mzrtsim: Raw Data Simulation for Reproducible Gas/Liquid Chromatography–Mass Spectrometry Based Non-targeted Metabolomics Data Analysis

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Abstract

Reproducibility of data analysis is pivotal in the context of non-targeted metabolomics based on mass spectrometry coupled with chromatography. While various algorithms have been proposed for feature or peak extraction, their validation often revolves around a limited set of known compounds or standards.While data simulation is widely used in other omics studies, simulation are focused on the feature level, neglecting uncertainties inherent in the feature or peak extraction process for metabolomics mass spectrometry data. In this technique note, we introduce a R package called 'mzrtsim' to simulate gas/liquid chromatography full scan raw data in the mzML format. Unlike simulations solely based on virtual features , our approach leverages experimental spectra data from the MassBank of North America(MoNA) and the human metabolome database(HMDB). We developed algorithms to simulate chromatography peaks, accounting for the tailing factor. The results of our study demonstrate the potential of this tool for comparing established metabolomics software (e.g., XCMS, mzMine, and OpenMS) against a ground truth. We found that the investigated software all introduced large amounts of false positive peaks (more than 75% of detected peaks by default setting) and loss compounds with less peaks. They also showed different sensitivity to tailing and leading peaks and we also found setting appropriate intensity cutoffs will reduce the influences from redundant peaks. This R package is free and available online(https://github.com/yufree/mzrtsim).

Introduction

Metabolomics is focused on the comprehensive identification and precise quantification of metabolites within a biological system[1]. To conduct metabolomics data analysis, researchers are required to fine-tune a multitude of software, settings, and parameters during the bioinformatic processing of raw data[2,3]. It is important to note that these adjustments do not occur in isolation, frequently leading to a cascade of repercussions across various facets of the experiment[4,5].

In mass spectrometry based metabolomics research, numerous software has been proposed to process the raw data and extract features[6–8]. However, past studies have illuminated significant disparities in the performance of these software tools and algorithmic parameters[9]. For instance, when applied to identical datasets, five distinct software packages yield comparable true positive peaks, yet exhibit variations in the relative quantification of true features. Furthermore, the task of evaluating these software solutions or algorithms is consistently challenging, given the multitude of parameters involved and the inherent uncertainties in non-targeted analysis. Previous approaches have often relied on the utilization of known compound standards[5], publicly available datasets[9] or manually checking[10] of peak shape to choose software, which might not be reproducible for other researchers. In this context, a “white-box” and reproducible compounds will be helpful to assess software performance or facilitate developmental efforts for metabolomics data analysis.

Proteomics and lipidomics research have used simulation data with a known ground truth[11,12]. LC-MSsim can simulate mzData files with FASTA files as input for proteomics studies[13]. MSSimulator extended the simulation to MS/MS data in data-dependent acquisition (DDA) modes considering isotope labeling technique[14]. Mspire-Simulator could generate data with post-translational modifications of peptides[15]. JAMSS provides a java based graphical user interface for proteomics data simulation[16] and Synthedia can cover data-independent acquisition (DIA) mode for proteomics data[12]. The Lipid DDA Simulator can perform in-silico simulation of lipidomics DDA on the Q-Exactive HF based on java[11]. To our knowledge, no R based simulation software has been reported for metabolomics data analysis while R is one of the most popular programming languages for metabolomics data analysis[17].

Prior efforts in simulating metabolomics data have predominantly concentrated on the compound-level data representation[18,19]. However, real experimental data frequently encompass redundant peaks, offering a glimpse into the intricate nature of

metabolomics datasets[20]. Many of these redundant peaks, including isotopologues, adducts, fragmental ions and multiple charged ions, often exhibit correlated intensities with base peaks and are susceptible to the influence of background noise with lower intensity[21]. Such redundant peaks might be useful for downstream annotation analysis[22]. Therefore, in the context of simulation, it becomes imperative to account for the presence of noisy background signals and incorporate the generation of redundant peaks that parallel the characteristics observed in actual experimental data.

Meanwhile, it's important to note that DDA or DIA modes can result in the loss of MS1 data coverage[23]. A regular workflow in metabolomics always begins with full-scan mode for the relative quantitative analysis, aiming to identify potential biomarkers. Subsequently, MS/MS spectra of selected ions are extracted for annotation purposes. Consequently, the majority of metabolomics software tools are primarily designed to extract MS1 peaks or features[24–27]. In this case, simulation of MS1 data for metabolomics will be helpful to evaluate metabolomics software.

