

# metaboPipe: a Modular Pipeline for Metabolomic Data Preprocessing



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## Final Work Card

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### Abstract

A maximum of 250 words, detailing the purpose, context of application, methodology, results and conclusions of the work.

*“Never let your sense of morality stop you from doing the right thing”*

Isaac Asimov

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# 1. Introduction

## 1.1 Problem description

Metabolomics, a powerful and evolving field within the realm of systems biology, plays a pivotal role in unraveling the intricate web of biochemical processes occurring within living organisms. As we delve into the molecular intricacies of biological systems, the generation of vast and complex datasets poses a significant challenge. Challenges in standardizing nutritional metabolomics include experimental design, sample preparation, and data analysis, which impact result validity and reproducibility. Efforts by the international community aim to establish standard procedures and infrastructure for advancing nutritional metabolomics research. This master thesis project aims for the creation of a modular pipeline designed to streamline the processing of targeted metabolomics data to a usable and meaningful dataset for further analysis and biological interpretation.

## 1.2 Context and justification

Metabolomics is a rapidly evolving field within biology that focuses on the comprehensive study of the metabolite composition of cell types, tissues, organs, or organisms [1–3]. It aims to measure, identify and (semi-)quantify those metabolites. Metabolites are chemical compounds that undergo analysis through conventional chemical assessment methods like [Mass Spectrometry \(MS\)](#) and [Nuclear Magnetic Resonance \(NMR\)](#) spectrometry. MS approaches are commonly integrated with [Gas Chromatography \(GC\)](#) and [Liquid Chromatography \(LC\)](#), leading to the development of two advanced techniques known as [Gas Chromatography-Mass Spectrometry \(GC-MS\)](#) and [Liquid Chromatography-Mass Spectrometry \(LC-MS\)](#). All of these analytical platforms and methodologies generate large amounts of high-dimensional and complex experimental raw data.

However, the statistical analysis of metabolomics data presents significant challenges, attributable not only to the inherent complexity of metabolomics as a research discipline but also to the intricate nature of the data itself. Notwithstanding that numerous studies have explored various methodologies for metabolomic data management, the field still lacks an accepted standard for preprocessing and pretreatment of such data.

One of the obstacles the field encounters is the lack of well defined terminology, as the terms “data preprocessing” and “data pretreatment” have not been used consistently in metabolomics literature [4].

The objectives of data preprocessing/pretreatment encompass two primary aims: firstly, to rectify or mitigate instrumental artifacts and extraneous biological variance, thereby amplifying the [Signal-to-Noise Ratio \(SNR\)](#); and secondly, to effectively transform the data into interpretable spectral profiles through processes such as centering, scaling, and dimensionality reduction [4, 5]. The choice of preprocessing and pretreatment methods can significantly im-

pact the downstream analysis and interpretation of metabolomic data [6] so the steps should be carefully selected based on the specific characteristics of the data and the research.

By establishing a standardized approach to preprocess and pretreat metabolomic data, the field can improve the quality, comparability, and reproducibility of metabolomic studies. This would facilitate data integration, enable the development of robust statistical models, and enhance our understanding of the complex metabolic processes underlying health and disease.

### 1.2.1 Preprocessing of data

Given the inherent dissimilarities in data acquisition techniques, unique preprocessing procedures are imperative before embarking on statistical analyses in metabolomics investigations. NMR spectra, for instance, often exhibit signal shifts along the axis due to factors like pH fluctuations [7]. Thus, meticulous preprocessing is indispensable to ensure robust statistical analyses and facilitate inter-spectral signal comparisons. This involves techniques such as binning, peak fitting with spectral databases, and exclusion of unstable or non-informative spectral regions (e.g., water peaks) [3, 4, 8]. By refining the dataset to a subset of relevant metabolites, statistical methods can effectively discern variations in signal intensity among sample groups [9].

The preprocessing workflows diverge between MS-based and NMR-based metabolomic analyses. In MS-based profiling, data are presented as three-dimensional (3D) tables, in contrast to the two-dimensional (2D) tables derived from GC-MS data preprocessing [4, 8]. GC-MS preprocessing entails deconvolution and peak integration to generate intensity profiles for each sample feature corresponding to RT/m/z pairs. Notably, metabolite identification strategies differ between GC-MS and LC-MS methodologies. While GC-MS relies on reproducible mass spectra and extensive databases for metabolite identification based on characteristic fragment ions, MS-based methods prioritize automation, accuracy, peak identification, integration, and annotation [10, 11].

While the primary objective of preprocessing is to render data comparable across samples despite instrumental discrepancies, the strategies employed in MS-based methodologies differ from those in NMR-based approaches. Moreover, variations exist between preprocessing methodologies utilized in GC-MS and LC-MS metabolomic analyses, underscoring the intricate nature of metabolomics data preprocessing.

#### MS-based data preprocessing

MS-based analysis involves the measurement of Mass-to-Charge Ratio (m/z). When combined with either LC or GC, the resulting raw GC/LC-MS data encompass three measured variables: m/z, chromatographic Retention Time (RT), and intensity count, thereby constituting a three-dimensional (3D) data structure. To streamline the data and eliminate spectral noise and irrelevant biological variability, a two-dimensional (2D) features table is generated through peak picking. This table encompasses all quantified metabolic features from the analyzed samples, with rows corresponding to samples and columns representing variables such as peak areas or intensities, characterized by m/z and retention time in minutes or scan number (m/z-RT pairs). The preprocessing of MS data involves several steps: 1) denoising and baseline correction; 2) alignment across all samples; 3) peak picking; 4) merging the peaks; and 5) creating a data

matrix [3, 4, 10, 12–17].

## NMR-based data preprocessing

Similar to MS-based analysis, NMR-based analysis generates a 2D structure of feature data matrix with the samples in the rows and the spectral data points in the columns. Also similar to MS-based analysis, the NMR-based analysis (e.g., 1H NMR analysis) requires data preprocessing to mitigate non-biologically relevant effects. The following data preprocessing steps could be performed: 1) baseline correction; 2) peak binning; 3) peak alignment; 4) quality control; 5) create a data matrix [4, 5, 15–20]. Preprocessing by either MS or NMR constructs a data matrix containing the relative abundances of a set of mass spectra for a group of samples or subjects under different conditions. The metabolomics data matrix are typically constructed in such a way that each row of the data matrix represents a subject and each column represents the mass spectra (metabolite intensities or metabolite relative abundances, peak or peak intensities).

