

生物資訊與數據分析

Metabolomics Data Analysis II

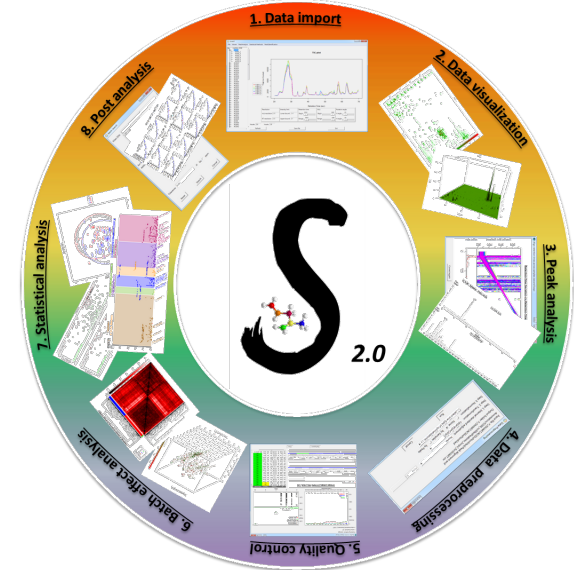
Yu-Jen Liang

Dr. Hsin-Chou Yang's Lab, ISS AS



Evolution & Work flow

SMART DEMOGRAPHY



iMAS 研發團隊

李易儒、梁佑任、楊智婷、林胤均、陳佳煒、李育誠

楊欣洲研究員 兼 統計所所長

團隊簡介

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梁佑任 (Yu-Jen Liang) 資深協同研究學者	專長：代謝體 & 基因體
楊智婷 (Chih-Ting Yang) 研究助理	專長：病毒基因體 & 資料科學
陳佳煒 (Chia-Wei Chen) 研究助理	專長：軟體開發 & 資料科學
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林舒瑤 (Shu-Yao Lin) 農生中心專案經理	專長：質譜代謝體 & 生物化學

合作者

台北醫學大學 公共衛生學院

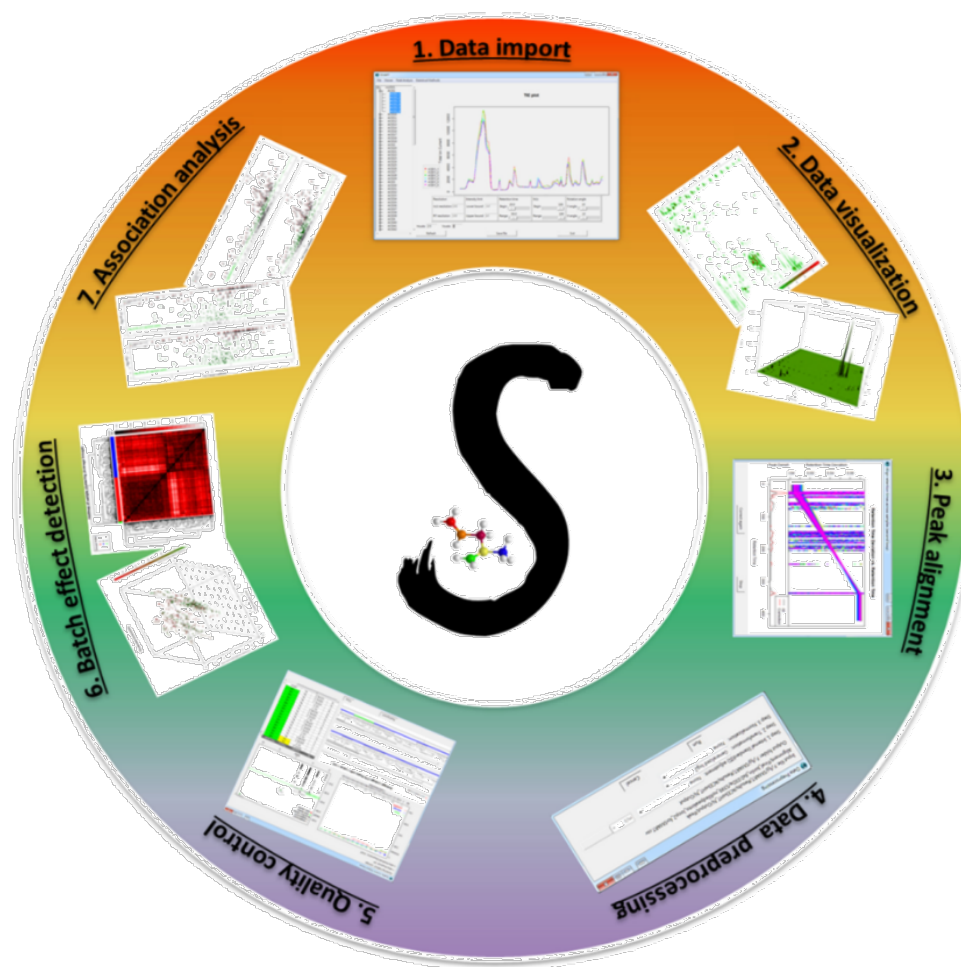
潘文涵 講座教授 專長：營養流行病學、營養生化、心臟血管流行病學

中央研究院 農業生物科技研究中心

徐麗芬 特聘研究員 專長：抗發炎藥用植物之抗癌暨代謝產物體學、酵素在農業生物科技之蛋白質工程研究

SMART description

- **SMART** (Statistical Metabolomics Analysis - an R Tool)
 - with a user-friendly interface was developed in R and R-GUI (Graphical User Interface) under the **Windows** and **Mac** operating system.



analytical chemistry Article
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SMART: Statistical Metabolomics Analysis—An R Tool
Yu-Jen Liang,^{1,2} Yu-Ting Lin,³ Chia-Wei Chen,² Chien-Wei Lin,⁵ Kun-Mao Chao,^{1,4,6} Wen-Harn Pan,^{1,6} and Hsin-Chou Yang^{1,2,3,4,6}

¹Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei 10617, Taiwan
²Institute of Statistical Science, Academia Sinica, Taipei 115, Taiwan
³Department of Computer Science and Information Engineering, National Taiwan University, Taipei 10617, Taiwan
⁴Institute of Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan
⁵Institute of Public Health, National Yang Ming University, Taipei 11221, Taiwan
⁶Department of Statistics, National Cheng Kung University, Tainan 701, Taiwan
⁷Institute of Statistics, National Tsing Hua University, Hsinchu 30013, Taiwan
⁸School of Public Health, National Defense Medical Center, Taipei 114, Taiwan

Supporting Information

ABSTRACT: Metabolomics data provide unprecedented opportunities to decipher metabolic mechanisms by analyzing hundreds to thousands of metabolites. Data quality concerns and complex batch effects in metabolomics must be appropriately addressed through statistical analysis. This study developed an integrated analysis tool for metabolomics studies to streamline the complete analysis flow from initial data preprocessing to downstream association analysis. We developed Statistical Metabolomics Analysis—An R Tool (SMART), which can analyze input files with different formats, visually represent various types of data features, implement peak alignment and annotation, conduct quality control for samples and peaks, explore batch effects, and perform association analysis. A pharmacometabolomics study of antihypertensive medication was conducted and data were analyzed using SMART. Neurexophilin N was identified as a metabolite significantly associated with angiotensin converting enzyme inhibitors in our metabolite-wide association analysis ($p = 1.56 \times 10^{-4}$ in an analysis of covariance (ANCOVA) with an adjustment for unknown latent groups and $p = 1.02 \times 10^{-3}$ in an ANCOVA with an adjustment for hidden substructures). This endogenous neuropeptide is highly related to renin-angiotensin and -angiotensin II, which are involved in blood pressure regulation and smooth muscle contraction. The SMART software, a user guide, and example data can be downloaded from <http://www.stat.sinica.edu.tw/hsinchou/metabolomics/SMART.htm>.

Keywords: metabolomics; data quality; batch effects; association analysis; R tool

INTRODUCTION Liquid chromatography–mass spectrometry (LC–MS) and gas chromatography–mass spectrometry (GC–MS) have high sensitivity and selectivity in rapidly separating and quantifying target or nontarget compounds, including metabolites and lipids, in biological fluids. Recently, metabolomics profiling has been used widely for exploring the associations of metabolites with disease states,¹ elucidating drug metabolism,² performing clinical diagnostics,³ understanding toxicity mechanisms,⁴ and investigating human nutrition.⁵ The results have facilitated understanding the roles of metabolites in biological mechanisms.^{6,7} As the frequency of metabolomics studies has increased, several analysis tools have become available. Commercial software for metabolomics data processing, peak alignment, and data visualization is provided with experimental instruments such as Mass Profiler Pro (Agilent Technologies, Palo Alto, CA, USA), ProfileAnalysis (Berkley, Illierica, Massachusetts, USA), and MarkerLynx (Waters, Milford, MA, USA). Moreover, free packages, such as MZmine,⁸ XCMS,⁹ and MultiAlign,¹⁰ have been developed. In addition, some web-based tools, such as MetaboAnalyst,¹¹ MetIDB,¹² and

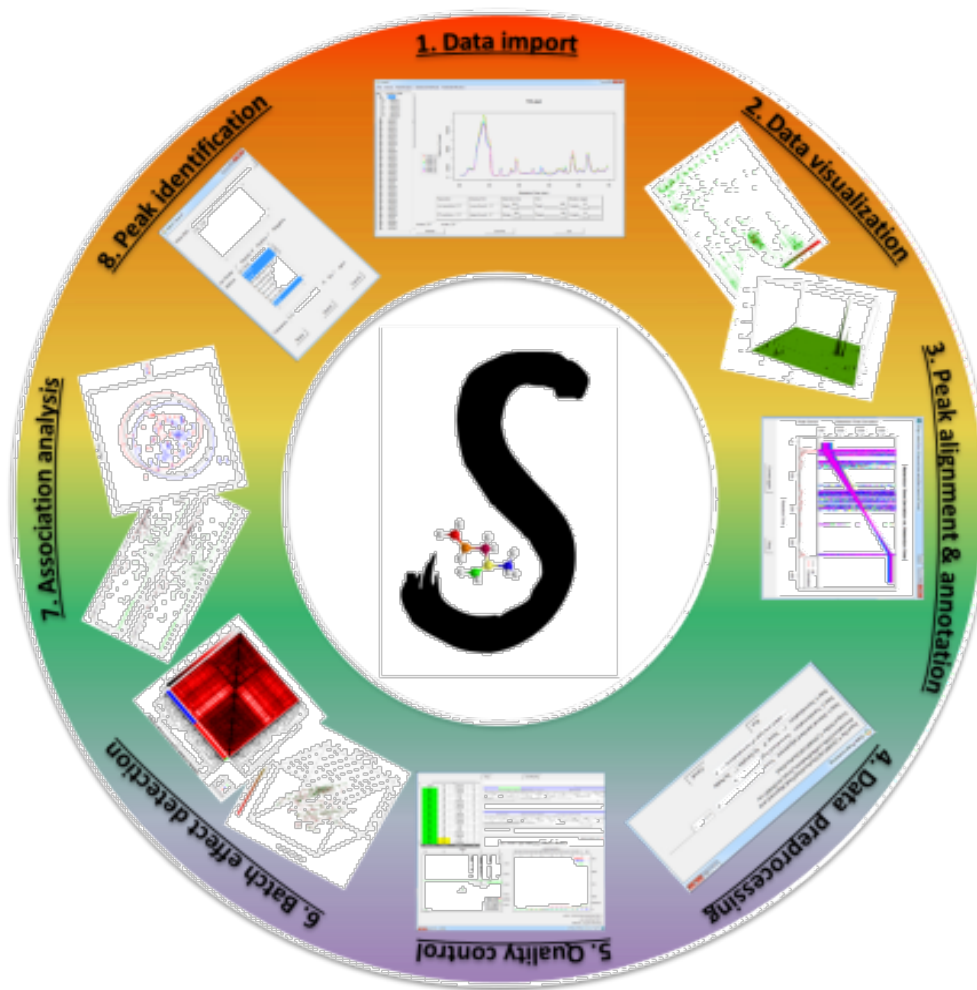
XCMS Online,^{13,14,15} are available for performing statistical and pathway analyses of metabolomics data. Recently, numerous large-scale metabolite-wide association studies (MWASs) have been conducted.^{16–18} Such studies are characterized by a long study time, complex experimental factors, and a high number of samples with multiple technical and biological replicates.^{19,20} Technical replicates are designed to detect system variability and biological replicates are used to increase the statistical power.^{21,22} The experiments of large-scale MWASs may be influenced by several sources of variation, including controllable and uncontrollable factors. The controllable factors, such as sample characteristics and known batch effects (e.g., a batch effect due to a change of an LC column²³ or a working solution of O-methylamine hydrochloride²⁴), can be considered during study design. Uncontrollable factors, such as specimen contamination and unknown batch effects

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Anal. Chem. 2016, 88, 6304–6311

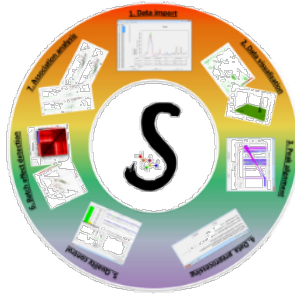
Statistical Metabolomics Analysis – an R Tool (SMART)



- 4. Data preprocessing
 - Pareto scaling (PS)
- 7. Association analysis
 - Partial least squares (PLS)
- 8. Peak identification
 - HMDB & Massbank

Website

<http://www.stat.sinica.edu.tw/hsinchou/metabolomics/SMART.htm>



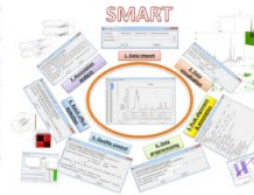
Version 1.0

SMART (Statistical Metabolomics Analysis - An R Tool)



Introduction:

SMART written in R and R GUI has been developed as user-friendly software for integrated analysis of metabolomics data. SMART streamlines the complete analysis flow from initial data preprocessing to downstream association analysis, including analyzing different data file formats (e.g., .raw, .d, and mzXML), visually representing various types of data features (e.g., total ion chromatogram and mass spectra), implementing peak alignment, conducting quality control for samples and peaks, exploring batch effects (e.g., known experimental conditions, unknown latent groups, or hidden substructures), and performing association analysis.



Software:

Version	System	Download File	File Type	File Size	Update Time
SMART 1.2 (beta)	Windows (64-bit)		7z	1.75 MB	2019/Mar/27
SMART 1.1	Windows (32-bit)		7z	75.0 KB	2016/Jul/26
	Windows (64-bit)		7z	79.7 KB	2016/Jul/26
SMART 1.0	Mac		7z	48.6 KB	2016/Jul/26
	Windows (32-bit)		7z	75.2 KB	2016/May/19
	Windows (64-bit)		7z	79.7 KB	2016/May/19
	Mac		7z	50.1 KB	2016/May/19

- Other required packages: (1) [R language and packages](#), (2) ActiveTcl 8.5 ([32-bit](#), [64-bit](#), [Mac](#)), and (3) [ProteoWizard](#).
- Software for unzipping *.7z files can be downloaded from the website: <http://www.7-zip.org/>

Example: An antihypertensive pharmacometabolomics study of 5 nonmedication and 5 ACEi medication patients

Version	Description	Download File	File Type	File Size	Update Time
SMART	Raw spectrum files		7z	853 Mb	2016/May/19
	Covariate file		CSV	4 Kb	2016/May/19
	Factor file		CSV	4 Kb	2016/May/19

User guide:

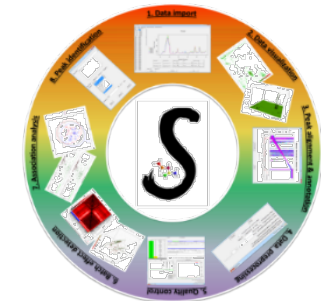
Version	Description	Download File	File Type	File Size	Update Time
SMART 1.2	Instructions		PDF	1.82 Mb	2019/Mar/27
SMART 1.1	Instructions		PDF	2.11 Mb	2016/Jul/26
SMART 1.0	Instructions		PDF	2.11 Mb	2016/May/19

Citation:

Yu-Jen Liang, Yu-Ting Lin, Chia-Wei Chen, Chien-Wei Lin, Kun-Mao Chao, Wen-Harn Pan and Hsin-Chou Yang (2016/05). SMART: Statistical Metabolomics Analysis – An R Tool. *Analytical Chemistry* **88**, 6334 – 6341.

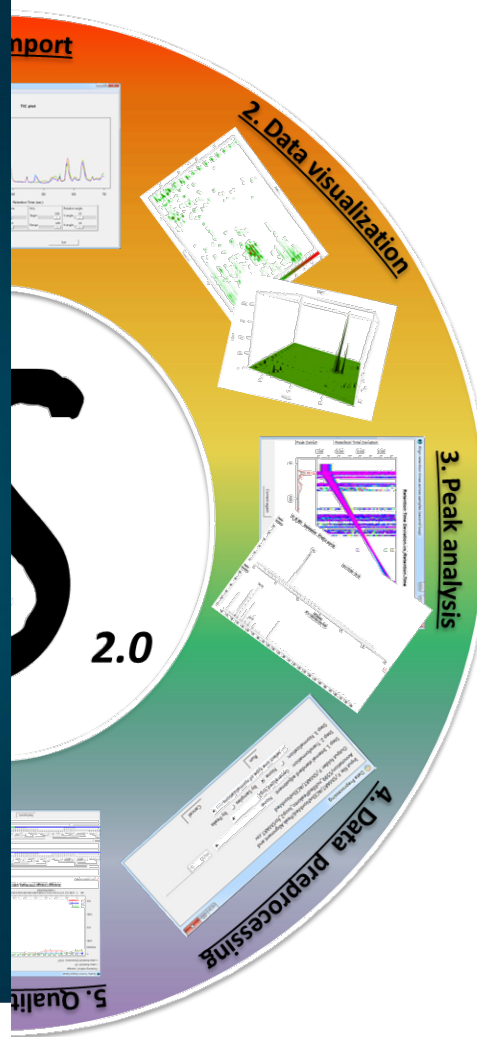
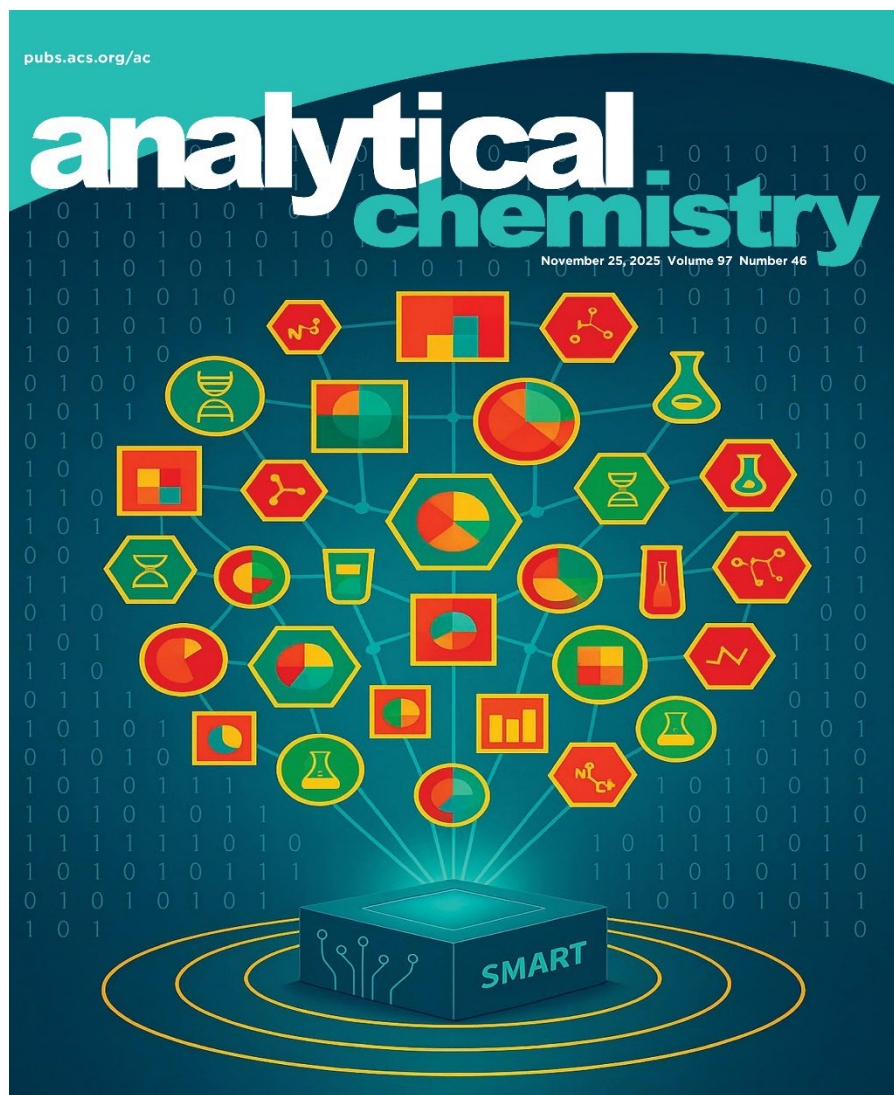
Correspondence:

Dr. Hsin-Chou Yang, Institute of Statistical Science, Academia Sinica, 128, Academia Road, Section 2 Nankang, Taipei 115, Taiwan. (Fax) 886-2-27831523; (Tel) 886-2-27889311 ext. 113; (E-mail) hsinchou@stat.sinica.edu.tw



Version 1.2

Statistical Metabolomics Analysis – an R Tool (SMART)



3. Peak analysis

- untargeted data analysis
- targeted data analysis

4. Data preprocessing

- Pareto scaling (PS)
- Inverse normal transformation

5. Quality control

- S/N ratio
- Clustering distance measures

7. Statistical analysis (~~Association analysis~~)

- ANCOVA
- Partial least squares (PLS)
- Integrative omics pathway analysis (IOPA)

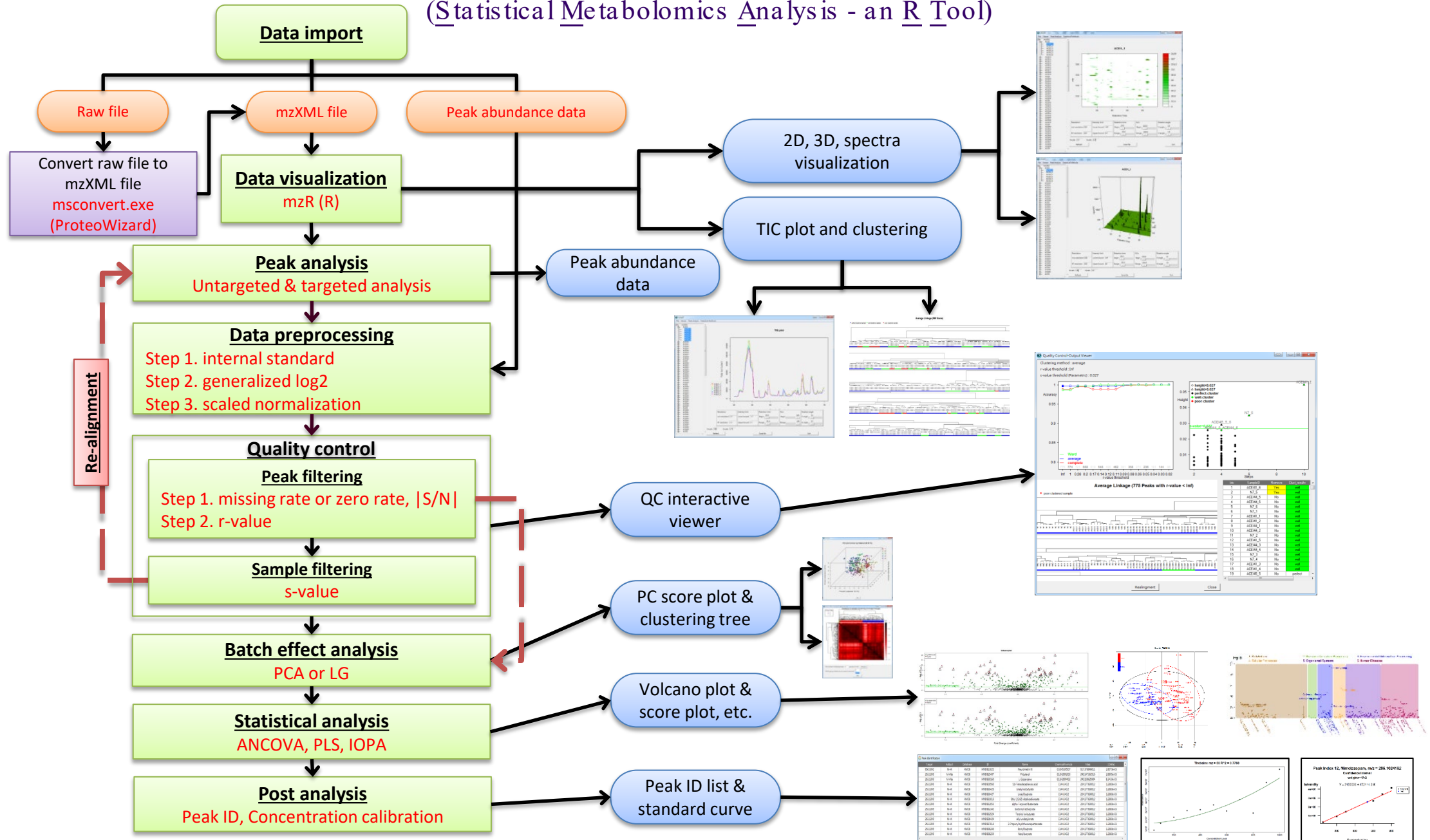
8. Post analysis

Peak identification

- HMDB & MassBank

Concentration calibration

- Calibration curve construction
- Concentration calculation



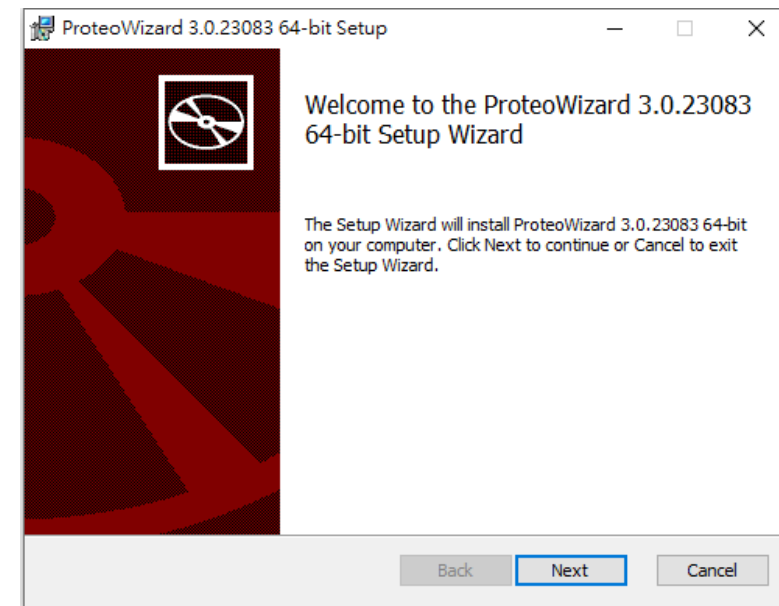
R, ActiveTCL, and ProteoWizard

SMART

SMART INSTALLATION

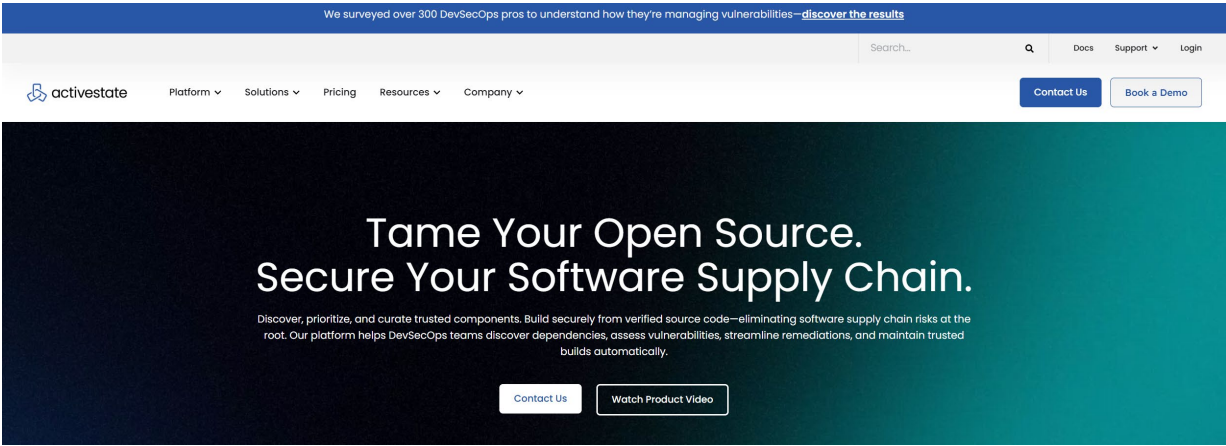
SMART installation

- Software requirements
 - **R 4.2.1+** (SMART 2.0, 1.2 R-code); **R 3.5.3** (SMART_V1.2.exe)
 - <https://cran.r-project.org/bin/windows/base/old/4.2.1/>
 - <https://cran.r-project.org/bin/macosx/>
 - **ActiveTcl 8.6**
 - <https://www.activestate.com/pricing/>
 - **ProteoWizard 3.0**
 - <https://proteowizard.sourceforge.io/download.html>



ActiveTcl

ActiveTcl 是一個由 ActiveState 公司開發的商業版本的 Tcl (Tool Command Language) 語言套件。(ChatGPT)



ActiveTcl 的功能與特色

功能類別	說明
Tcl 語言環境	提供完整的 Tcl 編譯器與執行環境（包含 Tcl 核心語法與函數庫）。
Tk 圖形界面庫	提供 Tk GUI 工具包，讓使用者可以開發跨平台的圖形介面應用程式。
擴充套件 (Packages)	內建大量 Tcl 擴充模組，如 Tcllib、Tklib、Expect、Thread、TLS、SQLite 等。
跨平台支援	支援 Windows、Linux、macOS 等作業系統。
商業支援	提供專業技術支援與安全更新（ActiveTcl Community Edition 免費，商業版提供完整支援）。
TEApot Package 管理	提供套件管理系統，方便安裝與更新 Tcl 擴充套件。
安全性與穩定性	ActiveState 維護，提供穩定、安全的官方版本。

ActiveTcl 應用領域

應用領域	說明
自動化腳本開發	系統管理、測試自動化、流程控制等。
資料處理與分析	撰寫快速數據處理與分析程式。
GUI 軟體開發	利用 Tk 開發跨平台的桌面應用程式。
資料庫操作	支援 SQLite 等資料庫存取。
網路程式開發	撰寫 TCP/IP 通訊程式、Socket 開發。
嵌入式系統	Tcl 常被嵌入在系統內作為控制語言。

ProteoWizard

ProteoWizard 是質譜 (Mass Spectrometry, MS) 領域非常有名且常用的開源工具套件，主要是針對質譜資料的處理與轉換。這是許多代謝體學 (Metabolomics) 、蛋白體學 (Proteomics) 、藥物分析 (Drug Analysis) 研究的標準工具之一。(ChatGPT)

ProteoWizard 主要功能與特色

功能類別	說明
資料轉檔	支援各大廠牌的原始質譜檔案格式轉換為開放格式，如 mzML、mzXML、MGF。
資料格式統一	統一各品牌質譜機 (Thermo, Sciex, Bruker, Agilent, Waters 等) 的資料格式。
檔案壓縮與最佳化	可將大型質譜檔案壓縮 (使用 zlib) 或最佳化 (保留關鍵資料)。
資料擷取	擷取特定的 MS1、MS2 資訊或指定範圍的質譜圖 (extract chromatograms, spectra)。
批次處理支援	支援命令列操作，可批次轉檔與資料處理 (適合高通量分析)。
資料檢視工具	提供 MSConvertGUI 與 SeeMS 等視覺化工具，方便檢視質譜資料。
軟體開發套件 (SDK)	提供 API 給開發者撰寫客製化的 MS 處理工具。



ProteoWizard 工具與用途

工具名稱	功能簡介
MSConvert	格式轉檔、壓縮、資料過濾
SeeMS	資料檢視工具
idConvert	資料庫搜尋結果轉換
peakPicker	資料峰值偵測
msaccess	MS 資料查詢工具

SMART installation (Mac)

- Mac OS (1)
— R 4.2.X

R for macOS

This directory contains binaries for a base distribution and packages to run on macOS. Releases for old Mac OS X systems (through Mac OS X 10.5) and PowerPC Macs can be found in the [old](#) directory.

Note: Although we take precautions when assembling binaries, please use the normal precautions with downloaded executables.

Package binaries for R versions older than 3.2.0 are only available from the [CRAN archive](#) so users of such versions should adjust the CRAN mirror setting (<https://cran-archive.r-project.org>) accordingly.

R 4.2.3 "Shortstop Beagle" released on 2023/03/15

Please check the integrity of the downloaded package by checking the signature:
pkgutil --check-signature R-4.2.3.pkg
in the *Terminal* application. If Apple tools are not available you can check the SHA1 checksum of the downloaded image:
openssl sha1 R-4.2.3.pkg

Latest release:

[R-4.2.3-arm64.pkg](#) (notarized and signed)
SHA1-hash: 99d1ad0480a67f8d40cd0195408e722f77b6b81
(ca. 86MB) for M1 and higher Macs only!

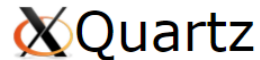
R 4.2.3 binary for macOS 11 (**Big Sur**) and higher, **Apple silicon arm64** build, signed and notarized package.
Contains R 4.2.3 framework, R.app GUI 1.79 for Apple silicon Macs (M1 and higher), Tcl/Tk 8.6.12 X11 libraries and Texinfo 6.8.
Important: this version does NOT work on older Intel-based Macs - see below for Intel version.

macOS Ventura users: there is a known bug in Ventura, if the installation fails, move the downloaded file away from the *Downloads* folder (e.g., to your home or Desktop)

Note: the use of X11 (including tcltk) requires [XQuartz](#) (version 2.8.1 or later). Always re-install XQuartz when upgrading your macOS to a new major version.

This release uses Xcode 13.1 and experimental GNU Fortran 12 arm64 fork. If you wish to compile R packages which contain Fortran code, you may need to download GNU Fortran for arm64 from <https://mac.R-project.org/tools>. Any external libraries and tools are expected to live in */opt/R/arm64* to not conflict with Intel-based software and this build will not use */usr/local* to avoid such conflicts (see the

—XQuartz 2.8.5



Home

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The XQuartz project is an open-source effort to develop a version of the [X.Org X Window System](#) that runs on macOS. Together with supporting libraries and applications, it forms the X11.app that Apple shipped with OS X versions 10.5 through 10.7.

Quick Download

Download	Version	Released	Info
XQuartz-2.8.5.pkg	2.8.5	2023-01-26	For macOS 10.9 or later

License Info

An XQuartz installation consists of many individual pieces of software which have various licenses. The X.Org software components' licenses are discussed on the [X.Org Foundation Licenses page](#). The [quartz-wm](#) window manager included with the XQuartz distribution uses the [Apple Public Source License Version 2](#).

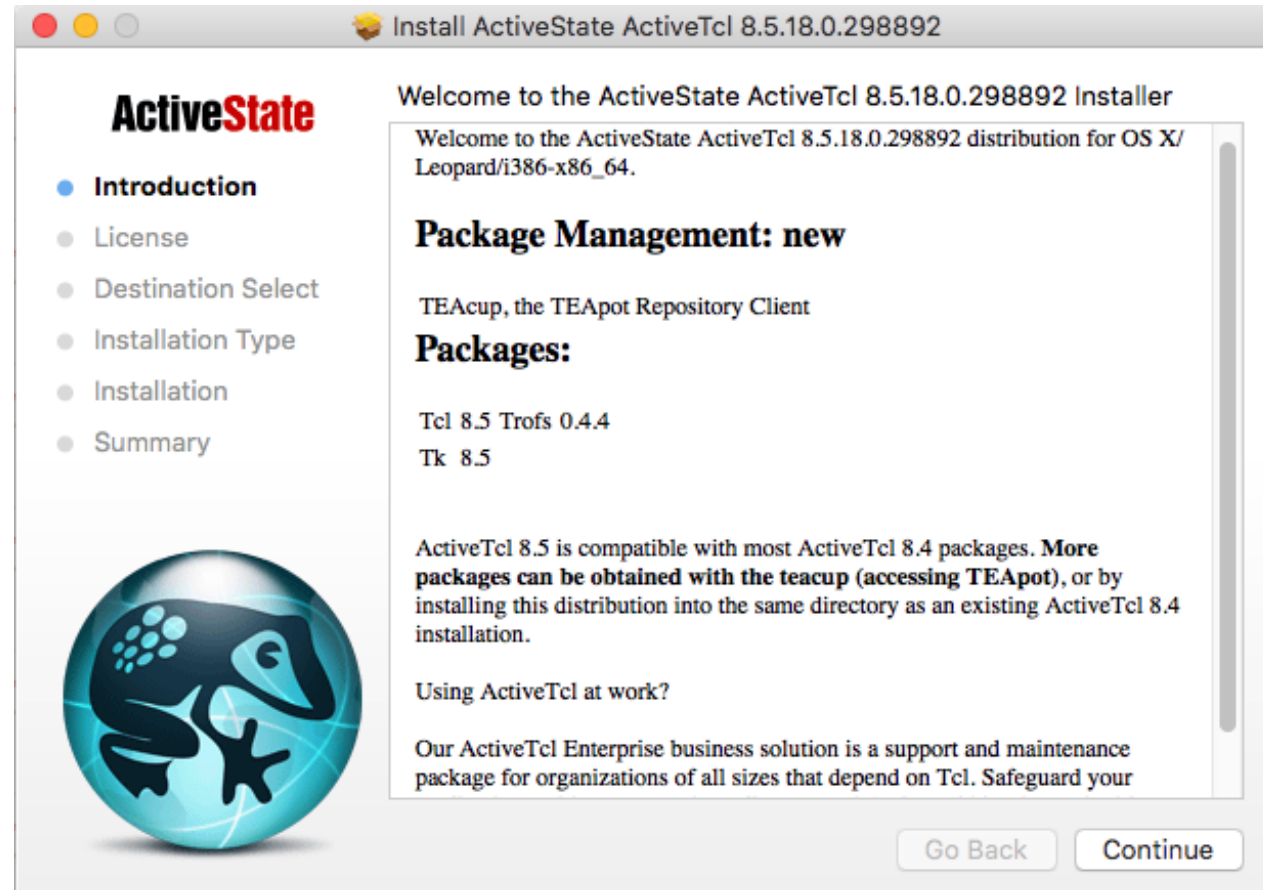
SMART installation (Mac)

- Mac OS (2)
 - ActiveTcl 8.6

macOS



ActiveTcl 8.6



SMART execution

- Execute SMART (v1.1 or 1.2)

- Windows OS

- SMART_V1.2_64bit.exe
- SMART_V1.1_32bit.exe

- Mac OS

- SMART_V1.1_Mac.r, SMART_V1.1_Mac_Gui.r, SMART_V1.1_Mac_Sub.r
- source('~/Documents/SMART/SMART_V1.1_Mac.r')

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Software:

Version	System	Download File	File Type	File Size	Update Time
SMART 1.2 (new)	Windows (64-bit)		7z	1.75 MB	2019/Mar/27
SMART 1.1	Windows (32-bit)		7z	75.0 KB	2016/Jul/26
	Windows (64-bit)		7z	79.7 KB	2016/Jul/26
SMART 1.0	Mac		7z	48.6 KB	2016/Jul/26
	Windows (32-bit)		7z	75.2 KB	2016/May/19
	Windows (64-bit)		7z	79.7 KB	2016/May/19
	Mac		7z	50.1 KB	2016/May/19

- Other required packages: (1) R language and packages, (2) ActiveTcl 8.5 (32-bit, 64-bit, Mac), and (3) ProteoWizard.
- Software for unzipping ".7z" files can be downloaded from the website: <http://www.7-zip.org/>

Example: An antihypertensive pharmacometabolomics study of 5 nonmedication and 5 ACEI medication patients

Version	Description	Download File	File Type	File Size	Update Time
SMART	Raw spectrum files		7z	863 Mb	2016/May/19
	Covariate file		CSV	4 Kb	2016/May/19
	Factor file		CSV	4 Kb	2016/May/19

User guide:

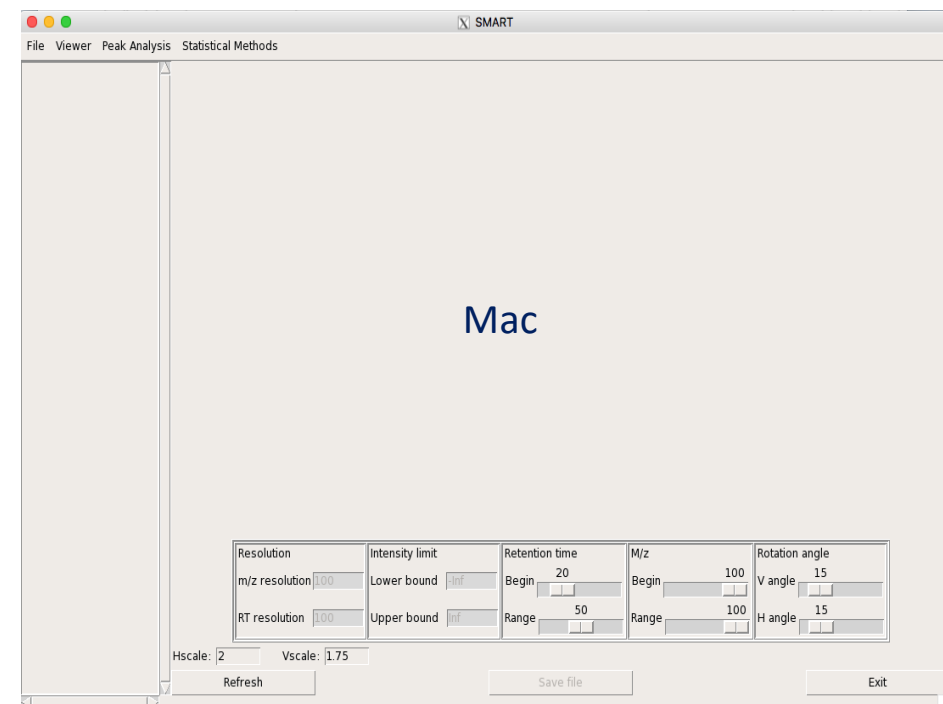
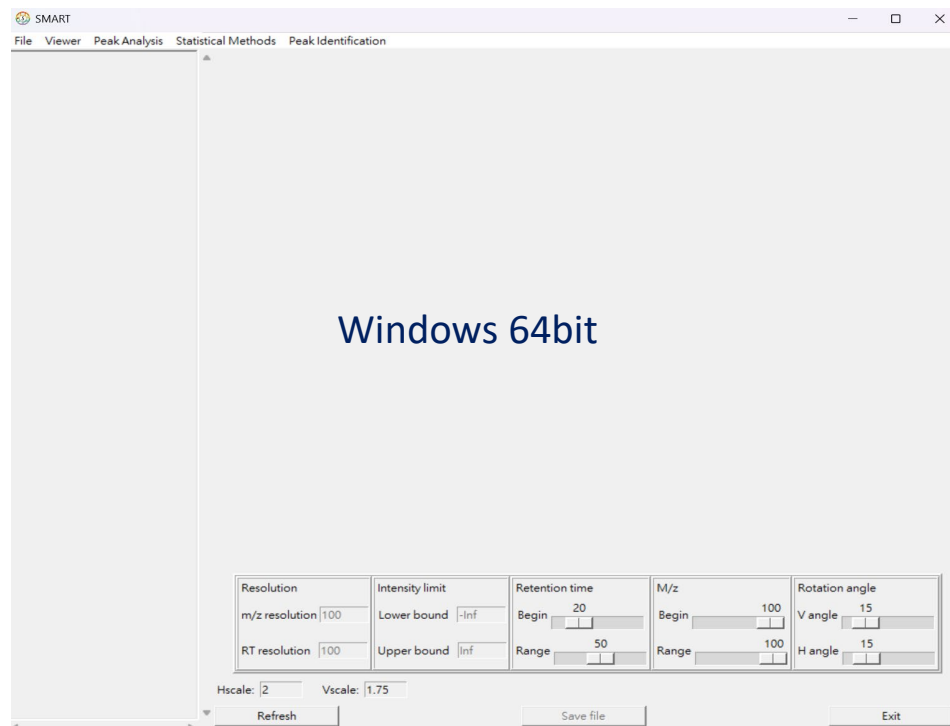
Version	Description	Download File	File Type	File Size	Update Time
SMART 1.2	Instructions		PDF	1.82 Mb	2019/Mar/27
SMART 1.1	Instructions		PDF	2.11 Mb	2016/Jul/26
SMART 1.0	Instructions		PDF	2.11 Mb	2016/May/19

Citation:

Yu-Jen Liang, Yu-Ting Lin, Chia-Viel Chen, Chien-Viel Lin, Kun-Mao Chao, Wen-Ham Pan and Hsin-Chou Yang (2016/05). SMART: Statistical Metabolomics Analysis – An R Tool. *Analytical Chemistry* **88**, 6334 – 6341.

