re-dissolved in 2 ml of 20% methanol in

5 $\mu\text{g L}^{-1}$ camphorsulfonic acid (CA) solution.” in to “The extract was then dissolved in 2 ml of 5 $\mu\text{g L}^{-1}$ camphorsulfonic acid (CA) solution, with 20% methanol solution as the solvent.”. Please see the line 175-176 in the revised MS.

3. Line 154: Please describe more about the dehumidification process.

Response: We modified the “After the sampling, the filter sample was subjected to dehumidification for 48 hours and weighed gravimetrically.” as “After the sampling, the filter sample was placed in a desicator for dehumidification for 48 hours and weighed gravimetrically.”. Please see the line 173 in the revised MS.

4. Lines 195-199: I don’t see any mass spectra shown in Figure 1. They are simply fragmentation schemes of those molecules under MS/MS. Please include product ion spectra to demonstrate resemblance to previous studies.

Response: Following the reviewer’s suggestion, we included the mass spectra of 9 organosulfates in Figure 2 to prove the accuracy of the standard substances identification. Please see the line 235 in the revised MS.

5. Lines 207-210: Please clarify the new elution program employed to achieve separation of 3MBS and PES. By looking at Figure 2, 3MBS clearly has a retention time longer than 10 min. Please also clarify the inserts for the BS and PES EIC in Figure 2 caption. It looks like the BS insert is simply a zoom-in view of the original EIC while the PES insert is the new EIC with shifted retention time.

Response: Following the reviewer’s suggestion, we clarified it in the revised MS. The mobile phase employed in the analysis consists of the following gradient elution program: during the initial 0–2 min, the mobile phase comprises 3% methanol containing 0.1% formic acid (Mobile Phase B). From 2–11 min, the proportion of Mobile Phase B is increased to 50%. This 50% B composition is maintained from 11–13 min. Subsequently, the proportion of Mobile Phase B is further elevated to 95% between 13–17 min. A rapid decrease in the proportion of Mobile Phase B back to 3% occurs from 17–17.1 min, and this 3% B composition is held constant from 17.1–20 min. Notably, both 3-methylbenzenesulfonic acid (3MBS) and phenylethyl sulfone (PES) elute during the 2–11 min segment of this gradient. Importantly, only PES exhibits a distinct peak at a retention time of 8.49 min, which enables the differentiation between PES and 3MBS. This optimized elution program was achieved through iterative adjustments of the mobile phase gradient during method development, and it effectively facilitates the separation and identification of these two isomers, PES and 3MBS.

We checked the time required for plotting and the sub-peak intensity data, and found

that the abscissa of the 3MBS substance plotting was wrongly selected. We then revised Figure 2. The extracted ion chromatograms (EIC) inserted for both BS and PES represent zoomed-in views of the original EIC. We have incorporated markers to clearly indicate the specific regions that have been magnified. Notably, the peak intensity of PES at 8.49 min is significantly lower compared to that at 9.20 min. Consequently, the peak at 8.49 min is barely discernible in the original chromatogram due to its relatively low intensity.

We noted these points in the revised MS (see lines 238-239, & Figure 3).

6. Lines 221-222: Can authors explain why ambient samples that may contain some of those reference OSs were used for recovery test instead of blank filters? Was a mixture of known quantity of reference OSs spiked on one filter or was each reference OS spiked on individual filter to determine the recoveries? Table 2. Please clarify why LOD and LOQ are the same for 3-methylbenzyl sulfate, octyl sulfate, and 4-nitrophenyl sulfate. Plus, the lower bound of the linear range is lower than the LOQ of these species.

Response: During the experimental procedure, a blank membrane was employed for the recovery test. The recoveries of nine organosulfates determined were: methyl sulfate: 59.0%, ethyl sulfate: 107.4%, phenyl sulfate: 75.6%, phenyl sulfate: 89.3%, benzyl sulfate: 93.0%, 3-methylbenzyl sulfate: 78.8%, phenethyl sulfate: 105.8%, octyl sulfate: 51.6%, 4-nitrophenyl sulfate: 103.8%. Environmental sample membranes were employed for evaluating matrix effects in analytical method. Specifically, a mixed solution containing known concentrations of nine standard substances was applied onto environmental sample membranes, and the concentrations of these standard substances were subsequently determined using analytical instruments. This approach enabled the assessment of enhancing or suppressive effects on the detection signals imposed by components present in the sample matrix other than the target analytes (such as proteins, lipids, salts, humic acids, etc.). Such interferences can significantly compromise the authenticity of quantitative results. In real samples, other matrix constituents may influence both the extraction efficiency of the target analytes and the detection signals. By utilizing sample membranes, a more realistic simulation of the conditions encountered in actual samples is achieved, thereby facilitating a more accurate evaluation of recovery rates. Consequently, we employed authentic environmental sample membranes for the determination of recovery rates in our study. For the determination of the limit of quantification (LOQ) and the limit of detection (LOD),