### 1.2.2 Pretreatment of Data

#### Handling Missing Values

Within datasets, missing values or zeros can arise due to a variety of factors, both biological and technical in nature. Categorizations by Sun Xia delineate these zeros into four distinct categories: 1) Structural zeros, 2) Sampling zeros, 3) Values below the limit of detection (LOD), and 4) Zeros derived from negative values that are automatically transformed.

1. **Structural zeros** pertain to peaks absent from a sample or chromatogram due to genuine biological absence rather than technical errors. For instance, if a compound is not present in a biological sample, the corresponding peak for that compound is deemed a structural zero.
2. **Sampling zeros** refer to peaks present in samples but missed during peak picking.
3. **Values below LOD** represent intensities or abundances falling below the detection limit of the mass spectrometer.
4. **Negative value zeros** result from negative intensity or abundance values, considered spectral artifacts or noise, and subsequently transformed to zero.

Identifying the origins of these zeros poses a challenge, and their prevalence presents a significant obstacle for statistical analyses [4, 21]. Hence, practical approaches for managing zeros include:

1. **Filtering** based on a threshold, such as the 80% rule.
2. **Imputation** techniques, which can involve substituting zeros with the mean, minimum (or half of the minimum) of non-missing values, or simply zero.
3. Utilizing **missing data estimation algorithms** to employ various methods for handling missing values.

However, it's crucial to recognize that valuable biological insights may be embedded within peaks containing missing values.

## Managing Outliers

Various methods exist for addressing outliers, including:

1. Assessing metabolite peak areas and comparing the ratio of mean to median, with the median often considered more robust in the presence of outliers.
2. Employing [Principal Component Analysis \(PCA\)](#) to identify outliers, followed by techniques such as [Principal Component Partial R-square \(PCPr2\)](#) and [Analysis of Variance \(ANOVA\)](#).
3. Recent advancements have introduced specialized algorithms for outlier identification in metabolomic data, such as cellwise outlier diagnostics using robust pairwise log ratios (cell-rPLR) and a kernel weight function-based biomarker identification technique.

## Normalization

Normalization is a crucial step in data preprocessing that seeks to eliminate unwanted variations between samples. By doing so, it ensures that samples can be directly compared to each other by eliminating or reducing systematic errors, biases, and experimental variance [22].

Normalization of data within metabolomic workflows can occur either during sample analysis (preanalytical normalization) or during postanalytical data processing. Normalization of samples is essential due to variations in composition influenced by factors like time of day, health status, and dietary intake.

For instance, blood samples may not require normalization due to the body's control over blood volume and composition. However, urine samples may necessitate normalization due to potential concentration variations [23].

## Centering and Scaling

Centering aims to shift metabolite concentrations to fluctuate around zero, while scaling adjusts for fold-change differences between metabolites. Both steps are crucial in data preprocessing.

## Transformation

Transformation becomes necessary to address data variance after scaling, aiming to correct for heteroscedasticity, convert multiplicative relations into additive ones, and normalize skewed distributions.

## 1.3 State of the art

Metabolomic data arrives to the researcher in different shapes and forms depending on the method, the instrument used and the company that analyses. Most of the time those companies do a preprocessing of the data, adjusting for baseline correction, peak picking, and alignment. The preprocessing of this data is a crucial step in the analysis, as it can significantly impact the results and the conclusions drawn from the data. For [MS](#)-based analysis, tools like XCMS

[24] and MZmine [25] facilitate preprocessing, while for NMR data, packages like BATMAN [26] and RAMSY [27] offer robust preprocessing capabilities. Pretreatment techniques include handling missing values, outlier detection, imputation and normalization.

Nevertheless the field lacks a standardized approach to metabolomic data preprocessing, with inconsistencies in terminology and methodologies. Stanstrup *et al.* in their “The metaR-bolomics Toolbox in Bioconductor and Beyond” made an extensive revision of both the scientific literature and the R landscape for packages relevant for metabolomic research.

Since there is no consensus on the order those pretreatment techniques should be applied, the researcher has to decide which steps to take and in which order. This can lead to inconsistencies in the results and the conclusions drawn from the data. Furthermore, the amount of packages that there are for performing the preprocessing and pretreatment of metabolomic data can be overwhelming. And thus preparing the data for analysis, changing the order of the steps, or even changing the parameters of the steps can be a time-consuming task.

The project aims to develop a modular pipeline for targeted metabolomic data pretreatment, implemented in R. The pipeline is designed to enhance efficiency and modularity compared to existing solutions. As a gift to the scientific community, this project is free and open source, with detailed documentation and code available in a public repository. It’s encouraged continuous community efforts to improve and expand the package.

Add hypothesis

### 1.3.1 Hypothesis

- It is possible to design and develop a modular pipeline for the pretreatment of targeted metabolomic data.
- Developing a standardized pretreatment pipeline for targeted metabolomics data will significantly enhance the comparability and reproducibility of results across different studies and laboratories.
- A standarization of the preprocessing and pretreatment of targeted metabolomic data can be achieved by
- A modular pipeline that allows researchers to customize preprocessing and pretreatment steps will improve the flexibility and efficiency of metabolomic data analysis,

## 2. Objectives

### 2.1 Main Objective

1. Develop a new pipeline for the preprocessing of targeted metabolomic data with the aim of improving efficiency and modularity compared to existing pipelines. This new pipeline will be implemented in R.

### 2.2 Specific Objectives

1. Design a new pipeline for the preprocessing of targeted metabolomic data.
2. Implement the preprocessing pipeline for targeted metabolomic data using the "targets" package to ensure replicability and efficient management of computational resources.
3. Select various targeted metabolomic datasets to validate and optimize the performance of the new pipeline, analyzing its quality and consistency.
4. Make the development accessible to the scientific community by creating detailed documentation and publishing the code in a public repository.

