# Responses to Comments on Manuscript amt-2020-267
# (Orbitool: A software tool for analyzing online Orbitrap mass spectrometry data)

We thank the reviewer for the constructive comments that help to improve this manuscript. The manuscript was revised according to these comments. We added more details in the revised manuscript and polished the language. The reviewer's comments are addressed in the following paragraphs. The comments are shown as sans-serif dark red texts and our responses are shown as serif black texts. Changes are highlighted in the revised manuscript and shown as "quoted underlined texts" in the responses. Line numbers, figures, and equations quoted in the responses correspond the revised manuscript. Reference are given at the end of the responses.

**Reviewer #3**

The authors present the application of a novel software solution (open source) for analyzing Thermo Fisher raw mass spectrometry files, which are not designed for online monitoring. This is of great value for the atmospheric science community, which increasingly applies Orbitrap technology for atmospheric applications. The paper certainly fits into the scope of AMT.

However, the paper requires quite some language editing and needs to explain some approaches more in detail. I am surprised that the major benefit of a HR-MS is not shown: the capability to resolve isobaric species.

**Response**: Thanks. We added more details in the revised manuscript. The terms used in this manuscript was revised according to the reviewer's comments to improve the accurateness and readability of this manuscript.

We do not put the high-resolution peak signals retrieved using Orbitool in the manuscript because the high resolution is an advantage of the Orbitrap rather than Orbitool and similar figures have been reported in our previous studies (Riva et al., 2019 and 2020; Lee et al., 2020).

Furthermore, the authors should comment on the data acquisition of the Orbitrap, which pre-filters data and should only record signals that are not attributed to (electronic) noise. Otherwise, the reader might be confused by the extensive effort of distinguishing noise from signal.

**Response**: Thanks. In the revised manuscript, we added the following text: "When converting electronic signals into a spectrum via the Fourier transform, the instrument removes signals below the noise threshold defined by the Orbitrap. However, there are still signals exceeding this noise threshold and they are converted into peaks recorded in the data file. In this study, "noise" refers to the noise peaks recorded in the data file."

The term "identification [of compounds]" is used in this manuscript quite often, but it rather describes the attribution of a molecular formula to a measured mass. An accurate mass measurement (incl. isotopic pattern) can only help in determining the sum formula, not the identification of compounds! For the correct use of "molecular identification" see Noziere et al., Chem. Rev., 2015. Therefore, the abstract needs rewriting, since the terms "identification" and "separation" are discussed in a wrong context.

**Response**: We thank the reviewer for this clarification. In the revised manuscript, we replaced "identification of chemical compounds" with "assignment of molecular formula". "Separate" was replaced by "distinguish" when it refers to two chemical formulae.

Since Orbitool is GUI-based software, some words about installation instruction on the website can be helpful for researchers which are not familiar with Python, but still are willing to use the software.

**Response**: We have added some description: "The users can either run Main.py via Python or Orbitool.exe without Python. The required Python environment (optional) for Orbitool is described in detail on the website" to ***Software availability***.

A brief instruction on the Python version and required packages has been added to the latest version of Orbitool.zip file, which can be found on the website.

Researchers who are not familiar with Python can directly run the Orbitool.exe file without a Python programming language on their computer.

Overall, the manuscript fits into AMT very well, the work is important for the community, but the paper needs additional information, major rewriting and some corrections, as partly listed under the following comments.

**Specific comments:**

p.2 l.8: Do you really mean "noise"? IUPAC definition of noise: "The random fluctuations occurring in a signal that are inherent in the combination of instrument and method." Maybe your analysis just discards noise with a more strict filter than the XCalibur acquisition software (which to my knowledge already applies a noise-filter during data acquisition)?

**Response**: We agree with the reviewer that "noise" in this manuscript does not follow the usual definition. It refers to the signal peaks generated by remained noises after the pre-filtering before the Fourier transform. To clarify it, we added the following text: "When converting electronic signals into a spectrum via the Fourier transform, Orbitrap applies a noise threshold to remove noises. However, there are still noises exceeding this noise threshold and they are converted into peaks recorded in the data file. In this study, "noise" refers to the noise peaks recorded in the data file. It is difficult to separate the signal peaks for some compounds with low concentrations from noise peaks."

p.2 l.9: The presented work does not show ozonolysis of monoterpenes, as atmospheric scientists would think of. You tested ozonolysis of orange peel emissions. These emissions contain monoterpenes, but not exclusively.

**Response**: We used the VOCs emitted from an orange peels to have a simple and user-friendly method to mass calibrate the Orbitrap (i.e., using the ambient $O_3$ to oxidize VOC, including monoterpenes emitted from an orange). We do not claim that an orange only emits monoterpenes, but the formation of HOMs has been mainly observed from the oxidation of monoterpenes (at least with high yields). As shown in Figure R1, the mass spectrum obtained from such mass calibration test is very similar to a laboratory-controlled monoterpene ozonolysis experiment, as reported in our initial study or by other groups (e.g., Jokinen et al., 2015). As a result, such simple approach can be very useful and easy to carry out without the need of dedicated experimental setup.