On the other hand, the performance of chromatography plays a pivotal role, and previous studies have often centered on assessing software based on chromatography peak quality[28,29]. Nevertheless, achieving consistent peak quality in real samples can be a challenging endeavor considering sample preparation and instrument analysis. Unlike targeted analysis where known ions are optimized, untargeted analysis doesn't have specific compounds to be optimized. Consequently, issues with peak quality can arise, particularly when dealing with unforeseen compounds. These fluctuations in peak quality might impact the effectiveness of peak picking algorithms. In this context, the inclusion of direct simulation of peak shapes, encompassing characteristics like leading and tailing peaks, can prove highly beneficial. Such simulations would not only provide a more comprehensive evaluation of software performance but also help account for the variability in peak quality encountered in real-world untargeted metabolomics studies.

In this work, we present the 'mzrtsim' package in R, designed specifically for the generation of mzML files with the primary aim of evaluating feature extraction or peak picking software. This tool is equipped to produce simulated experimental data that faithfully replicates the issues encountered in real metabolomics datasets. These include the incorporation of redundant peaks and background noise, mirroring the complexity of actual experimental data. Additionally, 'mzrtsim' is uniquely capable of simulating chromatography peak quality issues with reference to known ground truth, providing a comprehensive framework for assessing and enhancing the performance of metabolomics software.

Methods

The experimental full scan spectrum data from MassBank of North America(MoNA)[30] for liquid chromatography-mass spectrometry (LC-MS) and the human metabolome database(HMDB)[31] for gas chromatography-mass spectrometry (GC-MS) was extracted for simulation purpose with redundant peaks. In brief, MoNA database contains 6075 experimental compounds spectra from 1926 unique compounds. 2968 compound spectra from 1114 compounds are collected on high resolution mass spectrometry. Among those spectra, the average peak number is 43.5 and the median peak number is 15. As for HMDB, 10096 experimental spectra are collected from 2295 unique compounds. The average peak number for HMDB is 87.6 and the median peak number is 48. Those experimental data contain not only molecular ions, but also redundant peaks and background noises. Such full scan spectra have been included in the package and could be used for raw data simulation.

For each compound's spectra, a signal to noise('sn') ratio could be set for the base peaks. For example, a compound has two ions with one base peak and another isotopologue of the base peak. The intensity of the isotopologue peak is 5% of the base peaks. Then, the 'sn' of 100 with 'baseline' 100 for this compound means the base peak of the compound should have 10000 as intensity and 500 for the isotopologue peak. Mass accuracy in ppm can be set for the experimental spectrum with a default value of 5 ppm. To simulate data collected from time of flight mass spectrometry, such value can be set as 20ppm. The background peaks can also be simulated by inputting the peaks' mass to charge ratio. Meanwhile, a background peaks' database for LC-MS has been included in the package, which is extracted from previous studies' solvent blank samples[23].

Each ion in one spectrum will generate its own chromatography peaks by user defined scan rate and peak width. For example, a scan rate of 5 spectra per seconds with 10s as peak width will simulate a peak with 50 spectra. Without tailing, each ion in the mass spectra should follow a normal distribution, considering the relative intensity within each spectra. Normal distribution parameters could be set up with mean as 'baseline' and standard deviation as 'baselinesd' for each ion's chromatography peaks. The retention time could also be set for each compound. Meanwhile, the tailing factor was introduced to simulate peak quality for certain compounds as it's widely used in chromatography research[32]. Tailing peaks will show a tailing factor larger than 1 and leading peaks will show a tailing factor less than 1 by setting 'tailingfactor' in the software.

With simulation of single spectrum and chromatography peaks, mzrtsim package[33] could generate a mzML file through the Spectra package[34]. With control of peak width for base peaks and retention times, users could generate data sets samples from the same group or different groups. The output files should have mzML files and csv files with mass to charge ratio, retention time and compound name for each peak. Such csv files will be used to evaluate the performance of different software.

mzrtsim package could simulate both GC-MS and LC-MS data with inner databases. The fundamental differences between GC-MS and LC-MS are the ionization process for the compounds and different columns. GC-MS usually needs a hard ionization method to break down the structure of small molecules to generate structure information. However, LC-MS will apply a soft ionization process, which might contain limited information about the structure. Regarding the separation method, GC and LC are all fitting the plate theory. Depending on the separation performance, users can change the scan rate and peak quality for GC-MS or LC-MS raw data simulation.

The major function for mzML files simulation is 'simmzml'. By setting the compound index in the experimental spectra database, peaks width for the base peak of the compounds, retention time of each compound, signal to noise, and file name, mzrtsim package can generate a corresponding mzML file. To change the peak quality, tailing factors of each compound can be changed to generate tailing, leading or normal peaks.