En els objectius específics és on hauries de parlar de la forma en que ho faras (per exemple, fent servir el paquet "targets") i també introduir la selecció i anàlisi d'alguns datasets ilustratius. En aquest camp sovint els datasets ho son tot. Ja he vist que després parles deL "approach taken to achieve this objective", però es que això és el que haurien de ser les tasques i la metodologia.

Resumint, valdria la pena incloure lo del targets i els datasets en les tasques i deliverables i sobretot tenir en compte que caldrà agafar que ja estigui fet o que caldria fer, i per això serà bo que en la revisió de la literatura" hi incloguis, no només, treballs de metabolòmica sino paquets de R que ja estan implementant moltes d'aquestes coses.

### 3. Sustainable development goals

Our project aligns with multiple crucial Sustainable Development Goals (SDGs) set by the United Nations, fostering global sustainability and development. The primary objectives of our project focus on developing a pipeline to modulate the pretreatment of metabolomics data and creating an R implementation. This solution holds significant potential to support the following SDGs:

#### **SDG 3: Good Health and Well-being**

The use of our pipeline has the potential to reduce the time required for metabolomic data research, accelerating the investigation of rare diseases, cancer, and other medical conditions. By expediting research processes, our project contributes to advancing medical science, improving healthcare outcomes, and ultimately enhancing global health and well-being.

#### **SDG 9: Industry, Innovation, and Infrastructure:**

Our focus on developing an open-source, well-documented, and user-friendly pipeline fosters innovation and infrastructure development. By opening access to metabolomics research tools, our project empowers individuals from diverse backgrounds to engage in scientific inquiry and innovation, thus promoting inclusive economic growth and technological progress.

#### **SDG 10: Reduced Inequalities:**

Through our implementation, we prioritize inclusivity and accessibility, ensuring that individuals regardless of sex, gender, race, wealth, or ability can utilize, learn from, and contribute to our pipeline. By reducing barriers to entry and promoting equal opportunities for participation in scientific endeavors, our project contributes to reducing inequalities and promoting social inclusion.

While our project aims to bring about positive change, it is essential to consider potential negative impacts and ethical considerations. These may include concerns about data privacy and security, particularly in handling sensitive information. Additionally, there may be unintended consequences such as exacerbating existing inequalities in access to technology or inadvertently reinforcing biases in data analysis. Therefore, it is imperative to approach the development and implementation of our pipeline with careful consideration of ethical principles, transparency, and accountability to mitigate potential risks and maximize societal benefits.



## 4. Approach and methodology

### 4.1 Methodology

Mención de cuáles son las posibles estrategias para llevar a cabo el trabajo y cuál es la estrategia elegida (desarrollar un producto nuevo, adaptar un producto existente...). Hay que incluir una valoración de por qué esta es la estrategia más apropiada para conseguir los objetivos.

In the context of enhancing the field of metabolomics and improving the efficiency and accuracy of metabolomics reports, the choice of creating a tool for targeted metabolomic data pretreatment is a strategic decision. This tool will be developed in R, a widely used programming language in the field of bioinformatics and biostatistics. The tool will be designed to streamline the pretreatment of targeted metabolomic data, ensuring that the data is ready for further analysis and interpretation.

The tool will be modular, allowing users to select and apply specific pretreatment steps according to their needs and preferences. This modularity design makes the tool flexible, adaptable and more important expandable, as new pretreatment methods can be easily added to the pipeline and thus to the package using existent methods and tools from other packages.

In order to be modular, the package modules should take as inputs the same object type that outputs. Making it easier to chain the modules in any specific order. To achieve this objective, the package should use a main data structure to store the data. In omics data, the most used data structure is the **SummarizedExperiment** from the Bioconductor project [28]. However this data structure is not the most efficient for the pretreatment of the metabolomics data, as the **SummarizedExperiment** dataframes are designed to store the data features in a row-wise manner and the samples in a column-wise manner. Though this may fit well for other omics data, is not the most intuitive way to manipulate the data in the context of metabolomics.

Since targeted metabolomic data uses multiple dataframes to store the different types of data, the most intuitive and already developed data structure will be the **DatasetExperiment** from the **structToolbox** package [29]. This data structure is designed to store the data features in a column-wise manner and the samples in a row-wise manner, making it more intuitive to manipulate the data in the context of metabolomics. It also incorporate multiple methods for the manipulation of the data, making it easier to develop the package.

A deployment controller will be used to manage the order of the modules and the data flow between them. This controller will be created using the **targets** package [30], a pipeline toolkit for reproducible research. The **targets** package will ensure that the pipeline is reproducible, efficient and easy to manage.

The **targets** package only deploys the steps needed to complete the pipeline as previous steps that do not change its output will not be re-run. This makes the pipeline more efficient and faster, as only the steps that need to be re-run will be re-run.

The integration of modularity with a deployment controller creates 2 useful features:

- The first one is that the pipeline can be run in parallel, as the steps that do not depend on each other can be run at the same time. This will make the pipeline faster and more efficient.

In order to select the methods and tools that will be used in the pipeline, a deep review of the literature will be performed. This review will explore recent publications of metabolomics data and also comparative reviews of the most used methods and tools for the pretreatment of targeted metabolomic data to select the most used methods for metabolomic data pretreatment. This review will also explore and select datasets to validate and optimize the performance of the pipeline.