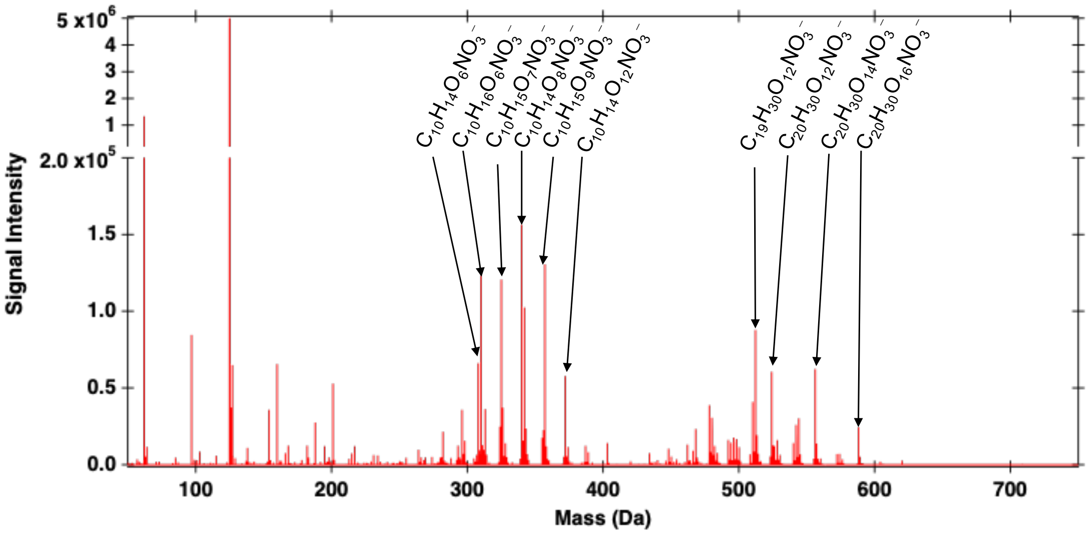

Figure R1, a mass spectrum from orange peeling experiment.

p.2 l.13: “Identification of unknown species” is not in line with the molecular identification defined by Noziere et al. – it is rather “sum formula attribution” than “identification”.

**Response**: We agree and replace “identification” with “assignment of molecular formula”. Molecular formula, by its definition, does not distinguish isomers.

p.2 l.15: In this case, do not use the term “separate” in order to avoid misunderstanding with chromatographic separation techniques.

**Response**: This phase was revised as “assign hundreds of molecular formulae”.

p.5 l.10: TofTools also requires the background data between all nominal masses, which are not recorded by XCalibur.

**Response**: We added “In addition, TofTools determines the noise level using the equally spaced data within a certain mass defect range, whereas such information is not recorded in the Orbitrap data” to this paragraph.

p.6 l.7: Before describing how data are handled with Orbitool, it should be described how data are recorded (E.g. experimental setup, ion source settings, data acquisition settings (scan rate, pre-averaging, use of a lock-mass, centroid mode, profile mode?), etc.).

**Response**: We thank the reviewer for this suggestion. The current version of Orbitool is developed for data recorded in the profile mode. Other parameters do not affect the analysis procedure with Orbitool, although they affect the analysis results and may influence the difficulty to analyze a spectrum data (for example, using a lock-mass may reduce the difficulties in attributing molecular formula to a peak). In the revised manuscript, we added the following text: “Most tuning parameters of Orbitrap, e.g., injection time of each micro scan and the number of micro scans for each single scan, do not affect the analysis with Orbitool. However, it should be specially clarified that Orbitool is compatible with the profile mode but not the centroid mode of Orbitrap, because the attribution of chemical formulas to measured signal peaks is based on fitting peak distributions to the profile data.”

p.7, l.9: I do not understand what the numbers of the mass defect range ([0.5, 0.8]) intend to express. What is the center of your mass defect and what is the isolation width. This does not become clear.

**Response**: If the mass defect corresponding to the peak mass of a fitted peak is no smaller than 0.5 and no larger than 0.8, it is selected in this step of data analysis. [0.5, 0.8] is a standard expression of a closed interval. The sentence was revised as follow: "Orbitool first takes all the detected peaks whose peak positions are located in the mass defect range of [0.5, 0.8] Da."

p.8, l.22: The concept of the lock mass is that the mass accuracy is stable for long time-series, making additional mass calibrations obsolete.

**Response**: We are not certain that the additional mass calibration is always redundant. For example, when using the reagent ions and its dimers of a CI-Orbitrap as lock masses, a majority of the measured species locates at larger masses. As a result, the mass accuracy of these measured species with large masses is improved by extrapolation rather than interpolation. An additional mass calibration with more species may contribute to the mass accuracy.

As a software tool to facilitate customized data analysis, Orbitool can skip the mass calibration procedure. This feature can be used when additional mass calibration is unnecessary.

The relevant sentence was revised as "The Orbitrap is able to maintain its mass accuracy (i.e., < 2 ppm) for long-time series..." and we added "The user can also skip the mass calibration."

p.9, l.9-16: For ion signals which are > 1e6 molecules cm$^{-3}$, the isotopic pattern can be used to verify / falsify sum formulas by calculating an isotopic pattern matching score. Is this feature possible with Orbitool?