With mzrtsim package (see figure 1), we could benchmark different metabolomics software. We simulate the same data set to compare XCMS[6], OpenMS[8], and MZmine 3[35]. We also test their performance on different types of chromatography peaks. Then we tested the influence of redundant peaks with lower intensity on XCMS. The overlapped peaks are defined as peaks with mass to charge ratio shift within 5 ppm and retention time shift within 5 seconds. All the simulated data and codes are available online.

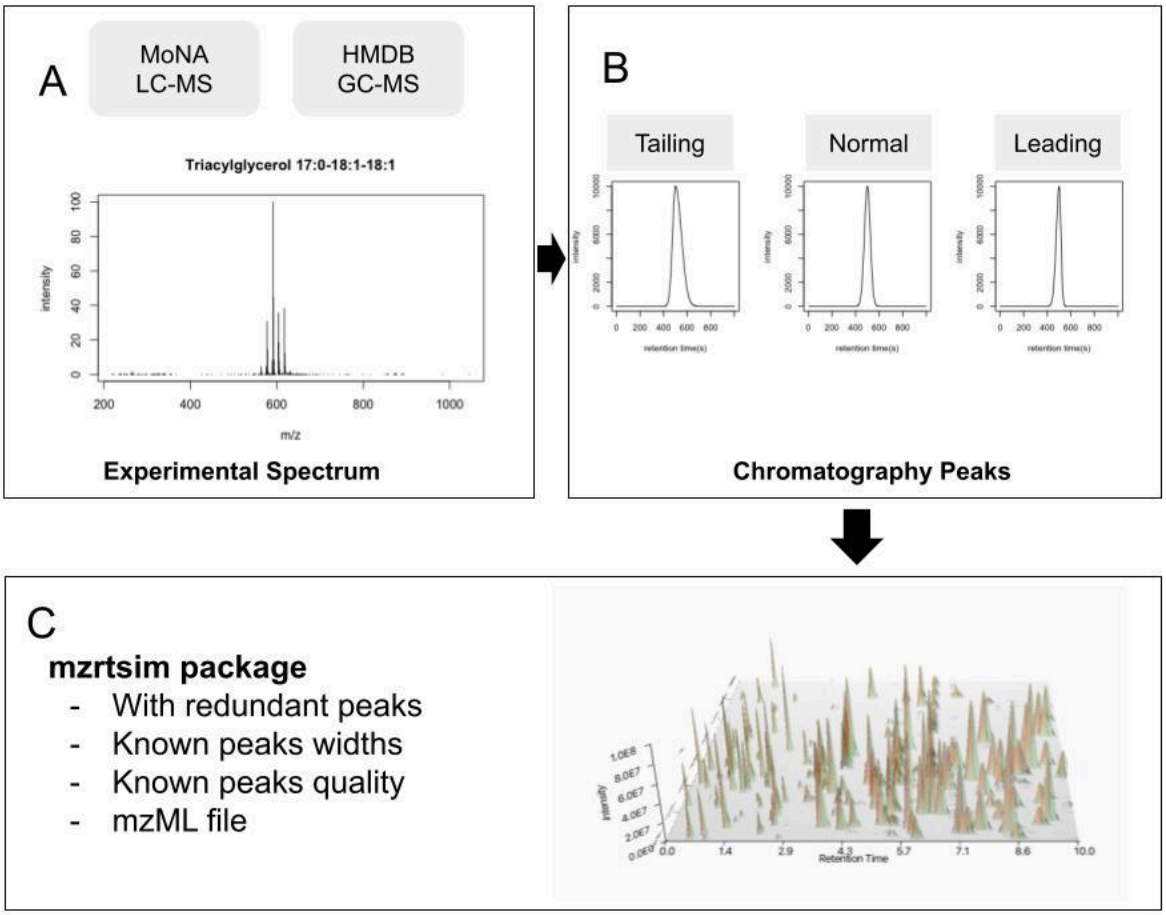

Figure1. mzML simulation process of mzrtsim package. (A)Experimental spectrum from publicly available databases(MoNA and HMDB) will be used for simulation and (B) each ion in the spectrum will generate chromatography peaks to (C) output the final mzML file with known information for each peak.