## 4.2 Planning and calendar

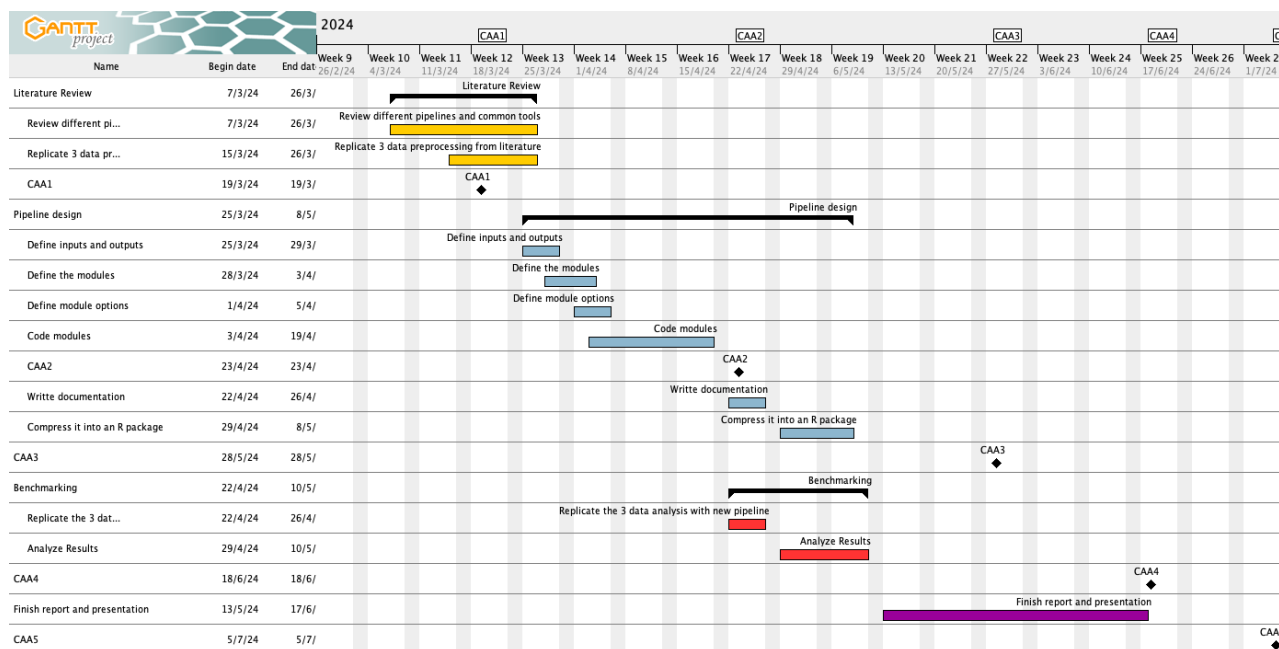


Figure 4.1: Gantt chart showing the project timeline and milestones.

### 4.2.1 Tasks

#### Main Tasks and prioritization

1. **Definition of the work plan:** Define the project's scope, objectives, methodology and expected outcomes. Create a project charter outlining the project's purpose and goals as well as a calendar with milestones and dates.
2. **Literature review:** Review multiple publications and replicate the data processing from 3 of them. Review most used methods and tools for metabolomics data processing like normalization, scaling, filtering, transformation, batch effect

3. **Pipeline design:** Propose and code in R a new pipeline to manage the processing of targeted metabolomics data.
4. **Benchmarking:** Replicate again the data processing of the 3 publications using the new pipeline and compare the results.

### Extra tasks

1. **Heavy-workload:** Optimize the pipeline to enable parallelization of the processing.
2. **Documentation:** Write a documentation for the package so its accessibility.
3. **R package implementation:** Pack the code for the pipeline in an R package to easy distribution.

## 4.3 Risk analysis

| Risk                        | Severity | Likelihood | Mitigation  |
|-----------------------------|----------|------------|---|
| Resource constraints        | Moderate | Moderate   | Develop a clear project timeline, incorporating milestones and allocating adequate time for each phase. Ensure contingency measures are in place to address unforeseen challenges or changes. |
| Technical challenges        | Moderate | High       | Perform proper exploration of packages and software and seek guidance and mentorship from professors or experts in relevant fields.   |
| User adoption and awareness | High     | Moderate   | Be sure to incorporate appropriate cautions regarding the correct application of the chosen modules and data.   |
| Pipeline branching          | Low      | Moderate   | Adopt new methods to interactively select the branching   |

Table 4.1: Risk analysis. This table presents various risks associated with the project, along with their severity, likelihood, and potential mitigation measures.

## 4.4 Final products

- A **pipeline** for targeted metabolomic data preprocessing designed to streamline data pre-treatment tasks, optimize workflows, and ensure reproducibility in metabolomic research

endeavors.

- A **R package** crafted to facilitate the seamless integration and modular implementation of the pipeline within the R environment, empowering researchers with flexible and efficient tools for metabolomic data analysis.
- A detailed **documentation** accompanying the pipeline and R package.
- A user-friendly **Shiny app** that enables researchers with varying levels of computational expertise to effortlessly pretreat targeted metabolomic data.
- A **PDF report** detailing the project's processes, including investigation, development, results, conclusions, and discussions.
- A **virtual presentation** providing a comprehensive overview of the project. This includes a video recording with explanatory narration to further enhance understanding.

## 5. Materials and methods

Metodes: Com Desenvolupo //  
Materials: Amb que ho fas

### 5.1 Literature review

The literature selected for the review was obtained from multiple journals. The search was conducted using the following keywords: "metabolomics", "data preprocessing", "data pretreatment", "metabolomics pipeline", "metabolomics tools", "metabolomics R packages", "targeted metabolomics", "nutrimetabolomics", "metabolomics proce". The search was limited to articles published in the last 20 years, with a focus on metabolomics data preprocessing and pretreatment methodologies. The review aimed to identify the most commonly used methods and tools for metabolomic data preprocessing and pretreatment, as well as to explore recent advancements in the field. The review also sought to identify gaps in the existing literature and to inform the development of the new pipeline.

Incloure taula amb els articles  
revisats?

### 5.2 Pipeline design

The methods selected to be included in the pipeline were based on the results of the literature review. The pipeline was designed to be as modular as possible so a variety of methods are included to perform the same step, allowing users to select and apply specific pretreatment steps according to the data characteristics and user needs and preferences.

### 5.3 Datasets

The datasets employed for the purpose of this study were obtained from public repositories. The datasets were selected based on the following criteria:

- Aim of the dataset
- Availability of raw data
- Targeted metabolomics data
- Diverse biological samples

The datasets selected were 2:

- **MTBLS79:** [31] This dataset represents a systematic evaluation of the reproducibility of a multi-batch direct-infusion mass spectrometry (DIMS)-based metabolomics study of cardiac tissue extracts. It comprises twenty biological samples (cow vs. sheep) that were analysed repeatedly, in 8 batches across 7 days, together with a concurrent set of quality

control (QC) samples. Data are presented from each step of the data processing workflow and are available through MetaboLights. This dataset was selected due to its importance in the field of metabolomics and its data availability.