we employed a mixed solution containing standard substances with known concentrations for testing. The respective concentrations of these standard substances in the mixture were as follows: 0.01 ng mL⁻¹, 0.1 ng mL⁻¹, 0.5 ng mL⁻¹, 1 ng mL⁻¹, 5 ng mL⁻¹, 10 ng mL⁻¹, 20 ng mL⁻¹, 50 ng mL⁻¹, 70 ng mL⁻¹, 100 ng mL⁻¹, 120 ng mL⁻¹, 150 ng mL⁻¹, 170 ng mL⁻¹, 200 ng mL⁻¹. We determined the concentration of the limit of detection (LOD) based on a signal-to-noise ratio (S/N) greater than 3, and the concentration of the limit of quantification (LOQ) was established when the S/N ratio exceeded 10. For 3-methylbenzyl sulfate, octyl sulfate, and 4-nitrophenyl sulfate, the S/N ratio was greater than 10 at a concentration of 0.1 ng mL⁻¹. However, at a concentration of 0.01 ng mL⁻¹, the S/N ratio did not surpass 3. Consequently, we set both the LOQ and LOD for these three substances at 0.1 ng mL⁻¹.

7. Line 250: It is worth noting that the cited measurements in the U.S. were conducted in summer and early fall. Wintertime measurements of methyl sulfate in the U.S. are available in the literature, which is more comparable to the current work. One example is: Chen, Y. et al Seasonal Contribution of Isoprene-Derived Organosulfates to Total Water-Soluble Fine Particulate Organic Sulfur in the United States. ACS Earth Space Chem. 5, 2419 – 2432 (2021).

Response: Following the reviewer's advice, we have incorporated the measurement results of methyl sulfate in the United States during winter to facilitate a more robust comparative analysis. Please see line 341 in the revised MS.

8. Line 266: this is the first time that phenyl methyl sulfate and 3-methyl phenyl sulfate were mentioned in the manuscript. Is it a typo? There are no corresponding labels for these two compounds in Figure 4.

Response: Benzyl sulfate is also known as phenyl methyl sulfate, the expression of 3-methylbenzyl sulfate in the text is unclear due to writing errors, which caused ambiguity in the text. These have been systematically corrected to benzyl sulfate (C₇H₇OSO₃⁻) and 3-methylbenzyl sulfate (C₈H₉OSO₃⁻), respectively, to align with standardized chemical terminology and ensure consistency with prior descriptions in the manuscript. This revision eliminates potential confusion regarding structural specificity and maintains coherence in the discussion of organosulfate derivatives. Please see lines 329-330 in the revised MS.

9. Line 274: Where are the total sulfur data and how are they obtained? Only SO₄ concentration is shown in Figure 4.

Response: The data of total sulfur was obtained from the measurement of inductively

coupled plasma optical emission spectrometer (ICP-OES, Agilent 5110, USA) in other study. Since this paper primarily focuses on the establishment and application of detection methodologies, comprehensive details regarding other relevant metrics are not elaborated here.

10. Line 281: The author claimed an improved synthetic procedure but the comparison with literature procedure was not discussed in detail.

Response: In the present study, we synthesized the specific sulfate derivatives such as 3-methylbenzyl sulfate, cyclohexyl sulfate, and phenethyl sulfate, which have not been reported in the literature, based on the procedures reported by Kundu et al. (2013), Olson et al. (2011), Staudt et al. (2014), and Li et al. (2010), with modifications to the reaction conditions. Notably, we employed a strong base, sodium hydride, instead of weaker bases such as dimethylaminopyridine (DMAP) or pyridine. This modification enabled the efficient synthesis of the target sulfate derivatives with high yields and purity within a short time frame. Furthermore, this method is also applicable to the synthesis of other derivatives, including ethyl sulfate and benzyl sulfate. These points are included in the revised MS (see lines 141-144).