Correspondence:

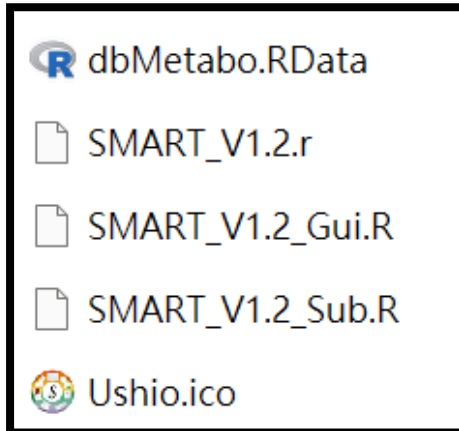
Dr. Hsin-Chou Yang, Institute of Statistical Science, Academia Sinica, 128, Academia Road, Section 2 Nankang, Taipei 115, Taiwan. (Fax) 886-2-27831523; (Tel) 886-2-27880311 ext. 113; (E-mail) hanchou@stat.sinica.edu.tw



SMART execution (R code)

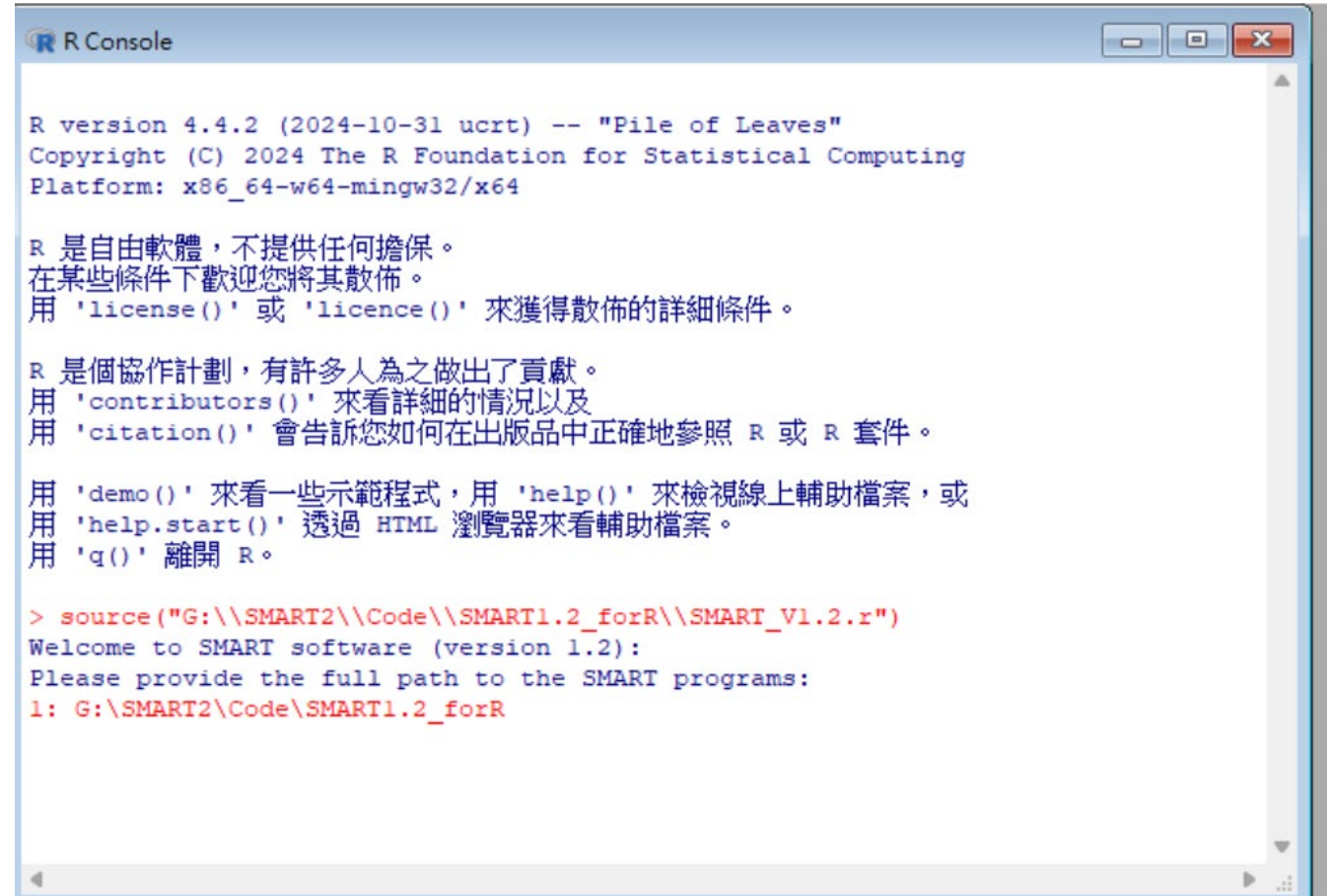
- Execute SMART 1.2
 - R 4.2.1 +
 - RStudio

SMART1.2_forR.zip



Open "SMART_V1.2.r"

```
cat("Welcome to SMART software (version 1.2): \n")
cat("Please provide the full path to the SMART programs:\n")
wd <- scan(n=1, what="character")
setwd(wd)
source("SMART_V1.2_Gui.r")
```



```
R Console

R version 4.4.2 (2024-10-31 ucrt) -- "File of Leaves"
Copyright (C) 2024 The R Foundation for Statistical Computing
Platform: x86_64-w64-mingw32/x64

R 是自由軟體，不提供任何擔保。
在某些條件下歡迎您將其散佈。
用 'license()' 或 'licence()' 來獲得散佈的詳細條件。

R 是個協作計劃，有許多人為之做出了貢獻。
用 'contributors()' 來看詳細的情況以及
用 'citation()' 會告訴您如何在出版品中正確地參照 R 或 R 套件。

用 'demo()' 來看一些示範程式，用 'help()' 來檢視線上輔助檔案，或
用 'help.start()' 透過 HTML 瀏覽器來看輔助檔案。
用 'q()' 離開 R。

> source("G:\\SMART2\\Code\\SMART1.2_forR\\SMART_V1.2.r")
Welcome to SMART software (version 1.2):
Please provide the full path to the SMART programs:
1: G:\\SMART2\\Code\\SMART1.2_forR
```

SMART execution

- Windows (details)

— Execute SMART*.exe

選取 C:\Users\lyj\Desktop\SMART_Demo\SMART_V1.1_64bit.exe

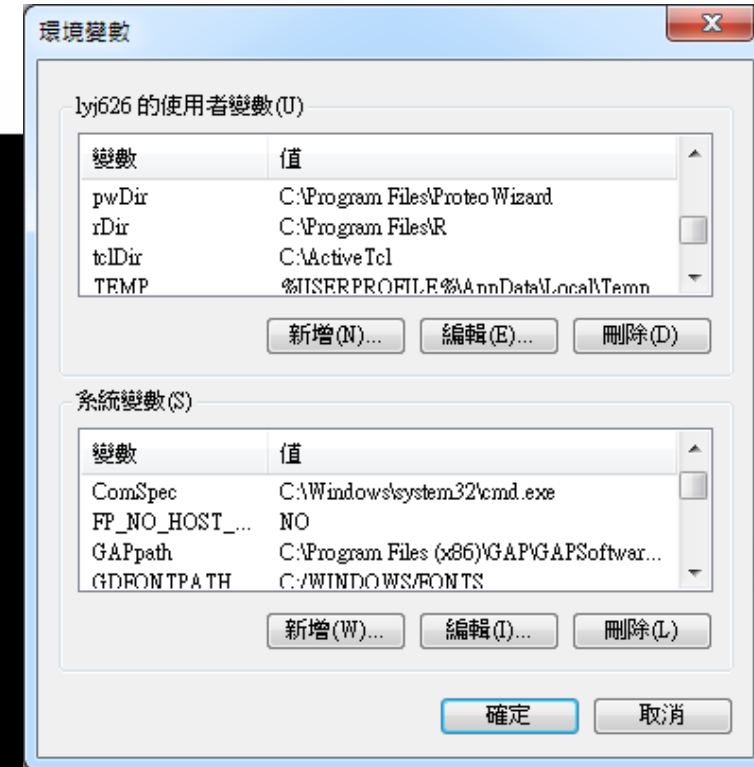
```
*****
SMART
*****
S-----S
| Welcome to SMART software | v1.1 | 30/Jun/2016 |
|-----|
| (C) 2016, GNU General Public License, v2.0 |
|-----|
| For documentation, citation & example instructions: |
| http://www.stat.sinica.edu.tw/hsinchou/metabolomics/SMART.htm |
S-----S
*****

*** Please provide the path of required software .....

R - "C:\Program Files\R"
Tcl - "c:\tcl"
ProteoWizard - "c:\program files (x86)\ProteoWizard"

*** Please wait for opening SMART... <(_ _)>

*** Please check the log file .....
```



Log file

OS: Windows 7 x64

R: R version 3.3.3 (2017-03-06) x86_64

Tcl: C:\ActiveTcl

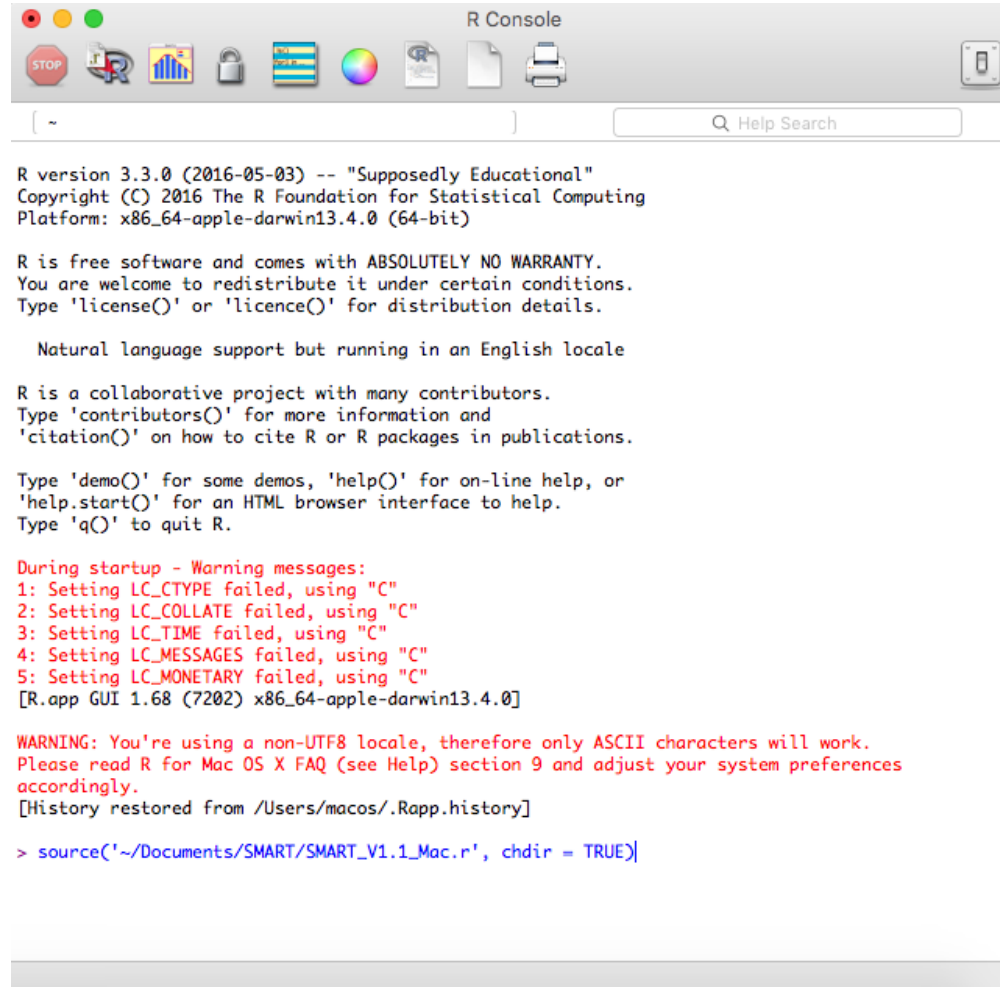
Ping cran.wu-wien.ac.at [137.208.57.37] (使用 32 位元組的資料)
回覆自 137.208.57.37: 位元組=32 時間=294ms TTL=43

137.208.57.37 的 Ping 統計資料:

封包: 已傳送 = 1, 已收到 = 1, 已遺失 = 0 (0% 遺失) .

SMART execution (Mac)

- Mac OS (details)
 - Open R
 - `source("~/Documents/SMART/SMART_V1.1_Mac.r")`



```
R Console

[ ~ ] [ Help Search ]

R version 3.3.0 (2016-05-03) -- "Supposedly Educational"
Copyright (C) 2016 The R Foundation for Statistical Computing
Platform: x86_64-apple-darwin13.4.0 (64-bit)

R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions.
Type 'license()' or 'licence()' for distribution details.

Natural language support but running in an English locale

R is a collaborative project with many contributors.
Type 'contributors()' for more information and
'citation()' on how to cite R or R packages in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

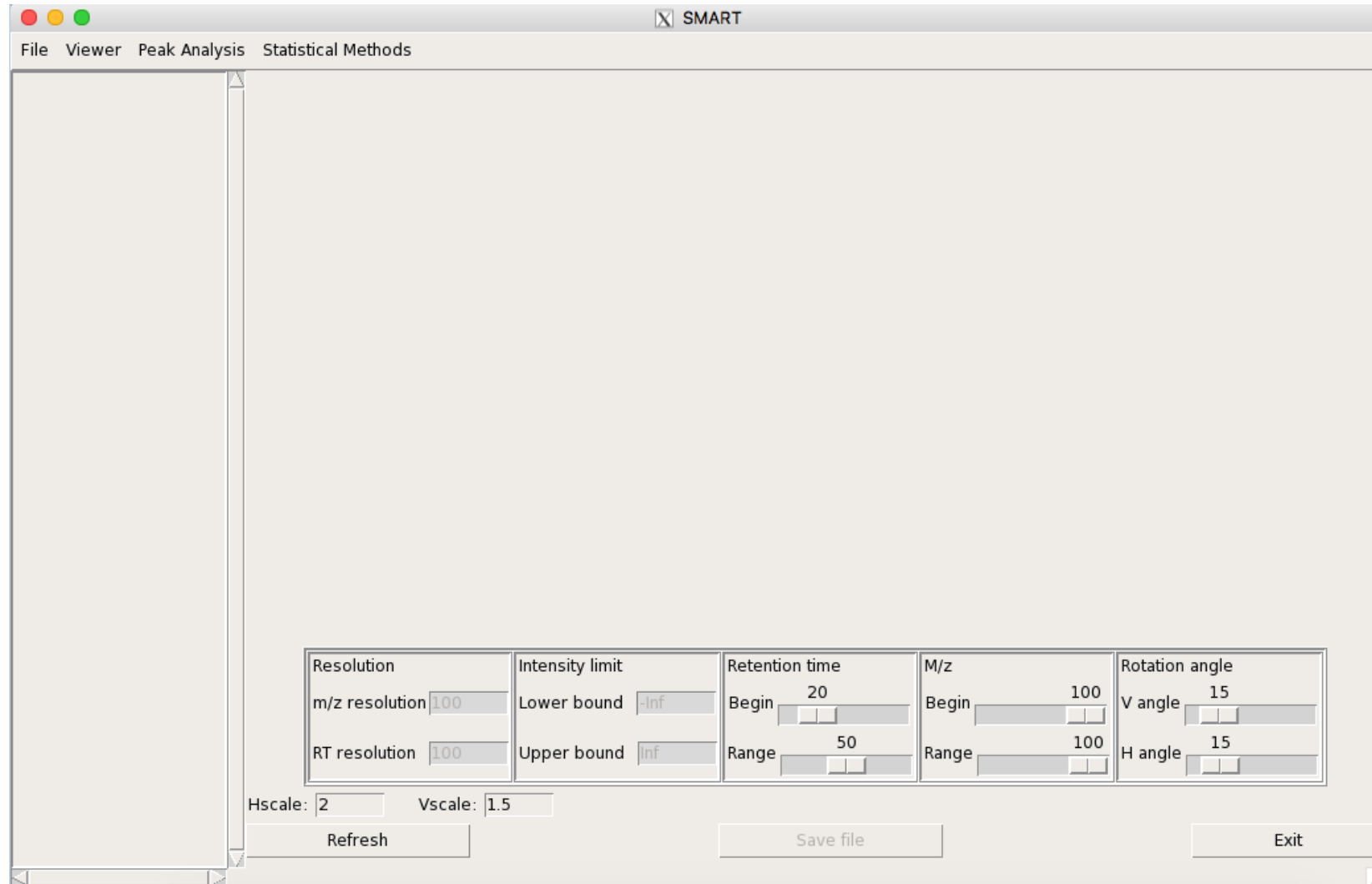
During startup - Warning messages:
1: Setting LC_CTYPE failed, using "C"
2: Setting LC_COLLATE failed, using "C"
3: Setting LC_TIME failed, using "C"
4: Setting LC_MESSAGES failed, using "C"
5: Setting LC_MONETARY failed, using "C"
[R.app GUI 1.68 (7202) x86_64-apple-darwin13.4.0]

WARNING: You're using a non-UTF8 locale, therefore only ASCII characters will work.
Please read R for Mac OS X FAQ (see Help) section 9 and adjust your system preferences
accordingly.
[History restored from /Users/macOS/.Rapp.history]

> source('~/.Documents/SMART/SMART_V1.1_Mac.r', chdir = TRUE)
```


SMART execution

- GUI



HT (hypertension), Drug, and BC (breast cancer)

DEMONSTRATION DATA

Demonstration data (HT)

Dr. Pan, Wen-Harn 潘文涵老師 – 生醫所

LC-MS

• Pharmacometabolomics study (Comput Struct Biotechnol J. 2022)

–250 hypertension patients

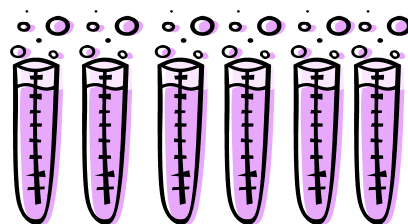
–Select 10 hypertension patients

- 5 ACEi treated patients
- 5 non-treated patients
- 60 replicate samples

ACEI1_1.raw
ACEI1_2.raw
ACEI1_3.raw
ACEI1_4.raw
ACEI1_5.raw
ACEI1_6.raw
ACEI2_1.raw
ACEI2_2.raw
ACEI2_3.raw
ACEI2_4.raw
ACEI2_5.raw
ACEI2_6.raw



6 replicates



Comput Struct Biotechnol J. 2022; 20: 6458–6466.

Published online 2022 Nov 17. doi: [10.1016/j.csbj.2022.11.030](https://doi.org/10.1016/j.csbj.2022.11.030)

PMCID: PMC9708446

PMID: [36467587](https://pubmed.ncbi.nlm.nih.gov/36467587/)

Pharmacometabolomic study of drug response to antihypertensive medications for hypertension marker identification in Han Chinese individuals in Taiwan

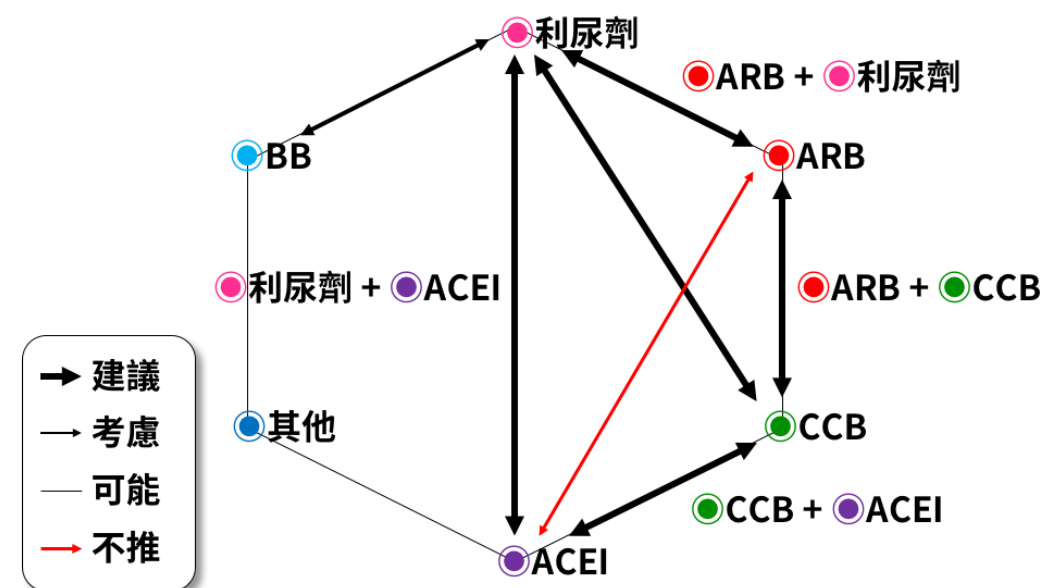
Yu-Jen Liang,^{a,1} Kuang-Mao Chiang,^{b,1} Li-li Xiu,^c Chia-Min Chung,^d Chi-Jen Lo,^{e,f} Ming-Shi Shiao,^{e,f} Mei-Ling Cheng,^{e,f} Cheng-Chin Kuo,^c Hsin-Chou Yang,^{a,g,h,i,*} and Wen-Harn Pan^{b,*}

► Author information ► Article notes ► Copyright and License information ► [Disclaimer](#)

共筆專用表 高血壓治療珍藏版

the New England
Journal of Medicine

不同機轉的高血壓藥品，可以併用嗎？請看最狂連連看...



資料來源: ESH/ESC Guidelines、JNC 8 Guidelines、JAMA. 2014;311:2216-24.

Demonstration data (Drug)

LC-MS/MS

Dr. Wang, Yi Sheng 王亦生老師 - 基因體研究中心

Drug Name	
1	海洛英 (Heroin) (Diacetylmorphine)
2	嗎啡 (Morphine)
3	古柯鹼 (Cocaine)
4	Thebaine
5	大麻 (delta9-THC) (delta9-Tetrahydrocannabinol)
6	安非他命 (Amphetamine)
7	甲基安非他命 (MA) (Methamphetamine)
8	搖頭丸 (MDMA) (3,4-Methylenedioxymethamphetamine)
9	Love Drug或Mellow Drug of America MDA (3,4-Methylenedioxyamphetamine)
10	愷他命 (Ketamine)
11	氟硝西洋 (Rohypnol) Flunitrazepam (FM2)
12	硝甲西洋 (硝甲氮平) Nimetazepam

.raw file		
12種毒品 (11 standard samples)		(1) 50 ppb
序列式稀釋混合		(2) 100 ppb
		(3) 200 ppb
		(4) 300 ppb
		(5) 400 ppb
		(6) 500 ppb (for test)
		(7) 600 ppb
		(8) 700 ppb
		(9) 800 ppb
		(10) 900 ppb
		(11) 1000 ppb

 D1_50_MS1.mzXML	 D1_50_MSMS.mzXML
 D2_100_MS1.mzXML	 D2_100_MSMS.mzXML
 D3_200_MS1.mzXML	 D3_200_MSMS.mzXML
 D4_300_MS1.mzXML	 D4_300_MSMS.mzXML
 D5_400_MS1.mzXML	 D5_400_MSMS.mzXML
 D6_500_MS1.mzXML	 D6_500_MSMS.mzXML
 D7_600_MS1.mzXML	 D7_600_MSMS.mzXML
 D8_700_MS1.mzXML	 D8_700_MSMS.mzXML
 D9_800_MS1.mzXML	 D9_800_MSMS.mzXML
 D10_900_MS1.mzXML	 D10_900_MSMS.mzXML
 D11_1000_MS1.mzXML	 D11_1000_MSMS.mzXML

Demonstration data (BC)



- Breast cancer study

132 samples

64 Black African-Americans and 68 White European-Americans.

—Gene expression (GEO GSE37751) 致癌代謝物
Affymetrix Human Gene 1.0 ST Array

- 108 samples. (53 B, 55 W)
 - Breast tumor samples (n = 61) and adjacent non-tumor tissue samples (n = 47).
- 20254 genes.

—Metabolomics (Supplemental Tables 1 and 2) GC-MS

- 132 samples. (64 B, 68 W)
 - 67 human breast tumors and 65 tumor-adjacent noncancerous tissues.
- 536 metabolites.
 - 32 metabolites are all zero.

J Clin Invest. 2014 Jan 2; 124(1): 398–412.

Published online 2013 Dec 9. doi: [10.1172/JCI71180](https://doi.org/10.1172/JCI71180)

MYC-driven accumulation of 2-hydroxyglutarate is associated with breast cancer prognosis

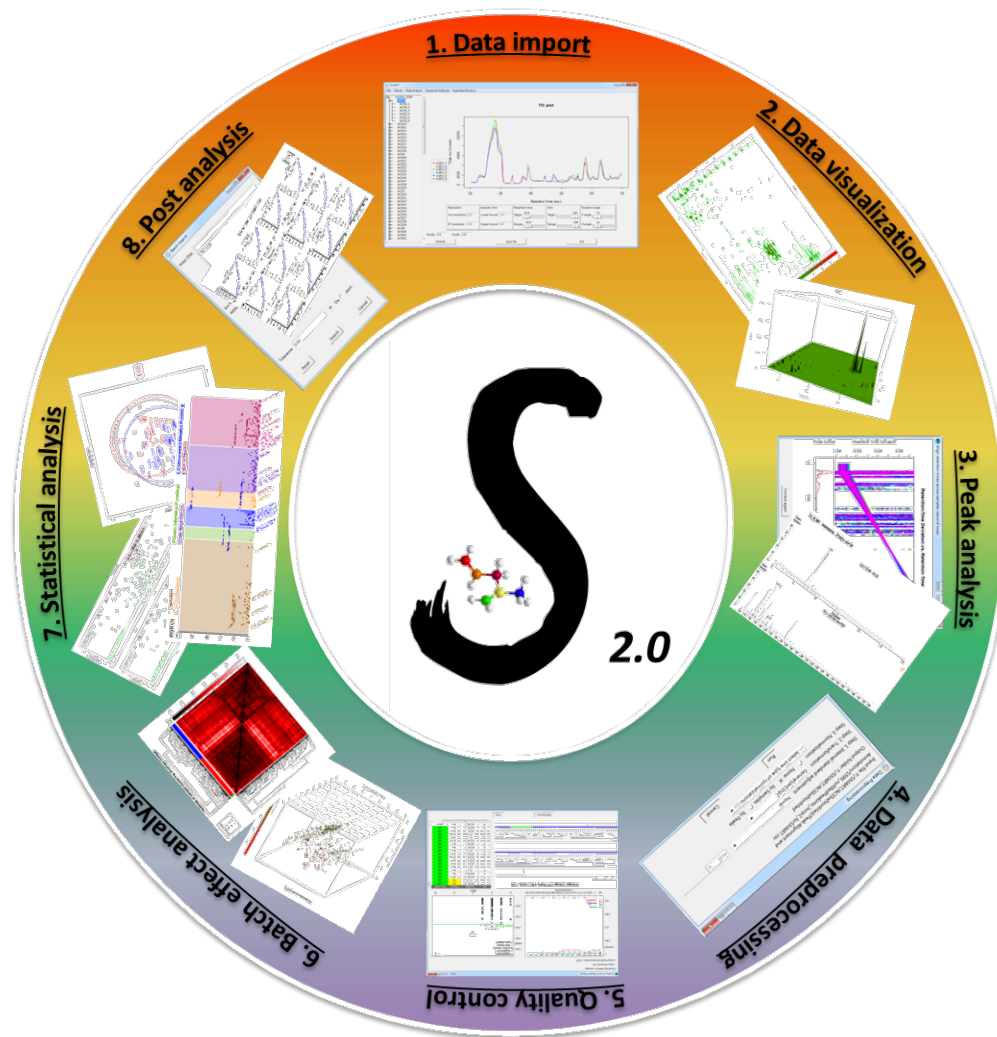
[Atsushi Terunuma](#),¹ [Nagireddy Putluri](#),² [Prachi Mishra](#),¹ [Ewy A. Mathé](#),¹ [Tiffany H. Dorsey](#),¹ [Ming Yi](#),³ [Tiffany A. Wallace](#),¹

PMCID: PMC3871244

PMID: [24316975](https://pubmed.ncbi.nlm.nih.gov/24316975/)

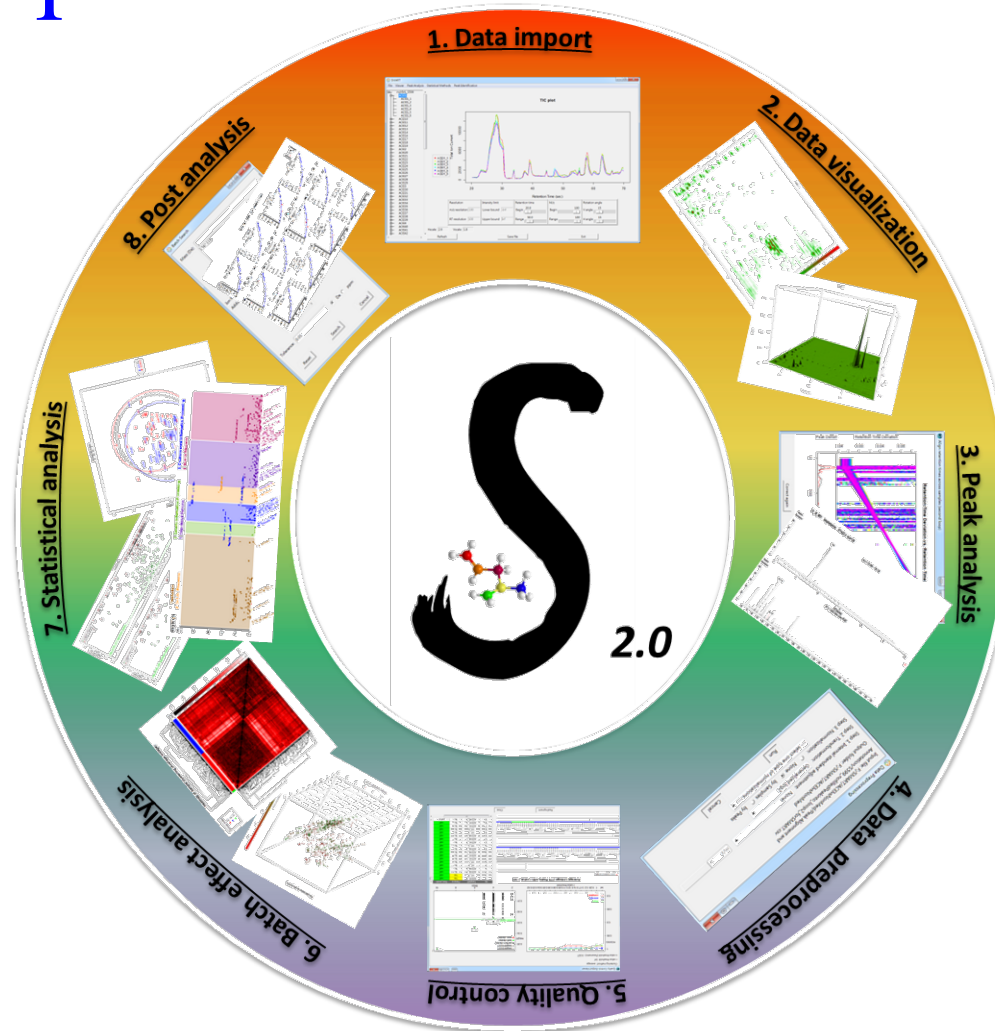
乳腺癌中，致癌代謝物
2-羥基戊二酸 (2HG) 的
濃度升高與 MYC
pathway之間有關聯。

SMART OPERATION



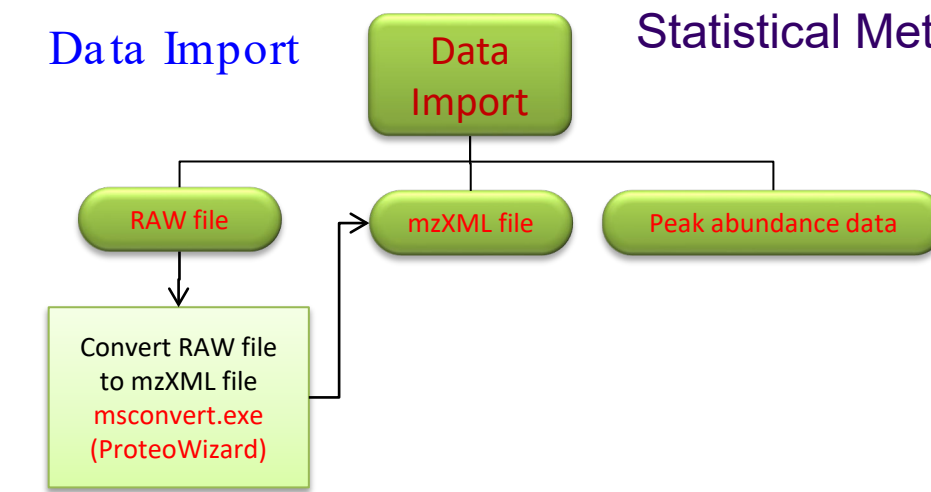
Statistical Metabolomics Analysis – an R Tool

1. Data import



Data Import

Statistical Metabolomics Analysis – an R Tool (SMART)



1

RAW file
(.raw or .d)

- ACEI1_1.raw
- ACEI1_2.raw
- ACEI1_3.raw
- ACEI1_4.raw
- ACEI1_5.raw
- ACEI1_6.raw
- ACEI2_1.raw
- ACEI2_2.raw
- ACEI2_3.raw
- ACEI2_4.raw
- ACEI2_5.raw
- ACEI2_6.raw

2

mzXML format file
(.mzXML)

- ACEI1_1.mzXML
- ACEI1_2.mzXML
- ACEI1_3.mzXML
- ACEI1_4.mzXML
- ACEI1_5.mzXML
- ACEI1_6.mzXML
- ACEI2_1.mzXML
- ACEI2_2.mzXML
- ACEI2_3.mzXML
- ACEI2_4.mzXML
- ACEI2_5.mzXML
- ACEI2_6.mzXML

3

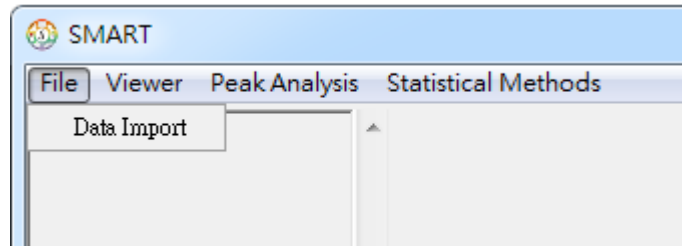
Peak abundance data file
(.csv)

Peak_Index	mz	Ret_Time.sec.	ACEI1_1	ACEI1_2	ACEI1_3
1	55.9369	336.6774	40.48807	49.20359	73.12604
2	56.9635	336.0434	26.2133	NA	34.25459
3	60.07568	29.1904	15.04558	18.71559	15.56744
4	61.01206	39.1728	NA	NA	NA
5	68.9834	23.9603	32.57462	39.53168	40.60577
6	69.07024	53.4746	NA	NA	NA
7	70.06568	32.2958	NA	NA	NA
8	72.08126	64.0674	NA	NA	NA
9	72.08131	37.8029	75.99819	59.4558	52.61279
10	77.03941	58.097	36.12143	39.46023	38.78575

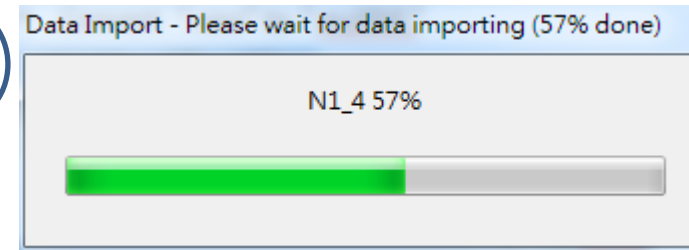
Data import (raw file)

- Data import (e.g., .raw file)
 - Input folder
 - indicate the directory of “.raw” files
 - Output folder
 - Create “mzXML” to be the directory name in the output folder
 - Convert “.raw” to “.mzXML”