- **ST000284:** [\[32\]](#) This dataset from MetaboWorkbench includes a study on colorectal cancer (CRC) using targeted liquid chromatography-tandem mass spectrometry. It examines 158 metabolites across 25 pathways in 234 serum samples (66 CRC patients, 76 polyp patients, 92 healthy controls). Blood samples were collected after fasting and bowel preparation. This dataset was selected due to its data availability, its diversity (3 groups) and amount of samples.

## 5.4 Packages

The pipeline was developed using the R programming language [\[33\]](#) and the packages described in [5.1](#).

| Package              | Version | Ref  |
|----------------------|---------|------|
| arrow                | 15.0.1  | [34] |
| base                 | 4.3.3   | [33] |
| BiocStyle            | 2.30.0  | [35] |
| caret                | 6.0.94  | [36] |
| cowplot              | 1.1.3   | [37] |
| crew                 | 0.9.2   | [38] |
| datasets             | 4.3.3   | [33] |
| dplyr                | 1.1.4   | [39] |
| DT                   | 0.33    | [40] |
| fst                  | 0.9.8   | [41] |
| ggforce              | 0.4.2   | [42] |
| graphics             | 4.3.3   | [43] |
| grDevices            | 4.3.3   | [33] |
| HotellingEllipse     | 1.1.0   | [44] |
| impute               | 1.76.0  | [45] |
| imputeLCMD           | 2.1     | [46] |
| knitr                | 1.46    | [47] |
| MetaboAnalystR       | 4.0.0   | [48] |
| methods              | 4.3.3   | [33] |
| missForest           | 1.5     | [49] |
| pcaMethods           | 1.94.0  | [50] |
| pmp                  | 1.14.1  | [51] |
| purrr                | 1.0.2   | [52] |
| renv                 | 1.0.7   | [53] |
| reshape2             | 1.4.4   | [54] |
| rmarkdown            | 2.27    | [55] |
| shiny                | 1.8.1.1 | [56] |
| shinyFiles           | 0.9.3   | [57] |
| stats                | 4.3.3   | [33] |
| structToolbox        | 1.14.0  | [58] |
| SummarizedExperiment | 1.32.0  | [28] |
| tarchetypes          | 0.9.0   | [59] |
| targets              | 1.7.0   | [60] |
| tidyverse            | 2.0.0   | [61] |
| tinytex              | 0.51    | [62] |
| tools                | 4.3.3   | [33] |
| usethis              | 2.2.3   | [63] |
| utils                | 4.3.3   | [33] |
| VIM                  | 6.2.2   | [64] |
| withr                | 3.0.0   | [65] |

Table 5.1: List of R packages with their versions used to develop `metaboPipe`

## 6. Results

### 6.1 Pipeline

add a summary of 1: Number of functions and 2: Number of lines of code

writte the pipeline

In this project, an R package named `metaboPipe` was developed to preprocess metabolomic data efficiently and accessibly. Metabolomic data preprocessing is a crucial step in ensuring the quality and reliability of downstream analyses. The transformations applied during preprocessing are primarily intended to remove unwanted effects unrelated to the study, but these tools can inadvertently delete meaningful biological data if used without caution. The `metaboPipe` package leverages the `targets` and `structToolbox` packages to create a modular and reproducible pipeline for preprocessing. This section details the components and functionality of `metaboPipe`.

### 6.2 Package Overview

The `metaboPipe` package is designed to streamline the preprocessing of metabolomic data by creating a series of user-accessible modules that translate into interconnected steps. These steps can be easily added, modified, and rearranged. The package utilizes the `targets` package to orchestrate task deployment and dependencies, ensuring that each step is executed in the correct order. The `structToolbox` package provides the `DatasetExperiment` object, which serves as the primary data structure throughout the pipeline, along with multiple functions for data transformation.

The package can be divided into three main categories:

- **Data manipulation:** These functions are the core of the package, as they transform the data into the desired formats and apply the diversity of methods to perform the preprocessing steps including the visualization.
- **Target factories:** They are the pipeline contruction functions for the users to interact with, as they use the `targets` package to create pipeline nodes that use the data manipulation functions. Each factory is intended to represent a specific pretreatment step.
- **Shiny app:** This is a user-friendly interface that allows users to create and run the pipeline without the need for R programming knowledge.

### 6.3 Data manipulation

For the pretreatment of the data, the package includes the following functions:

- A function to create a `DatasetExperiment` object from a list of dataframes.



- A function to filter the data based on the percentage of missing values both in the features and the samples.
- A function to filter the outliers based on a Hotelling's T2 distribution ellipse.
- 8 functions to impute the missing values based on the following methods: *Mean*, *Median*, *Random Forest (RF)*, *Quantile Regression Imputation of Left-Censored data (QRILC)*, *k-Nearest Neighbors (kNN)*, *Singular Value Decomposition (SVD)*, *Bayesian Principal Component Analysis (BPCA)* and *Probabilistic Principal Component Analysis (PPCA)*.
- 2 functions to remove the batch effect using either the *Quality Control-Robust Spline Correction (QC-RSC)* method or the **ComBat** method.
- A function to normalize, scale and transform the data based on the methods provided by the **metaboAnalystR** package.
- 12 data visualization functions to create plots.
- 17 utility functions to perform different tasks.

add references to the methods

## 6.4 Target factories

For the pipeline construction, the package includes the following 9 functions:

- The **load\_data** function is implemented to efficiently load and read data from specified files into data frames. It dynamically creates six different nodes based on the presence of the **variableMetadata** parameter.
- The **create\_experiment** function is the pivotal component in the data processing pipeline, enabling the creation of a **DatasetExperiment** S4 object that will be used in almost all of the pipeline. This facilitates efficient modularity.
- The **factorize\_cols** function that makes the specified columns of the **sampleMetadata** as factors.
- The **filter\_step** function that creates up to 5 nodes depending on the parameters provided. These nodes filter the data based on the percentage of missing values in the features and samples, filter outliers using a Hotelling's T2 distribution ellipse and creates a plot for visualizing the data missing values before and after the filtering is done.
- The **batch\_correct** function that creates a node to remove the batch effect using the QC-RSC method.
- The **impute** function that creates a node to impute the missing values using the method provided.
- The **normalize** function that creates a node to normalize, scale and/or transform the data using the methods provided.
- The **export\_data** function that creates a node to export the data to a specified directory.