11. Lines 283-284: The only improvement seems to be the separation of PES and 3MBS with a potential compromise on the PES sensitivity (Figure 3). As mentioned in my earlier comment, author did not provide detailed discussion on the improved UPLC-ESI- MS/MS method. How is it compared to previous studies using similar RPLC methods (e.g., Want et al, <https://doi.org/10.1021/acs.est.3c01422> and Ding et al, <https://doi.org/10.1016/j.scitotenv.2022.155314>)?

Response: The gradient elution program was optimized using the HSS T3 chromatographic column, and the isomers PES (phenethyl sulfate) and 3MBS (3-methylbenzyl sulfate) with the same molecular weight (m/z 201) were successfully separated (with retention times of 8.49 min and 9.20 min, respectively). In fact, previous studies have not been reported the effective separation of such isomers. Further the limit of detection (LOD) of all target OSs reached 0.10 ng mL^{-1} , and the limit of quantization (LOQ) was $0.10\text{-}0.50 \text{ ng mL}^{-1}$, which are substantially higher than those reported by Ding et al. (2022) (LOD: 1.0 ng mL^{-1} for methyl sulfate) and Hettiyadura et al. (2015) (LOD and LOQ of methyl sulfate ethyl sulfate and benzyl sulfate: 2.6, 3.4, 3.9 and 8.6, 11.2, 13.2 ng mL^{-1} , respectively).

Furthermore, we studied the comparison in retention between the experiments with the

BEH amide column and the HSS T3 column. The experimental results indicated that the separation effect of the BEH amide column was not good, and all OSs peaks emerged within 2 minutes. On the contrary, the retention time of the HSS T3 column was longer. Although the retention time for low-molecular-weight organosulfates (methyl sulfates, ethyl sulfates) is very short, there are no significant interference peaks. Please see section 3.2 in the revised MS.

Technical corrections:

1. Lines 188-189: mass-to-charge ratio Line 251: the sentence is incomplete before the parenthetical citation.

Response: We have corrected these statements. Please see the line 201 and 321 in the revised MS.

2. SI Figure S1: the labeling for (b) and (c) in the caption is incorrect.

Response: There is no mistakes for the labeling of Figure S1. In the caption of Figure S1, the components have been explicitly annotated as follows: (a) ethyl sulfate, (b) benzyl sulfate, (c) cyclohexyl sulfate, and (d) 3-methylbenzyl sulfate.

References:

Blair, S. L., MacMillan, A. C., Drozd, G. T., Goldstein, A. H., Chu, R. K., Paša-Tolić, L., Shaw, J. B., Tolić, N., Lin, P., Laskin, J., Laskin, A., and Nizkorodov, S. A.: Molecular characterization of organosulfur compounds in biodiesel and diesel fuel secondary organic aerosol, *Environ Sci Technol*, 51, 119-127, <https://doi.org/10.1021/acs.est.6b03304>, 2017.

Hettiyadura, A. P. S., Stone, E. A., Kundu, S., Baker, Z., Geddes, E., Richards, K., and Humphry, T.: Determination of atmospheric organosulfates using HILIC chromatography with MS detection, *Atmospheric Measurement Techniques*, 8, 2347-2358, <https://amt.copernicus.org/articles/8/2347/2015/>, 2015.

Hettiyadura, A. P. S.: Quantification of organosulfates and their application in source apportionment of atmospheric organic aerosols, University of Iowa, University of Iowa, 2018.

Huang, L., Liu, T., and Grassian, V. H.: Radical-initiated formation of aromatic organosulfates and sulfonates in the aqueous phase, *Environ Sci Technol*, 54, 11857-11864, <https://doi.org/10.1021/acs.est.0c05644>, 2020.

Riva, M., Tomaz, S., Cui, T., Lin, Y.-H., Perraudin, E., Gold, A., Stone, E. A., Villenave, E., and Surratt, J. D.: Evidence for an unrecognized secondary anthropogenic source of organosulfates and sulfonates: Gas-phase oxidation of polycyclic aromatic hydrocarbons in the presence of sulfate aerosol, *Environ Sci Technol*, 49, 6654-6664, <https://doi.org/10.1021/acs.est.5b00836>, 2015.