Results and Discussion

Software comparison for peak picking

We simulated 10 mzML files with 100 compounds' high resolution spectra. Those compounds' spectra were from MoNA experimental data and peak shape followed a normal distribution. Those 100 compounds generated 593 detectable chromatography peaks. Then peak picking was performed using XCMS, OpenMS, and MZmine 3 with default settings. To fit the simulated data, the peak width range of XCMS was changed to (5,15) while the other two software' default settings should cover the simulated peaks.Results showed that those three software found 41 compounds with slightly different profiles. However, their peak profiles are quite different. XCMS found 495

peaks and 121 peaks are real peaks. OpenMS found 926 peaks and 179 peaks are from simulated compounds. MZmine 3 detected 776 peaks with 186 true positive peaks. More than 75% of the detected peaks are false positive peaks in all of those three software. However, a closer examination using a Venn diagram (figure 2) revealed that only 105 peaks were shared among the true positives. This finding suggests that while the software tools exhibited varying performance in detecting false positive peaks, there was a substantial overlap in their identification of true peaks. Therefore, in practical applications, employing multiple software tools for peak extraction and subsequently focusing on the overlapping peaks is advisable to mitigate the impact of false positives and optimize analysis efficiency.

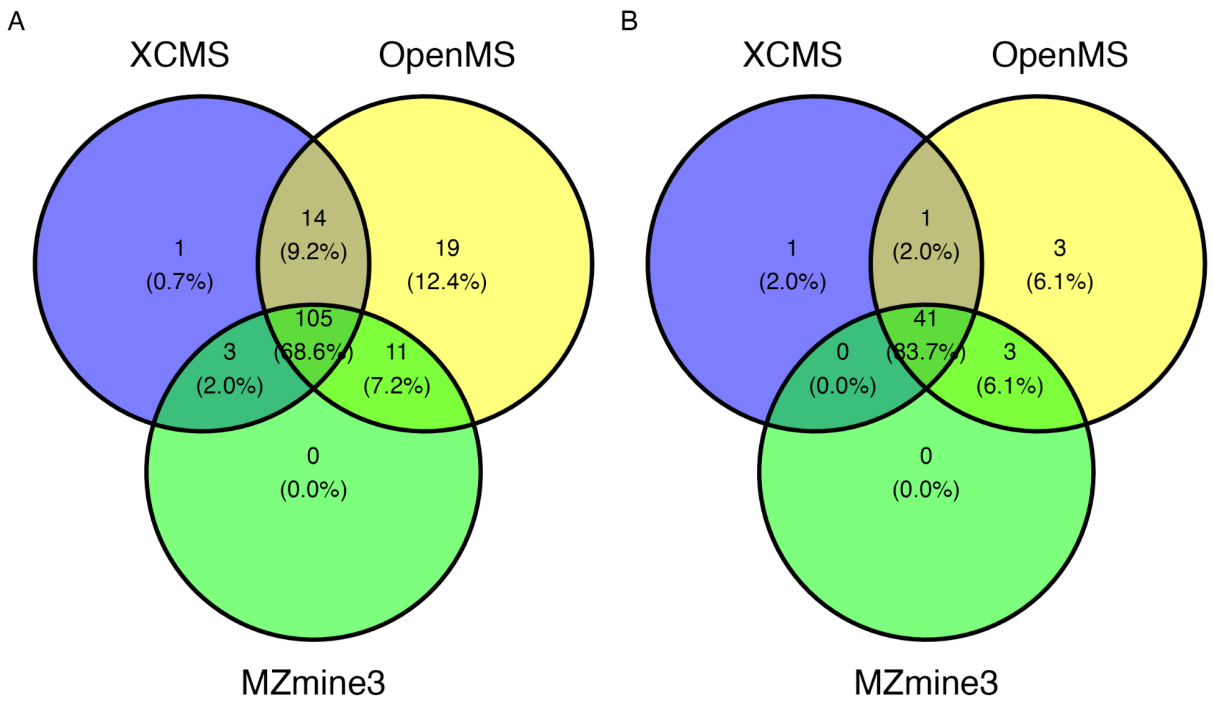

Figure 2. Venn diagram for real detected peaks(left) and detected compounds(right) among XCMS, OpenMS, and MZmine 3.

Since more than half of the compounds are missing for all those three compounds, we also checked the missed compounds. Only 75 missing peaks are not captured by peak picking software while they are from 51 unique compounds. In this case, when the compounds generate less peaks, the software might miss them.

Software comparison for tailing/leading peaks

We also simulate the tailing and leading chromatography peaks by changing the tailing factor of all peaks. As shown in table 1, different software also displays different sensitivity. XCMS's performance is quite stable across different types of peaks while finding the minimal numbers of true peaks. MZmine also showed a stable performance with a similar false positive rate(75%-77%) compared with XCMS. OpenMS, on the other hand, finds more false positive peaks in leading peaks than tailing peaks. In this case, for data with known leading or tailing peaks, it's better to use MZmine 3 for peak picking.