## 6.5 Documentation

## 6.6 Shiny app

A shiny app was developed to allow users to interact with the pipeline without the need for R programming knowledge. The app provides 3 main tabs:

- The **Data upload** tab allows users to either upload their data or use one of the example data provided.
- The **Data Config** tab allows users to set the global parameters for the data, like the `sample_ID` column, the column to be used as QC or Sample identifier, and the main study factor. It also allows users to visualize the data tables they are working with to ensure the data is correctly loaded.
- The **Process Selector** tab allows users to select and configure the different steps of the pipeline. Each step is represented by a card that can be expanded to show the parameters of the step. The user can select the steps they want to include in the pipeline and configure them according to their needs. The app will then generate the `_targets.R` file and execute the pipeline from

## 7. Discussion

## 8. Conclusion and future vision

The second one is that it enables the pipeline to be used as a pretreatment method comparable tool. As the easy of use and order can be easily changed, the user can test different pretreatment methods and orders to compare the changes in the data.

Este capítulo tiene que incluir:

- Una descripción de las conclusiones del trabajo:
  - Una vez se han obtenido los resultados, ¿qué conclusiones se extraen?
  - ¿Estos resultados son los esperados? ¿O han sido sorprendentes? ¿Por qué?
- Una reflexión crítica sobre el logro de los objetivos planteados inicialmente:
  - ¿Hemos logrado todos los objetivos? Si la respuesta es negativa, ¿por qué motivo?
- Un análisis crítico del seguimiento de la planificación y metodología a lo largo del producto:
  - ¿Se ha seguido la planificación?
  - ¿La metodología prevista ha sido suficientemente adecuada?
  - ¿Ha habido que introducir cambios para garantizar el éxito del trabajo? ¿Por qué?
- De los impactos previstos en 3, ético-sociales, de sostenibilidad y de diversidad, evaluar/mencionar si se han mitigado (si eran negativos) o si se han conseguido (si eran positivos).
- Si han aparecido impactos no previstos a 3, evaluar/mencionar cómo se han mitigado (si eran negativos) o que han aportado (si eran positivos).
- Las líneas de trabajo futuro que no se han podido explorar en este trabajo y han quedado pendientes.
- Glossary test: [LaTeX](#)

# Glossary

**LaTeX** A typesetting system used for document preparation. [27](#)

# Acronyms

**ANOVA** Analysis of Variance. [12](#)

**GC** Gas Chromatography. [9](#), [10](#)

**GC-MS** Gas Chromatography-Mass Spectrometry. [9](#), [10](#)

**LC** Liquid Chromatography. [9](#), [10](#)

**LC-MS** Liquid Chromatography-Mass Spectrometry. [9](#), [10](#)

**m/z** Mass-to-Charge Ratio. [10](#)

**MS** Mass Spectrometry. [9–12](#)

**NMR** Nuclear Magnetic Resonance. [9–11](#), [13](#)

**PCA** Principal Component Analysis. [12](#)

**PCPr2** Principal Component Partial R-square. [12](#)

**RT** Retention Time. [10](#)

**SNR** Signal-to-Noise Ratio. [9](#)

# Bibliography

1. Patti, G. J., Yanes, O. & Siuzdak, G. Metabolomics: The Apogee of the Omics Trilogy. *Nature Reviews Molecular Cell Biology* **13**, 263–269. ISSN: 1471-0080 (Apr. 2012).
2. Zhang, A., Sun, H. & Wang, X. Serum Metabolomics as a Novel Diagnostic Approach for Disease: A Systematic Review. *Analytical and Bioanalytical Chemistry* **404**, 1239–1245. ISSN: 1618-2650 (Sept. 1, 2012).
3. Chen, Y., Li, E.-M. & Xu, L.-Y. Guide to Metabolomics Analysis: A Bioinformatics Workflow. *Metabolites* **12**, 357. ISSN: 2218-1989 (4 Apr. 2022).
4. Sun, J. & Xia, Y. Pretreating and Normalizing Metabolomics Data for Statistical Analysis. *Genes & Diseases* **11**, 100979. ISSN: 2352-3042 (May 1, 2024).
5. Martin, M. *et al.* PepsNMR for <sup>1</sup>H NMR Metabolomic Data Pre-Processing. *Analytica Chimica Acta* **1019**, 1–13. ISSN: 1873-4324. pmid: [29625674](#) (Aug. 17, 2018).
6. Karaman, I. in *Metabolomics: From Fundamentals to Clinical Applications* (ed Sussulini, A.) 145–161 (Springer International Publishing, Cham, 2017). ISBN: 978-3-319-47656-8.
7. Bhinderwala, F., Roth, H., Noel, H., Feng, D. & Powers, R. Chemical Shift Variations in Common Metabolites. *Journal of magnetic resonance (San Diego, Calif. : 1997)* **345**, 107335. ISSN: 1090-7807. pmid: [36410060](#) (Dec. 2022).
8. Stanstrup, J. *et al.* The metaRbolomics Toolbox in Bioconductor and Beyond. *Metabolites* **9**, 200. ISSN: 2218-1989 (10 Oct. 2019).
9. Qiu, S. *et al.* Small Molecule Metabolites: Discovery of Biomarkers and Therapeutic Targets. *Signal Transduction and Targeted Therapy* **8**, 1–37. ISSN: 2059-3635 (Mar. 20, 2023).
10. Xiao, J. F., Zhou, B. & Ressom, H. W. Metabolite Identification and Quantitation in LC-MS/MS-based Metabolomics. *Trends in analytical chemistry : TRAC* **32**, 1–14. ISSN: 0165-9936. pmid: [22345829](#) (Feb. 1, 2012).
11. Kiseleva, O., Kurbatov, I., Ilgisonis, E. & Poverennaya, E. Defining Blood Plasma and Serum Metabolome by GC-MS. *Metabolites* **12**, 15. ISSN: 2218-1989. pmid: [35050137](#) (Dec. 24, 2021).
12. Defernez, M. & Le Gall, G. in *Advances in Botanical Research* (ed Rolin, D.) 493–555 (Academic Press, Jan. 1, 2013).
13. Troisi, J., Troisi, G., Scala, G. & Richards, S. M. in *Metabolomics Perspectives* (ed Troisi, J.) 287–379 (Academic Press, Jan. 1, 2022). ISBN: 978-0-323-85062-9.
14. Burton, L. *et al.* Instrumental and Experimental Effects in LC-MS-based Metabolomics. *Journal of Chromatography B. Hyphenated Techniques for Global Metabolite Profiling* **871**, 227–235. ISSN: 1570-0232 (Aug. 15, 2008).