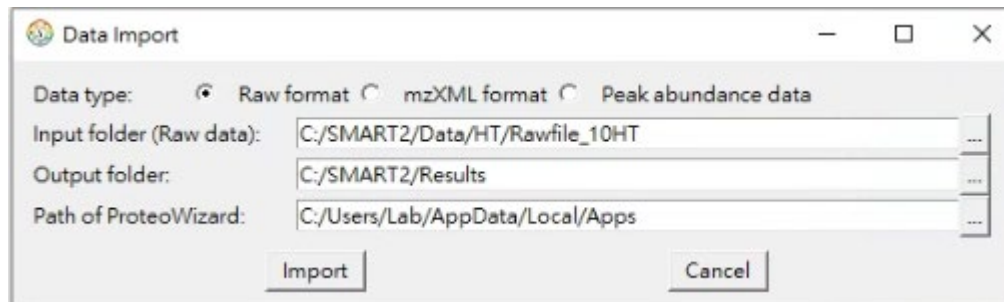
1



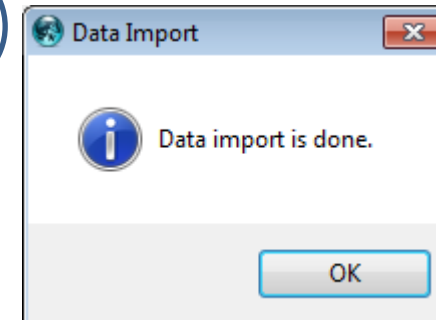
3



2



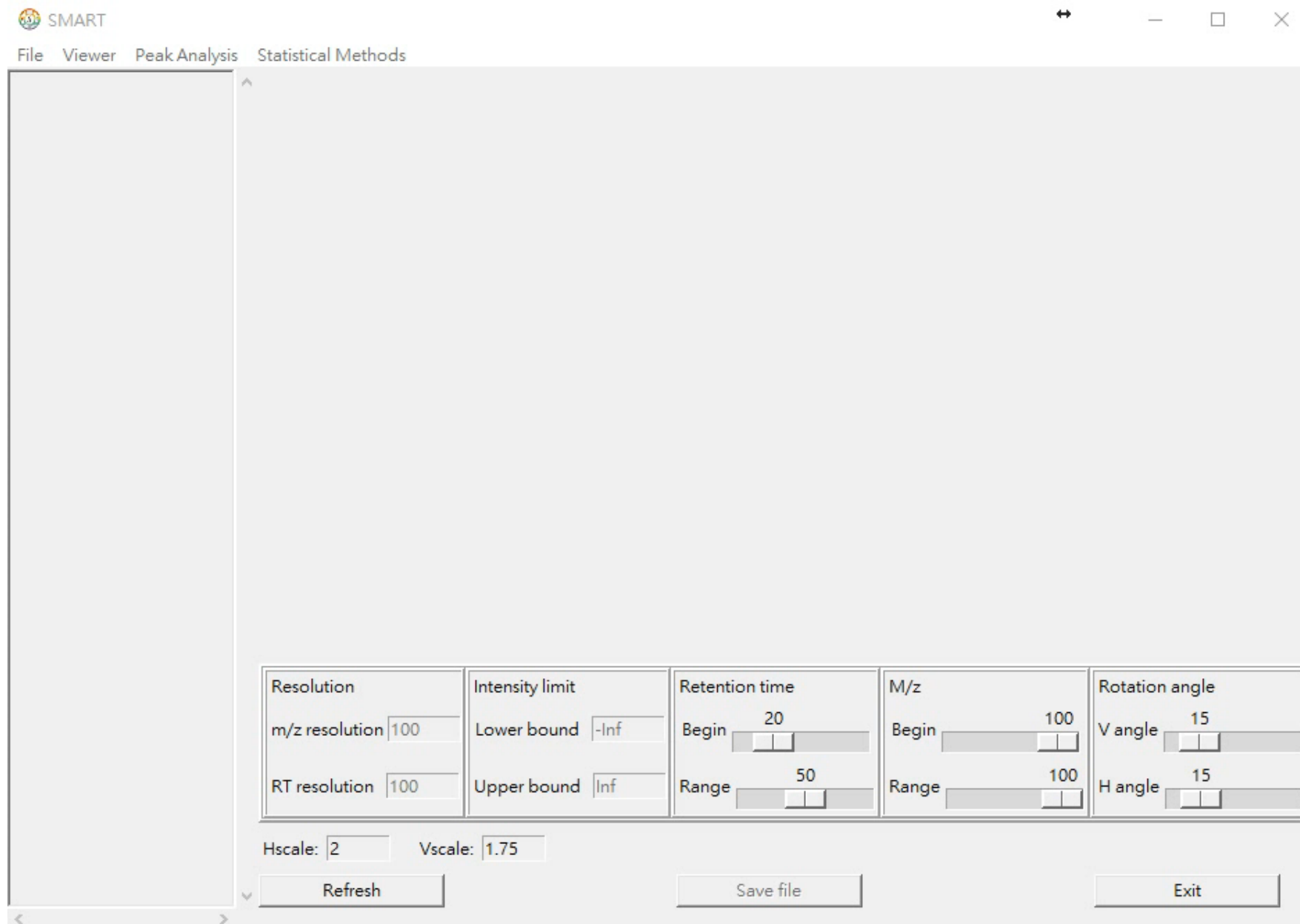
4



Note: only “.raw” file can be in the input directory

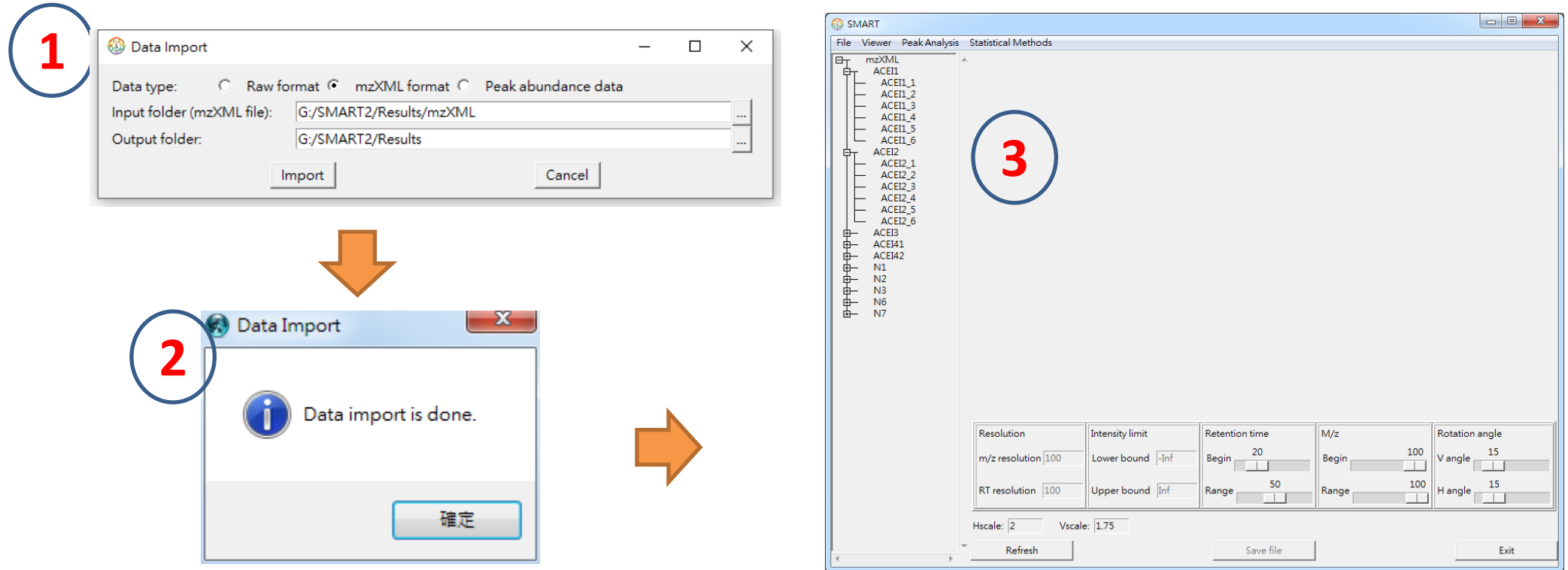
ProteoWizard:

C:\Users\username\AppData\Local\Apps



Data import (mzXML file)

- Data import (for mzXML file)
 - Folder setting
 - Note: only “mzXML” file can be in the input folder



SMART

File Viewer Peak Analysis Statistical Methods

Resolution

m/z resolution 100

RT resolution 100

Intensity limit

Lower bound -Inf

Upper bound Inf

Retention time

Begin 20

Range 50

M/z

Begin 100

Range 100

Rotation angle

V angle 15

H angle 15

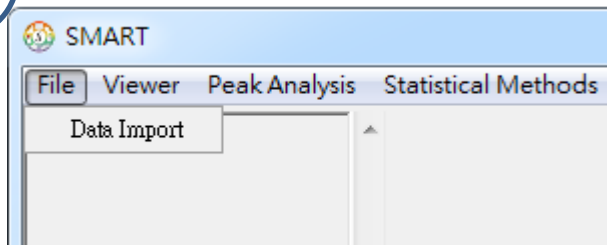
Hscale: 2 Vscale: 1.75

Refresh Save file Exit

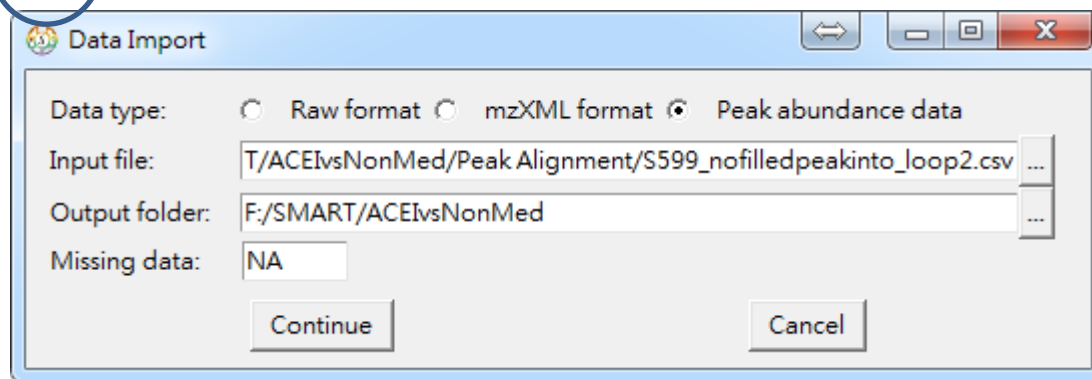
Data import (peak abundance data)

- Data import (for peak abundance data)

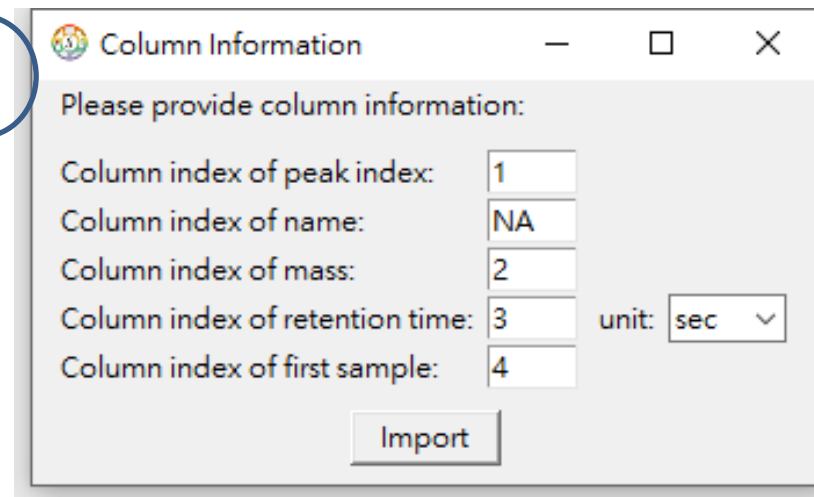
1



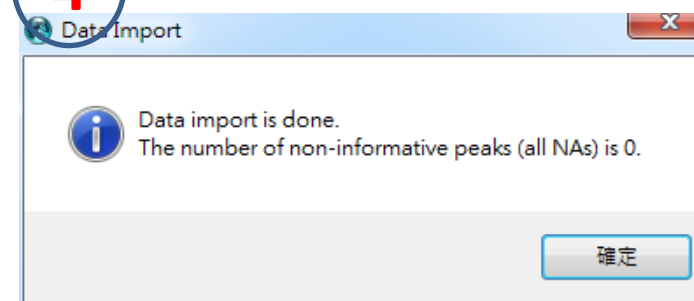
2



3

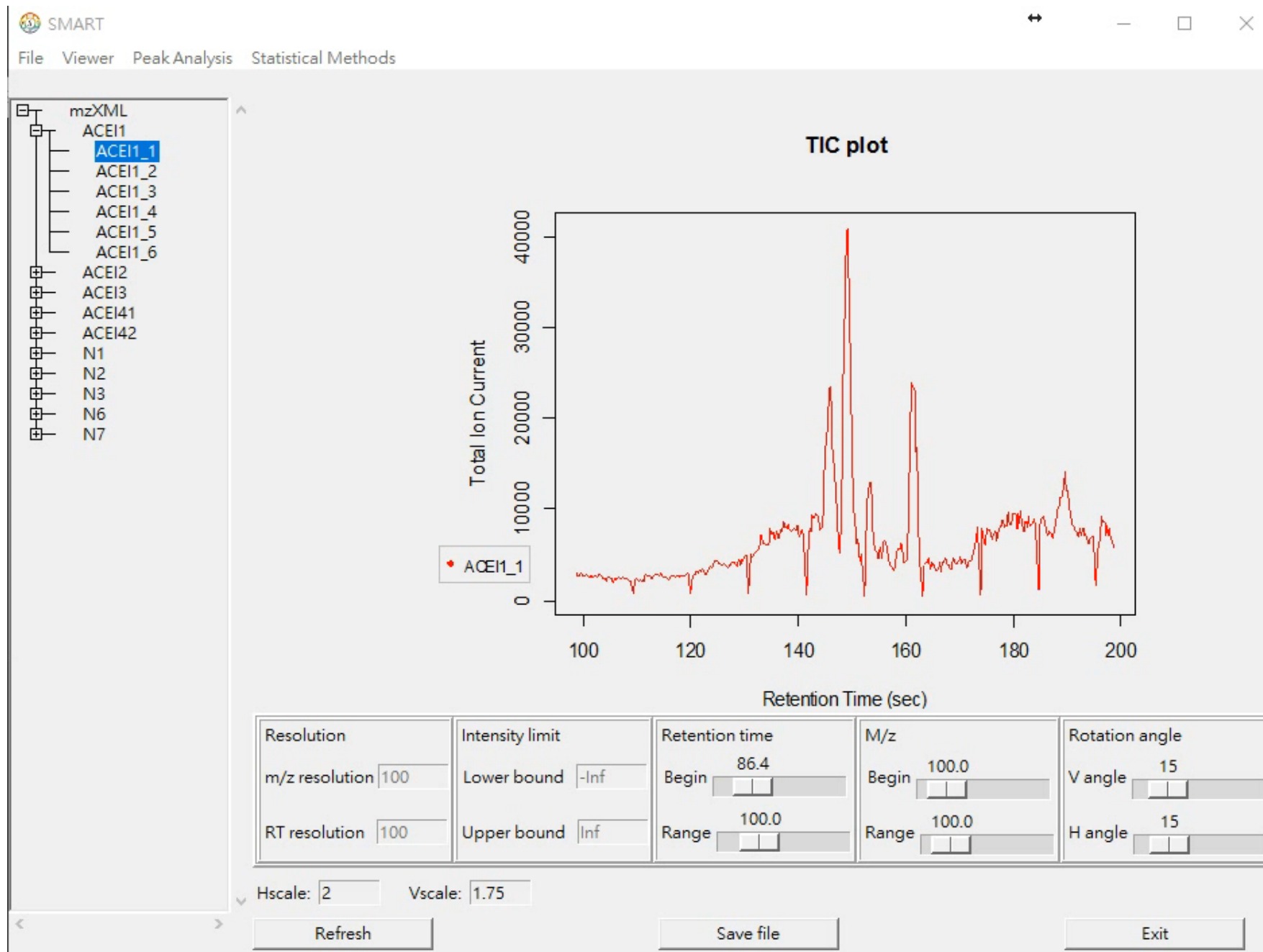


4



5

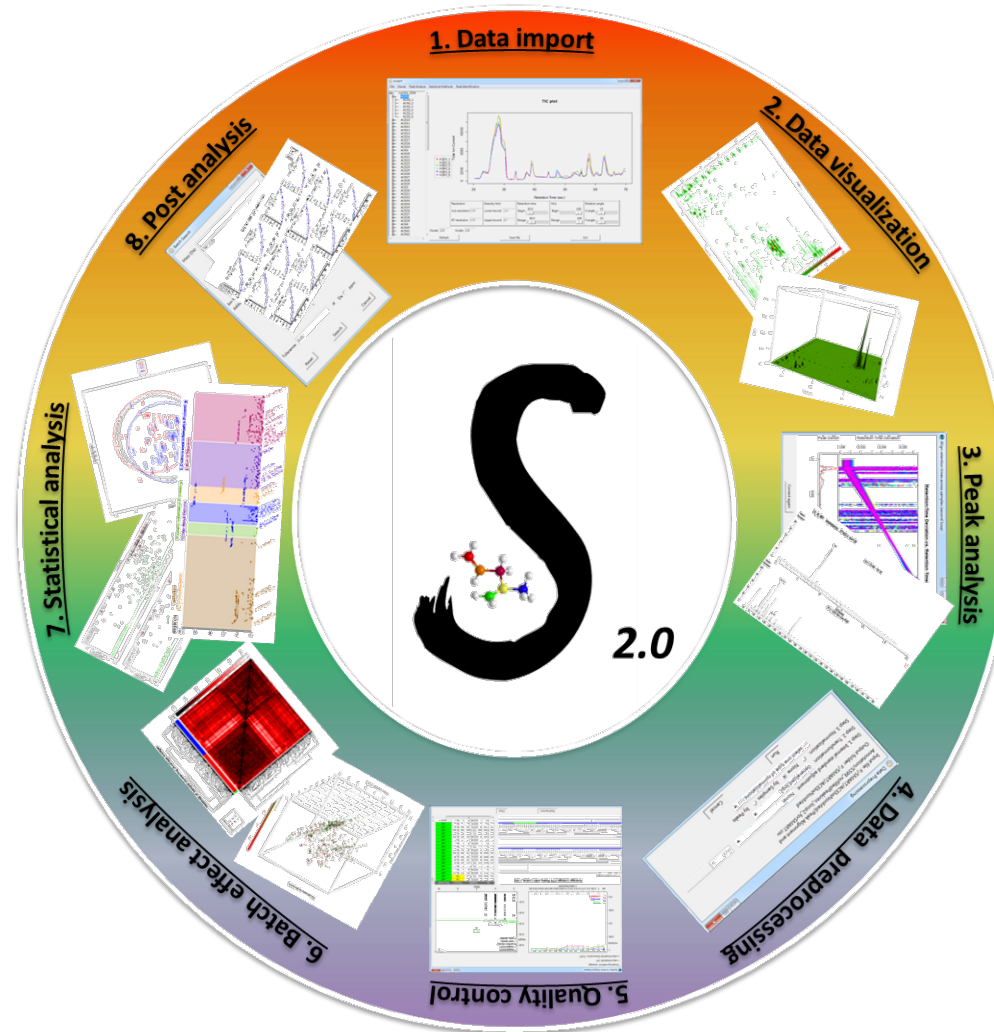
S60_nofilledpeakinto_loop2.csv
S60_nofilledpeakinto_loop2_forSMART.csv



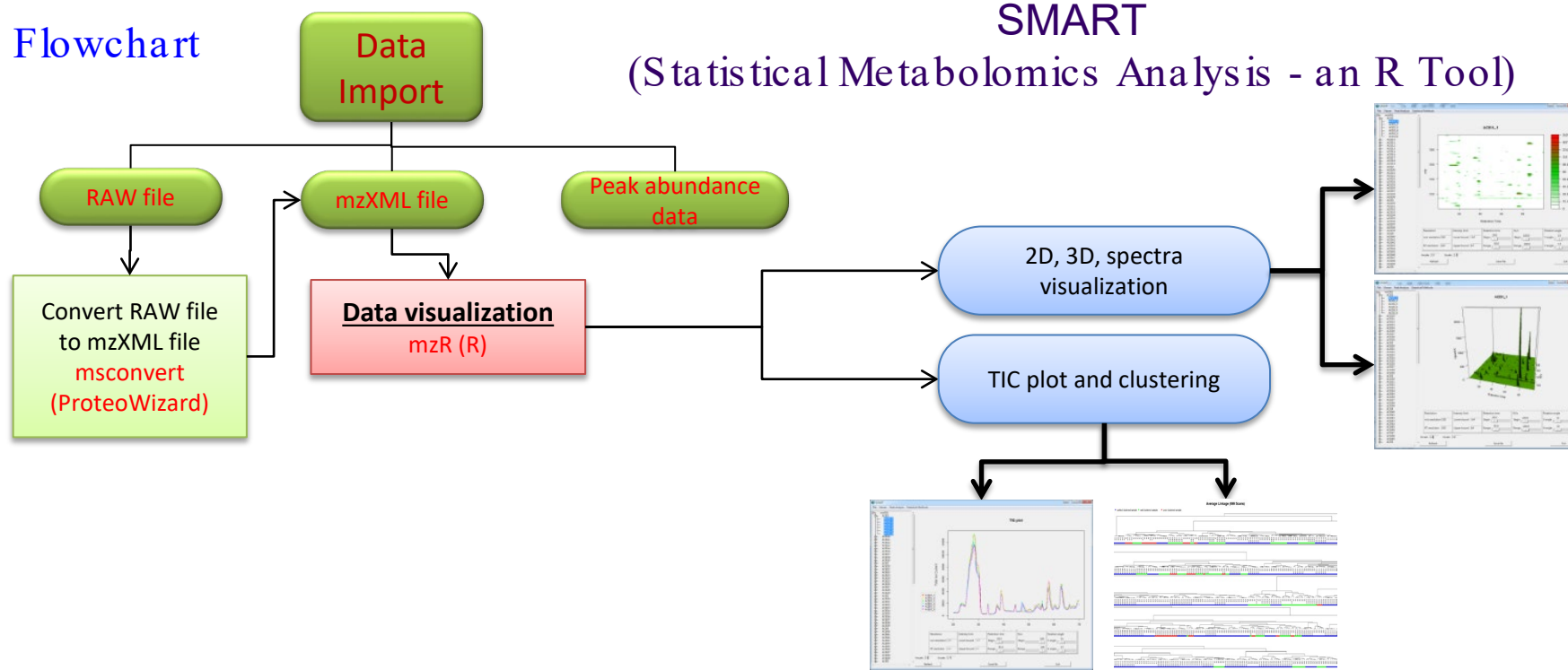
Data import (for peak abundance data)

Statistical Metabolomics Analysis – an R Tool

2. Data visualization

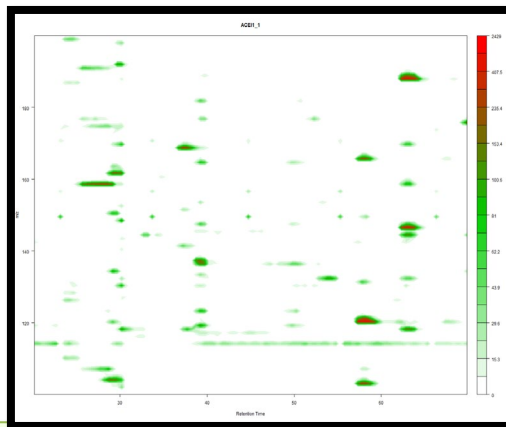
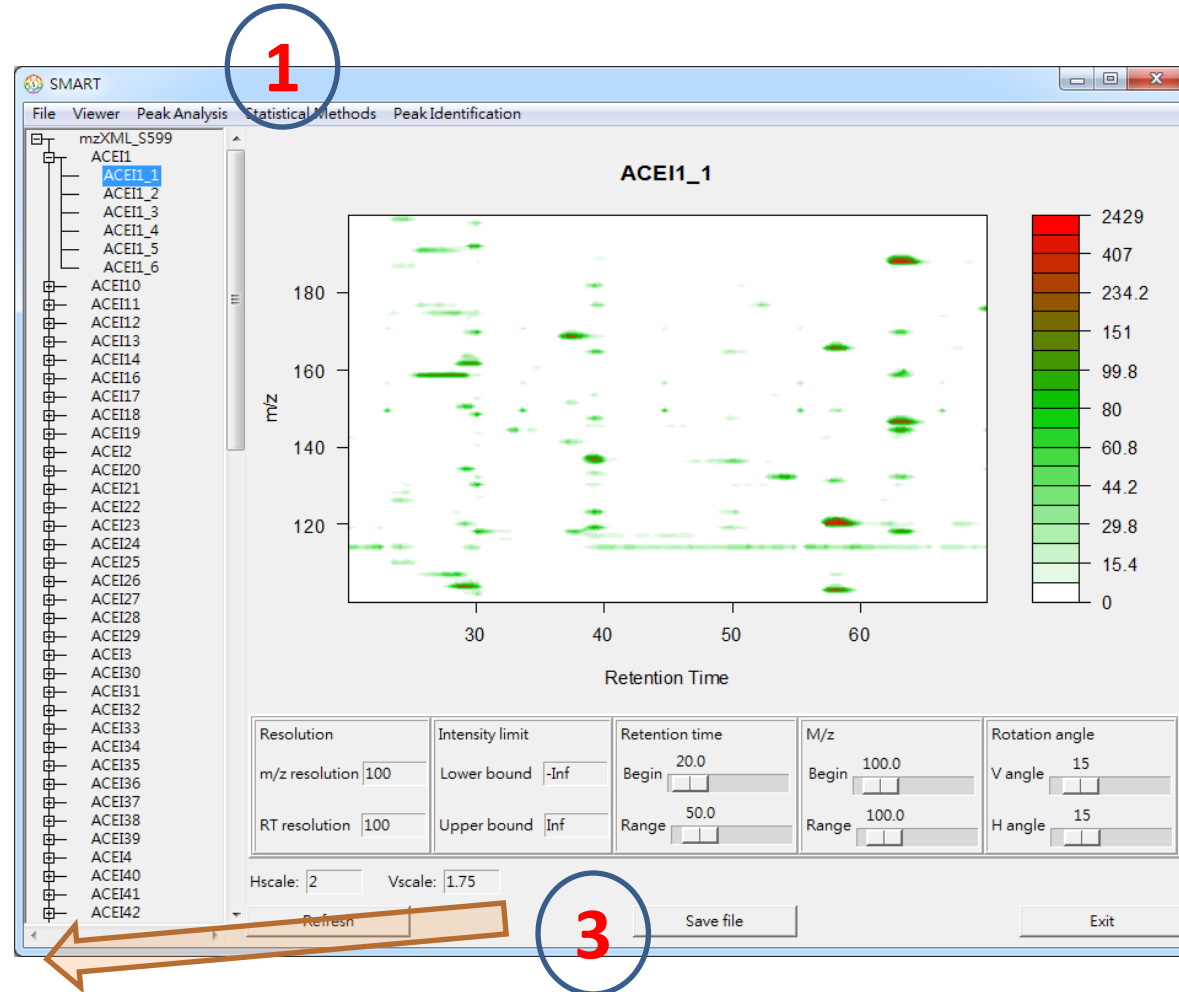
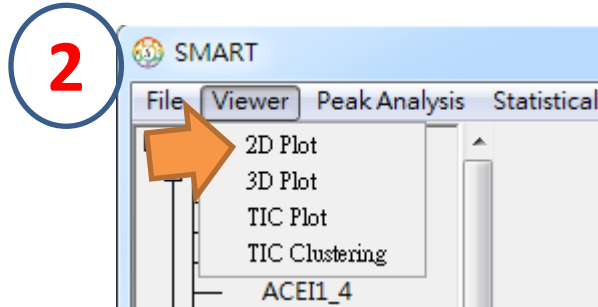


Flowchart



Data visualization

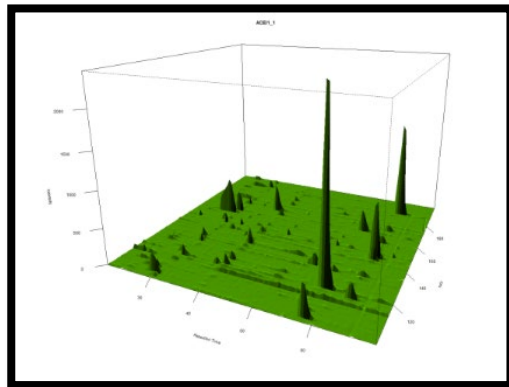
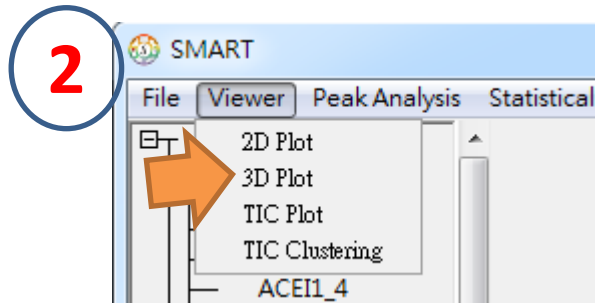
- 2D plot



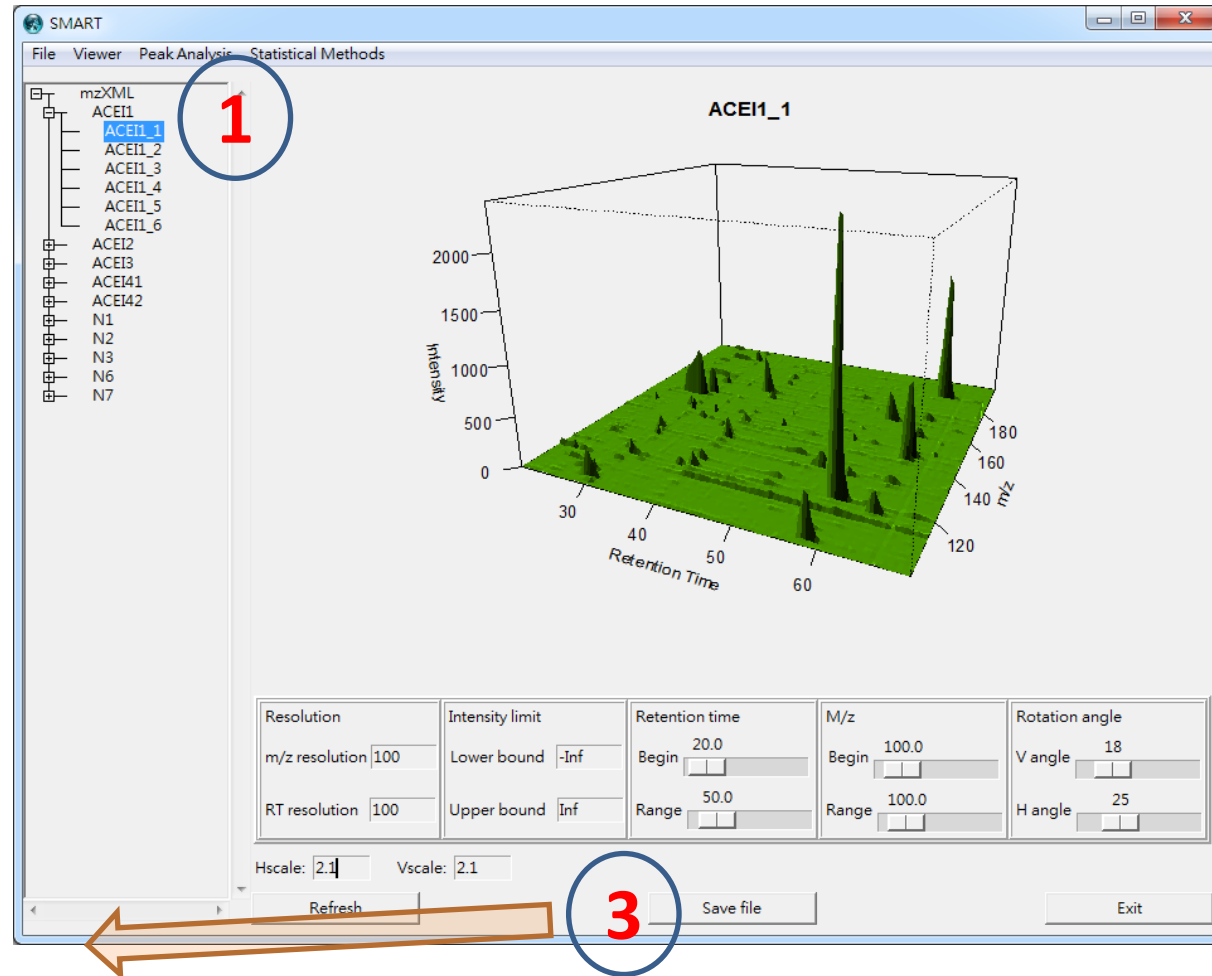
outpath\2D plot\ACEI1_1_RT20(50)_mz100(100).png

Data visualization

- 3D plot

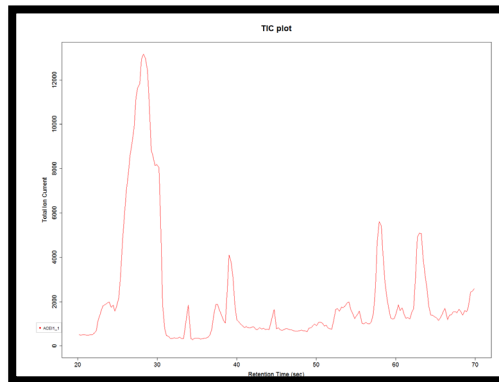
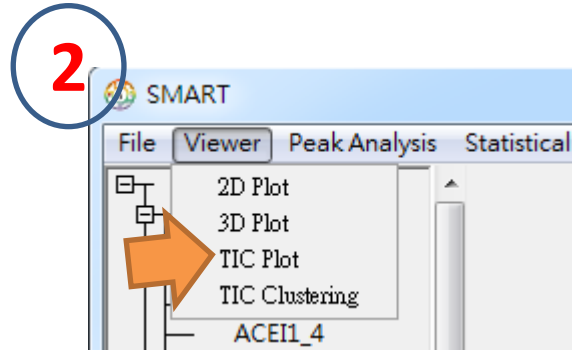


outpath\3D plot\ACEI1_1_RT20(50)_mz100(100)_h25_v18.png

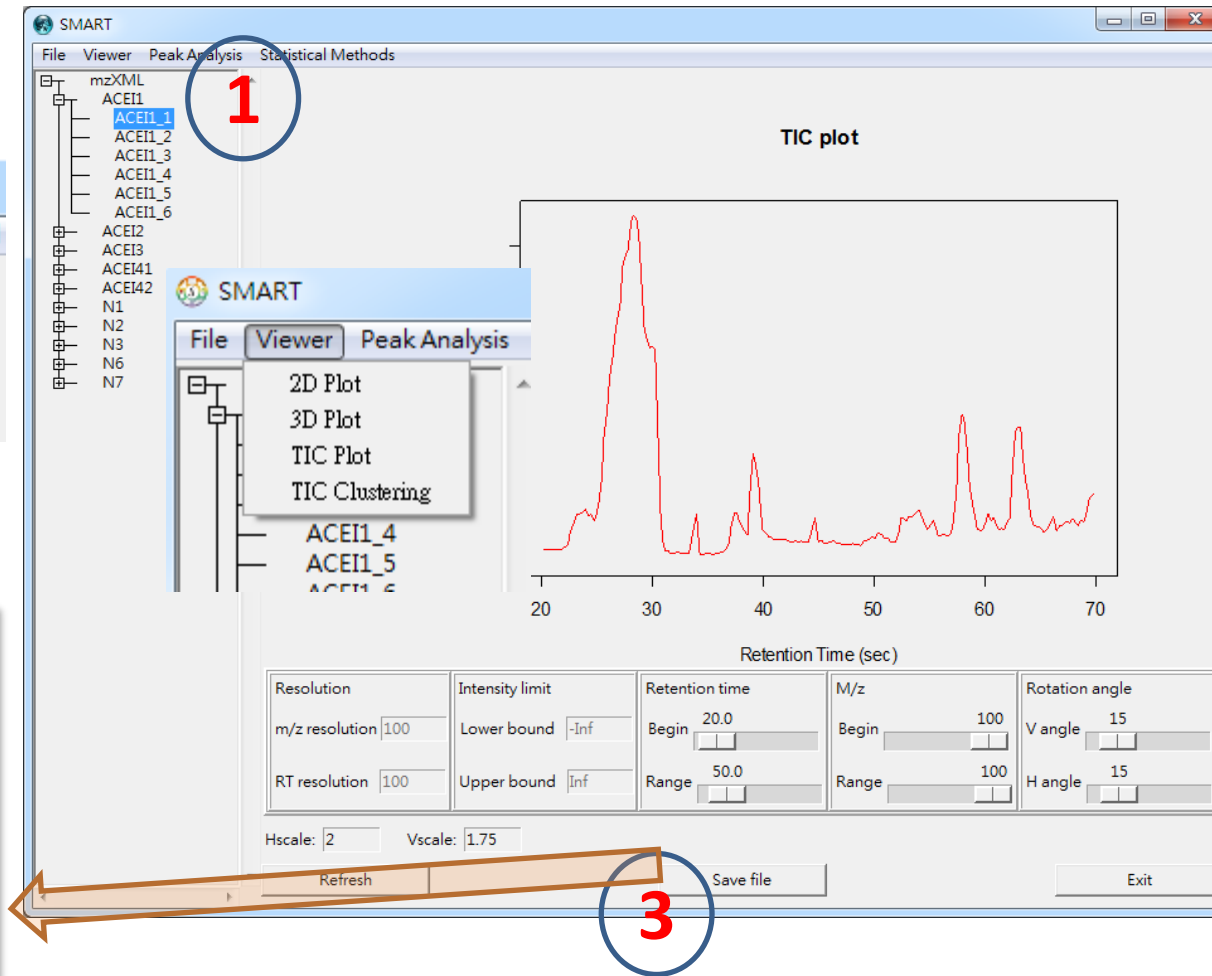


Data visualization

- TIC plot
 - For each replicate sample



outputpath\TIC plot\ACEI1_1_RT20(50).png



在質譜中，總離子電流 **total ion current (TIC)**顯示在各個保留時間 **retention time (RT)**的總離子電流，提供了樣本組成的全面視圖。TIC色譜圖代表在分析的每一點上檢測到的整個質量範圍的總強度。

SMART

File Viewer Peak Analysis Statistical Methods

mzXML

- ACEI1
 - ACEI1_1
 - ACEI1_2
 - ACEI1_3
 - ACEI1_4
 - ACEI1_5
 - ACEI1_6
- ACEI2
- ACEI3
- ACEI41
- ACEI42
- N1
- N2
- N3
- N6
- N7