Table 1. Extracted peaks/True positive peaks/Detected compounds of XCMS, OpenMS, MZmine 3 peak picking algorithms for 100 simulated compounds with 593 normal (tailing factor 1), leading(tailing factor 0.8) and tailing(tailing factor 1.5) chromatography peaks.

	XCMS	OpenMS	MZmine 3
normal	495/121/43	926/179/48	776/186/44
leading	495/122/43	1182/188/51	789/185/44
tailing	489/120/43	772/159/48	787/193/45

Redundant peaks' influences on statistical analysis

Redundant peaks, characterized by their lack of new biological information and interdependence with other peaks from the same compounds, can affect the efficiency of data analysis. To address this, we conducted experiments involving two groups of samples, each containing known compounds with altered intensities. Importantly, we applied different intensity cutoffs to each set of spectra, with the rationale of reducing the volume of real data for expedited high-throughput analysis.

In a scenario where 30 out of 100 peaks exhibited changes between the two groups of files, we employed intensity cutoffs of 0 and 0.05 to examine their impact on statistical analysis. When the cutoff is set at 0, all peaks derived from the same compounds were retained in the analysis. However, when the cutoff was increased to 0.05, peaks with relative intensities lower than 5% of the base peaks were filtered out. This adjustment resulted in a notable reduction in the typical file size of mzML files, shrinking from 185MB to 34.5MB for the same compounds.

Specifically, using a cutoff of 0, we identified 3,400 real peaks from 100 compounds, whereas with a cutoff of 0.05, only 533 peaks remained. Subsequently, employing XCMS for peak extraction, we obtained 2,546 peaks for cutoff 0 and 496 peaks for cutoff 0.05. Among these, 914 peaks from 26 compounds exhibited changes in files with a cutoff of 0, and 817 peaks were significant according to t-tests with Benjamini-Hochberg procedure[36] to control false discovery rate, with 15 compounds classified as false positives. For the cutoff of 0.05, 154 peaks changed from 27 compounds, and 154 peaks were significant using t-tests with false discovery control, with 8 compounds identified as false positives.

This analysis demonstrates that an increase in redundant peaks leads to a higher occurrence of false positives for differential analysis. In practical applications, a suitable cutoff to remove redundant peaks is crucial to avoid bias on compounds with more peaks.

Other potential applications

In addition to comparing various peak-picking algorithms, the simulation of mzML files holds significant potential for enhancing existing algorithms in a broader context within the field of metabolomics. For instance, researchers can utilize this methodology to introduce matrix ions as a form of background noise into their data. This allows for the investigation of how these additional ions may impact subsequent statistical analyses, providing valuable insights into data robustness and the potential need for noise mitigation strategies.Furthermore, when dealing with known compounds with predefined retention times, mzML file simulation offers a unique opportunity to assess the influence of co-elution effects and evaluate the effectiveness of peak alignment algorithms. These insights are crucial for refining data processing pipelines and improving the accuracy of biomarker discovery. Beyond these applications, mzML file simulation can also serve as a valuable tool for validating emerging data analysis methods, such as those based on deep learning techniques and image analysis. By generating controlled datasets with known properties, researchers can rigorously test and fine-tune these innovative approaches, ultimately advancing the state-of-the-art in metabolomics data analysis.

This package also contains limitations. Though we applied experimental data for simulation, the real samples might contain complex matrix effects. The lack of response factor database makes it hard to simulate real samples under certain metabolites profile. Meanwhile, we also left the simulation of retention time to users' inputs as the elution order of compounds will change on different columns.

Conclusion

In summary, we have introduced a dedicated raw file simulation tool designed specifically for chromatography coupled with mass spectrometry data. Our initial findings found the presence of false positive peaks and missing true positive peaks. Additionally, our investigation has shed light on the role of redundant peaks, which can introduce false positives into the analysis. The mzrtsim package highlights critical insights into the challenges and opportunities for metabolomics data analysis software development. Such challenges and opportunities included, but are not limited to the optimization for compounds with less peaks, intensity cutoff selection for each spectrum, peak shape adaptation for different column conditions, as well as simulation of various matrix effects.

Author Contributions

M. Y.: Conceptualization, software development, data curation, visualization, writing-original draft, writing-review and editing; V.P.: writing review and editing, supervision. All authors read, reviewed, and accepted the final manuscript.

Data Availability Statement

All the data associated with the present study can be accessed on Zenodo (https://doi.org/10.5281/zenodo.8299782) and software could be downloaded from GitHub (https://github.com/yufree/mzrtsim)

Conflicts of Interest

The authors declare no conflict of interests. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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