15. Trygg, J., Gabrielsson, J. & Lundstedt, T. in *Comprehensive Chemometrics* (eds Brown, S. D., Tauler, R. & Walczak, B.) 1–8 (Elsevier, Oxford, Jan. 1, 2009). ISBN: 978-0-444-52701-1.
16. Alonso, A., Marsal, S. & Julià, A. Analytical Methods in Untargeted Metabolomics: State of the Art in 2015. *Frontiers in Bioengineering and Biotechnology* **3**. ISSN: 2296-4185 (Mar. 5, 2015).
17. Bloemberg, T. G., Gerretzen, J., Lunshof, A., Wehrens, R. & Buydens, L. M. C. Warping Methods for Spectroscopic and Chromatographic Signal Alignment: A Tutorial. *Analytica Chimica Acta* **781**, 14–32. ISSN: 0003-2670 (June 5, 2013).
18. Bork, C., Ng, K., Liu, Y., Yee, A. & Pohlscheidt, M. Chromatographic Peak Alignment Using Derivative Dynamic Time Warping. *Biotechnology Progress* **29**, 394–402. ISSN: 1520-6033 (2013).
19. Veselkov, K. A. *et al.* Recursive Segment-Wise Peak Alignment of Biological (1)h NMR Spectra for Improved Metabolic Biomarker Recovery. *Analytical Chemistry* **81**, 56–66. ISSN: 1520-6882. pmid: [19049366](https://pubmed.ncbi.nlm.nih.gov/19049366/) (Jan. 1, 2009).
20. Sawall, M. *et al.* Multi-Objective Optimization for an Automated and Simultaneous Phase and Baseline Correction of NMR Spectral Data. *Journal of Magnetic Resonance* **289**, 132–141. ISSN: 1090-7807 (Apr. 1, 2018).
21. Martín-Fernández, J. A., Palarea-Albaladejo, J. & Olea, R. A. in *Compositional Data Analysis* 43–58 (John Wiley & Sons, Ltd, 2011). ISBN: 978-1-119-97646-2.
22. Zacharias, H. U., Altenbuchinger, M. & Gronwald, W. Statistical Analysis of NMR Metabolic Fingerprints: Established Methods and Recent Advances. *Metabolites* **8**, 47. ISSN: 2218-1989 (3 Sept. 2018).
23. Ulaszewska, M. M. *et al.* Nutrimetabolomics: An Integrative Action for Metabolomic Analyses in Human Nutritional Studies. *Molecular Nutrition & Food Research* **63**, 1800384. ISSN: 1613-4133 (2019).
24. Smith, C. A. *et al.* *xcms: LC-MS and GC-MS Data Analysis* R package version 4.0.2 (2024). <https://bioconductor.org/packages/xcms>.
25. Schmid, R. *et al.* Integrative Analysis of Multimodal Mass Spectrometry Data in MZmine 3. *Nature Biotechnology* **41**, 447–449. ISSN: 1546-1696 (Apr. 2023).
26. Keyes, O. *et al.* *batman: Convert Categorical Representations of Logicals to Actual Logicals* R package version 0.1.0 (2015). <https://CRAN.R-project.org/package=batman>.
27. Gu, H., Gowda, G. A. N., Neto, F. C., Opp, M. R. & Raftery, D. RAMSY: Ratio Analysis of Mass Spectrometry to Improve Compound Identification. *Analytical Chemistry* **85**, 10771–10779. ISSN: 0003-2700 (Nov. 19, 2013).
28. Morgan, M., Obenchain, V., Hester, J. & Pagès, H. *SummarizedExperiment: SummarizedExperiment container* R package version 1.32.0 (2023). <https://bioconductor.org/packages/SummarizedExperiment>.
29. Lloyd, G. R., Jankevics, A. & Weber, R. J. M. struct: an R/Bioconductor-based framework for standardized metabolomics data analysis and beyond. *Bioinformatics* **36**, 5551–5552. <https://doi.org/10.1093/bioinformatics/btaa1031> (2020).