**Response**: Orbitool supports this feature. For each assigned molecular formula, its theoretical relative abundance is displayed in the widget for peak fitting and molecular formula assignment. If this molecular formula contains a less abundant isotope and its corresponding molecular formula with the more abundant isotope exists in the spectrum, the measured relative abundance is also displayed. The value of the measured relative abundance is by default used as a criterion during the peak assignment. The measured relative abundance cannot exceed of certain ratio of the theoretical relative abundance, otherwise Orbitool will not assign this molecular formula. Meanwhile, Orbitool provides a separate peak assignment algorithm that does not use the isotope abundance to filer possible molecular formulae.

In the revised manuscript, we added a paragraph to discuss the details of this feature related to isotope abundance: "Orbitool also supports the identification of isotopes. The users can adjust the possible isotopes in an isotope list and all the isotopes in this list will be considered. Using the default algorithm for peak determination, the chemical formula containing a less abundant isotope can be determined only when its corresponding formula containing the abundant isotope is found in the spectrum. In addition, this default algorithm also checks whether the abundance ratio of these two species is consistent with their natural abundances within the uncertainty range. To facilitate studies such as isotope labeling experiments, Orbitool also provides an algorithm that does not restrict isotope abundances during peak assignment."

p.11, l.16: Additionally to the mass defect plot, other visualizations might be also informative, such as the aromaticity index or the Kendrick mass defect.

**Response**: Thanks. We will add these features in future versions of the Orbitool.

p.11, l.22: Again, I do not understand the mass defect range of [0.5, 0.8] as a filter for determining the noise level. Does this rather broad range of 0.5 amu requires only one main ion signal within this range? My experience, is that in such a large mass range on can usually find more than five-to-ten different (baseline-separated) ion signals. I think the text needs a more detailed explanation.

**Response**: The reviewer is correct that multiple peaks may be found in this broad mass range. This range is expressed in a closed interval of [0.5, 0.8] and hence the width is 0.3 amu for each unit mass. The corresponding sentence in the manuscript is "Orbitool first takes all the detected peaks whose peak positions are located in the mass defect range of [0.5, 0.8] Da", which should have no ambiguity. The aim of this step is to the potential noise peaks instead of a single noise peak. The noise level is determined by statistics on these selected peaks, as elaborated in section 2.2.

p.12, l.10-12: ". . . number of peaks after noise reduction with the 50th percentile is insensitive to the averaging time." –> I cannot see this in the data: After 5 min averaging time the number of peaks converges to _1000, after 30 min to _1300, and after 60 min to _2000. Hence, your reasoning appears questionable.

**Response**: We revised this sentence as: "This is consistent with the observed trend of the total number of peaks after noise reduction in Fig. 3, in which the total number of peaks after noise reduction with the 50th percentile increases slightly with an increasing averaging time." This observed relationship between the number of peaks and averaging time is consistent with the previous sentence "Considering that some species may not be detected in a single scan (i.e., present at very low concentration), the total number of peaks in the averaged spectrum should grow with the increasing averaging time when the period is considerably short, and then it should converge to a certain value when the averaging period is sufficiently long."

Figure 4: Can you also demonstrate the Orbitrap technology really has an advantage over ToF-MS by resolving several gas-phase signals on one nominal mass? My experience is that with $NO_3$-CIMS (using ToF) of monoterpene ozonolysis peaks are already well fitted, and show little evidence for isobaric interference. A zoom in on the x-scale of both experiments would be worth to show.

**Response**: As discussed earlier in this review, the manuscript focuses on the software part (i.e., Orbitool) but not on the advantages of the hardware (i.e., Orbitrap). In addition, such figures have already been reported in all of our previous studies (Riva et al., 2019 and 2020; Lee et al., 2020).

**Technical corrections:**

Consider to minimize the use of "greatly" (used in p.2 l. 8, l. 13)

p.2 l.3: "wildly-used"? I think you rather intended to say "widely-used"

p.2 l.6: maintaining –> improving?

p.2 l.14: consider: . . . ambient gas-phase measurements in urban Shanghai.

p.3 l. 2: produce . . . into the atmosphere? Needs rephrasing. E.g. Biogenic and anthropogenic sources emit a wide variety of VOCs into the atmosphere.

**Response**: Thanks. We revised this manuscript according to the above comments.

p.4. l. 22-23: What do you mean with ". . .overcome the interference of noise and accumulate signals."?

**Response**: This sentence was revised as: "The measured spectra must be averaged over a long period to decrease the noise level so that these low signals can be unambiguously identified among noises."

p.8. l.20: It is m/z which is determined, not the mass.

**Response**: We prefer to avoid using m/z (i.e., mass/charge) as mass and charge are both dimensional quantities. Because they have different dimensions, mass/charge is a dimensional quantity. As a result, we prefer to keep a meaningful annotation and decide to use mass (Da) which is also consistent with our previous published recently in AMT (Riva et al., 2019).

Figure 5: blue and purple are very hard to distinguish from each other.

**Response**: Thanks. We changed the colors of markers.

## References

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