Resolution

m/z resolution 100

RT resolution 100

Intensity limit

Lower bound -Inf

Upper bound Inf

Retention time

Begin 20

Range 50

M/z

Begin 100

Range 100

Rotation angle

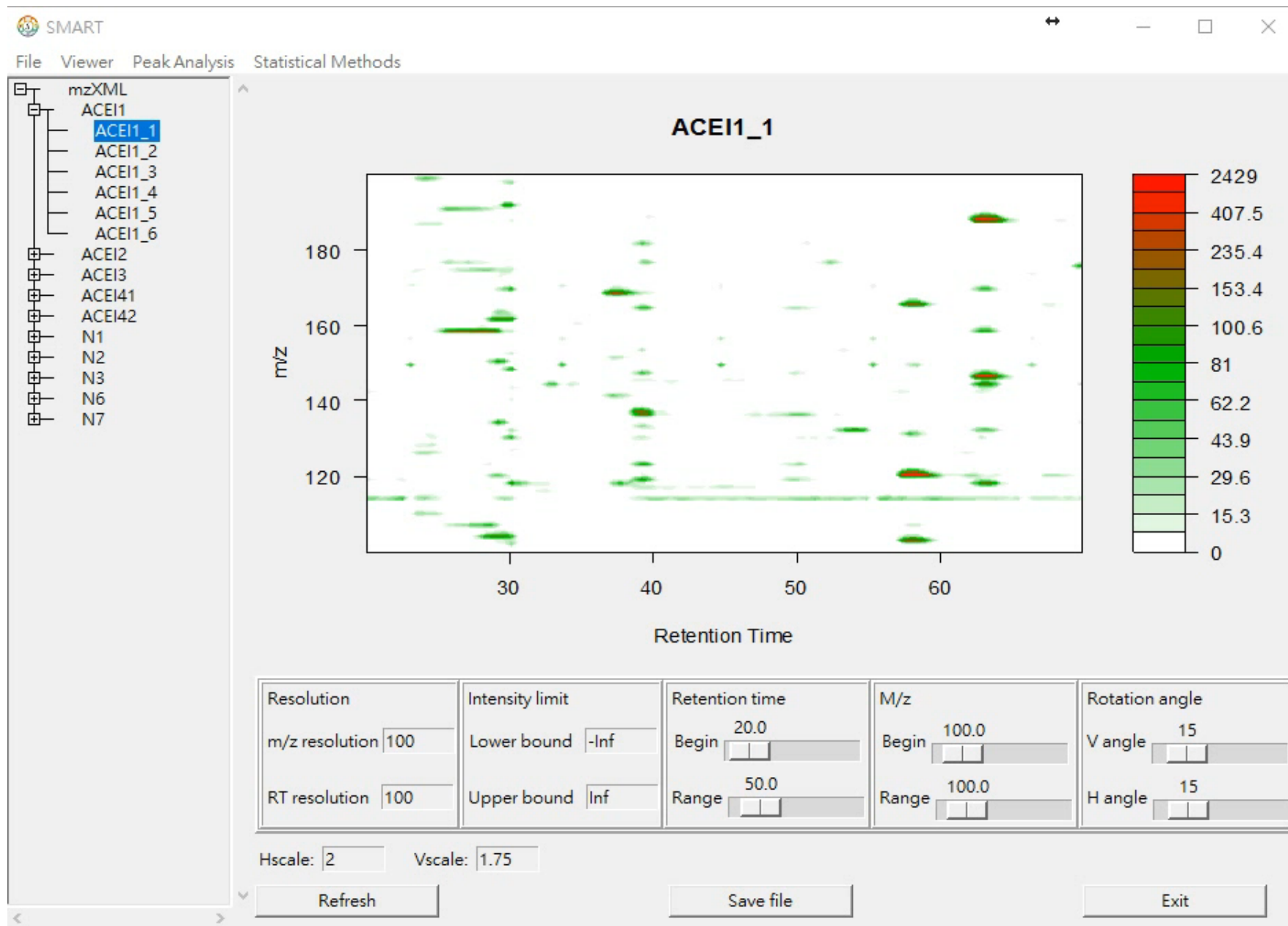
V angle 15

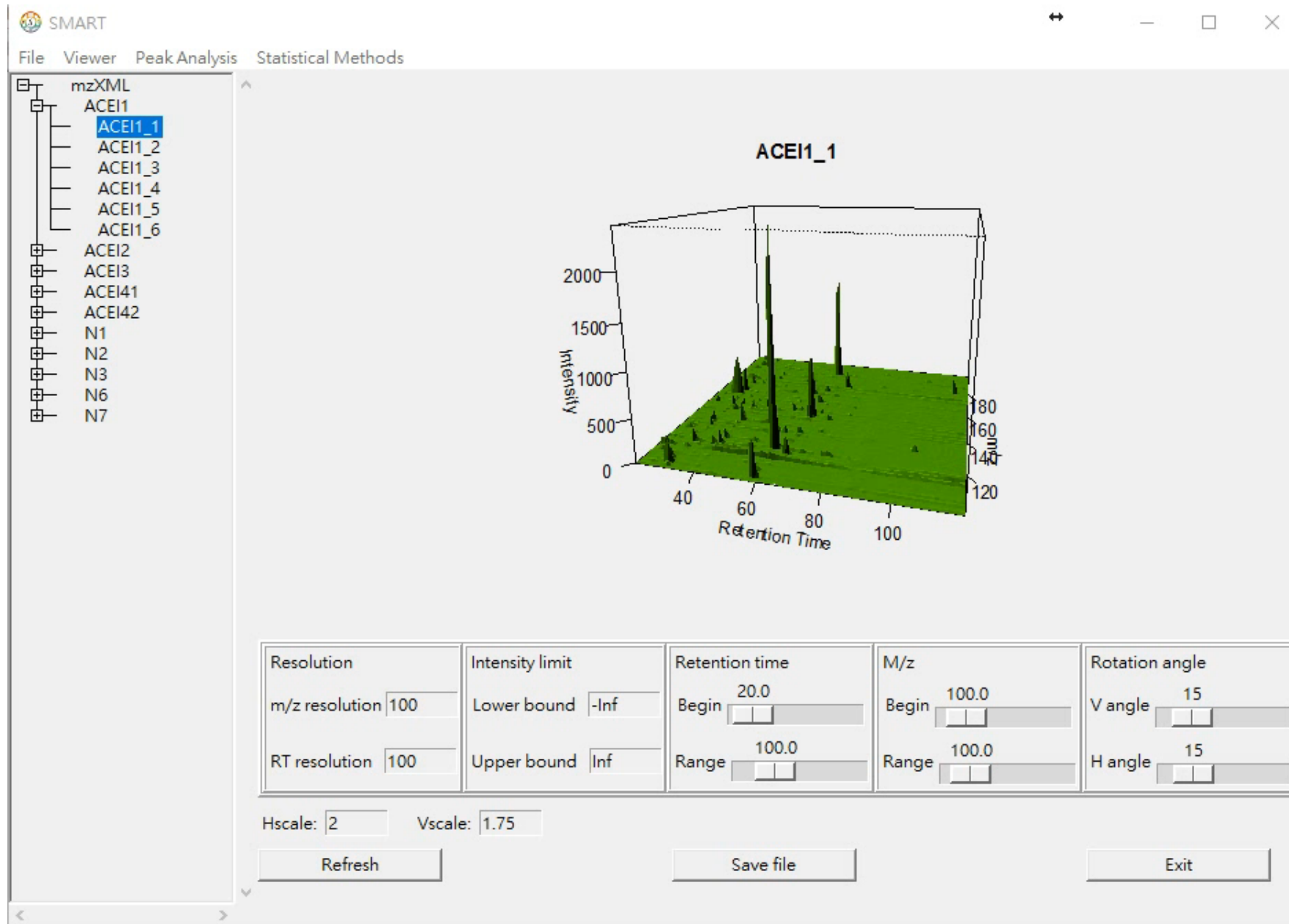
H angle 15

Hscale: 2 Vscale: 1.75

Refresh Save file Exit

2D plot



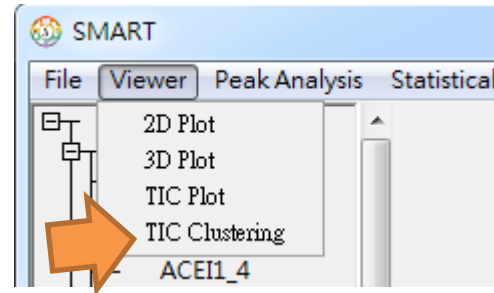


TIC plot

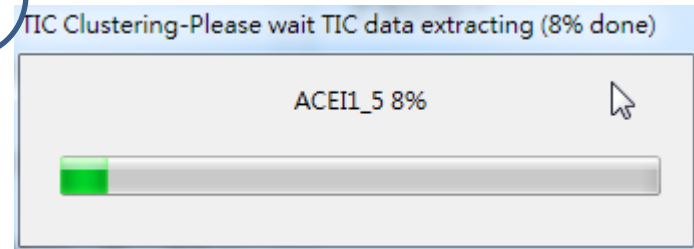
Data visualization

- TIC clustering

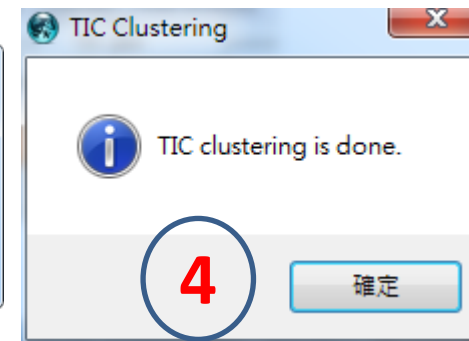
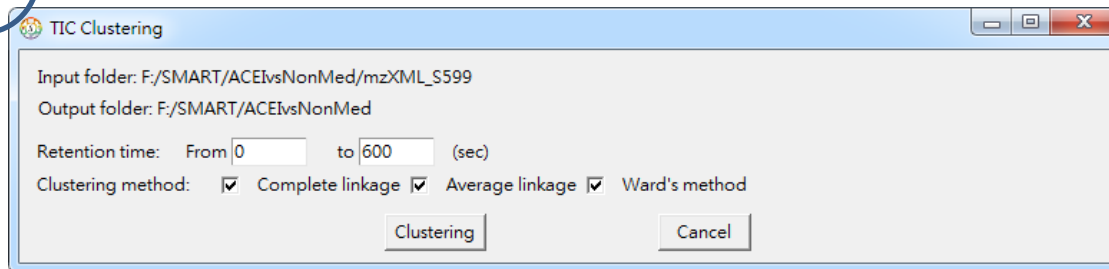
1



3



2



Statistical Metabolomics Analysis – an R Tool

- Data visualization
 - TIC clustering
 - output

outpath\TIC clustering\TICtable.csv

	A	B	C	D	E	F	G	H	I	J	K
1	Scan_index	Ret_Time	ACEI1_1	ACEI1_2	ACEI1_3	ACEI1_4	ACEI1_5	ACEI1_6	ACEI2_1	ACEI2_2	ACEI2_3
2	1	1.611703	1840.316	1883.235	1999.769	1929.929	2029.317	2244.63	1590.959	1761.858	2098.871
3	2	1.914309	525.8319	513.814	509.7652	502.4335	512.8351	492.2585	480.4986	472.9005	519.8399
4	3	2.154968	562.8346	509.1748	539.4163	467.0927	511.4119	472.8939	543.9075	475.7264	537.953
5	4	2.395501	512.4183	508.6098	636.6686	503.0518	503.2116	464.3456	503.8277	548.2333	528.2615
6	5	2.635341	538.5349	520.6004	542.8813	545.037	507.3488	507.485	439.9774	494.179	526.823
7	6	2.875018	591.0371	545.6836	536.9702	542.5765	497.7138	488.2345	509.0443	535.7909	588.3315
8	7	3.114768	532.316	481.7373	572.3064	488.0685	531.7565	461.464	522.0878	490.4316	580.4105
9	8	3.35471	552.8936	549.1855	555.149	482.2098	464.2407	499.5836	528.0374	554.635	544.5468
10	9	3.594811	557.4039	485.2134	568.3623	560.4765	529.6015	451.1695	506.7829	518.2427	559.6974
11	10	3.83523	543.802	487.1033	569.2555	466.8808	459.995	447.294	493.1324	537.6599	556.3434
12	11	4.075452	540.6479	551.7796	583.8124	488.8864	514.7813	502.7545	502.8375	476.9177	565.6591
13	12	4.315626	520.1061	566.7769	545.4417	574.0717	494.1166	516.8445	468.7639	495.1702	515.7346
14	13	4.555596	565.7251	514.366	553.2607	503.1035	488.1889	516.7361	526.1669	523.666	564.338
15	14	4.795548	498.9484	464.8117	522.7676	527.2203	511.1384	473.0254	518.6355	529.5582	497.3565
16	15	5.035726	521.6926	494.5057	550.8154	514.8136	481.3621	501.2584	507.0366	539.1605	545.1652

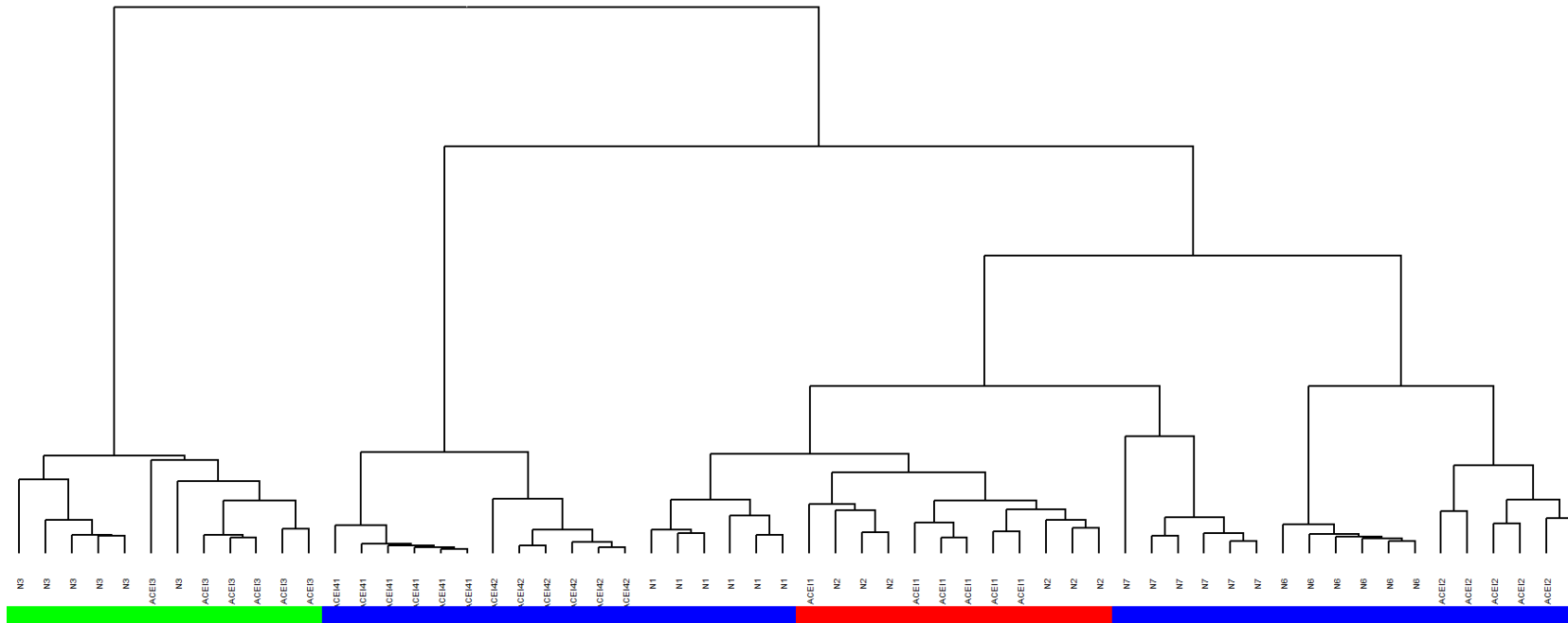
Statistical Metabolomics Analysis – an R Tool

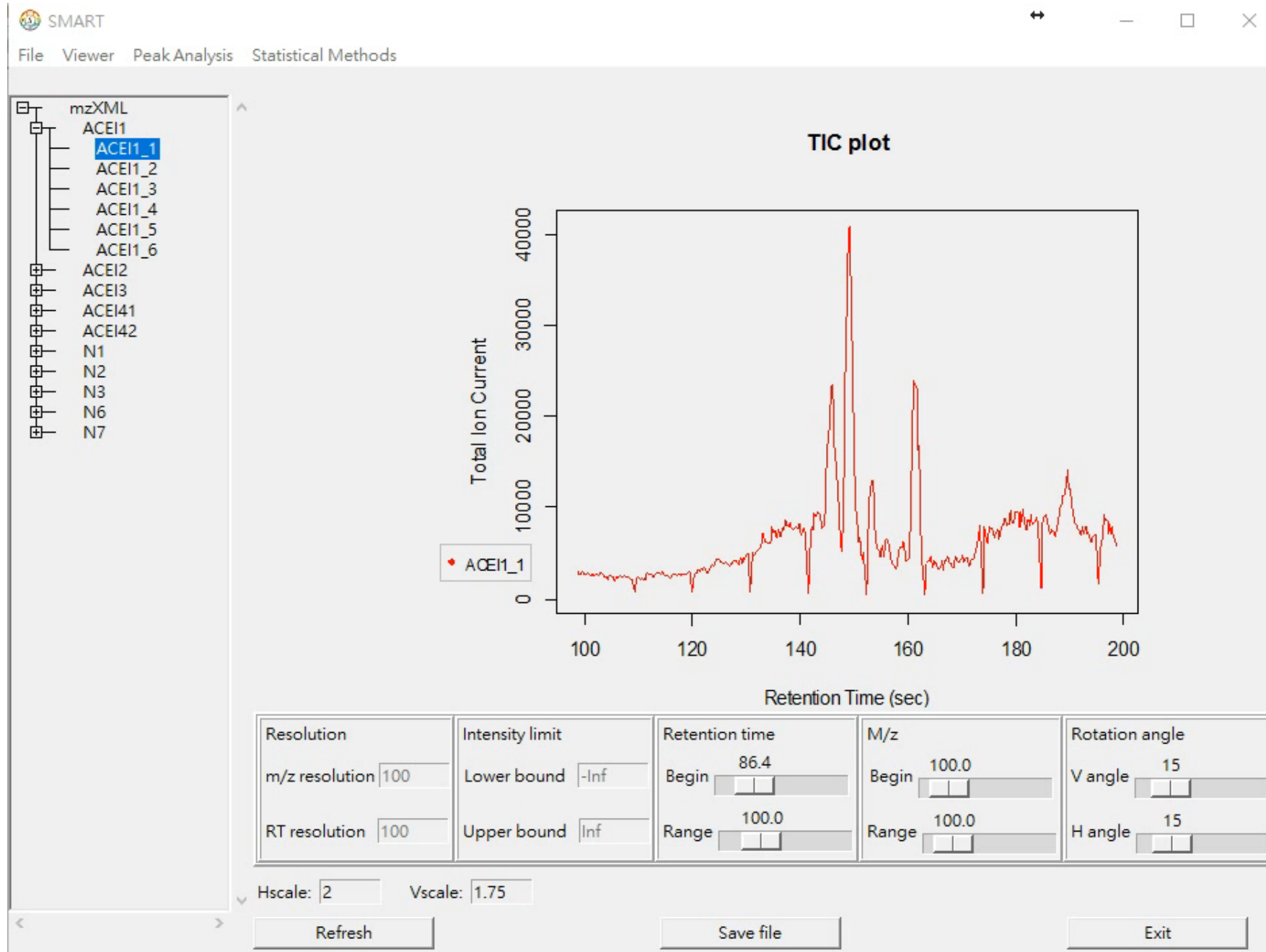
- Data visualization
 - TIC clustering
 - output

outpath\TIC clustering\Average\Clustering.png

Average Linkage (1674 Scans)

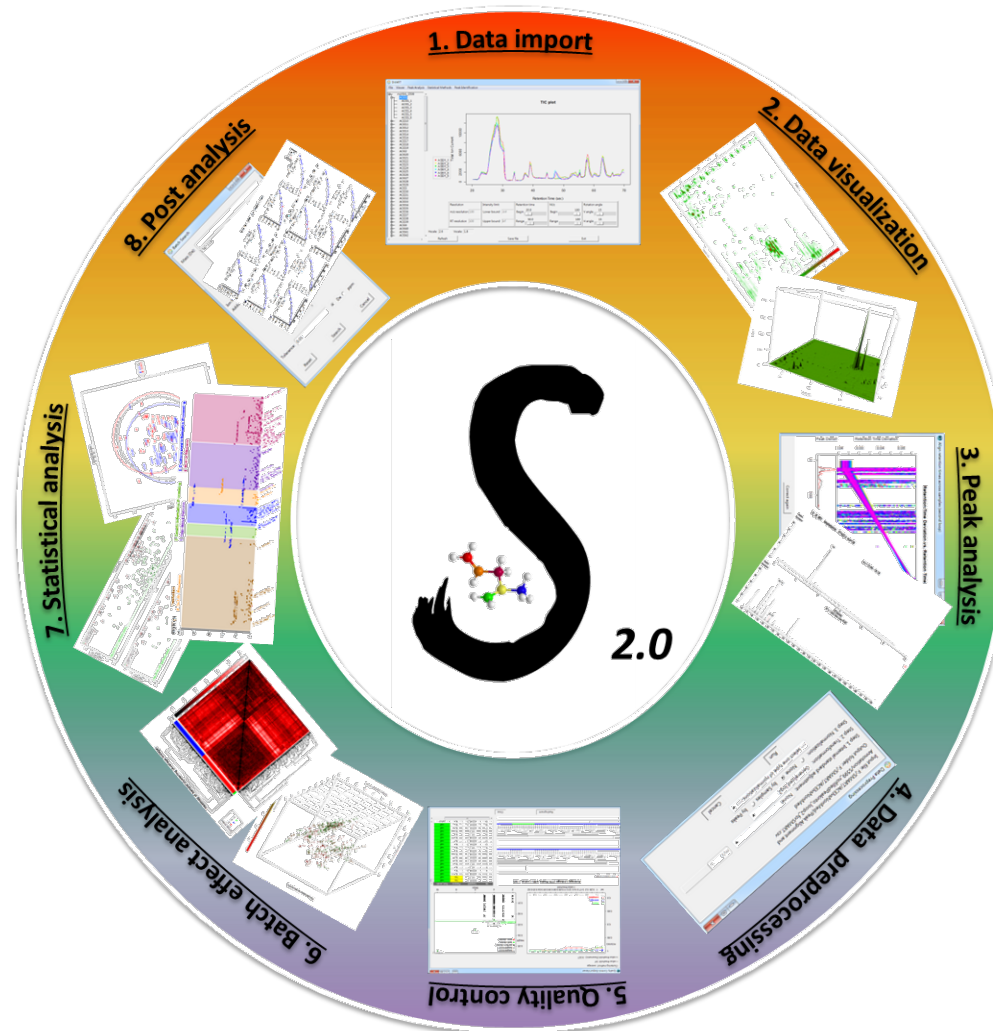
■ perfectly-clustered sample ■ well-clustered sample ■ poorly-clustered sample





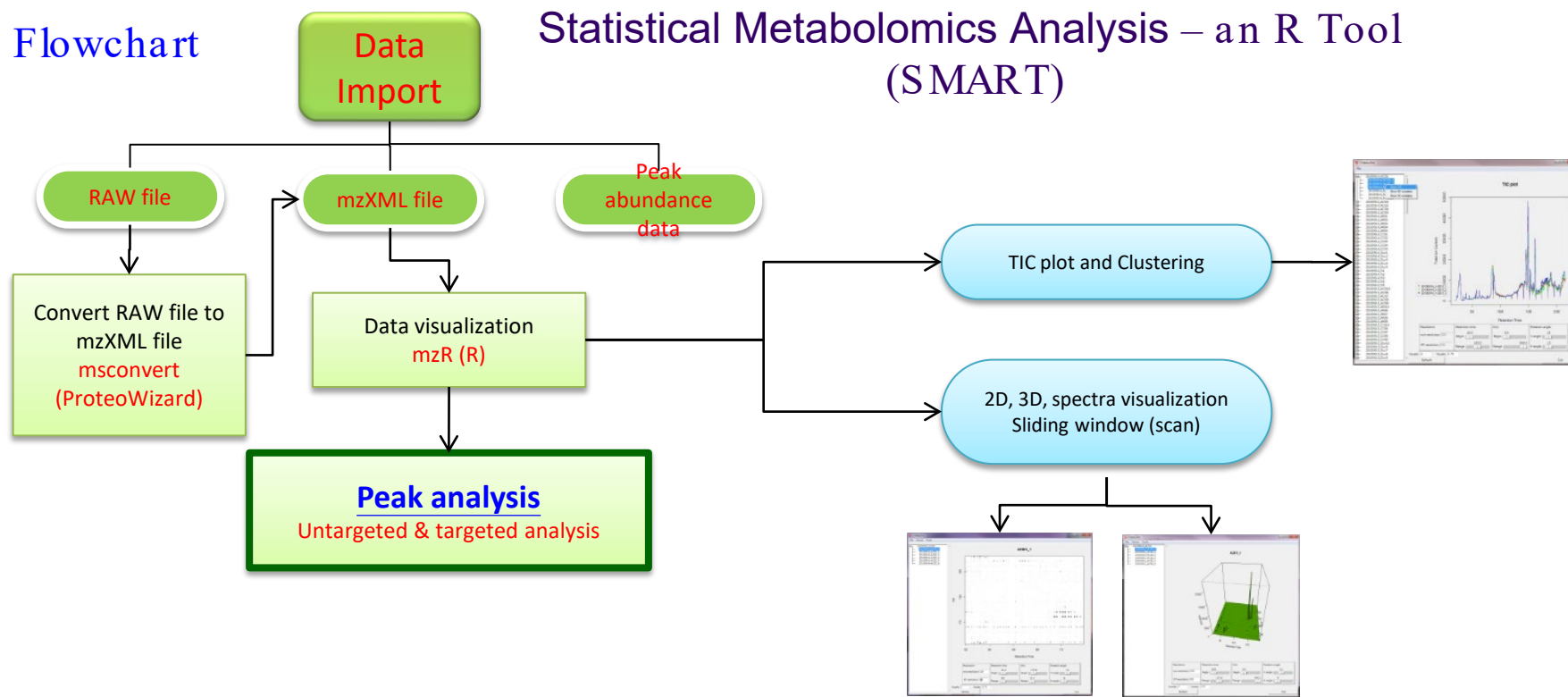
Statistical Metabolomics Analysis – an R Tool

3. Peak analysis (untargeted)



Flowchart

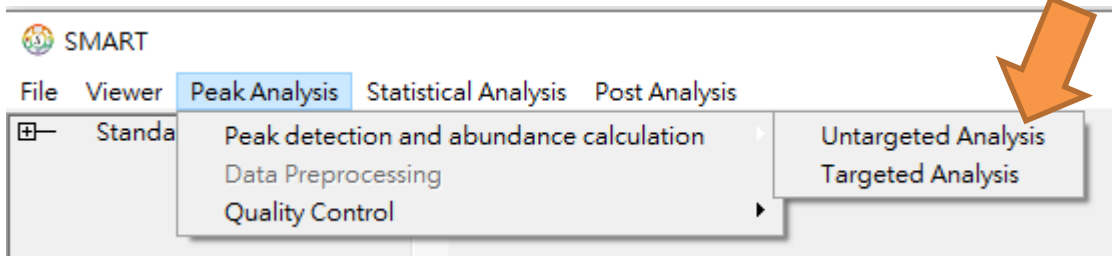
Statistical Metabolomics Analysis – an R Tool (SMART)



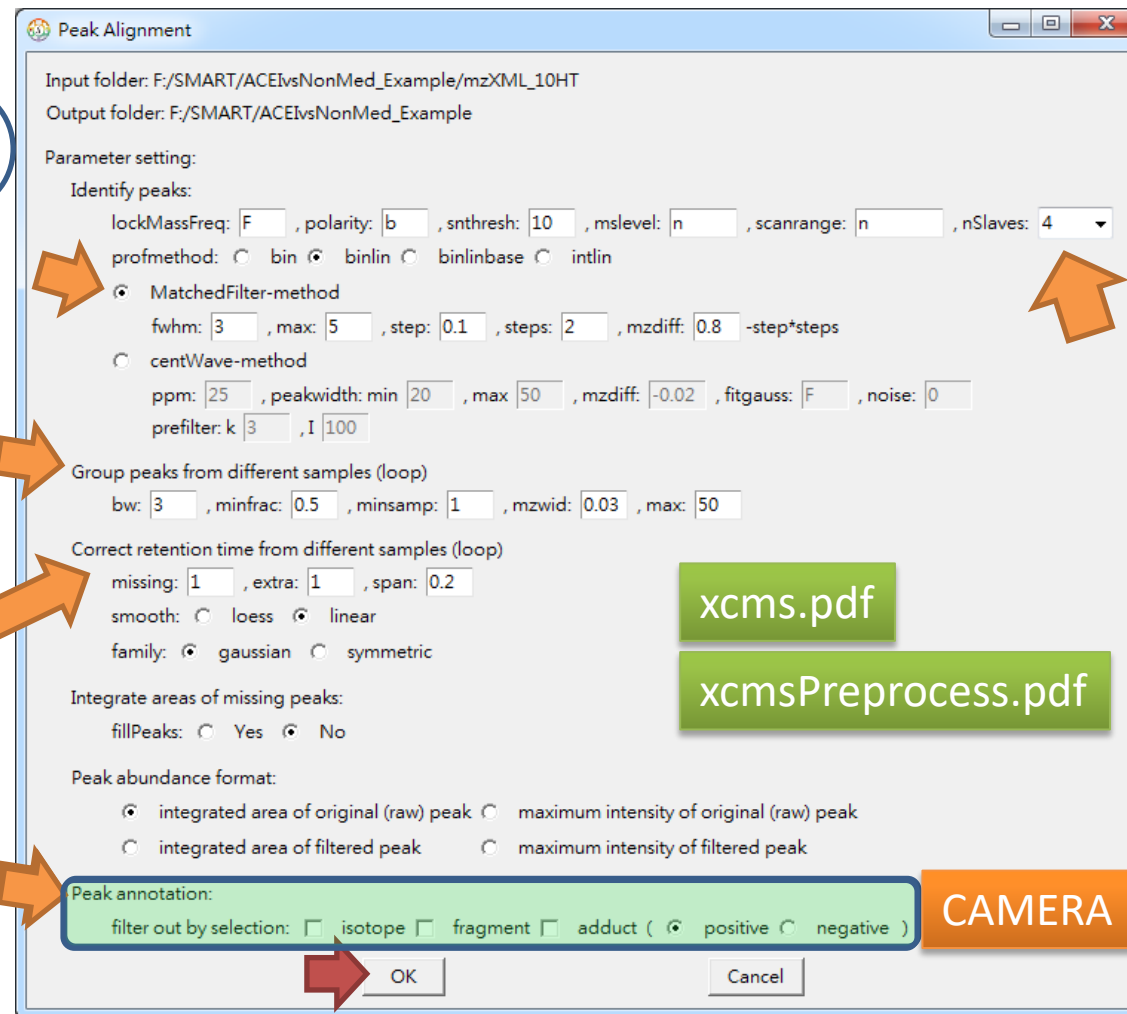
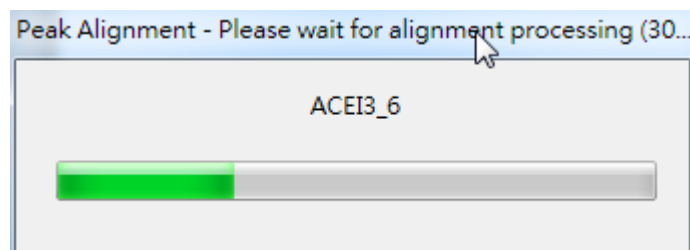
Peak analysis (untargeted)

- Untargeted analysis
 - Step 1: import mzXML files
 - Step 2: execute XCMS
 - (Peak alignment)

1

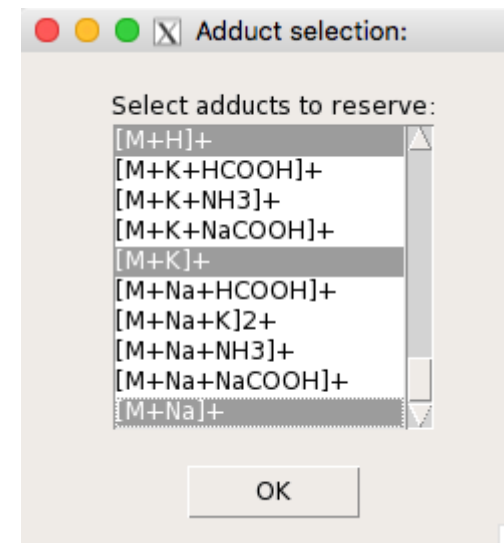
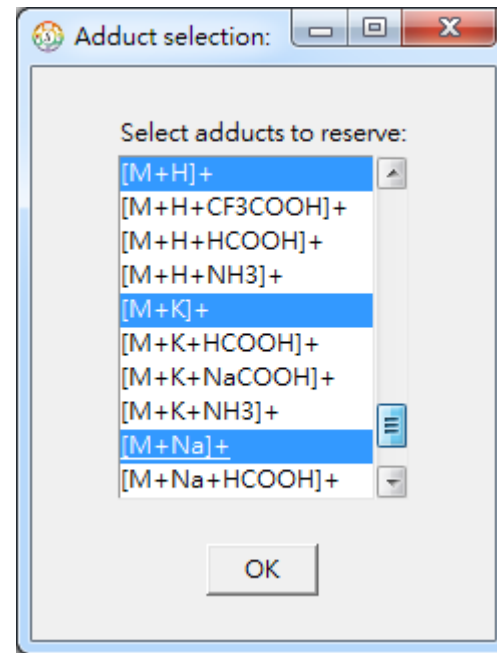


2



Peak analysis (untargeted)

- Step 3: execute CAMERA (Peak annotation)



Peak analysis (untargeted)

- 3. Peak alignment and annotation -- output file (peak abundance table)

outpath\Peak Alignment\S60_nofilledPeakinto_loop2.csv

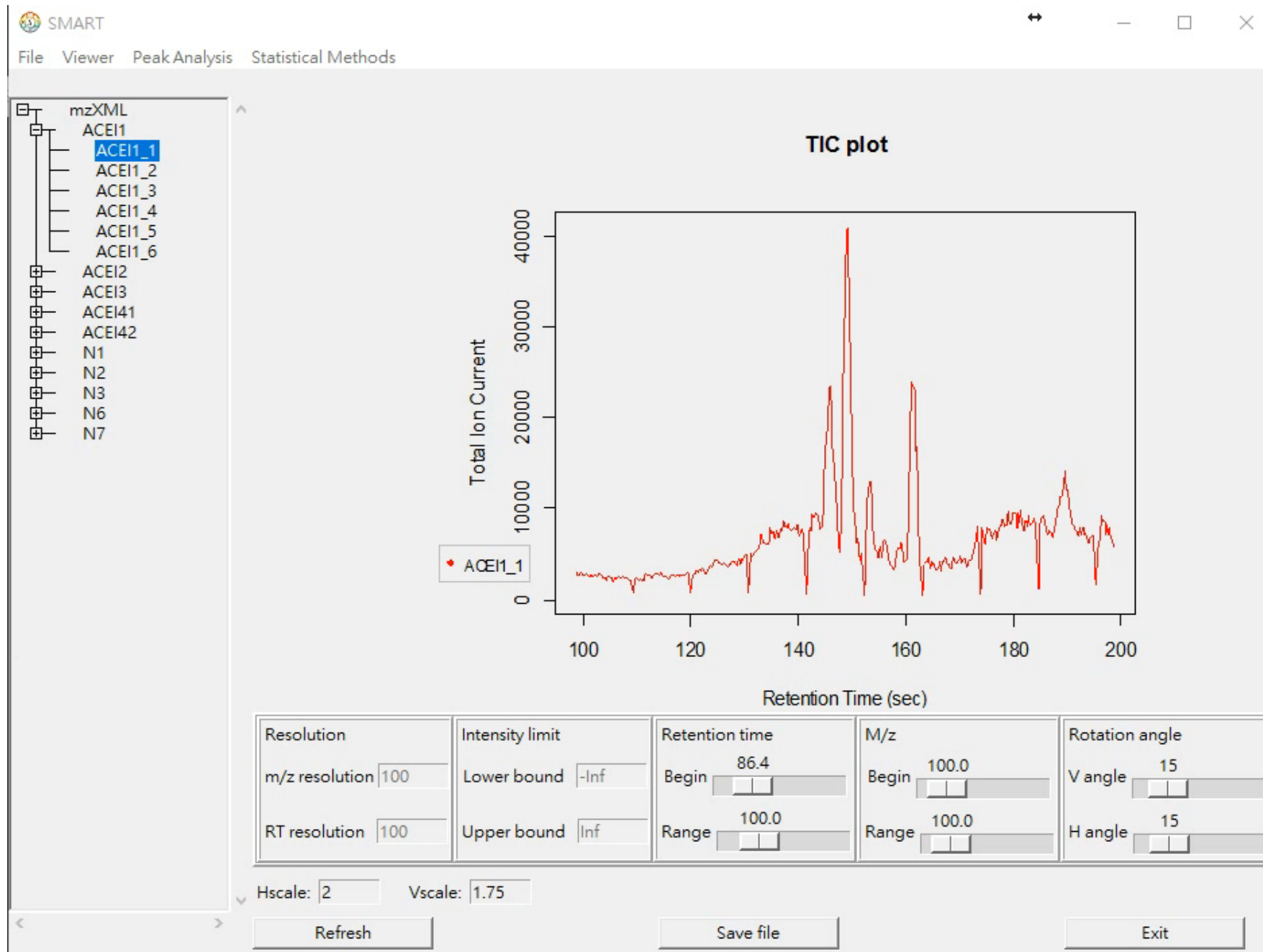
Peak_Index	mz	Ret_Time.sec	ACEI1_1	ACEI1_2	ACEI1_3	ACEI1_4	ACEI1_5	ACEI1_6	ACEI2_1	ACEI2_2	ACEI2_3
1	55.9302	336.7816	40.49721	48.4055	68.5233	131.6603	153.4566	68.20429	98.66187	101.203	125.084
2	56.95207	335.9918	25.78232	NA	33.81898	31.96166	34.43379	36.10391	NA	21.12795	NA
3	57.93077	335.9468	25.30278	26.6211	29.52791	37.52189	34.32671	20.62405	19.76005	23.86996	29.22897
4	60.07351	29.2962	15.05838	19.10104	15.67263	20.25476	14.3002	19.61156	NA	17.66053	16.79027
5	68.97672	24.1171	32.99825	39.72613	41.26156	24.86147	37.08042	29.47383	48.53648	51.11766	60.06144
6	70.05911	32.3207	NA	NA	NA	NA	NA	NA	88.67861	71.20517	77.2781
7	72.07474	37.8148	77.36168	60.19769	53.10426	45.49223	52.31326	45.89031	266.037	248.2481	192.2031
8	72.07494	64.0368	NA	NA	NA	NA	NA	NA	61.4457	57.43003	54.12979
9	72.93235	336.7707	54.64176	NA	116.1767	105.3885	268.4261	NA	NA	116.3039	208.1015
10	74.93326	336.1583	NA	46.74469	53.22282	46.98308	44.75656	40.26063	50.46011	42.93352	57.50089
11	77.03265	57.9572	36.87367	38.9539	38.91873	31.10987	37.97594	34.42077	131.6498	148.9135	208.078
12	79.04987	57.9244	23.16134	15.64894	19.70598	19.23182	20.40361	15.09588	70.72101	60.65076	71.75067
13	82.00799	24.1165	22.04538	26.83705	28.74175	32.02328	22.70863	20.06136	41.06131	34.26637	40.51016
14	84.03872	30.0699	38.46723	45.41489	54.63141	50.46705	52.82711	49.82799	127.9401	147.1337	136.5419
15	84.03838	48.6612	33.55734	30.40641	21.84934	26.916	35.54772	27.8324	42.54551	47.40438	44.89901
16	84.95268	24.1224	245.3396	218.0391	247.8054	261.728	190.1302	217.5573	324.1442	256.0635	321.4737
17	84.95221	335.6901	301.2523	195.8762	316.6637	302.4194	272.9157	542.9502	232.0436	295.5475	193.4221
18	86.08994	53.8669	238.2553	219.8844	231.6355	185.6219	186.2405	190.8221	1209.354	1210.146	1024.845
19	86.98677	24.1066	17.84491	19.26533	NA	17.23194	20.42927	NA	33.63286	32.69955	25.17788
20	87.09331	53.7237	15.80857	NA	16.59535	NA	NA	NA	73.34223	76.04747	63.93756

Peak analysis (untargeted)

- 3. Peak alignment and annotation -- output file (peak abundance table)

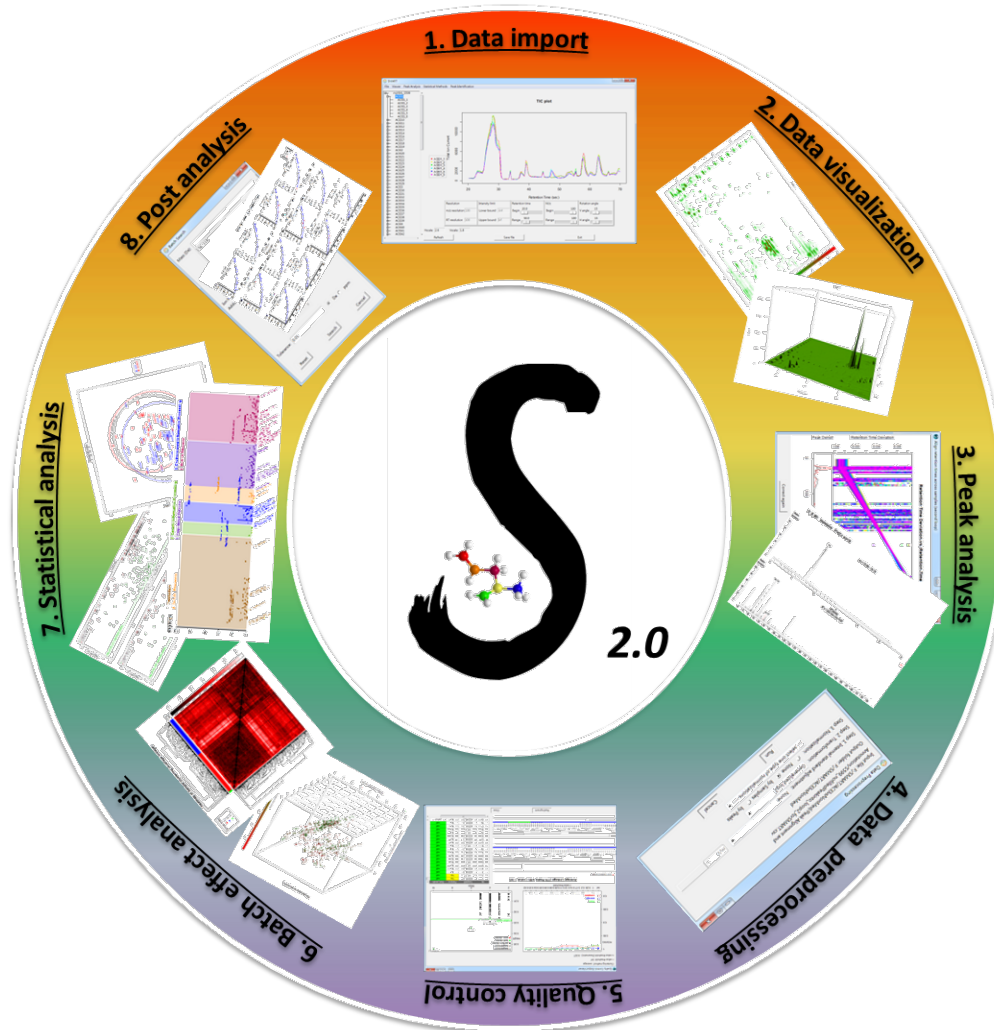
outpath\Peak Alignment\S60_nofilledPeakinto_loop2_annoinfo.csv

Peak_Index	mz	Ret_Time.sec	isotopes	adduct	pcgroup
1	22.9849305	27.7127			35
2	55.92994829	336.8763			319
3	56.95336493	335.936		[M+K]+ 17.9854	29
4	57.9299371	335.8817		[M+H]+ 56.9236	7
5	59.92473806	335.8649			220
6	60.07189387	29.25			336
7	68.97673761	24.1198		[M+Na]+ 45.9928	46
8	69.06404612	52.8241			182
9	70.05927741	32.3222			166
10	72.07471273	37.7522		[M+K]+ 33.1119 [M+Na]+ 49.0859 [M+H]+ 71.0677	44
11	72.07482812	64.0093			162
12	72.93202428	336.803		[M+Na]+ 49.9429	58
13	74.93307144	336.1642		[2M+K]+ 17.9854	29
14	76.92734109	336.1336		[3M+Na]+ 17.9854 [3M+Na+2K-H]2+ 17.9854	29
15	77.03232255	57.8967		[M+Na]+ 54.0431	4
16	79.04787429	57.9087			4
17	82.00795337	24.1116			46
18	84.03810375	30.1203			13
19	84.03824131	48.7586			252
20	84.07434463	38.9898		[M+H]+ 83.0689	23



Statistical Metabolomics Analysis – an R Tool

3. Peak analysis (targeted)



Peak analysis (targeted)