30. Landau, W. M. The targets R package: a dynamic Make-like function-oriented pipeline toolkit for reproducibility and high-performance computing. *Journal of Open Source Software* **6**, 2959. <https://doi.org/10.21105/joss.02959> (2021).
31. Kirwan, J. A., Weber, R. J. M., Broadhurst, D. I. & Viant, M. R. Direct Infusion Mass Spectrometry Metabolomics Dataset: A Benchmark for Data Processing and Quality Control. *Scientific Data* **1**, 140012. ISSN: 2052-4463 (June 10, 2014).
32. Zhu, J. *et al.* Colorectal Cancer Detection Using Targeted Serum Metabolic Profiling. *Journal of Proteome Research* **13**, 4120–4130. ISSN: 1535-3907. pmid: [25126899](https://pubmed.ncbi.nlm.nih.gov/25126899/) (Sept. 5, 2014).
33. R Core Team. *R: A Language and Environment for Statistical Computing* R Foundation for Statistical Computing (Vienna, Austria, 2024). <https://www.R-project.org/>.
34. Richardson, N. *et al.* arrow: Integration to Apache 'Arrow' R package version 15.0.1 (2024). <https://github.com/apache/arrow/>.
35. Oleś, A. *BiocStyle: Standard styles for vignettes and other Bioconductor documents* R package version 2.30.0 (2023). <https://bioconductor.org/packages/BiocStyle>.
36. Kuhn, M. *caret: Classification and Regression Training* R package version 6.0-94 (2023). <https://github.com/topepo/caret/>.
37. Wilke, C. O. *cowplot: Streamlined Plot Theme and Plot Annotations for ggplot2* R package version 1.1.3 (2024). <https://wilkelab.org/cowplot/>.
38. Landau, W. M. *crew: A Distributed Worker Launcher Framework* R package version 0.9.2 (2024). <https://wlandau.github.io/crew/>.
39. Wickham, H., François, R., Henry, L., Müller, K. & Vaughan, D. *dplyr: A Grammar of Data Manipulation* R package version 1.1.4 (2023). <https://dplyr.tidyverse.org>.
40. Xie, Y., Cheng, J. & Tan, X. *DT: A Wrapper of the JavaScript Library DataTables* R package version 0.33 (2024). <https://github.com/rstudio/DT>.
41. Klik, M. *fst: Lightning Fast Serialization of Data Frames* R package version 0.9.8 (2022). <http://www.fstpackage.org>.
42. Pedersen, T. L. *ggforce: Accelerating ggplot2* R package version 0.4.2, <https://github.com/thomasp85/ggforce> (2024). <https://ggforce.data-imaginist.com>.
43. Gentleman, R., Whalen, E., Huber, W. & Falcon, S. *graph: A package to handle graph data structures* R package version 1.80.0 (2023). <https://bioconductor.org/packages/graph>.
44. Goueguel, C. L. *HotellingEllipse: Hotelling T-Square and Confidence Ellipse* R package version 1.1.0 (2022). <https://github.com/ChristianGoueguel/HotellingEllipse>.
45. Hastie, T., Tibshirani, R., Narasimhan, B. & Chu, G. *impute: Imputation for microarray data* R package version 1.76.0 (2023). <https://bioconductor.org/packages/impute>.
46. Lazar, C. & Burger, T. *imputeLCMD: A Collection of Methods for Left-Censored Missing Data Imputation* R package version 2.1 (2022). <https://CRAN.R-project.org/package=imputeLCMD>.
47. Xie, Y. *knitr: A General-Purpose Package for Dynamic Report Generation in R* R package version 1.46 (2024). <https://yihui.org/knitr/>.

48. Xia, J., Chong, J. & Pang, Z. *MetaboAnalystR: An R Package for Comprehensive Analysis of Metabolomics Data* R package version 4.0.0 (2024).
49. Stekhoven, D. J. *missForest: Nonparametric Missing Value Imputation using Random Forest* R package version 1.5 (2022). <https://www.r-project.org>.
50. Stacklies, W., Redestig, H. & Wright, K. *pcaMethods: A collection of PCA methods* R package version 1.94.0 (2023). <https://bioconductor.org/packages/pcaMethods>.
51. Jankevics, A., Lloyd, G. R. & Weber, R. J. M. *pmp: Peak Matrix Processing and signal batch correction for metabolomics datasets* R package version 1.14.1 (2024).
52. Wickham, H. & Henry, L. *purrr: Functional Programming Tools* R package version 1.0.2 (2023). <https://purrr.tidyverse.org/>.
53. Ushey, K. & Wickham, H. *renv: Project Environments* R package version 1.0.5 (2024). <https://rstudio.github.io/renv/>.
54. Wickham, H. *reshape2: Flexibly Reshape Data: A Reboot of the Reshape Package* R package version 1.4.4 (2020). <https://github.com/hadley/reshape>.
55. Allaire, J. *et al.* *rmarkdown: Dynamic Documents for R* R package version 2.26, <https://pkgs.rstudio.com/rmarkdown/> (2024). <https://github.com/rstudio/rmarkdown>.
56. Chang, W. *et al.* *shiny: Web Application Framework for R* R package version 1.8.1.1 (2024). <https://shiny.posit.co/>.
57. Pedersen, T. L., Nijs, V., Schaffner, T. & Nantz, E. *shinyFiles: A Server-Side File System Viewer for Shiny* R package version 0.9.3 (2022). <https://github.com/thomasp85/shinyFiles>.
58. Lloyd, G. R. & Weber, R. J. M. *structToolbox: Data processing & analysis tools for Metabolomics and other omics* R package version 1.14.0 (2023). <https://bioconductor.org/packages/structToolbox>.
59. Landau, W. M. *tarchetypes: Archetypes for Targets* R package version 0.9.0, <https://github.com/ropensci/tarchetypes> (2024). <https://docs.ropensci.org/tarchetypes/>.
60. Landau, W. M. *targets: Dynamic Function-Oriented Make-Like Declarative Pipelines* R package version 1.6.0, <https://github.com/ropensci/targets> (2024). <https://docs.ropensci.org/targets/>.
61. Wickham, H. *tidyverse: Easily Install and Load the Tidyverse* R package version 2.0.0, <https://github.com/tidyverse/tidyverse> (2023). <https://tidyverse.tidyverse.org>.
62. Xie, Y. *tinytex: Helper Functions to Install and Maintain TeX Live, and Compile LaTeX Documents* R package version 0.51 (2024). <https://github.com/rstudio/tinytex>.
63. Wickham, H., Bryan, J., Barrett, M. & Teucher, A. *usethis: Automate Package and Project Setup* R package version 2.2.3 (2024). <https://usethis.r-lib.org>.
64. Templ, M., Kowarik, A., Alfons, A., de Cillia, G. & Rannetbauer, W. *VIM: Visualization and Imputation of Missing Values* R package version 6.2.2 (2022). <https://github.com/statistikat/VIM>.
65. Hester, J. *et al.* *withr: Run Code With Temporarily Modified Global State* R package version 3.0.0 (2024). <https://withr.r-lib.org>.

Listado de apartados que son demasiado extensos para incluir dentro de la memoria y tienen un carácter autocontenido (por ejemplo, manuales de usuario, manuales de instalación, etc.)  
Dependiendo del tipo de trabajo, es posible que no haya que añadir algún anexo.