- Targeted analysis
 - Step 1: import mzXML files & true m/z information

1 mzXML files

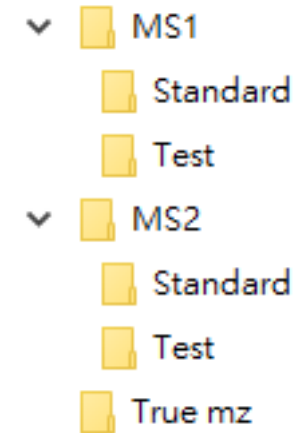
D1_50_MS1.mzXML
D2_100_MS1.mzXML
D3_200_MS1.mzXML
D4_300_MS1.mzXML
D5_400_MS1.mzXML
D6_500_MS1.mzXML
D7_600_MS1.mzXML
D8_700_MS1.mzXML
D9_800_MS1.mzXML
D10_900_MS1.mzXML
D11_1000_MS1.mzXML

2 true m/z

Name	mz	Ret_Time.sec	tol_time.sec
Heroin	370	685.2	5
Morphine	286	147	5
Cocaine	304	702	5
Thebaine	312	678.6	5
delta-9 THC	315	1513.8	5
Amphetamine	136	422.4	20
MA	150	495.6	10
MDMA	194	539.4	5
Love Drug	180	506.4	10
Ketamine	238	600	5
FM2	314	1044	5
Nimetazepam	296	1041	5

MS1

targeted_MS1_tol_time.csv



Name	parent_ion	daughter_ion	tol_time.sec
Heroin	370	370;328;268; 211;193;58	10
Morphine	286	286; 268; 229; 201	10
Cocaine	304	304; 182; 150; 105; 82	10
Thebaine	312	312; 281; 266; 251; 221; 58	10
delta-9 THC	315	315; 259; 193; 135; 123; 107; 93	10
Amphetamine	136	136; 119; 91	20
MA	150	150; 119; 91	10
MDMA	194	194; 163; 135; 133; 105	10
Love Drug	180	180; 163; 135; 133; 105	10
Ketamine	238	238; 220; 179; 163; 152; 125	10
FM2	314	314; 200; 286; 268	15
Nimetazepam	296	296; 268; 250; 222; 193; 165	10

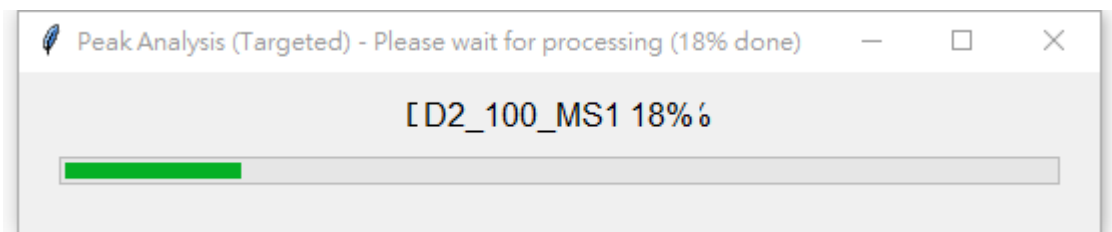
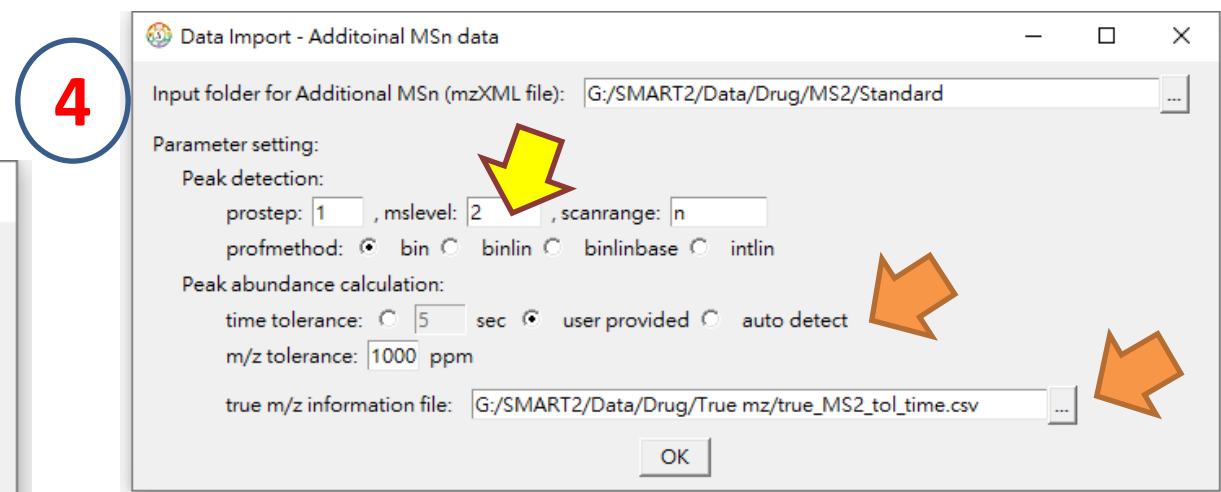
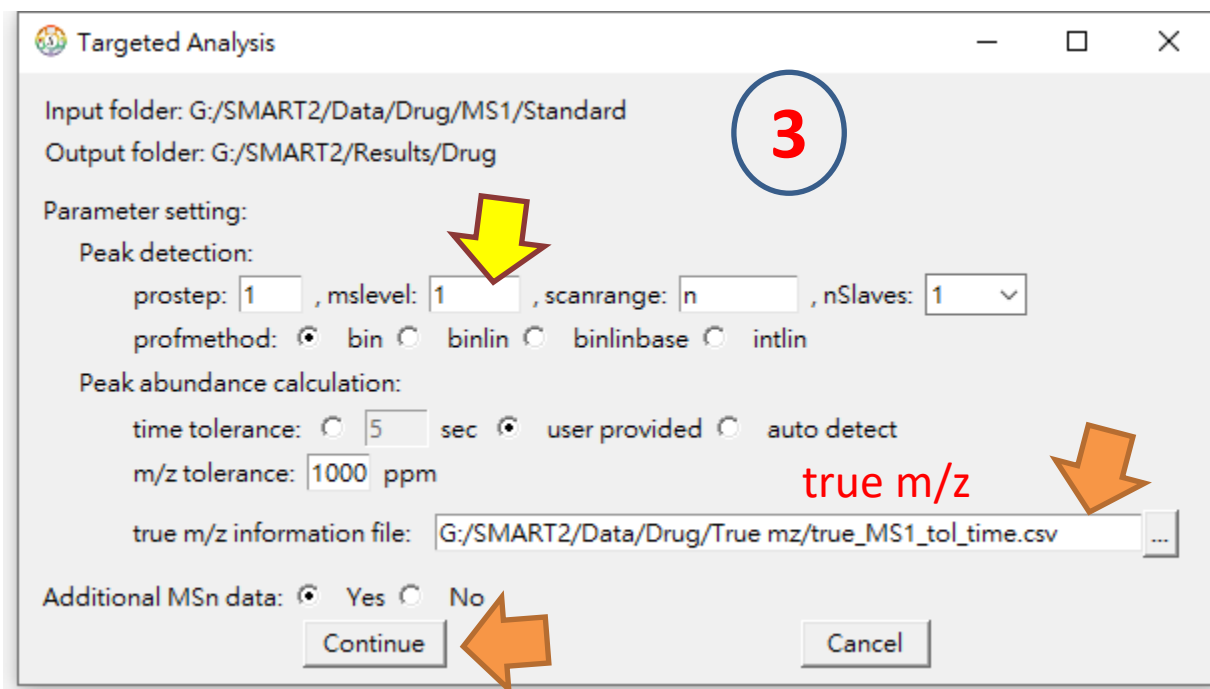
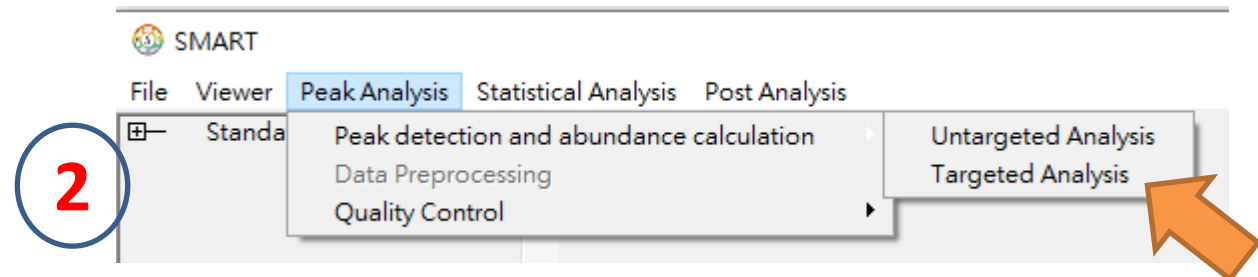
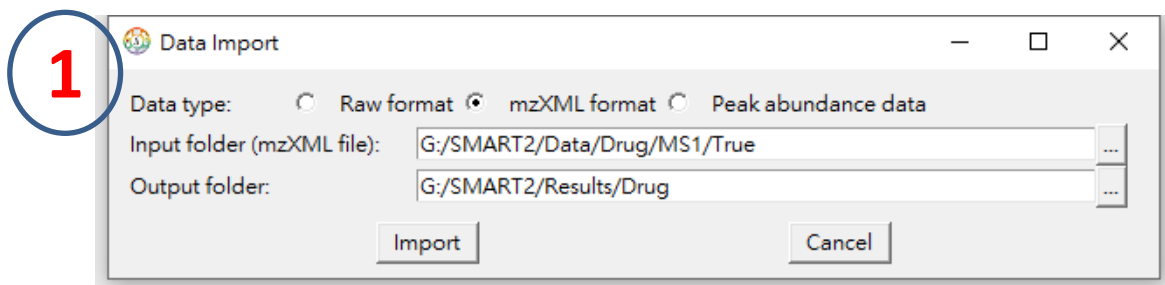
MS2

targeted_MS2_tol_time.csv

- Targeted analysis

- Step 1: import mzXML files & correct m/z
- Step 2: peak detection & abundance calculation

Peak analysis (targeted)



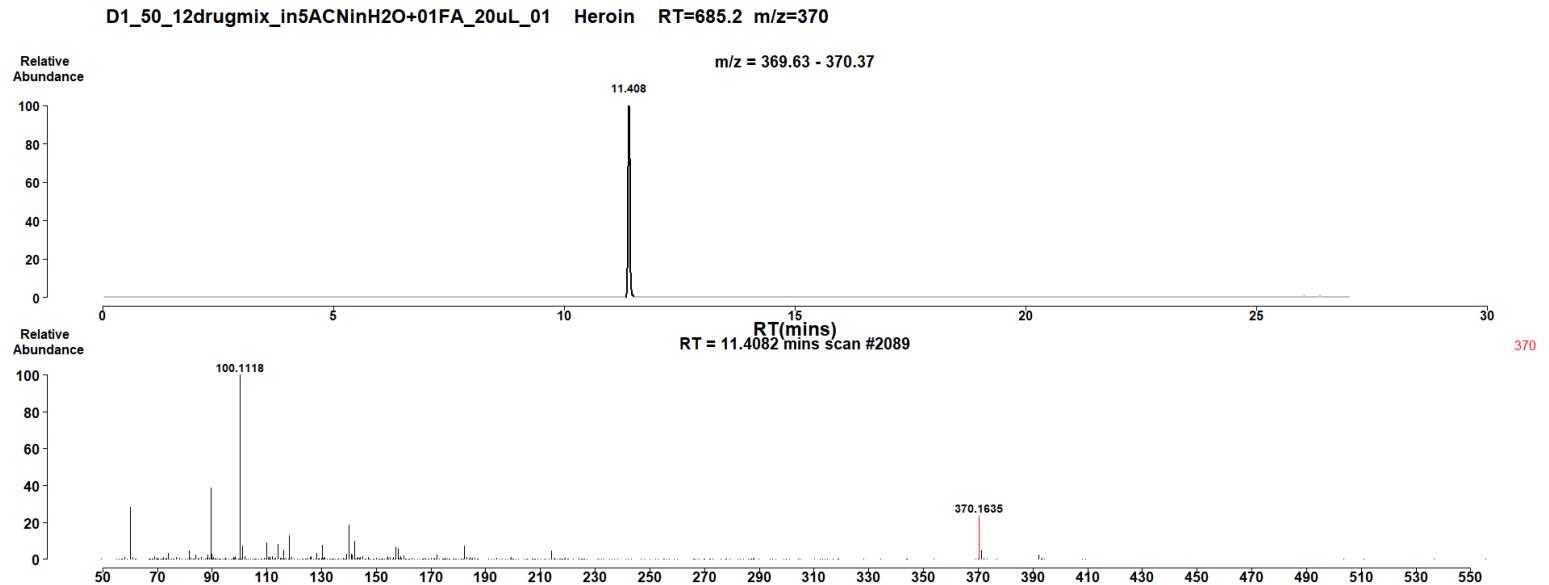
Peak analysis (targeted)

- Step 3. output file (peak abundance table; MS, MSMS spectrum)

outpath\Peak Analysis (Targeted)\S11_peak_abundance_MS.csv

D1_50_MS1
D1_50_MSMS
D2_100_MS1
D2_100_MSMS
D3_200_MS1
D3_200_MSMS
D4_300_MS1
D4_300_MSMS
D5_400_MS1
D5_400_MSMS
D6_500_MS1
D6_500_MSMS
D7_600_MS1
D7_600_MSMS
D8_700_MS1
D8_700_MSMS
D9_800_MS1
D9_800_MSMS
D10_900_MS1
D10_900_MSMS
D11_1000_MS1
D11_1000_MSMS

S11_mz_MS1.csv
S11_mz_MS2.csv
S11_peak_abundance_MS1.csv
S11_peak_abundance_MS1_forSMART...
S11_peak_abundance_MS2.csv
S11_peak_abundance_MS2_forSMART...
S11_relative_abundance_MS2.csv
S11_retention_time_MS1.csv
S11_retention_time_MS2.csv
S11_sn_MS1.csv
S11_sn_MS2.csv



Peak analysis (targeted)

Output file (Peak abundance table of all samples)

Peak abundance (MS1)

Peak_Index	Name	mz	Ret_Time .sec	Ret_Time _est.sec	mz_est	D1_50_MS 1	D10_900_ MS1	D11_1000_ MS1	D2_100_M S1	D3_200_M S1	D4_300_M S1	D5_400_M S1	D6_500_M S1	D7_600_M S1	D8_700_M S1	D9_800_M S1
1	Heroin	370	685.2	685.3724	370.1643	20454233	4.85E+08	5.37E+08	33246830	65461545	1.08E+08	1.12E+08	2.2E+08	2.18E+08	3.07E+08	3.79E+08
2	Morphine	286	147	146.2696	286.1431	41215408	5.53E+08	6.23E+08	55632394	1.11E+08	1.59E+08	1.66E+08	2.97E+08	3.05E+08	4.01E+08	4.69E+08
3	Cocaine	304	702	701.136	304.1538	67391965	1.91E+09	2.16E+09	1.09E+08	2.8E+08	4.54E+08	4.79E+08	9.74E+08	9.95E+08	1.32E+09	1.57E+09
4	Thebaine	312	678.6	678.8176	312.1588	13918057	6.54E+08	7.9E+08	22156818	57057964	1.12E+08	1.14E+08	2.89E+08	3.04E+08	4.41E+08	5.71E+08
5	delta-9 THC	315	1513.8	1513.319	315.2302	584689	12324894	10750614	1040859	3017275	4419165	4255491	6385888	8052278	9572914	11385840
6	Amphetamine	136	422.4	419.1944	136.0804	28233349	38237498	37707935	30552141	36745807	37802678	38425018	38130787	39861533	37695091	38418264
7	MA	150	495.6	496.8365	150.1021	88366857	4.84E+08	5.19E+08	1.14E+08	1.84E+08	2.48E+08	2.57E+08	3.68E+08	3.78E+08	4.43E+08	4.61E+08
8	MDMA	194	539.4	538.9991	194.1173	57005641	1.36E+09	1.61E+09	77917415	1.73E+08	3.32E+08	3.11E+08	6.96E+08	7.22E+08	9.57E+08	1.14E+09
9	Love Drug	180	506.4	505.6407	180.0866	2977060	32927321	40623589	3508615	5215888	7552811	8060482	14862760	15982588	21387478	24410233
10	Ketamine	238	600	599.9313	238.0988	65361457	1.9E+09	2.12E+09	1.1E+08	3.04E+08	5.13E+08	5.38E+08	1.07E+09	1.13E+09	1.41E+09	1.64E+09
11	FM2	314	1044	1044.274	314.093	23235201	4.47E+08	5.37E+08	37148568	83573478	1.28E+08	1.31E+08	2.39E+08	2.43E+08	3.1E+08	3.84E+08
12	Nimetazepam	296	1041	1040.522	296.1024	27111684	5.03E+08	5.88E+08	41037216	91247724	1.44E+08	1.48E+08	2.57E+08	2.67E+08	3.45E+08	4.14E+08

Peak analysis (targeted)

Output file (Peak abundance table of all samples)

Peak abundance (MS2)

Peak_Index	Name	parent_ion	mz	Ret_Time.sec	Ret_Time_est.sec	mz_est	D1_50_MS S	D10_900_MS MS	D11_1000_M SMS	D2_100_MS MS	D3_200_MS MS	D4_300_MS MS	D5_400_MS MS	D6_500_MS MS	D7_600_MS MS	D8_700_MS MS	D9_800_MS MS
1	Heroin	370	370	NA	685.5322	370.1636	34936037	4.31E+08	4.74E+08	45887062	80773701	1.19E+08	1.85E+08	2.31E+08	2.75E+08	2.99E+08	3.65E+08
2	Heroin	370	328	NA	685.5322	328.1531	5994749	79250544	88352065	8205245	14903335	22352355	34701357	40951023	49833482	55034774	67134243
3	Heroin	370	268	NA	685.5322	268.1322	5966182	80881111	96696971	8852504	15962231	23508212	36036774	44988809	54698078	57847453	70564375
4	Heroin	370	211	NA	685.5322	211.0747	3361656	46230644	54063830	4770158	8791204	12473739	20439931	25000126	31336412	33862691	40365021
5	Heroin	370	193	NA	685.5322	193.0641	1510510	20921156	23617575	2064699	3996665	5545420	8870916	9727212	12878930	14027716	17201483
6	Heroin	370	58	NA	686.438	57.99157	NA	258.2116	NA	273.661	NA	NA	638.7396	NA	NA	NA	NA
7	Morphine	286	286	NA	145.5	286.143	16131323	2.1E+08	2.22E+08	34800087	63534132	84246919	1.1E+08	1.36E+08	1.46E+08	1.61E+08	1.97E+08
8	Morphine	286	268	NA	145.5	268.1305	430782.9	6575950	6785043	969792.4	1816443	2392807	3283501	4052850	4471966	4903943	6066029
9	Morphine	286	229	NA	145.5	229.0854	1381069	19277741	20679948	3056907	5480279	7688644	10079360	11702549	13521425	14964256	17815511
10	Morphine	286	201	NA	145.5	201.0879	1967459	28574132	28953802	4338963	8250409	11269557	14353928	17231385	19143587	21127442	25307275
11	Cocaine	304	304	NA	700.1591	304.1536	1869106	18330609	20144319	4651245	7718511	9179664	12901861	13487416	15494409	16525349	17790183
12	Cocaine	304	182	NA	699.8602	182.117	43752791	5.02E+08	5.37E+08	1.18E+08	1.99E+08	2.42E+08	3.23E+08	3.65E+08	4.08E+08	4.44E+08	4.85E+08
13	Cocaine	304	150	NA	699.8602	150.091	4216007	54187027	58594892	12328872	21144421	26080012	33884162	37684106	42701985	46630588	51139926
14	Cocaine	304	105	NA	699.8602	105.0332	3613007	50772687	56131246	10074624	17121746	21047970	29647120	34590745	35860013	40174301	46894619
15	Cocaine	304	82	NA	699.8602	82.0649	5027314	69164979	78411520	14017254	23880243	29053601	40693701	46962217	51254207	56796007	64853128
16	Thebaine	312	312	NA	678.0616	312.1517	1095431	9256829	11341677	1766889	3150661	3824506	5264614	6189062	7429242	7942980	8623290
17	Thebaine	312	281	NA	678.0616	281.1163	534162.5	4365164	4921961	804912.5	1398028	1823320	2546612	2982317	3328803	3572010	3994250
18	Thebaine	312	266	NA	678.0616	266.1032	1866305	16921521	19156966	3263283	5384742	6937593	9418883	11512846	12833181	13809395	15751537
19	Thebaine	312	251	NA	678.0616	251.0695	3056593	26949412	31532800	5253820	8837063	11769548	15247149	18110674	21036849	23342167	25431602
20	Thebaine	312	221	NA	678.0616	221.0838	1812587	17278121	19202136	3174271	5577776	7159779	9476728	11502196	12895684	14336249	15762883
21	Thebaine	312	58	NA	678.7908	58.04143	NA	5888.264	7090.007	4134.432	286.0357	4603.693	14751.52	6566.844	12127.54	11897.7	7630.39

Peak analysis (targeted)

Output file

Relative abundance (MS2)

Peak_Index	Name	parent_ion	mz	Ret_Time.sec	Ret_Time_est.sec	mz_est	D1_50_MS MS	D10_900_ MSMS	D11_1000_ MSMS	D2_100_M SMS	D3_200_M SMS	D4_300_M SMS	D5_400_M SMS	D6_500_M SMS	D7_600_M SMS	D8_700_M SMS	D9_800_M SMS
1	Heroin	370	370	NA	685.5322	370.1636	100	100	100	100	100	100	100	100	100	100	100
2	Heroin	370	328	NA	685.5322	328.1531	17.15921	18.39443	18.6417	17.88139	18.45073	18.73004	18.74169	17.76055	18.09249	18.37667	18.39403
3	Heroin	370	268	NA	685.5322	268.1322	17.07744	18.77289	20.40242	19.29194	19.76167	19.69859	19.46293	19.51175	19.85863	19.31585	19.33385
4	Heroin	370	211	NA	685.5322	211.0747	9.622315	10.73035	11.40711	10.39543	10.88375	10.45231	11.0393	10.84261	11.37696	11.3071	11.05956
5	Heroin	370	193	NA	685.5322	193.0641	4.323645	4.855901	4.983152	4.499524	4.947978	4.646757	4.79105	4.218714	4.675811	4.683997	4.713014
6	Heroin	370	58	NA	686.438	57.99157	NA	5.99E-05	NA	0.000596	NA	NA	0.000345	NA	NA	NA	NA
7	Morphine	286	286	NA	145.5	286.143	100	100	100	100	100	100	100	100	100	100	100
8	Morphine	286	268	NA	145.5	268.1305	2.670475	3.125105	3.062768	2.786753	2.859004	2.840231	2.987035	2.972688	3.06945	3.042394	3.075344
9	Morphine	286	229	NA	145.5	229.0854	8.561415	9.161407	9.334928	8.784194	8.625725	9.126321	9.169298	8.583596	9.280783	9.283786	9.032074
10	Morphine	286	201	NA	145.5	201.0879	12.19651	13.57935	13.06975	12.46825	12.98579	13.37682	13.05792	12.63889	13.1397	13.10741	12.83023
11	Cocaine	304	304	NA	700.1591	304.1536	4.271969	3.654745	3.751795	3.939854	3.871665	3.786899	3.996785	3.692513	3.800112	3.720228	3.665711
12	Cocaine	304	182	NA	699.8602	182.117	100	100	100	100	100	100	100	100	100	100	100
13	Cocaine	304	150	NA	699.8602	150.091	9.635973	10.80377	10.91305	10.44322	10.60621	10.75882	10.49676	10.31695	10.47296	10.4976	10.53751
14	Cocaine	304	105	NA	699.8602	105.0332	8.257776	10.12303	10.45421	8.533747	8.588402	8.682946	9.184191	9.47007	8.794919	9.04414	9.662752
15	Cocaine	304	82	NA	699.8602	82.0649	11.49027	13.79007	14.60382	11.87337	11.97852	11.98552	12.60624	12.85707	12.57045	12.78606	13.36315

Peak analysis (targeted)

Output file
m/z (MS1)

Peak_In dex	Name	mz	Ret_Time.s ec	Ret_Time_est. sec	mz_est	D1_50_MS 1	D10_900_ MS1	D11_1000_ MS1	D2_100_M S1	D3_200_M S1	D4_300_M S1	D5_400_M S1	D6_500_M S1	D7_600_M S1	D8_700_M S1	D9_800_M S1
1	Heroin	370	685.2	685.3724	370.1643	370.1641	370.1642	370.1642	370.1643	370.1644	370.1643	370.1643	370.1644	370.1643	370.1642	370.1643
2	Morphine	286	147	146.2696	286.1431	286.1432	286.1431	286.143	286.1432	286.1432	286.1432	286.1432	286.1432	286.1431	286.1431	286.1431
3	Cocaine	304	702	701.136	304.1538	304.1537	304.1538	304.1538	304.1539	304.154	304.1538	304.1537	304.1539	304.1538	304.1539	304.1538
4	Thebaine	312	678.6	678.8176	312.1588	312.1588	312.1587	312.1586	312.1588	312.1588	312.1588	312.1588	312.1588	312.1588	312.1587	312.1587
5	delta-9 THC	315	1513.8	1513.319	315.2302	315.2193	315.2314	315.2317	315.2313	315.2314	315.2313	315.2312	315.2313	315.2311	315.2312	315.2313
6	Amphetamine	136	422.4	419.1944	136.0804	136.0589	136.0881	136.0891	136.0615	136.0743	136.0787	136.0822	136.0876	136.0871	136.088	136.0886
7	MA	150	495.6	496.8365	150.1021	150.0957	150.1096	150.1148	150.0878	150.0931	150.0988	150.0962	150.1067	150.0988	150.1092	150.1121
8	MDMA	194	539.4	538.9991	194.1173	194.1173	194.1172	194.1172	194.1171	194.1173	194.1173	194.1173	194.1173	194.1173	194.1173	194.1172
9	Love Drug	180	506.4	505.6407	180.0866	180.0712	180.0972	180.101	180.0718	180.084	180.0921	180.0782	180.0869	180.0861	180.0898	180.094
10	Ketamine	238	600	599.9313	238.0988	238.0988	238.099	238.0989	238.0988	238.0988	238.0988	238.0988	238.0988	238.0988	238.0988	238.099
11	FM2	314	1044	1044.274	314.093	314.0929	314.093	314.093	314.0932	314.0931	314.093	314.0931	314.0929	314.093	314.093	314.093
12	Nimetazepam	296	1041	1040.522	296.1024	296.1024	296.1025	296.1025	296.1024	296.1024	296.1024	296.1025	296.1022	296.1024	296.1023	296.1026

Peak analysis (targeted)

Output file
m/z (MS2)

Peak_Index	Name	parent_ion	mz	Ret_Time.sec	Ret_Time_est.sec	mz_est	D1_50_MS MS	D10_900_ MSMS	D11_1000_ MSMS	D2_100_M SMS	D3_200_M SMS	D4_300_M SMS	D5_400_M SMS	D6_500_M SMS	D7_600_M SMS	D8_700_M SMS	D9_800_M SMS
1	Heroin	370	370 NA		685.5322	370.1636	370.1635	370.1637	370.1635	370.1635	370.1635	370.1634	370.1635	370.1636	370.1638	370.1635	370.1636
2	Heroin	370	328 NA		685.5322	328.1531	328.1533	328.1531	328.1528	328.1533	328.1532	328.1531	328.1531	328.153	328.1531	328.1531	328.153
3	Heroin	370	268 NA		685.5322	268.1322	268.1324	268.1322	268.132	268.1324	268.1323	268.1323	268.1322	268.1322	268.1322	268.1321	268.1322
4	Heroin	370	211 NA		685.5322	211.0747	211.0746	211.0746	211.0746	211.0745	211.0747	211.0748	211.0748	211.0748	211.0746	211.0746	211.0746
5	Heroin	370	193 NA		685.5322	193.0641	193.0642	193.0642	193.064	193.0641	193.0642	193.0641	193.0641	193.0641	193.0641	193.0641	193.0642
6	Heroin	370	58 NA		686.438	57.99157 NA		57.9707 NA		57.9983 NA		NA	58.0057 NA		NA	NA	NA
7	Morphine	286	286 NA		145.5	286.143	286.1429	286.1428	286.1428	286.1431	286.143	286.1431	286.1431	286.143	286.1431	286.1429	286.1429
8	Morphine	286	268 NA		145.5	268.1305	268.1101	268.1326	268.1325	268.1327	268.1326	268.1326	268.1326	268.1325	268.1326	268.1325	268.1325
9	Morphine	286	229 NA		145.5	229.0854	229.0853	229.0854	229.0854	229.0854	229.0854	229.0854	229.0854	229.0854	229.0855	229.0854	229.0854
10	Morphine	286	201 NA		145.5	201.0879	201.0616	201.0905	201.0905	201.0905	201.0905	201.0904	201.0905	201.0905	201.0906	201.0906	201.0904
11	Cocaine	304	304 NA		700.1591	304.1536	304.1536	304.1536	304.1536	304.1537	304.1537	304.1534	304.1538	304.1538	304.1537	304.1537	304.1535
12	Cocaine	304	182 NA		699.8602	182.117	182.1171	182.117	182.117	182.117	182.117	182.1169	182.117	182.117	182.1171	182.117	182.1171
13	Cocaine	304	150 NA		699.8602	150.091	150.0909	150.091	150.0909	150.091	150.091	150.0909	150.091	150.091	150.091	150.0909	150.0909
14	Cocaine	304	105 NA		699.8602	105.0332	105.0332	105.0332	105.0332	105.0332	105.0332	105.0332	105.0333	105.0332	105.0332	105.0332	105.0332
15	Cocaine	304	82 NA		699.8602	82.0649	82.0649	82.0649	82.0649	82.0649	82.0649	82.0649	82.0649	82.0649	82.0649	82.0649	82.0649

Peak analysis (targeted)

Output file
RT (MS1)

Peak_Index	Name	mz	Ret_Time.sec	Ret_Time_est.sec	mz_est	D1_50_MS 1	D10_900_ MS1	D11_1000_ MS1	D2_100_M S1	D3_200_M S1	D4_300_M S1	D5_400_M S1	D6_500_M S1	D7_600_M S1	D8_700_M S1	D9_800_M S1
1	Heroin	370	685.2	685.3724	370.1643	684.492	685.62	685.626	685.392	684.96	685.428	685.062	685.68	685.632	685.89	685.314
2	Morphine	286	147	146.2696	286.1431	144.954	146.202	147.018	145.902	146.196	146.196	146.202	146.154	146.796	146.85	146.496
3	Cocaine	304	702	701.136	304.1538	699.912	701.256	701.226	700.8	701.274	701.034	701.286	701.43	701.394	701.586	701.298
4	Thebaine	312	678.6	678.8176	312.1588	678.264	678.828	678.858	678.504	678.786	678.672	678.948	678.744	679.002	679.314	679.074
5	delta-9 THC	315	1513.8	1513.319	315.2302	1513.5	1513.308	1513.782	1512.63	1513.332	1512.768	1513.47	1513.41	1513.632	1513.422	1513.26
6	Amphetamine	136	422.4	419.1944	136.094	421.452	415.902	414.804	423.384	420.396	418.092	417.762	416.316	430.752	416.67	415.608
7	MA	150	495.6	496.8365	150.1021	495.9	496.668	496.824	496.854	496.818	496.458	496.794	495.954	498.144	497.148	497.64
8	MDMA	194	539.4	538.9991	194.1173	538.212	539.214	539.298	538.506	538.752	538.824	539.424	539.172	539.496	538.992	539.1
9	Love Drug	180	506.4	505.6407	180.0866	504.756	505.356	506.364	504.726	505.014	505.836	506.154	505.44	506.694	505.932	505.776
10	Ketamine	238	600	599.9313	238.0988	599.22	599.994	600.06	599.826	599.922	599.88	600.156	599.796	600.072	600.12	600.198
11	FM2	314	1044	1044.274	314.093	1044.162	1044.828	1044.162	1043.718	1044.252	1044.438	1043.772	1044.33	1044.582	1044.99	1043.778
12	Nimetazepam	296	1041	1040.522	296.1024	1039.692	1041.312	1040.952	1040.52	1040.508	1040.46	1040.1	1040.442	1040.688	1040.538	1040.532

Peak analysis (targeted)

Output file
RT (MS2)

Peak_Index	Name	parent_ion	mz	Ret_Time.sec	Ret_Time_est.sec	mz_est	D1_50_MS MS	D10_900_ MSMS	D11_1000_ MSMS	D2_100_M SMS	D3_200_M SMS	D4_300_M SMS	D5_400_M SMS	D6_500_M SMS	D7_600_M SMS	D8_700_M SMS	D9_800_M SMS
1	Heroin	370	370	NA	685.5322	370.1636	683.292	686.46	686.472	683.16	683.1	686.37	686.376	686.4	686.376	686.424	686.424
2	Heroin	370	328	NA	685.5322	328.1531	683.292	686.46	686.472	683.16	683.1	686.37	686.376	686.4	686.376	686.424	686.424
3	Heroin	370	268	NA	685.5322	268.1322	683.292	686.46	686.472	683.16	683.1	686.37	686.376	686.4	686.376	686.424	686.424
4	Heroin	370	211	NA	685.5322	211.0747	683.292	686.46	686.472	683.16	683.1	686.37	686.376	686.4	686.376	686.424	686.424
5	Heroin	370	193	NA	685.5322	193.0641	683.292	686.46	686.472	683.16	683.1	686.37	686.376	686.4	686.376	686.424	686.424
6	Heroin	370	58	NA	686.438	57.99157	NA	679.944	NA	689.724	NA	NA	689.646	NA	NA	NA	NA
7	Morphine	286	286	NA	145.5	286.143	145.524	145.518	145.494	145.47	145.5	145.488	145.482	145.5	145.5	145.524	145.5
8	Morphine	286	268	NA	145.5	268.1305	145.524	145.518	145.494	145.47	145.5	145.488	145.482	145.5	145.5	145.524	145.5
9	Morphine	286	229	NA	145.5	229.0854	145.524	145.518	145.494	145.47	145.5	145.488	145.482	145.5	145.5	145.524	145.5
10	Morphine	286	201	NA	145.5	201.0879	145.524	145.518	145.494	145.47	145.5	145.488	145.482	145.5	145.5	145.524	145.5
11	Cocaine	304	304	NA	700.1591	304.1536	700.038	699.876	703.164	699.906	699.816	699.816	699.828	699.84	699.792	699.84	699.834
12	Cocaine	304	182	NA	699.8602	182.117	700.038	699.876	699.876	699.906	699.816	699.816	699.828	699.84	699.792	699.84	699.834
13	Cocaine	304	150	NA	699.8602	150.091	700.038	699.876	699.876	699.906	699.816	699.816	699.828	699.84	699.792	699.84	699.834
14	Cocaine	304	105	NA	699.8602	105.0332	700.038	699.876	699.876	699.906	699.816	699.816	699.828	699.84	699.792	699.84	699.834
15	Cocaine	304	82	NA	699.8602	82.0649	700.038	699.876	699.876	699.906	699.816	699.816	699.828	699.84	699.792	699.84	699.834

Peak analysis (targeted)

Output file
S/N (MS1)

Peak_IndexName	mz	Ret_Time.sec	Ret_Time_ est.sec	mz_est	D1_50_MS 1	D10_900_ MS1	D11_1000_ MS1	D2_100_M S1	D3_200_M S1	D4_300_M S1	D5_400_M S1	D6_500_M S1	D7_600_M S1	D8_700_M S1	D9_800_M S1
1 Heroin	370	685.2	685.3724	370.1643	1.258	1.3451	1.3331	1.2637	1.2006	1.2379	1.2124	1.3227	1.2697	1.2882	1.3332
2 Morphine	286	147	146.2696	286.1431	0.8601	0.8815	0.8624	0.7993	0.7657	0.7593	0.7614	0.8325	0.821	0.889	0.8425
3 Cocaine	304	702	701.136	304.1538	1.0586	1.1744	1.1824	1.0686	1.1593	1.1368	1.1814	1.1737	1.1954	1.1696	1.1643
4 Thebaine	312	678.6	678.8176	312.1588	1.2042	1.3537	1.3344	1.1617	1.2331	1.3413	1.3679	1.4252	1.4004	1.35	1.39
5 delta-9 THC	315	1513.8	1513.319	315.2302	0.4827	0.7615	0.786	0.6074	0.7792	0.7682	0.7972	0.812	0.8056	0.7853	0.8336
6 Amphetamine	136	422.4	419.1944	136.0804	1.4295	0.8961	0.8624	1.4094	1.2505	1.1382	1.1193	0.9806	0.9936	0.9322	0.9072
7 MA	150	495.6	496.8365	150.1021	1.2172	1.0342	1.0247	1.1924	1.1465	1.1408	1.1538	1.1191	1.1219	1.0873	1.0896
8 MDMA	194	539.4	538.9991	194.1173	0.8665	1.0204	1.0133	0.8288	0.9271	1.0063	0.974	1.064	1.0822	1.0516	1.085
9 Love Drug	180	506.4	505.6407	180.0866	1.324	1.5555	1.5448	1.2935	1.303	1.3073	1.4168	1.4861	1.4673	1.7218	1.4975
10 Ketamine	238	600	599.9313	238.0988	1.1623	1.1566	1.1886	1.1839	1.259	1.2549	1.2607	1.2335	1.2798	1.2108	1.2105
11 FM2	314	1044	1044.274	314.093	0.9901	0.9353	0.9865	0.9695	0.9464	0.9406	0.9512	0.9684	0.9777	0.9584	0.9852
12 Nimetazepam	296	1041	1040.522	296.1024	0.9651	0.9587	0.9532	0.9341	0.9048	0.9264	0.9336	0.9345	0.9144	0.9565	0.9393

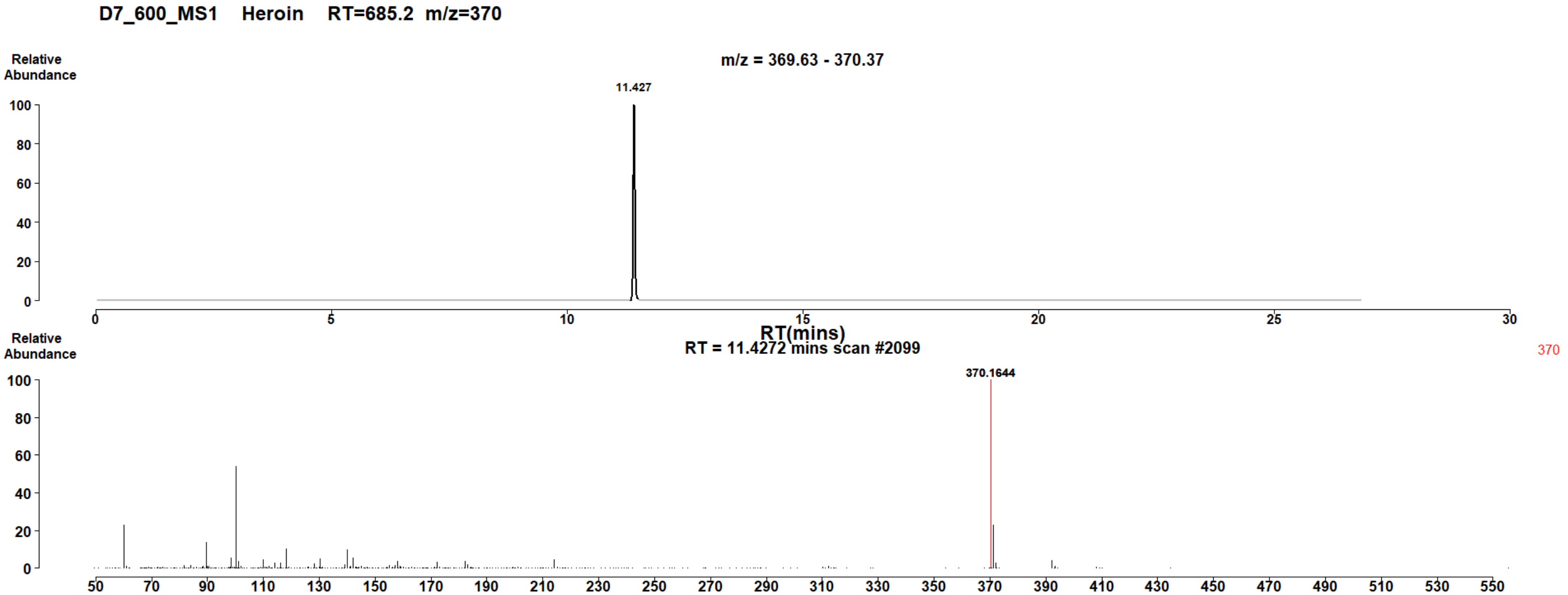
Peak analysis (targeted)

Output file
S/N (MS2)

Peak_Index	Name	parent_ion	mz	Ret_Time.sec	Ret_Time_est.sec	mz_est	D1_50_MS MS	D10_900_ MSMS	D11_1000_ MSMS	D2_100_M SMS	D3_200_M SMS	D4_300_M SMS	D5_400_M SMS	D6_500_M SMS	D7_600_M SMS	D8_700_M SMS	D9_800_M SMS
1	Heroin	370	370	NA	685.5322	370.1636	2.2754	2.1575	2.2095	1.7426	1.5182	1.5774	1.8792	1.919	1.9821	1.9042	2.0616
2	Heroin	370	328	NA	685.5322	328.1531	2.2624	2.1629	2.2075	1.7496	1.5132	1.5627	1.8573	1.9065	1.9546	1.8699	2.0653
3	Heroin	370	268	NA	685.5322	268.1322	2.2613	2.1554	2.2105	1.7726	1.5105	1.57	1.8721	1.9139	1.9731	1.8614	2.0704
4	Heroin	370	211	NA	685.5322	211.0747	2.2738	2.1484	2.2039	1.7586	1.5087	1.5658	1.8721	1.9124	1.9829	1.8725	2.0448
5	Heroin	370	193	NA	685.5322	193.0641	2.2736	2.1775	2.2153	1.7398	1.5218	1.5767	1.8779	1.8741	1.9691	1.8508	2.0683
6	Heroin	370	58	NA	686.438	57.99157	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7	Morphine	286	286	NA	145.5	286.143	1.5084	1.498	1.4622	1.6833	1.794	1.6962	1.7063	1.6977	1.6228	1.5853	1.5373
8	Morphine	286	268	NA	145.5	268.1305	1.4903	1.5157	1.4851	1.7056	1.8104	1.7309	1.7479	1.7242	1.6543	1.6184	1.5653
9	Morphine	286	229	NA	145.5	229.0854	1.5004	1.5458	1.506	1.7119	1.7769	1.7119	1.7187	1.6686	1.6353	1.6151	1.561
10	Morphine	286	201	NA	145.5	201.0879	1.48	1.5655	1.5007	1.7254	1.7964	1.7166	1.7216	1.6632	1.6224	1.5915	1.5471
11	Cocaine	304	304	NA	700.1591	304.1536	1.9155	1.4562	1.4427	2.0541	1.9777	1.8857	1.7429	1.6952	1.6246	1.5651	1.4984
12	Cocaine	304	182	NA	699.8602	182.117	1.8864	1.5056	1.4639	2.0907	2.0307	1.9291	1.8093	1.7527	1.6855	1.6817	1.5992
13	Cocaine	304	150	NA	699.8602	150.091	1.8077	1.5238	1.4723	2.0971	2.0433	1.9661	1.8037	1.7921	1.6852	1.6823	1.5917
14	Cocaine	304	105	NA	699.8602	105.0332	1.7233	1.6279	1.5504	2.1	2.0632	1.9639	1.8819	1.8348	1.7372	1.7087	1.6749
15	Cocaine	304	82	NA	699.8602	82.0649	1.7085	1.623	1.5539	2.1039	2.0543	1.97	1.8798	1.8226	1.7479	1.7198	1.6732

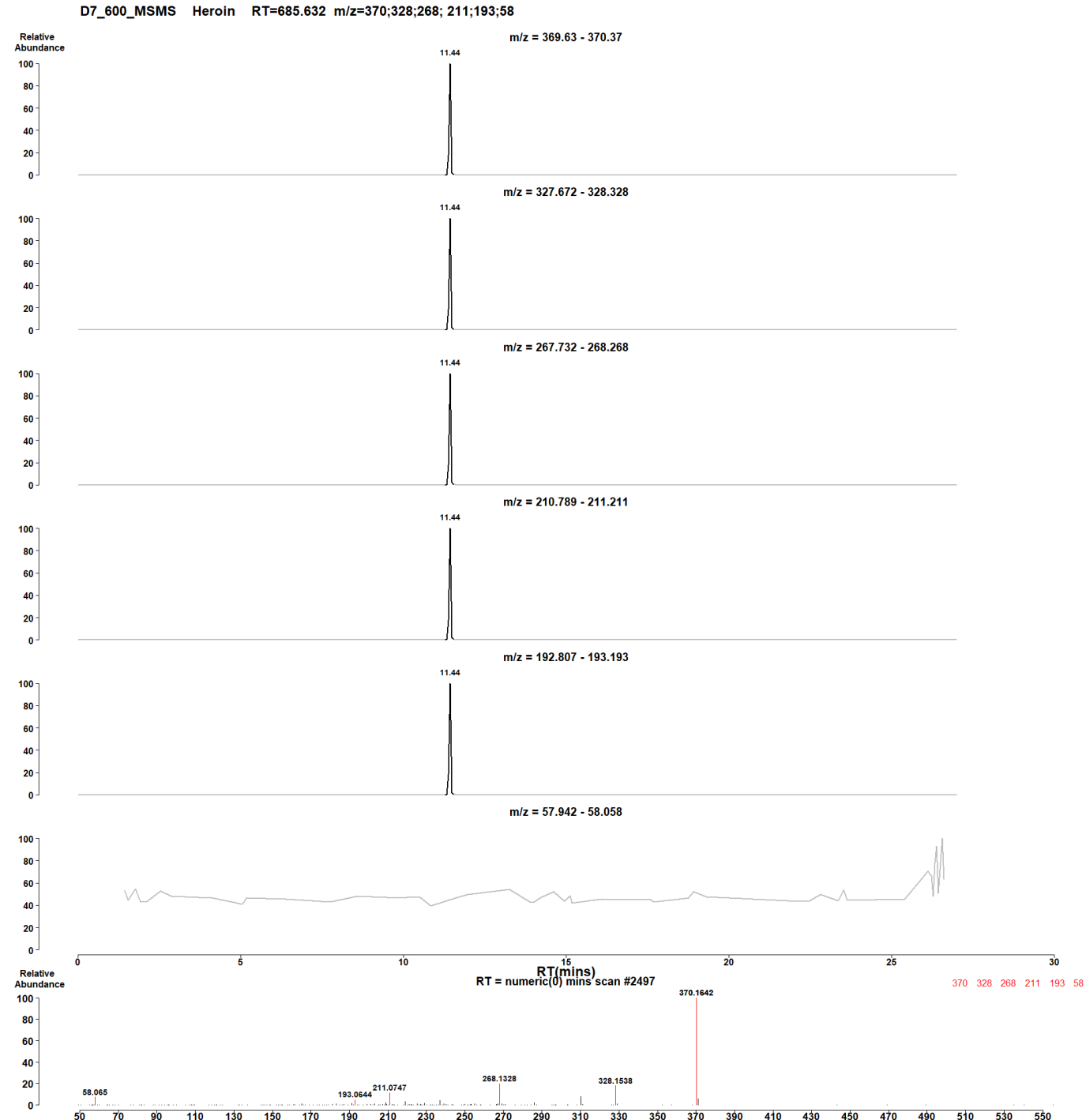
Peak analysis (targeted)

Figure (MS1)



Peak analysis (targeted)

Figure (MS2)



Auto detection

Targeted Analysis

Input folder: G:/SMART2/Data/Drug/MS1/Standard
Output folder: G:/SMART2/Results/Drug

Parameter setting:

Peak detection:
prostep: 1 , mslevel: 1 , scanrange: n , nSlaves: 1

Peak abundance calculation:
time tolerance: 5 sec user provided auto detect
m/z tolerance: 1000 ppm
true m/z information file: G:/SMART2/Data/Drug/True mz/true_MS1_wo_time.csv

Additional MSn data: Yes No

Continue Cancel

1	Name	mz	Ret_Time.sec
2	Heroin	370	NA
3	Morphine	286	NA
4	Cocaine	304	NA
5	Thebaine	312	NA
6	delta-9 THC	315	NA
7	Amphetamine	136	NA
8	MA	150	NA
9	MDMA	194	NA
10	Love Drug	180	NA
11	Ketamine	238	NA
12	FM2	314	NA
13	Nimetazepam	296	NA



true_MS1_wo_time.csv

Data Import - Additional MSn data

Input folder for Additional MSn (mzXML file): G:/SMART2/Data/Drug/MS2/Standard

Parameter setting:

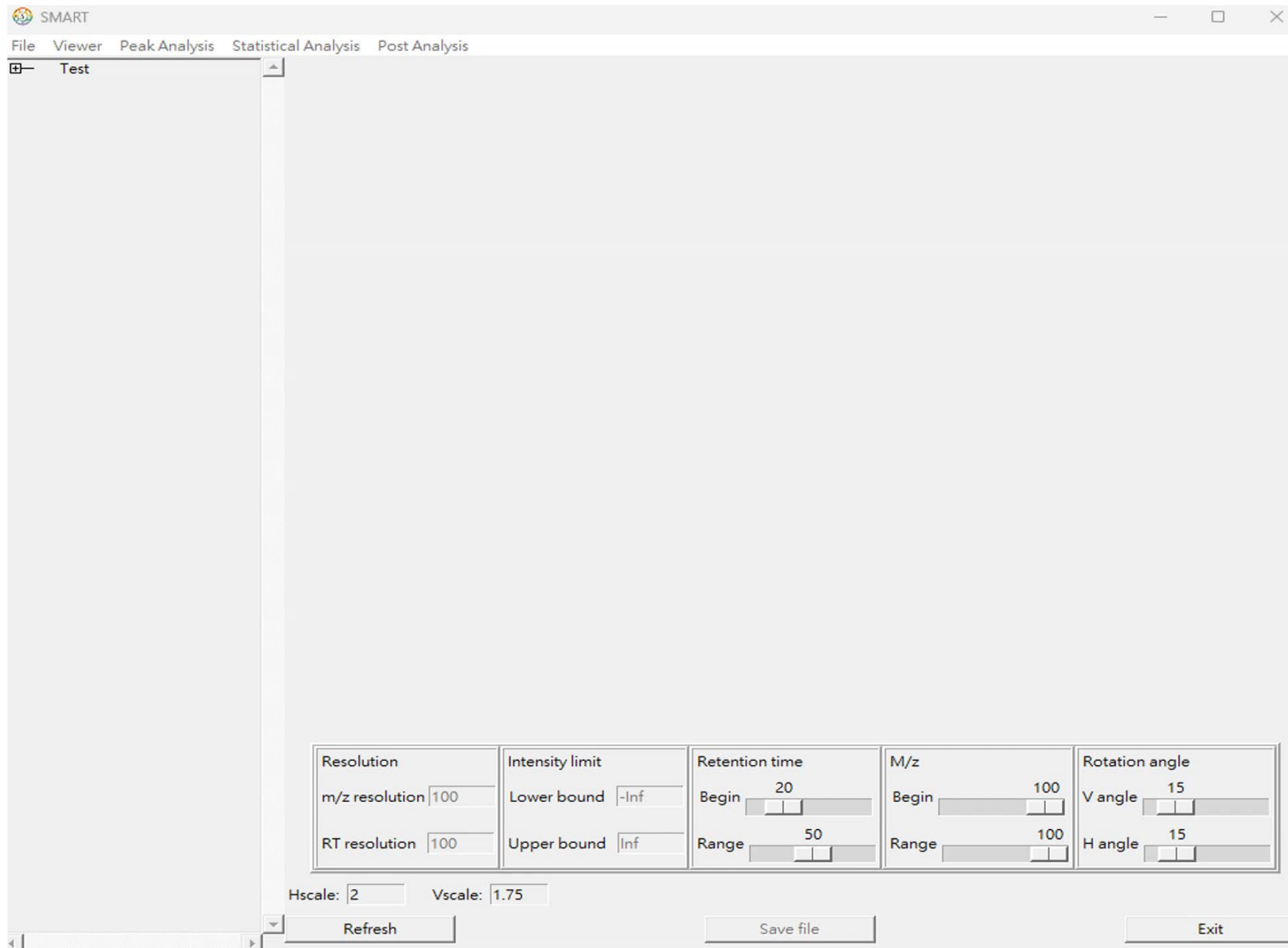
Peak detection:
prostep: 1 , mslevel: 2 , scanrange: n

Peak abundance calculation:
time tolerance: 5 sec user provided auto detect
m/z tolerance: 1000 ppm
true m/z information file: G:/SMART2/Data/Drug/True mz/true_MS2.csv

OK

true_MS2.csv

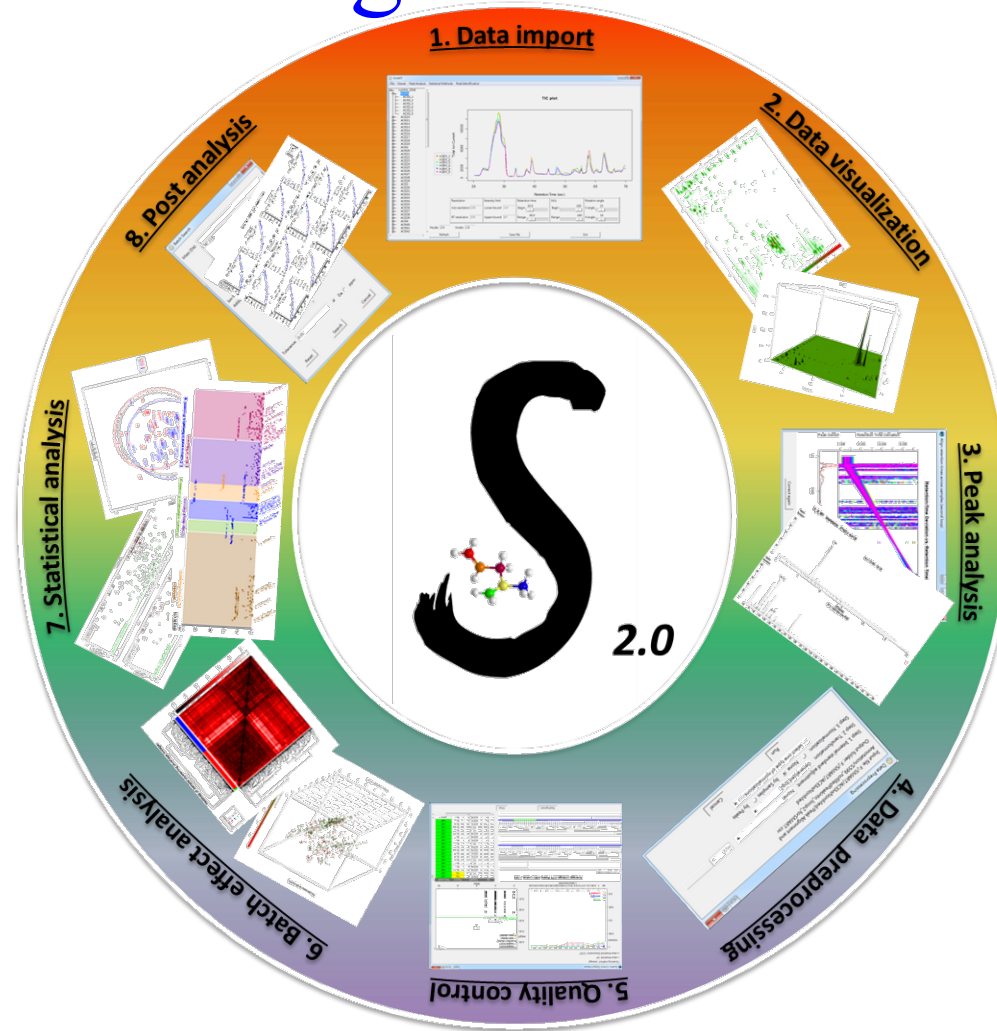
1	Name	parent_ion	daughter_ion
2	Heroin	370	370;328;268; 211;193;58
3	Morphine	286	286; 268; 229; 201
4	Cocaine	304	304; 182; 150; 105; 82
5	Thebaine	312	312; 281; 266; 251; 221; 58
6	delta-9 THC	315	315; 259; 193; 135; 123; 107; 93
7	Amphetamine	136	136; 119; 91
8	MA	150	150; 119; 91
9	MDMA	194	194; 163; 135; 133; 105
10	Love Drug	180	180; 163; 135; 133; 105
11	Ketamine	238	238; 220; 179; 163; 152; 125
12	FM2	314	314; 200; 286; 268
13	Nimetazepam	296	296; 268; 250; 222; 193; 165



Peak analysis (targeted)

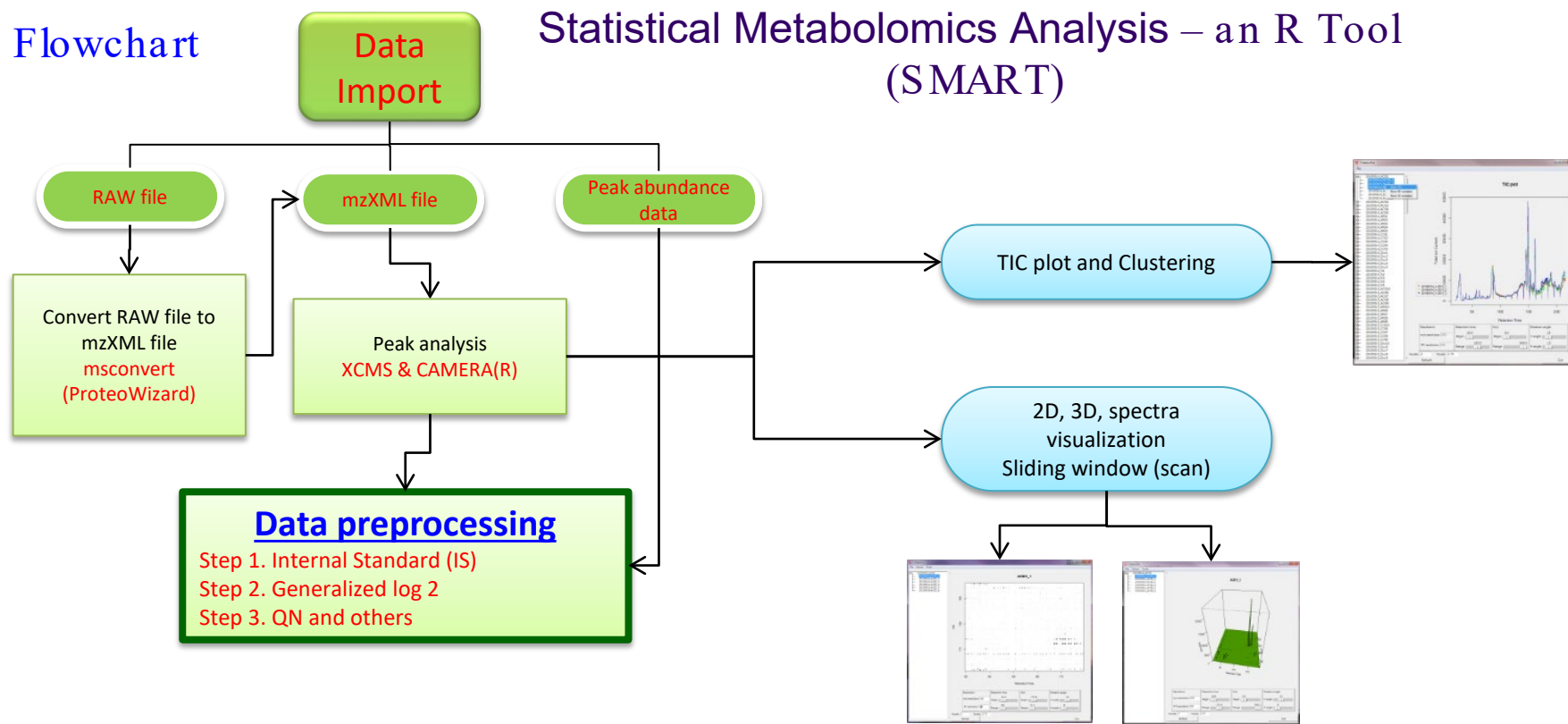
Statistical Metabolomics Analysis – an R Tool

4. Data preprocessing



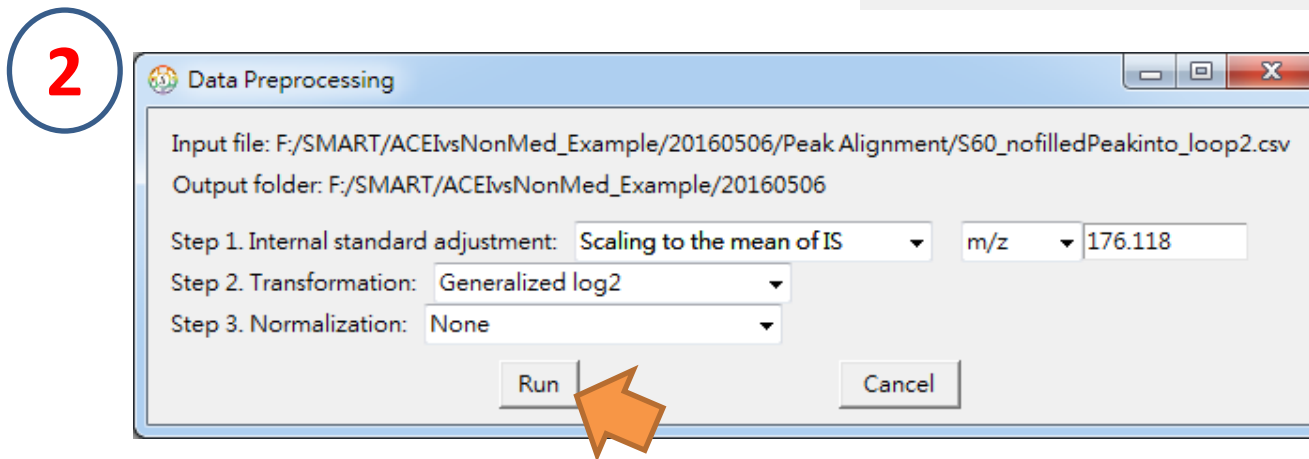
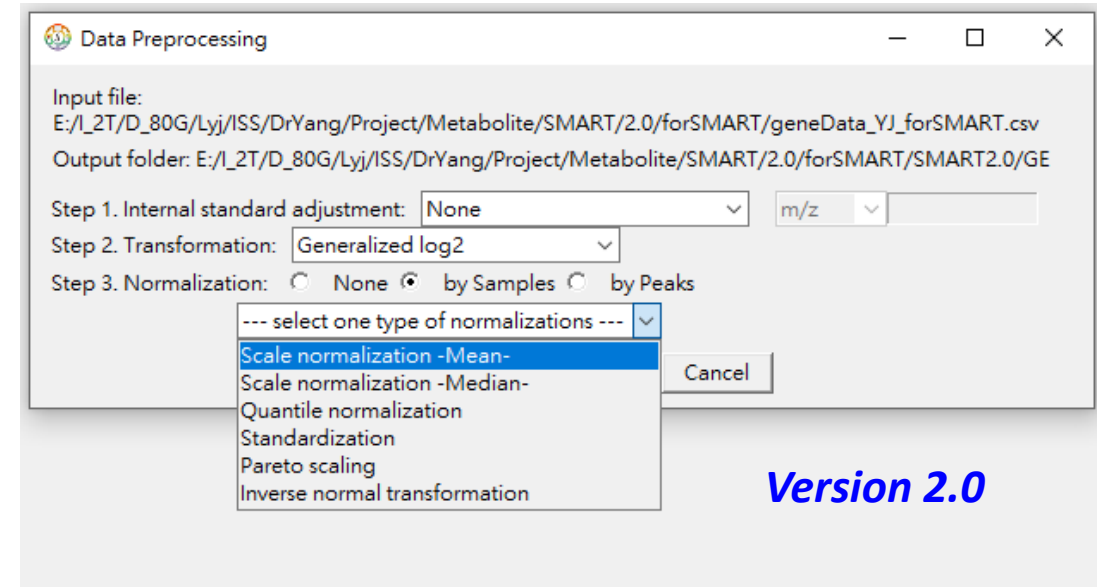
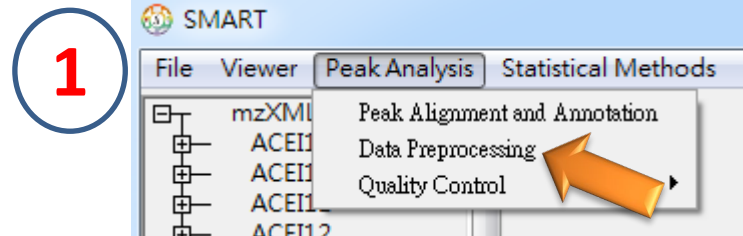
Flowchart

Statistical Metabolomics Analysis – an R Tool (SMART)



Statistical Metabolomics Analysis - an R Tool

- 4. Data preprocessing

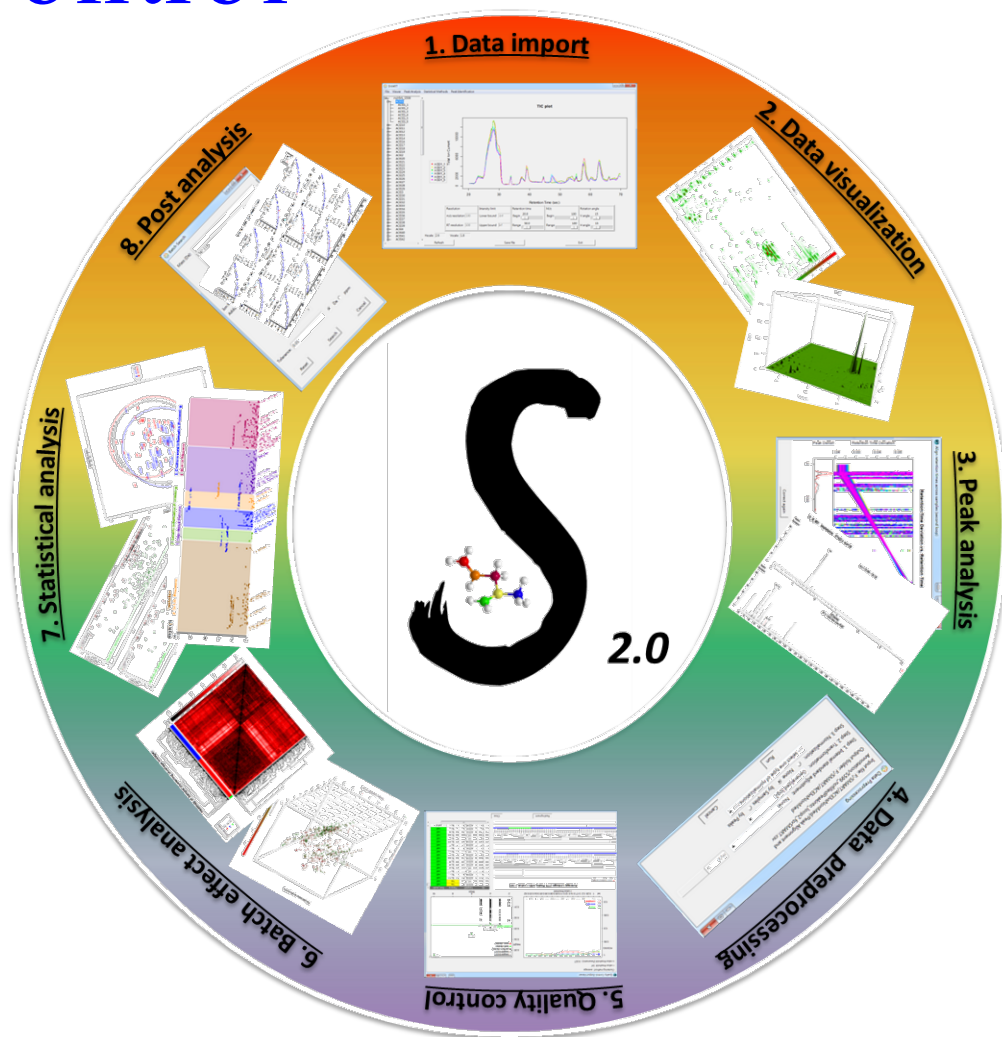




Data preprocessing

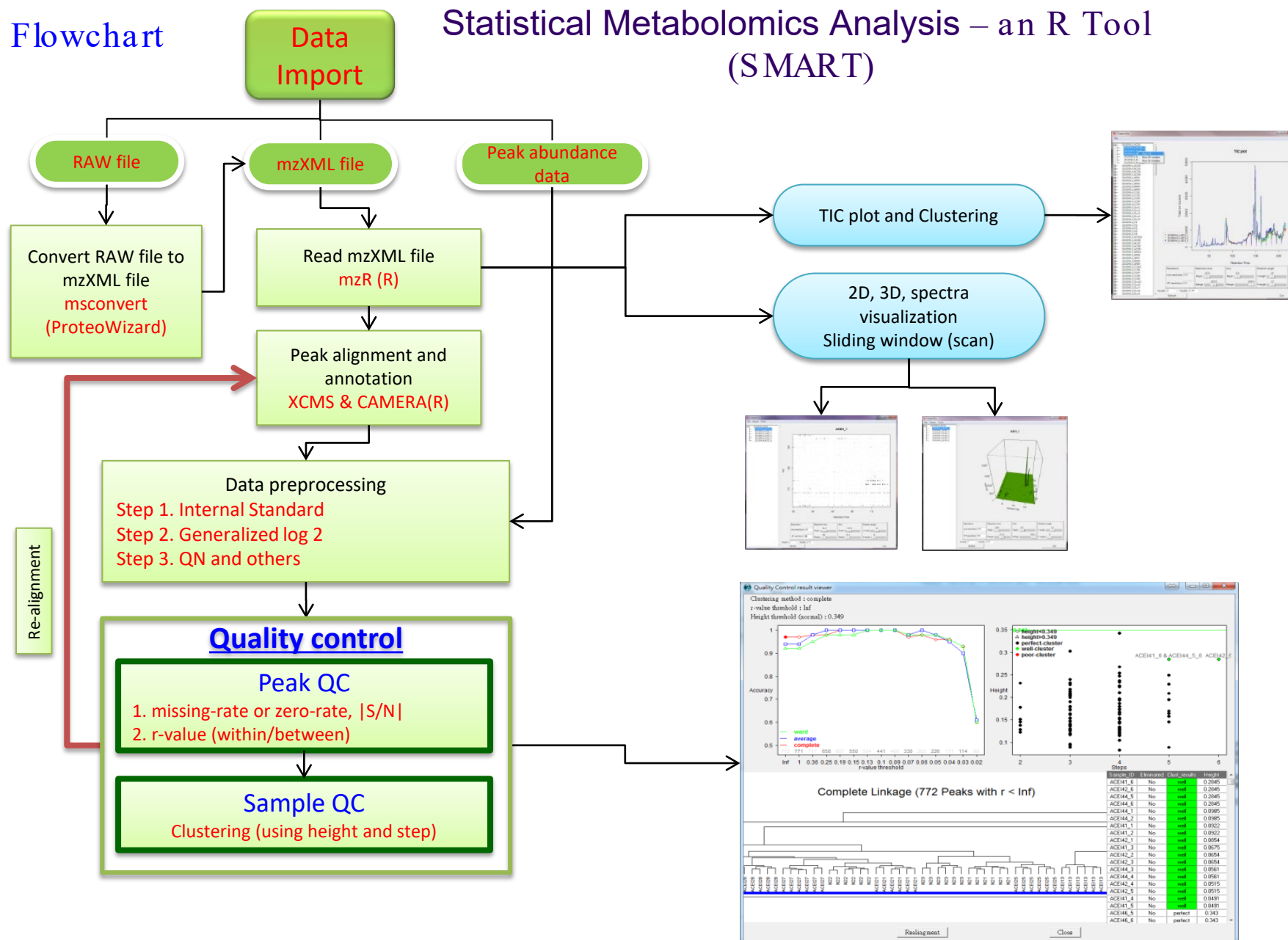
Statistical Metabolomics Analysis – an R Tool

5. Quality control

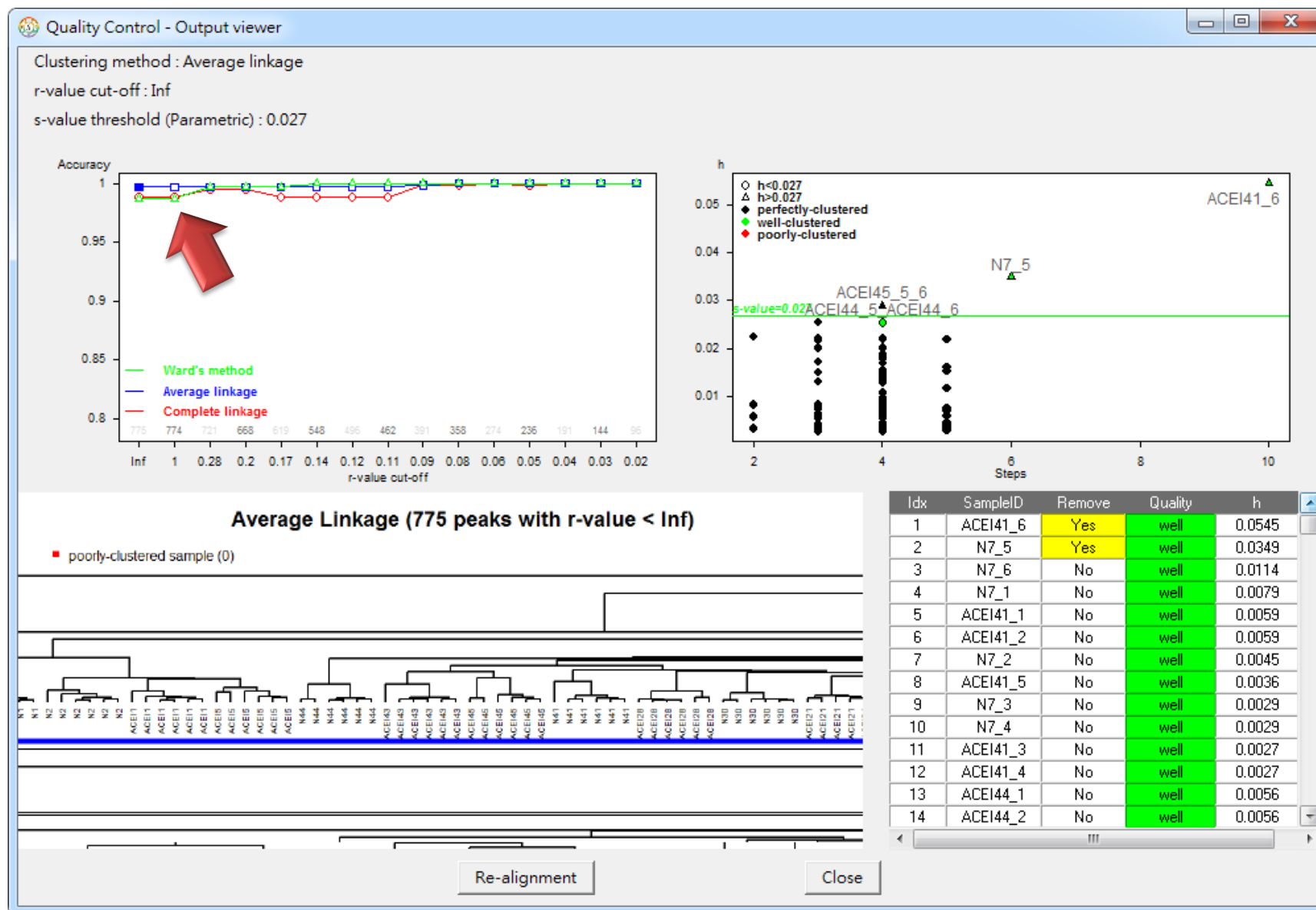
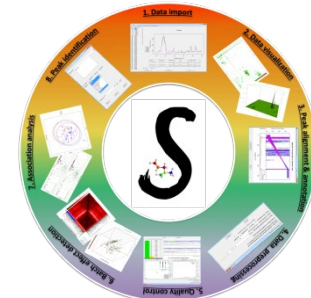


Flowchart

Statistical Metabolomics Analysis – an R Tool (SMART)



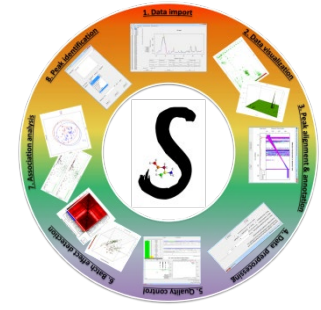
5. Quality control -- sample filtering (1)



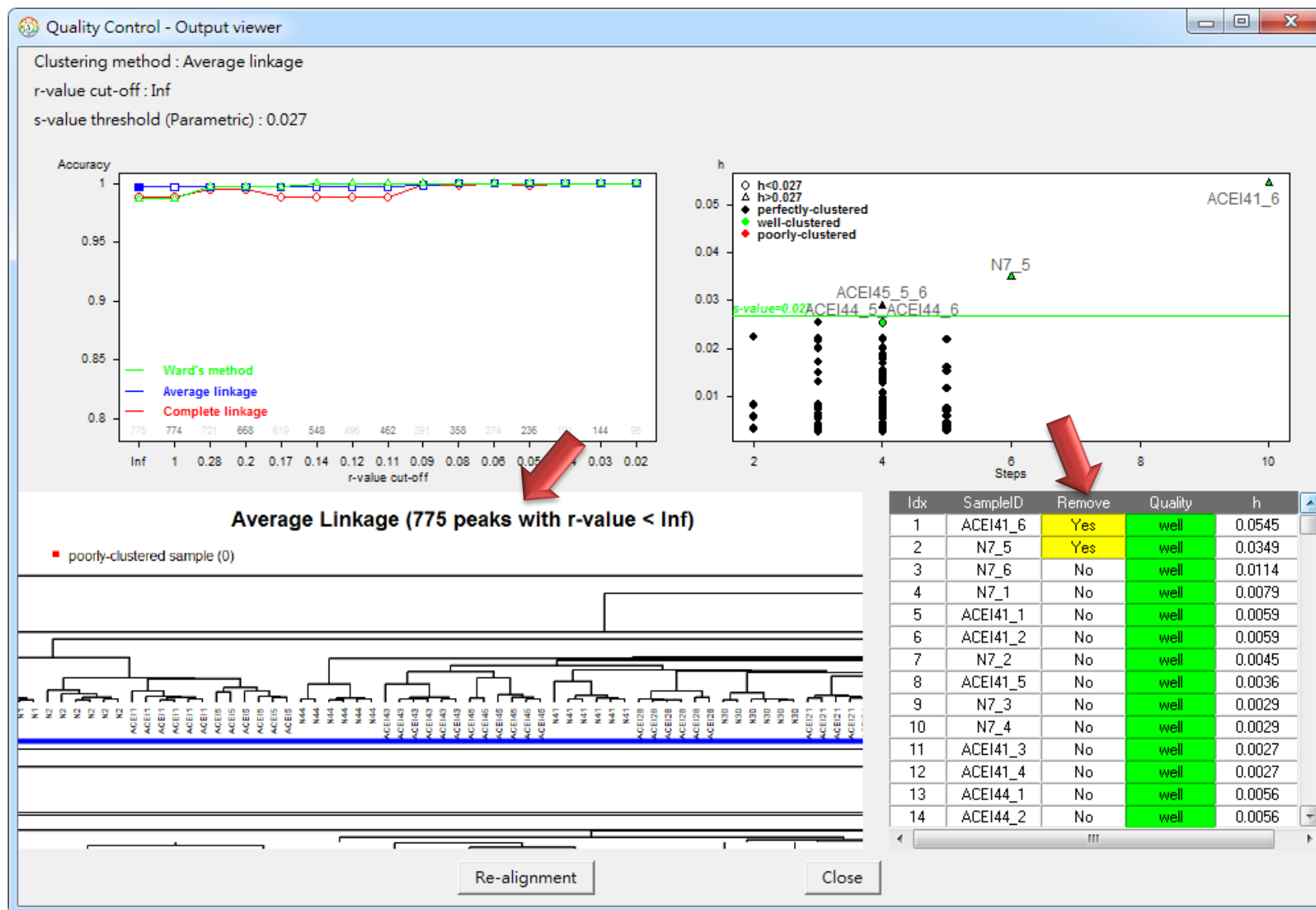
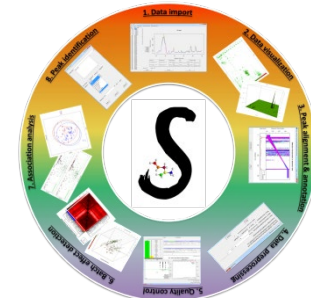
The diagram illustrates the 7 steps of the data science process in a circular flow:

- 1. Data impact**: Represented by a line graph showing a peak.
- 2. Data collection**: Represented by a document with a green arrow pointing to it.
- 3. Data cleaning & manipulation**: Represented by a document with a purple arrow pointing to it.
- 4. Data processing**: Represented by a document with a green arrow pointing to it.
- 5. Quality control**: Represented by a document with a green arrow pointing to it.
- 6. Data interpretation**: Represented by a document with a green arrow pointing to it.
- 7. Data visualization**: Represented by a document with a green arrow pointing to it.

The central circle contains a large black 'S' and a molecular structure.

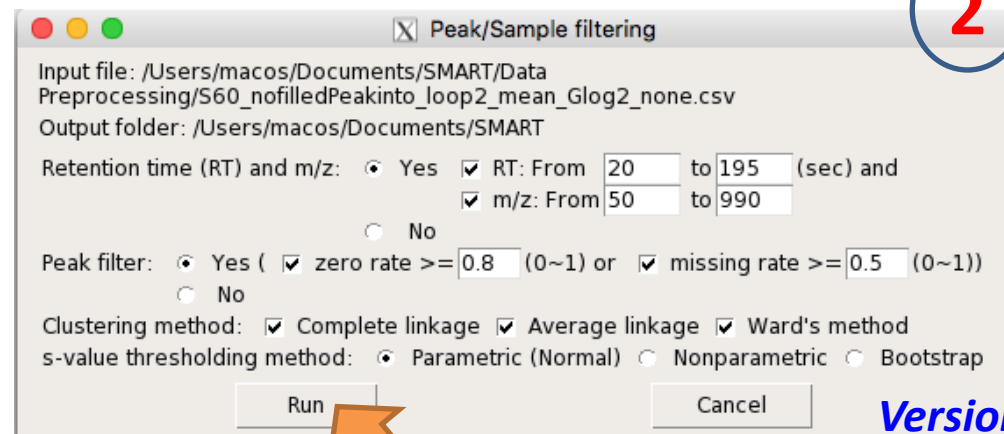
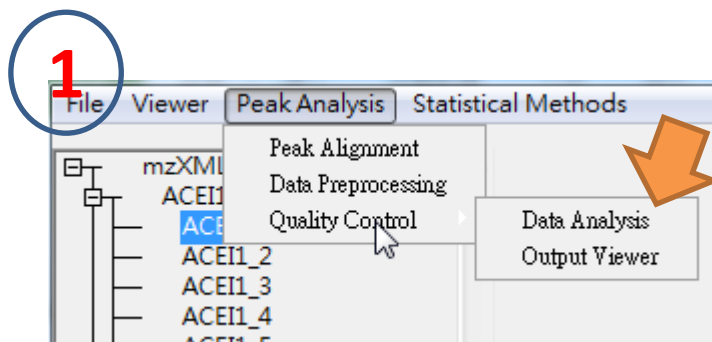


5. Quality control -- sample filtering (3)



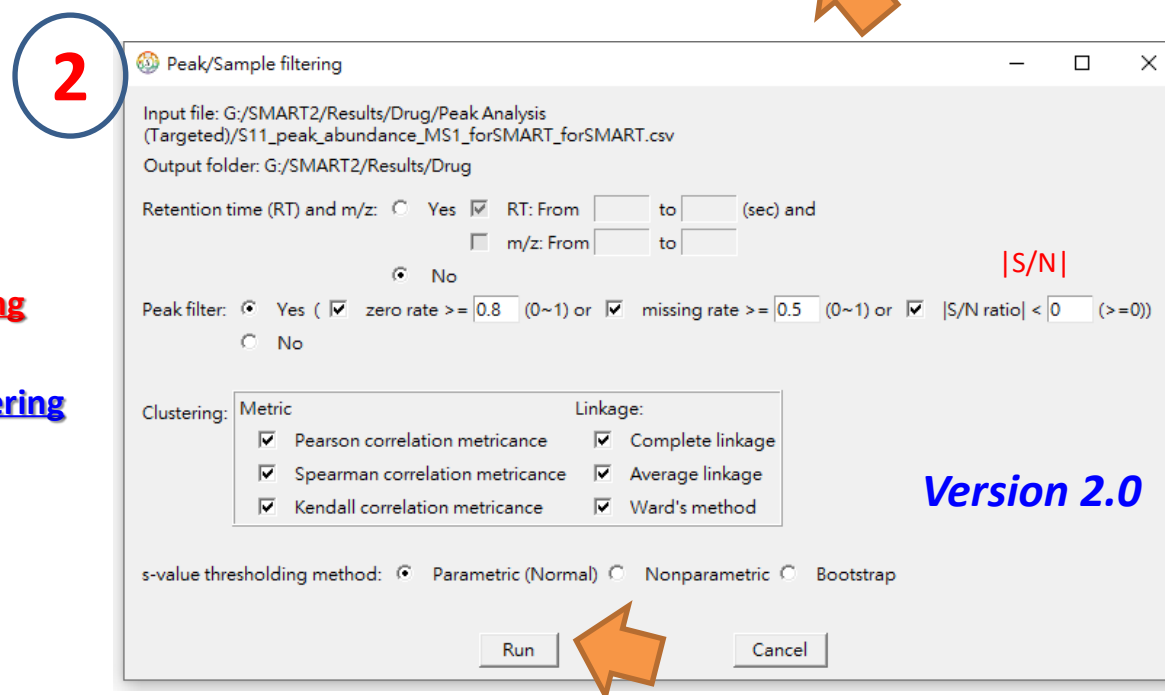
Statistical Metabolomics Analysis – an R Tool

- 5. Quality control



Peak filtering

Sample filtering



Statistical Metabolomics Analysis – an R Tool

- 5. Quality control

Peak/Sample filtering

Input file: G:/SMART2/Results/HT/Data
Preprocessing/S60_nofilledPeakinto_loop2_forSMART_mean_Glog2_none_forSMART.csv
Output folder: G:/SMART2/Results/HT

Retention time (RT) and m/z: ☒ Yes ☒ RT: From 20 to 195 (sec) and ☒ m/z: From 50 to 990
☐ No

Peak filter: ☒ Yes (☒ zero rate >= 0.8 (0~1) or ☒ missing rate >= 0.5 (0~1) or ☒ |S/N ratio| < 0 (>=0))
☐ No

Clustering: Metric Linkage:
☒ Pearson correlation metricance ☒ Complete linkage
☒ Spearman correlation metricance ☒ Average linkage
☒ Kendall correlation metricance ☒ Ward's method

s-value thresholding method: ☒ Parametric (Normal) ☐ Nonparametric ☐ Bootstrap

Run Cancel

Version 2.0

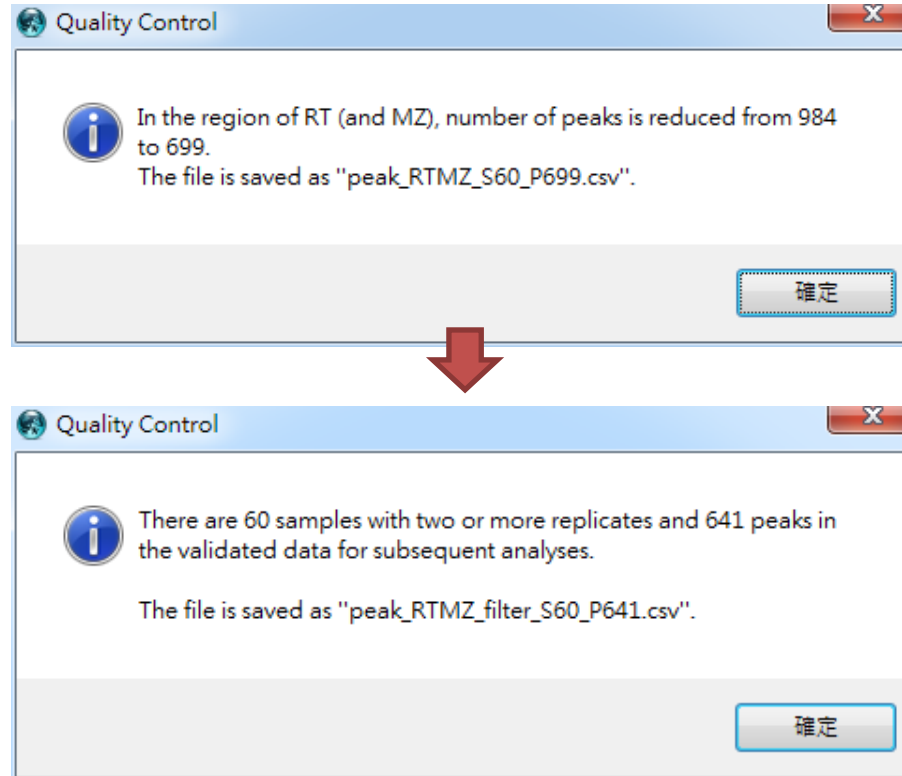
3

QC result – PLEASE CHOOSE ONE RData

QC_results-2023-06-14-1612 (Parametric - KendallCorrDist)
QC_results-2023-06-14-1620 (Parametric - KendallCorrDist)
QC_results-2023-06-14-1611 (Parametric - PearsonCorrDist)
QC_results-2023-06-14-1619 (Parametric - PearsonCorrDist)
QC_results-2023-06-14-1612 (Parametric - SpearmanCorrDist)
QC_results-2023-06-14-1619 (Parametric - SpearmanCorrDist)

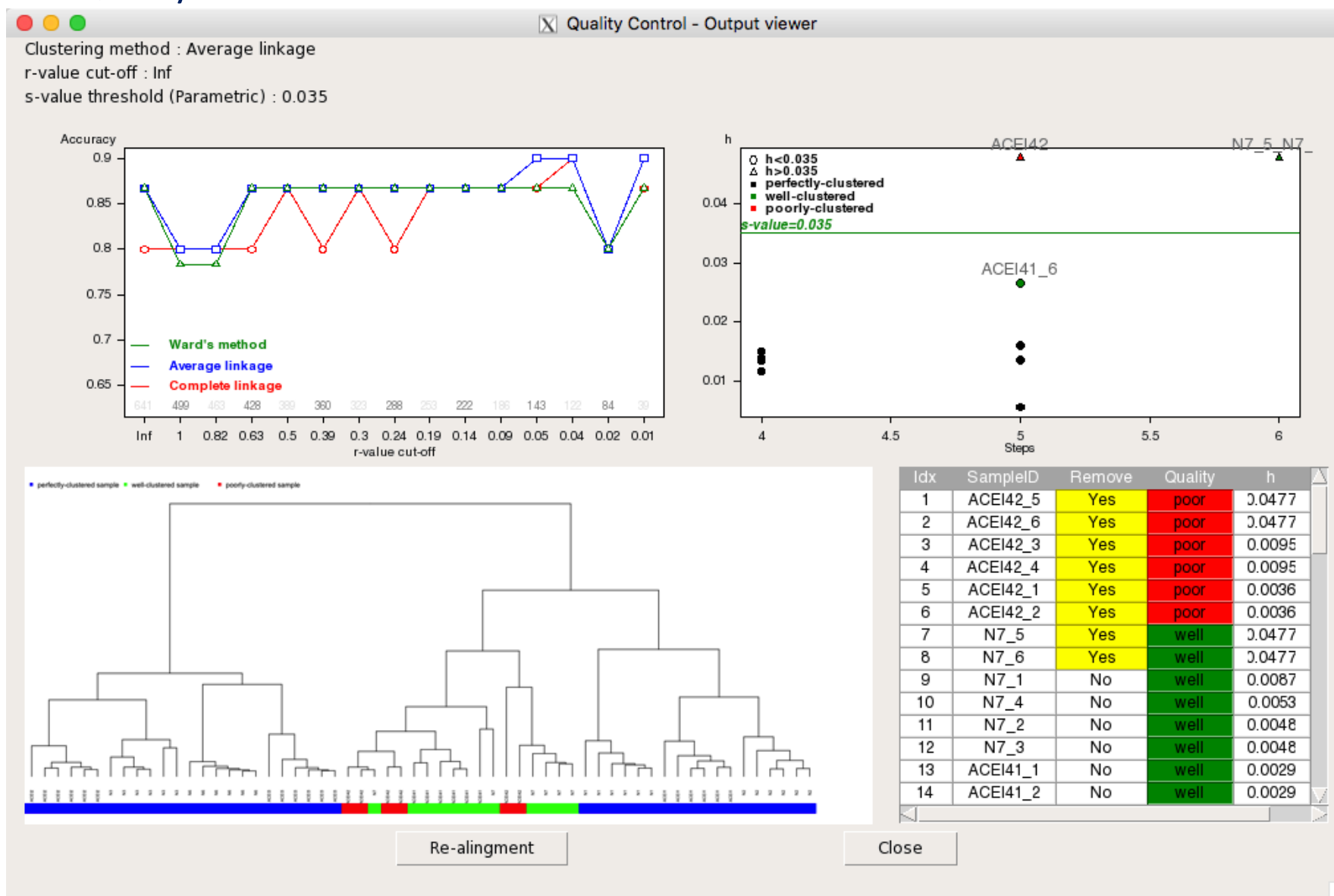
Statistical Metabolomics Analysis – an R Tool

- 5. Quality control



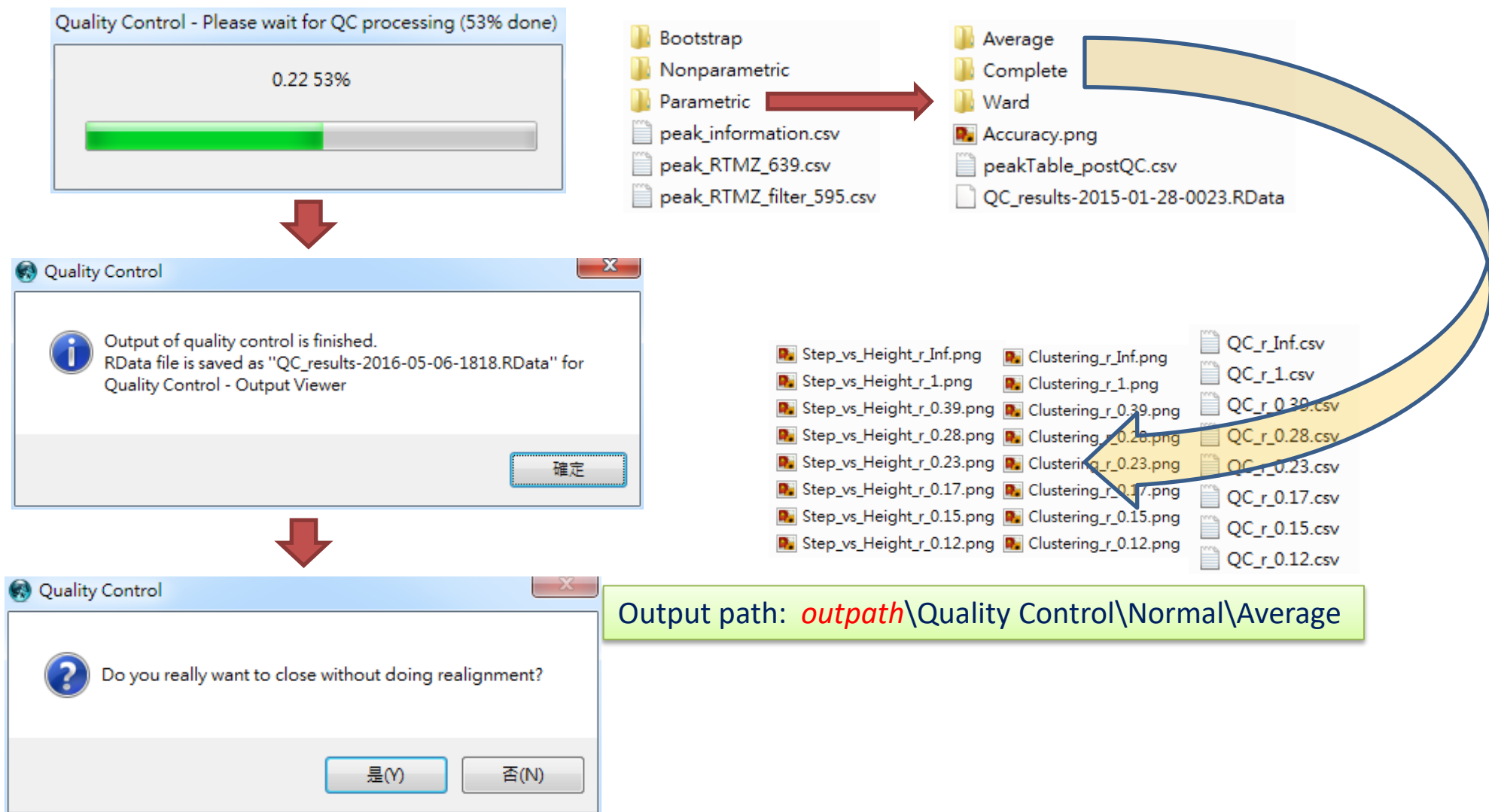
Statistical Metabolomics Analysis – an R Tool

- 5. Quality control



Statistical Metabolomics Analysis – an R Tool

- 5. Quality control

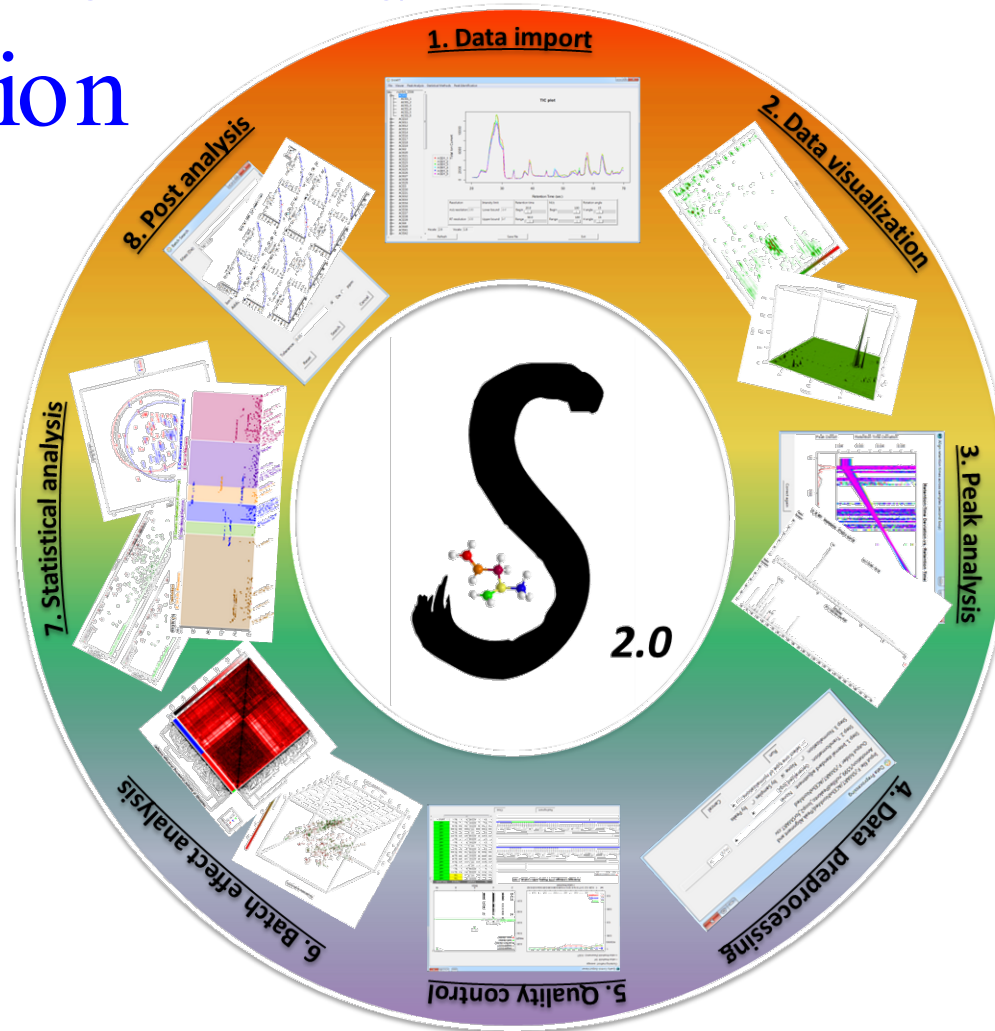




Quality control (1st)

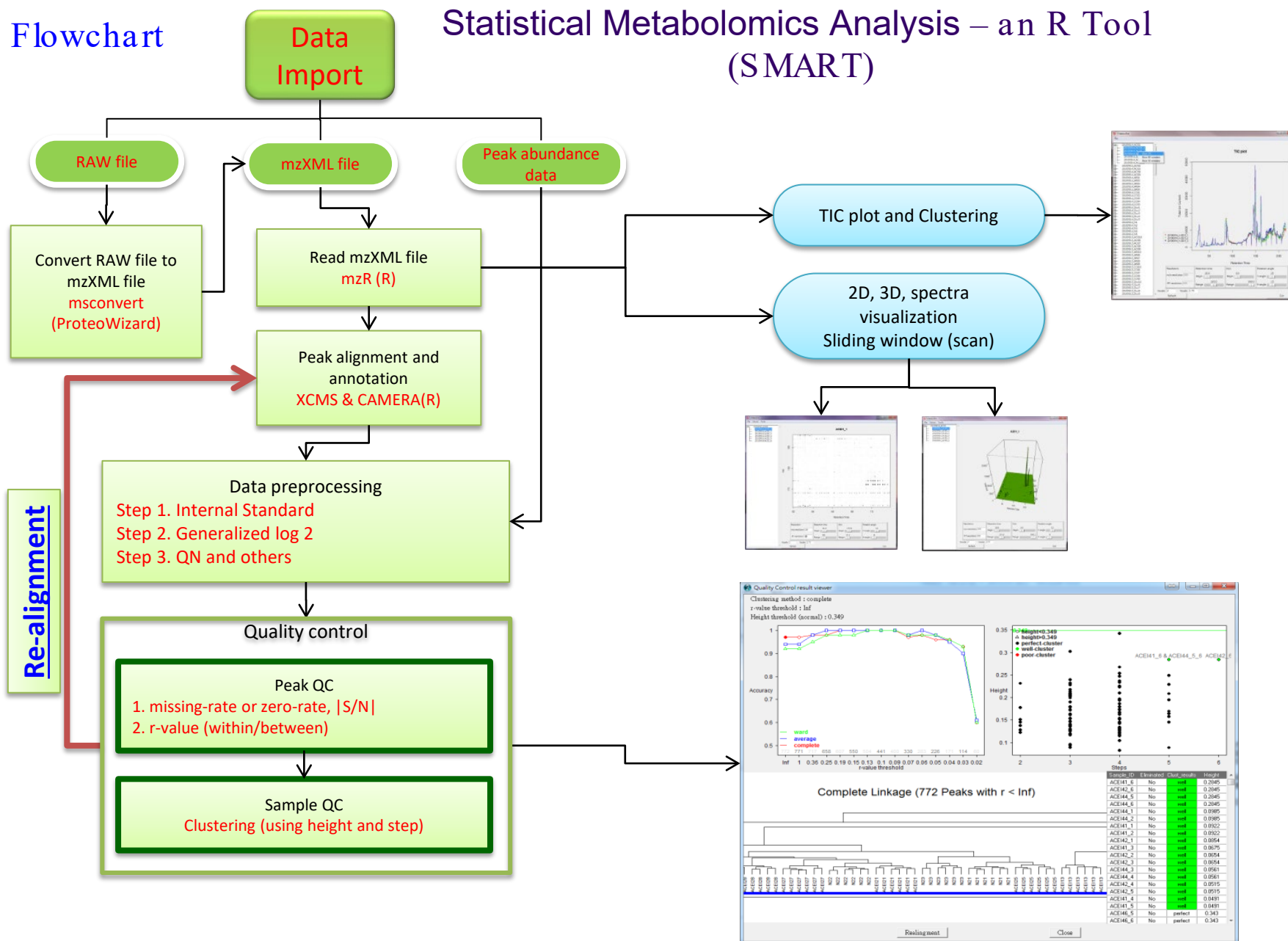
Statistical Metabolomics Analysis – an R Tool

Peak re-alignment and annotation



Flowchart

Statistical Metabolomics Analysis – an R Tool (SMART)



Re-alignment & annotation

- Re-alignment & annotation

Peak Re-alignment and Annotation

Input folder: F:/SMART/ACEIvsNonMed_Example/mzXML_10HT
Output folder: F:/SMART/ACEIvsNonMed_Example

Parameter setting:

Identify peaks:

lockMassFreq: , polarity: , snthresh: , mslevel: , scanrange: , nSlaves:

profmethode: ☐ bin ☒ binlin ☐ binlinbase ☐ intlin

☒ MatchedFilter-method

fwhm: , max: , step: , steps: , mzdif: -step*steps

☐ centWave-method

ppm: , peakwidth: min , max , mzdif: , fitgauss: , noise:

prefilter: k , I

Group peaks from different samples (loop)

bw: , minfrac: , minsamp: , mzwid: , max:

Correct retention time from different samples (loop)

missing: , extra: , span:

smooth: ☐ loess ☒ linear

family: ☒ gaussian ☐ symmetric

Integrate areas of missing peaks:

fillPeaks: ☐ Yes ☒ No

Peak abundance format:

☒ integrated area of original (raw) peak ☐ maximum intensity of original (raw) peak

☐ integrated area of filtered peak ☐ maximum intensity of filtered peak

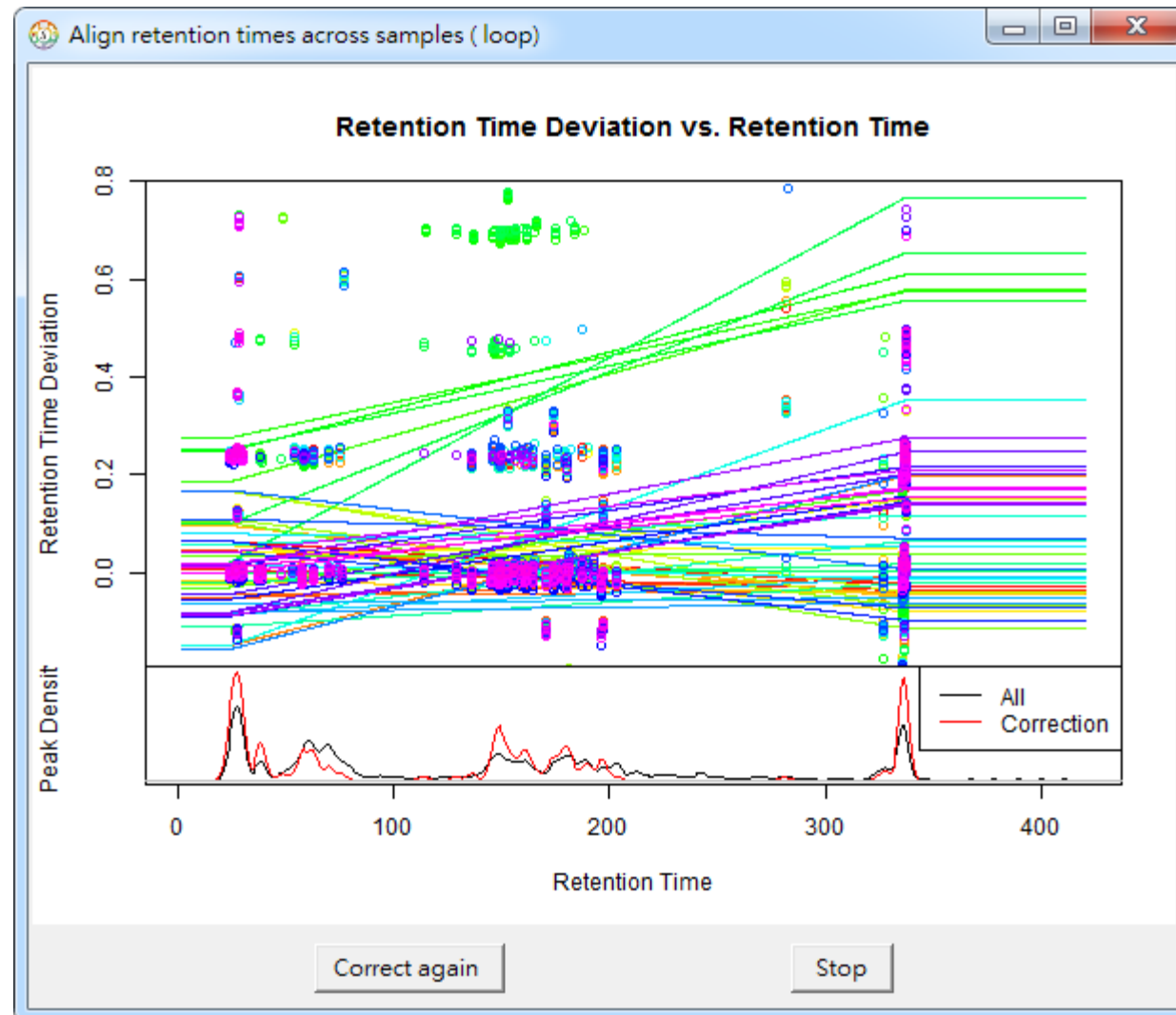
Peak annotation:

filter out by selection: ☒ isotope ☒ fragment ☒ adduct (☒ positive ☐ negative)

OK Cancel

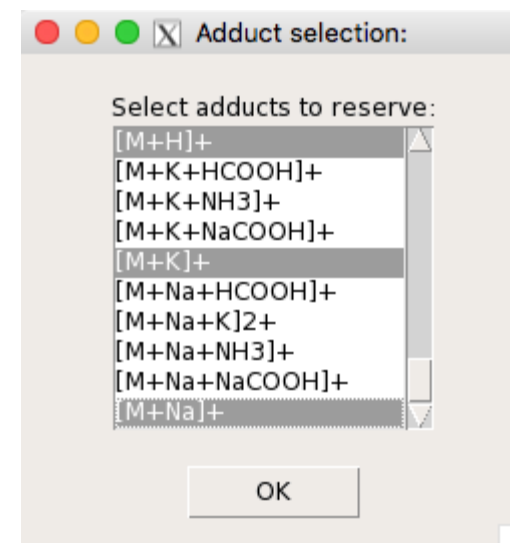
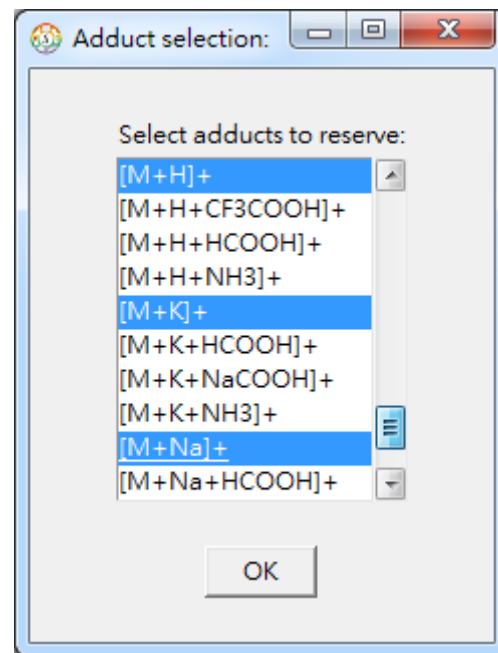
Statistical Metabolomics Analysis – an R Tool

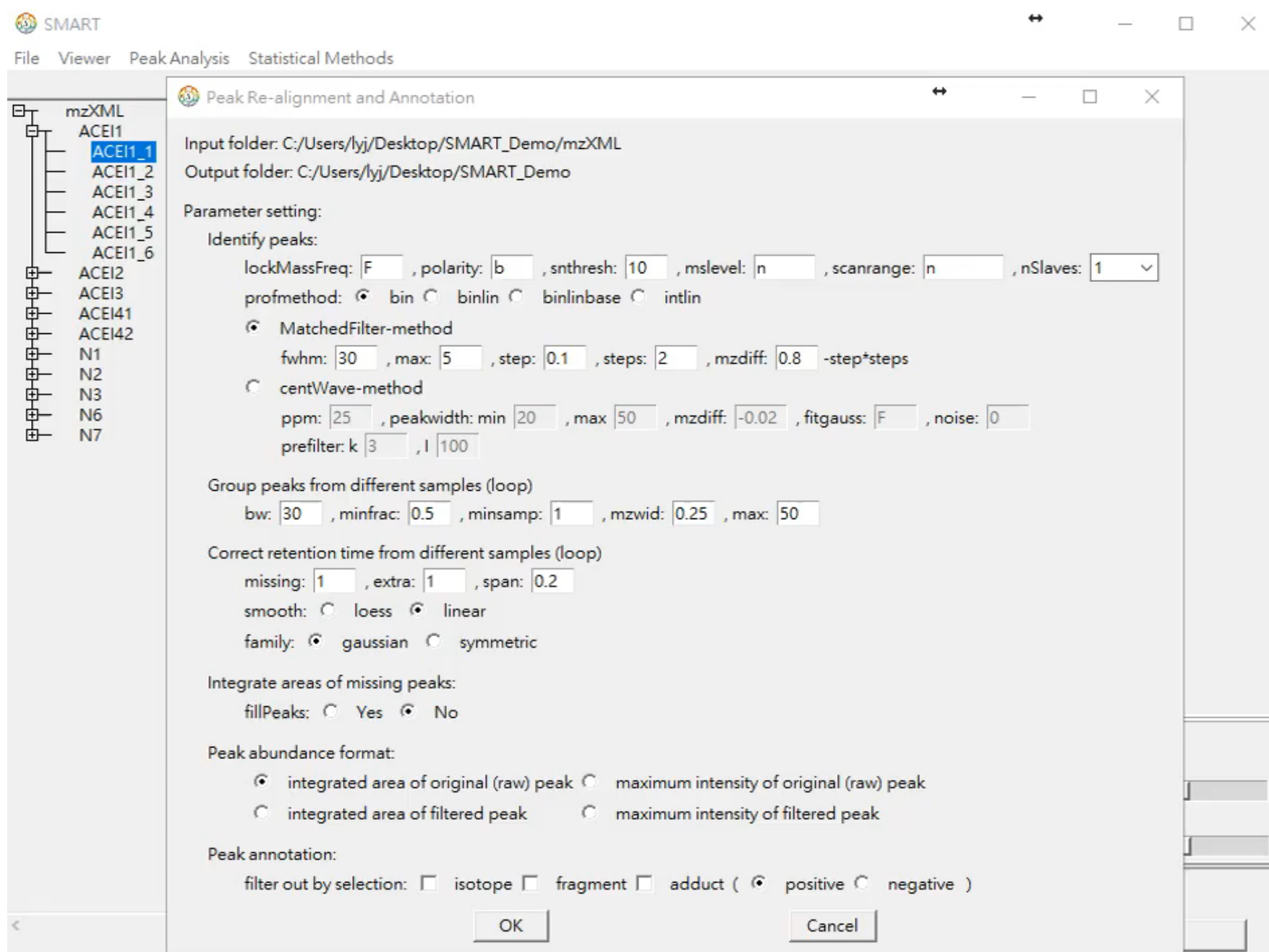
- Re-alignment
& annotation



Statistical Metabolomics Analysis – an R Tool

- Re-alignment
– CAMERA

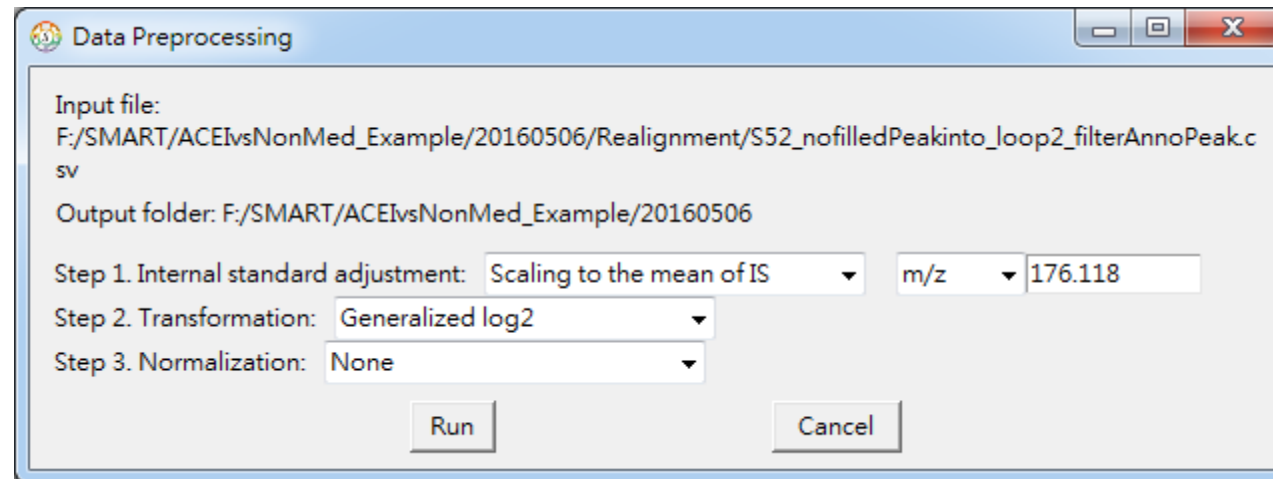




Peak re-alignment

Statistical Metabolomics Analysis – an R Tool

- Data preprocessing (after re-alignment)



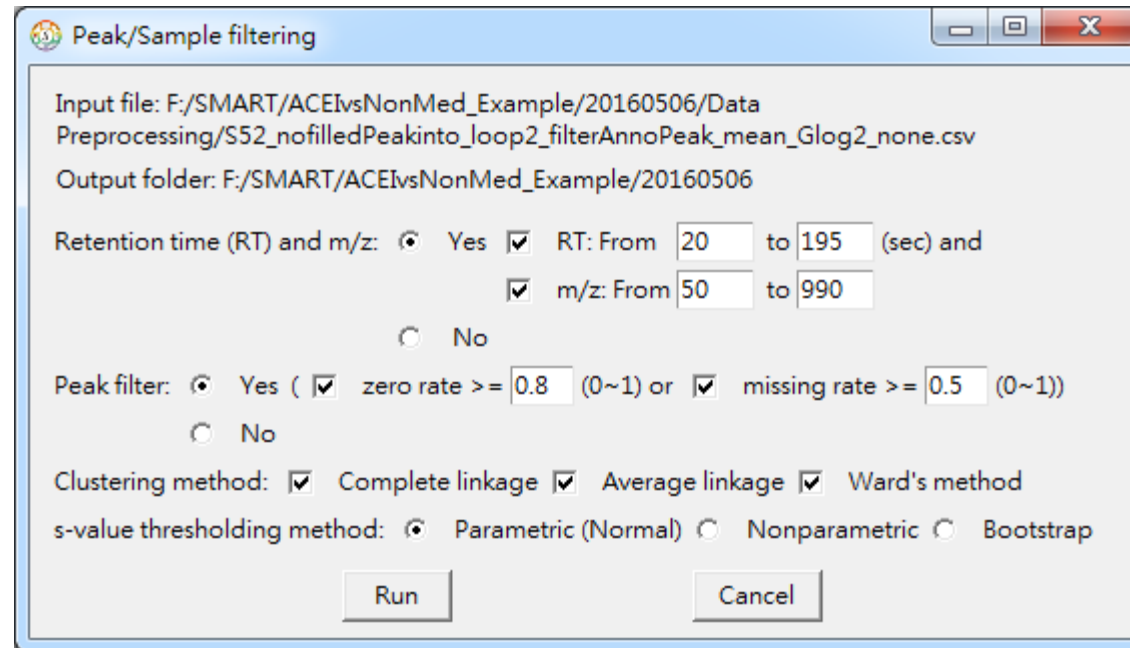
The screenshot shows a 'Data Preprocessing' dialog box with the following settings:

- Input file:** F:/SMART/ACEIvsNonMed_Example/20160506/Realignment/S52_nofilledPeakinto_loop2_filterAnnoPeak.csv
- Output folder:** F:/SMART/ACEIvsNonMed_Example/20160506
- Step 1. Internal standard adjustment:** Scaling to the mean of IS (dropdown menu), m/z (dropdown menu), 176.118 (text input)
- Step 2. Transformation:** Generalized log2 (dropdown menu)
- Step 3. Normalization:** None (dropdown menu)

Buttons: Run, Cancel

Statistical Metabolomics Analysis – an R Tool

- Quality control (after re-alignment)
 - Peak filtering



Peak/Sample filtering

Input file: F:/SMART/ACEIvsNonMed_Example/20160506/Data
Preprocessing/S52_nofilledPeakinto_loop2_filterAnnoPeak_mean_Glog2_none.csv
Output folder: F:/SMART/ACEIvsNonMed_Example/20160506

Retention time (RT) and m/z: ☒ Yes ☒ RT: From to (sec) and
☒ m/z: From to
☐ No

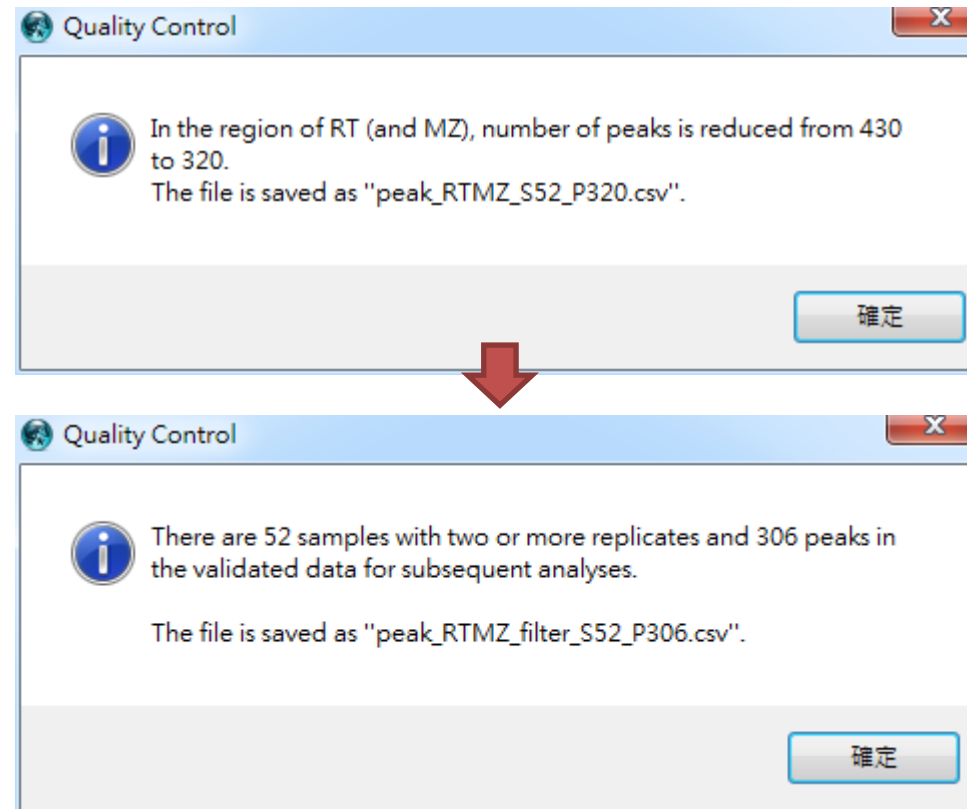
Peak filter: ☒ Yes (☒ zero rate $\geq$ (0~1) or ☒ missing rate $\geq$ (0~1))
☐ No

Clustering method: ☒ Complete linkage ☒ Average linkage ☒ Ward's method

s-value thresholding method: ☒ Parametric (Normal) ☐ Nonparametric ☐ Bootstrap

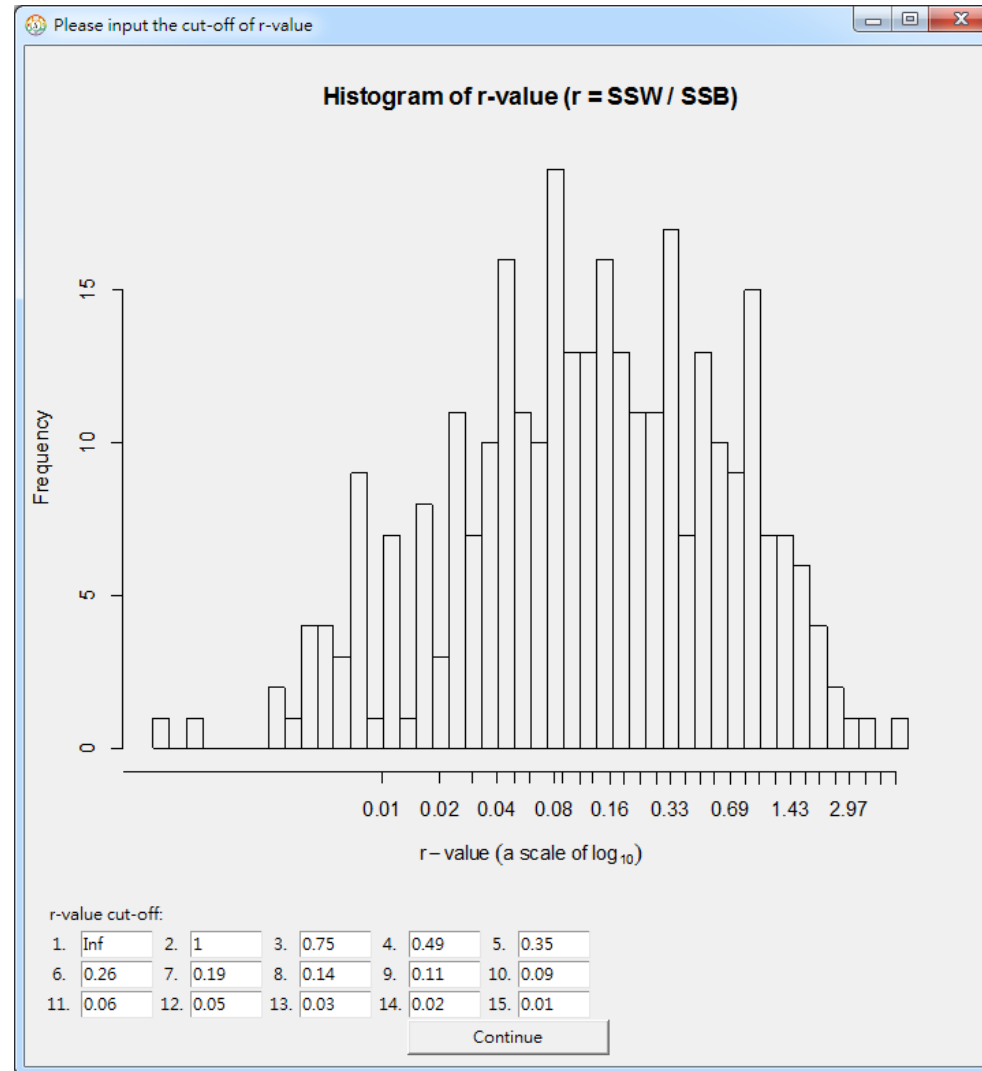
Statistical Metabolomics Analysis – an R Tool

- Quality control (after re-alignment)
 - Peak filtering



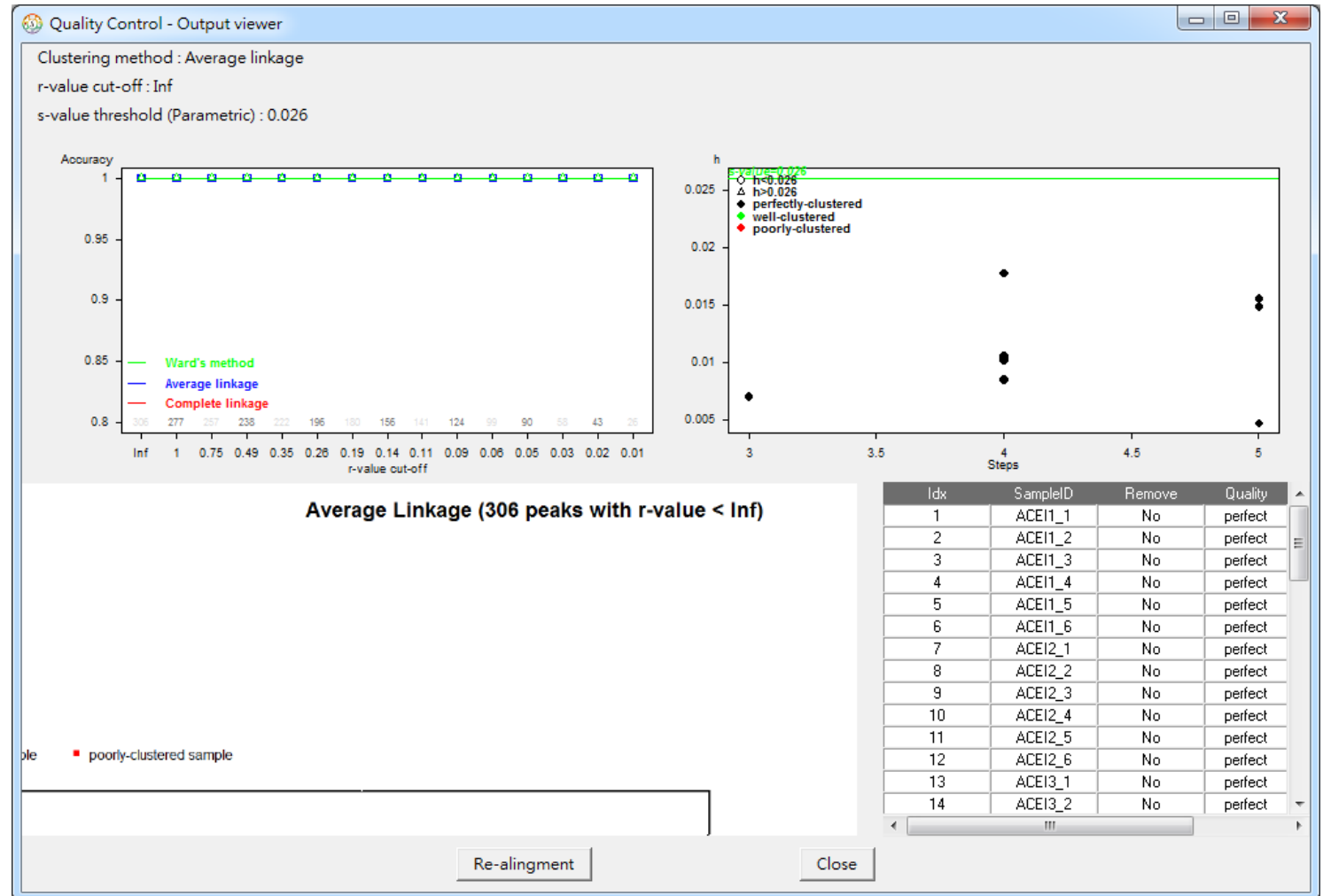
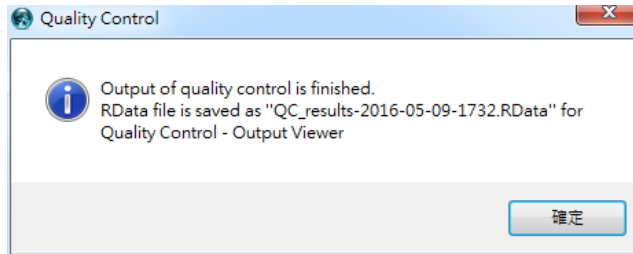
Statistical Metabolomics Analysis – an R Tool

- Quality control (after re-alignment)
 - Peak filtering



Statistical Metabolomics Analysis – an R Tool

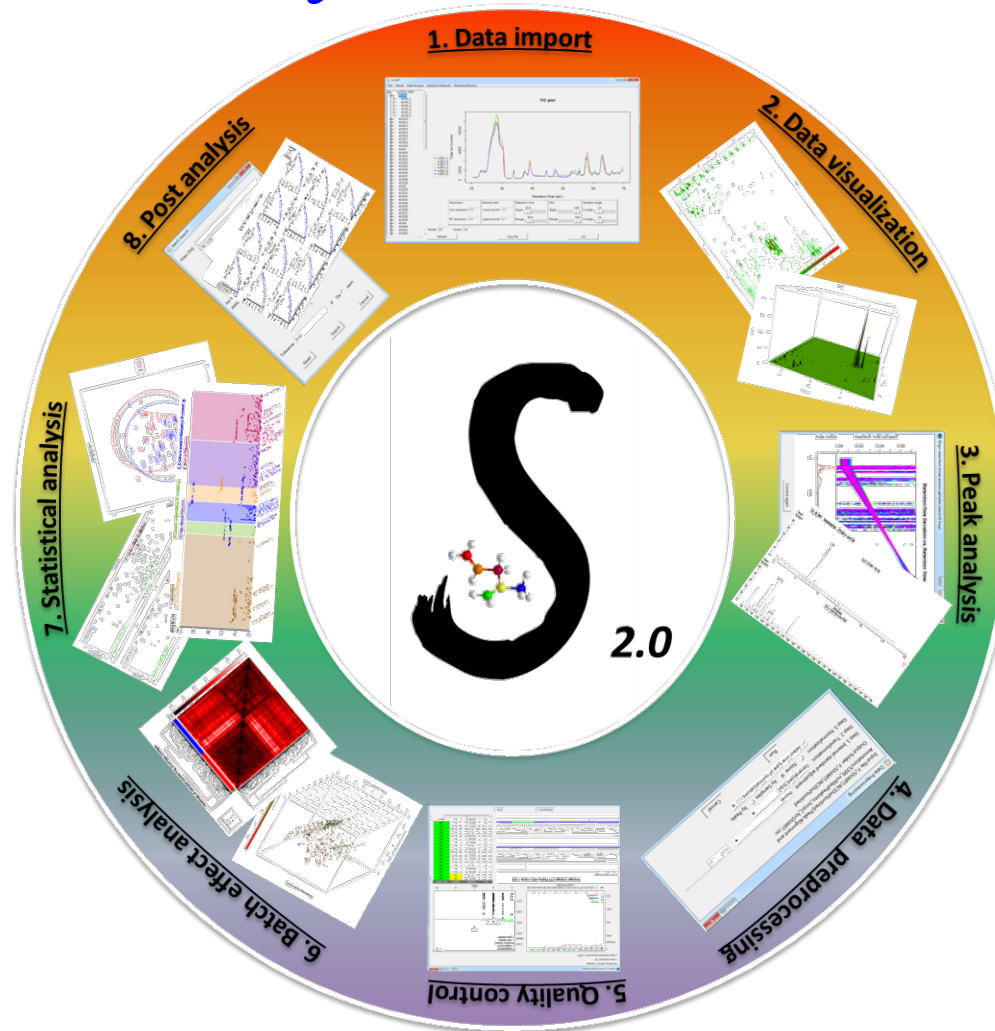
- Quality control (after re-alignment)
 - Peak filtering





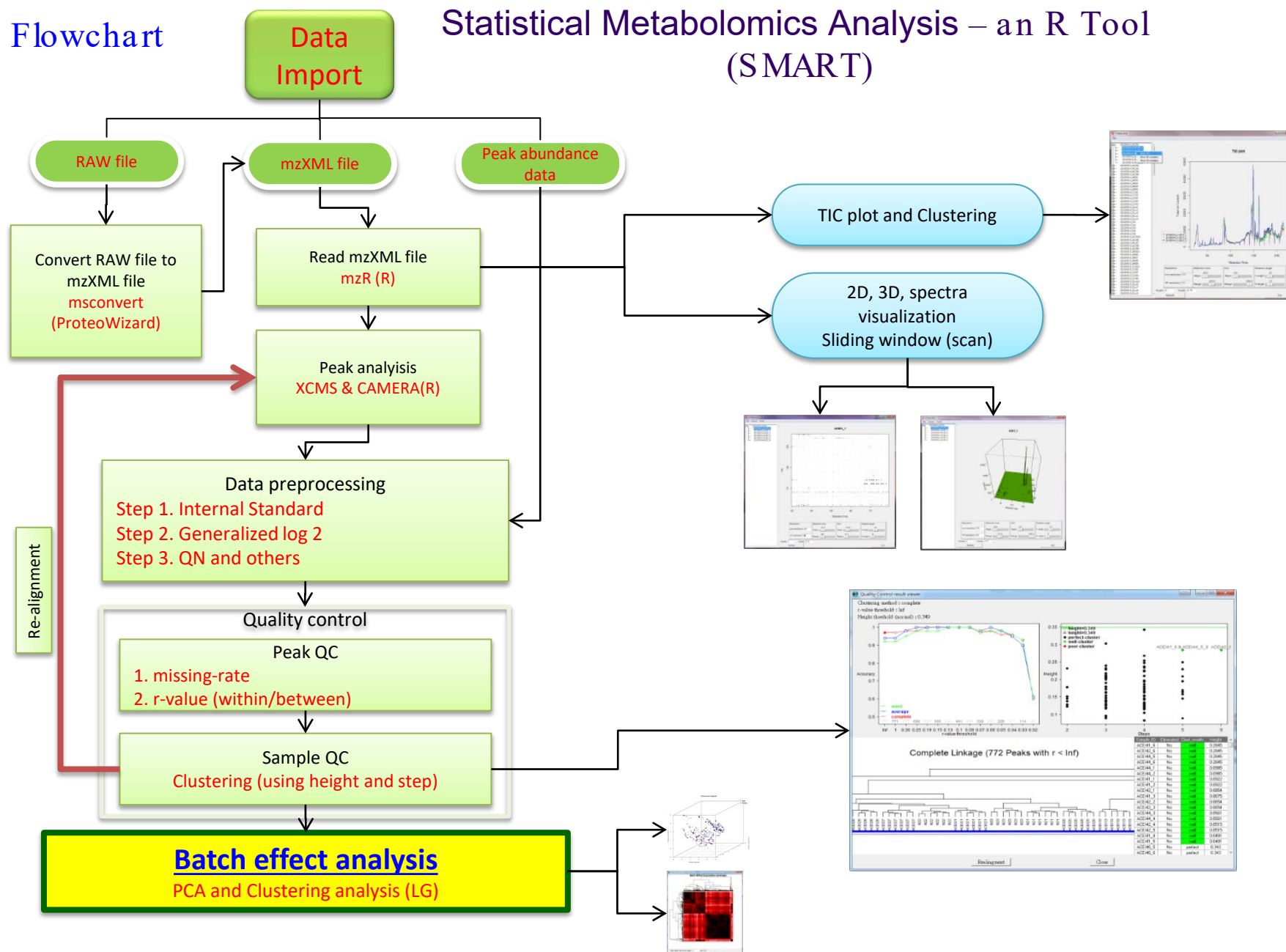
Statistical Metabolomics Analysis – an R Tool

6. Batch effect analysis



Flowchart

Statistical Metabolomics Analysis – an R Tool (SMART)



Batch effect analysis

- Batch effect (latent group)
 - Clustering analysis

1

Statistical Methods

Batch Effect Analysis
Analysis of Covariance (ANCOVA)

Principal Component Analysis (PCA)
Latent Group

2

Batch Effect Detection - LG

Input file: F:/SMART/ACEIvsNonMed_Example/20160506/Quality Control/S52_P430/Parametric/peakTable_postQC_S52_P306.csv

Output folder: F:/SMART/ACEIvsNonMed_Example/20160506

Covariates file: onMed_Example/20160506/Covariate/ACEI_N_HT10_cov.csv

Method: ☐ Complete linkage ☒ Average linkage ☐ Ward's method

OK Cancel

Peak abundance data (peakTable_postQC.csv)

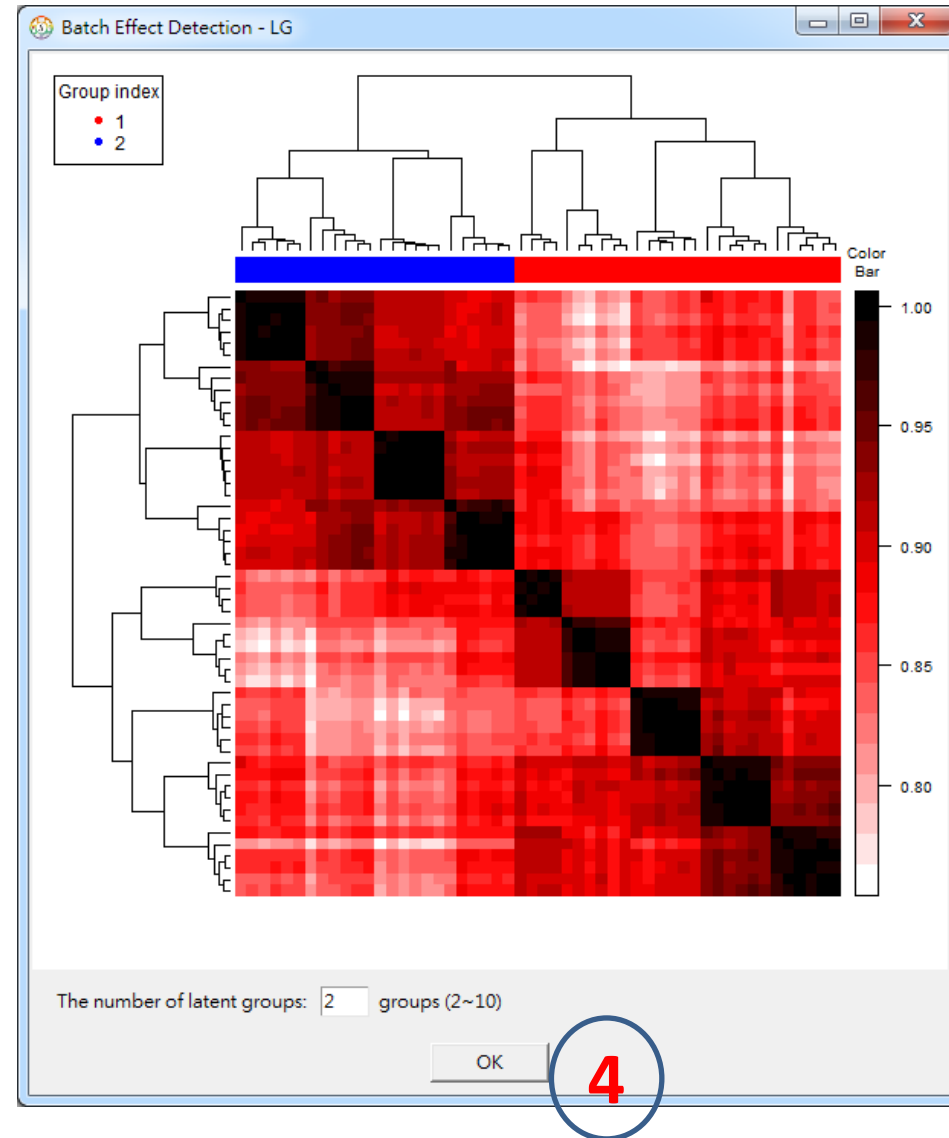
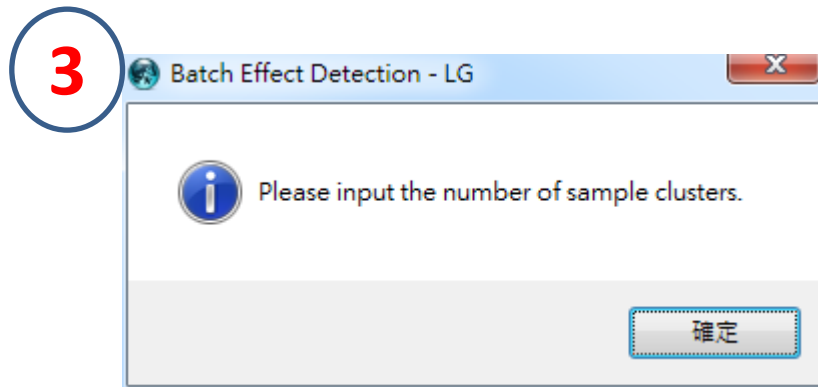
Peak_Index	Mass	Ret_time(sec)	N7_1	N7_6
3	60.0756	29.1905	4.9484	NA
4	61.0121	39.1727	NA	NA
5	68.9834	23.9602	7.40761	7.19992
			NA	NA
			NA	NA
			NA	NA
9	72.0813	37.8041	6.70913	5.64685
10	77.0394	58.0969	6.34075	5.80933
11	79.0526	58.1402	NA	4.92574

Cov file

SampleID	Ddataset	Dgender	Cage	Cbmi	Ddate
ACEI1	1	1	46	26.08	D0914
ACEI10	1	1	46	26.65	D0915
ACEI11	2	1	46	23.41	D0920
ACEI12		1	47	27.32	D0920
ACEI13		1	46	23.76	D0920
ACEI14	5	1	42	30.81	D1014
ACEI16	2	1	41	23.29	D0924
ACEI17	2	1	23	25.45	D0924
ACEI18	2	1	24	29	D0924
ACEI19	2	2	50	26.08	D0924

Batch effect analysis

- Batch effect (latent group)
 - Clustering analysis

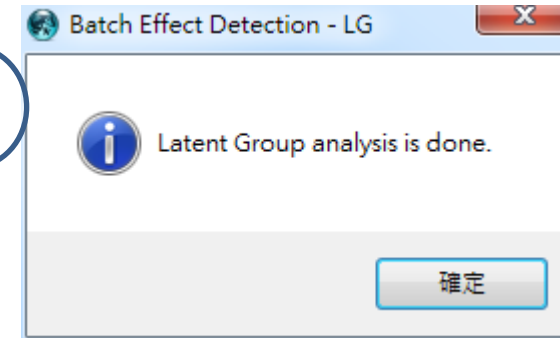


Batch effect analysis

- Batch effect (latent group)
 - Clustering analysis (output files)

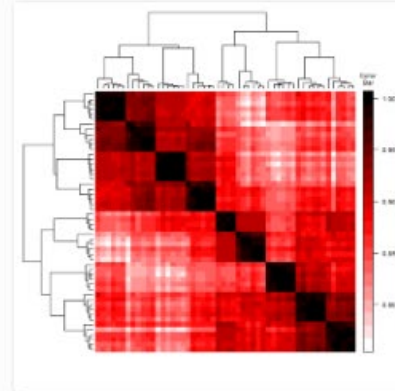
outpath\Batch Effect Analysis\Latent Group (2)

5

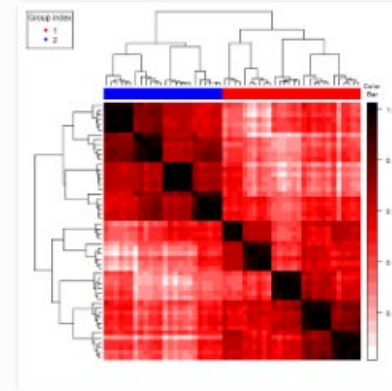


1	Sample_ID	group_idx
2	N7_1	1
3	N7_6	1
4	ACEI41_1	1
5	N7_2	1
6	ACEI41_5	1
7	ACEI41_4	1
8	N7_3	1
9	N7_4	1
10	ACEI41_2	1
11	ACEI41_3	1
12	ACEI45_5	1
13	ACEI45_6	1
14	N41_6	1
15	ACEI46_4	2
16	ACEI46_5	2
17	ACEI46_6	2

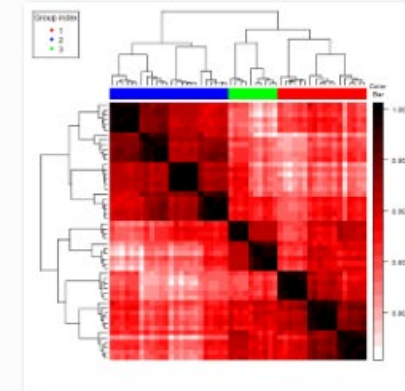
latent_group_Average_2.csv



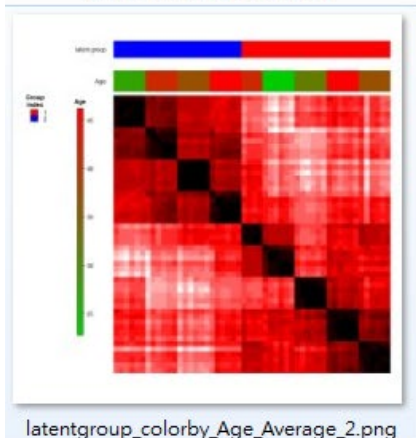
latent_group_Average.png



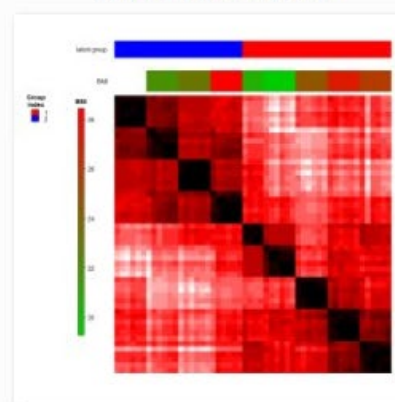
latent_group_Average_2.png



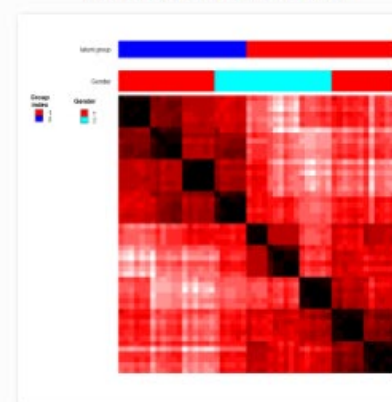
latent_group_Average_3.png



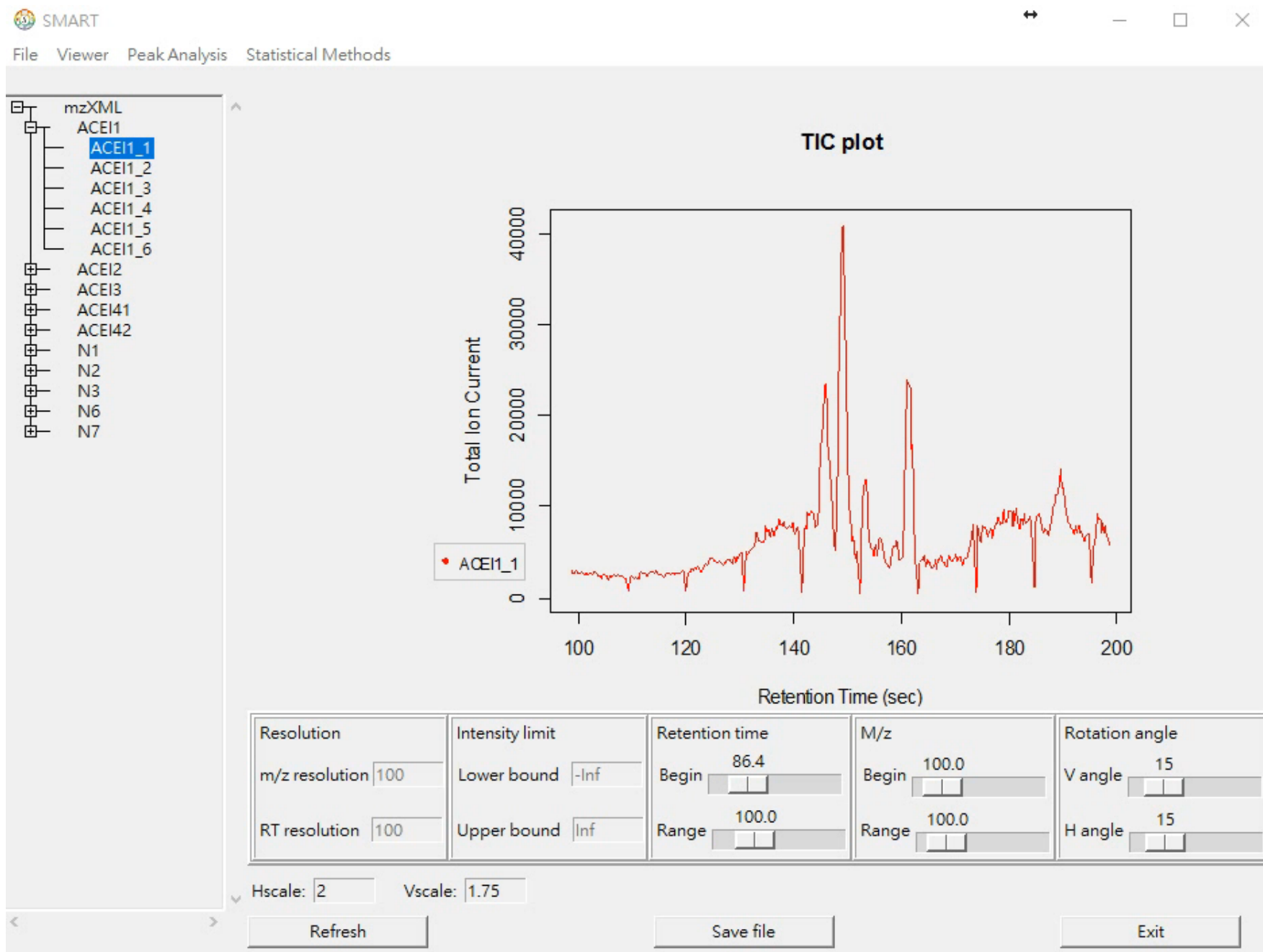
latentgroup_colorby_Age_Average_2.png



latentgroup_colorby_BMI_Average_2.png



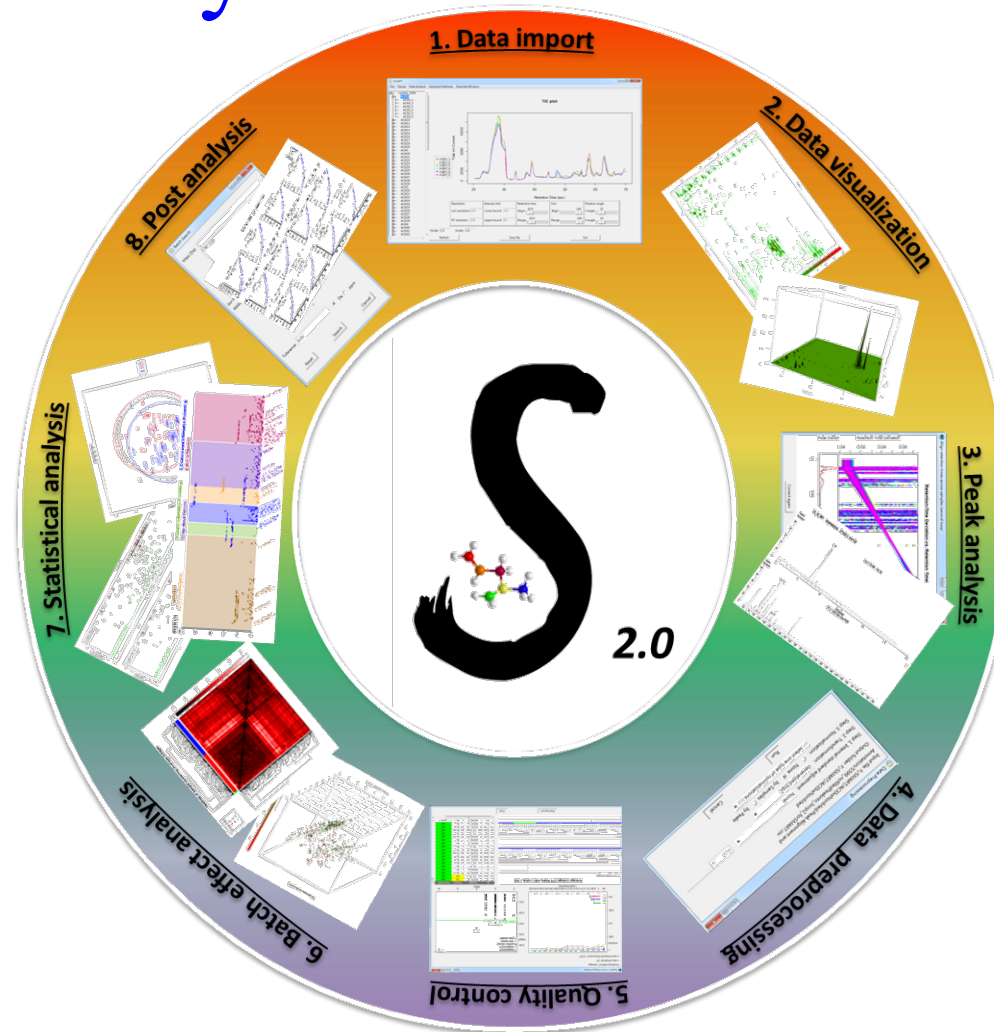
latentgroup_colorby_Gender_Average_2.png



Batch effect (latent group)

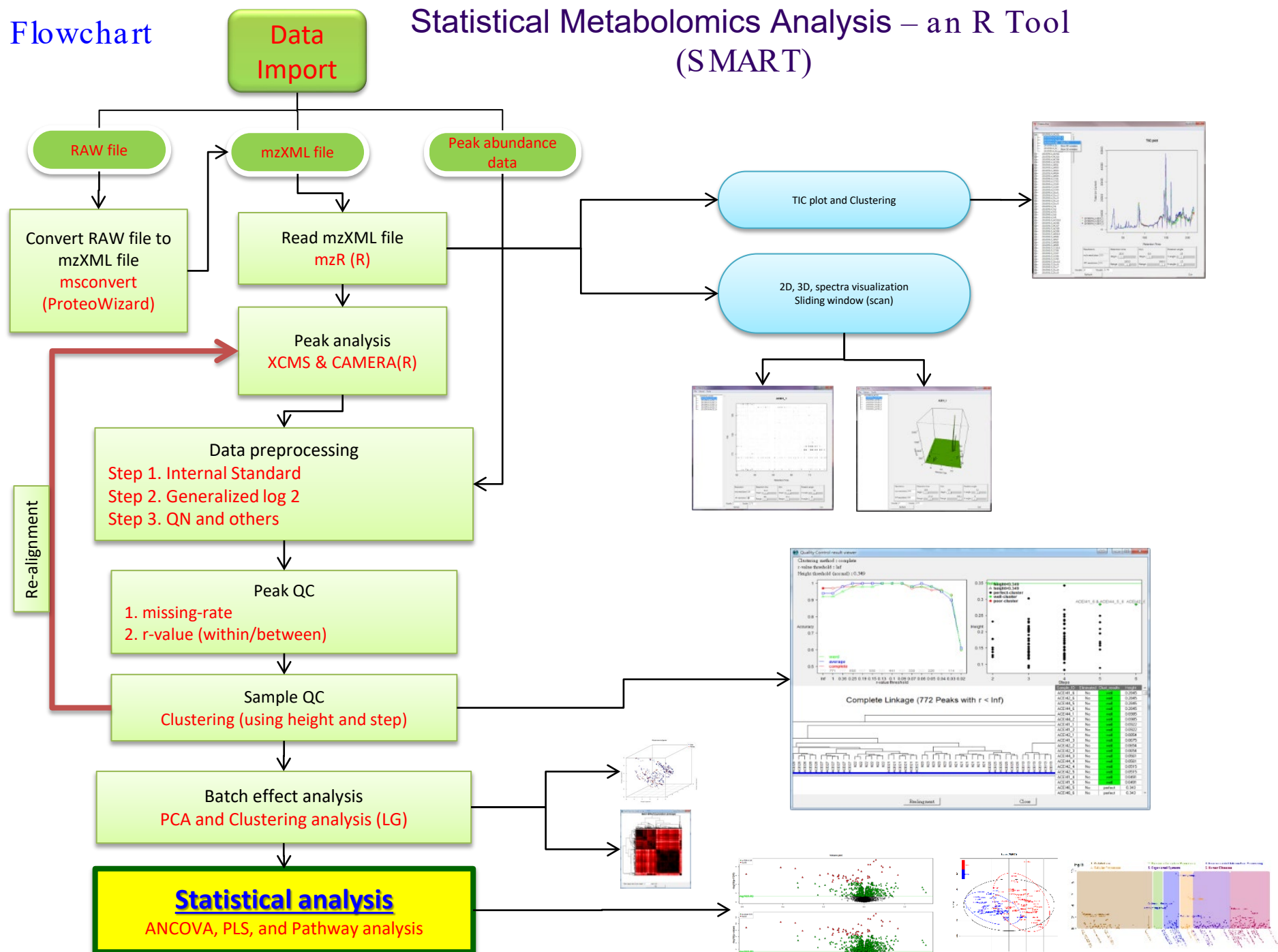
Statistical Metabolomics Analysis – an R Tool

7. Statistical analysis



Flowchart

Statistical Metabolomics Analysis – an R Tool (SMART)



ANCOVA

STATISTICAL ANALYSIS (1)

Statistical analysis (ANCOVA)

Latent group

- Association analysis

- ANCOVA

- Batch effect adjustment (Latent group)

1

Statistical Analysis Post Analysis

Batch Effect Detection
Analysis of Covariance (ANCOVA)
Partial Least Squares (PLS/PLS-DA)
Pathway Analysis

Analysis of Covariance (ANCOVA)

Input file: F:/SMART/ACEIvsNonMed_Example/20160506/Quality Control/S52_P430/Parametric/peakTable_postQC_S52_P306.csv

Output folder: F:/SMART/ACEIvsNonMed_Example/20160506

Covariates file (optional): ART/ACEIvsNonMed_Example/20160506/Covariate/ACEI_N_HT10_cov.csv

Batch effects file (optional): 0506/Batch Effect Detection/Latent Group (2)/latent_group_Average_2.csv

Factor file (required): T/ACEIvsNonMed_Example/20160506/Covariate/ACEI_N_HT10_factor.csv

Label for the control group: NonMed

☒ Run permutation test # of permutations: 10000 Cut-off of nominal p-value: 0.05

Number of cores: 4

OK Cancel

Factor (variable of interest) file

2

Cov file

SampleID	DDataset	Dgender	Cage	Cbmi	Ddate
ACEI1	1	1	46	26.08	D0914
ACEI10	1	1	46	26.65	D0915
ACEI11	2	1	46	23.41	D0920
ACEI12	2	1	47	27.32	D0920
ACEI13	2	1	46	23.76	D0920
ACEI14	5	1	42	30.81	D1014
ACEI16	2	1	41	23.29	D0924
ACEI17	2	1	23	25.45	D0924
ACEI18	2	1	24	29	D0924
ACEI19	2	2	50	26.08	D0924

Batch effect file
(latent group)

	Sample_ID	group_idx
1	N7_1	1
2	N7_6	1
3	ACEI41_1	1
4	N7_2	1
5	ACEI41_5	1
6	ACEI41_4	1
7	N7_3	1
8	N7_4	1
9	ACEI41_2	1
10	ACEI41_3	1
11	ACEI41_3	1

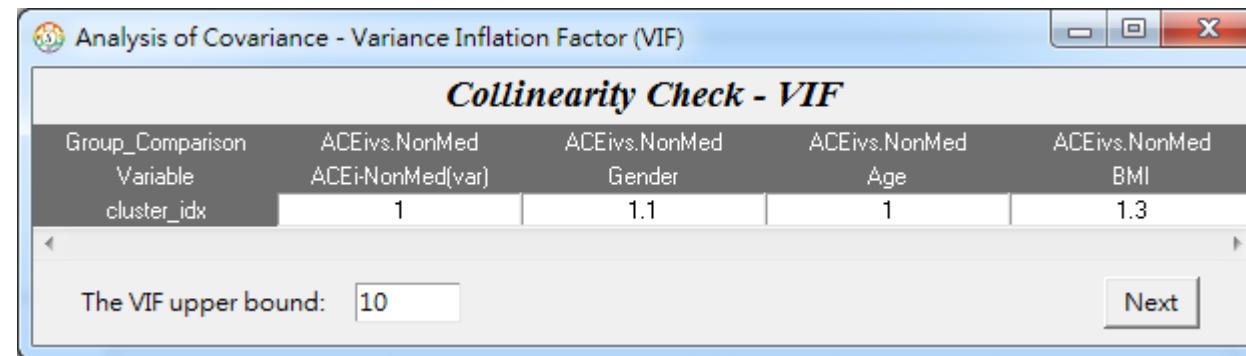
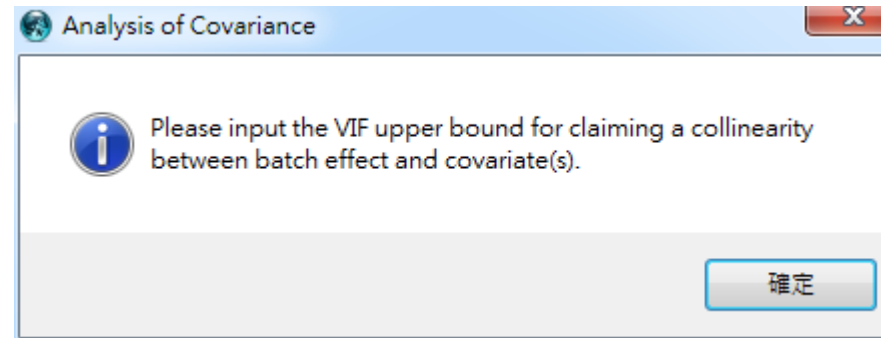
Factor file

SampleID	Group
ACEI1	ACEI
ACEI2	ACEI
ACEI3	ACEI
ACEI4	ACEI
ACEI5	ACEI
ACEI6	ACEI
ACEI7	ACEI
ACEI8	ACEI
ACEI9	ACEI
ACEI10	ACEI

Statistical analysis (ANCOVA)

Latent group

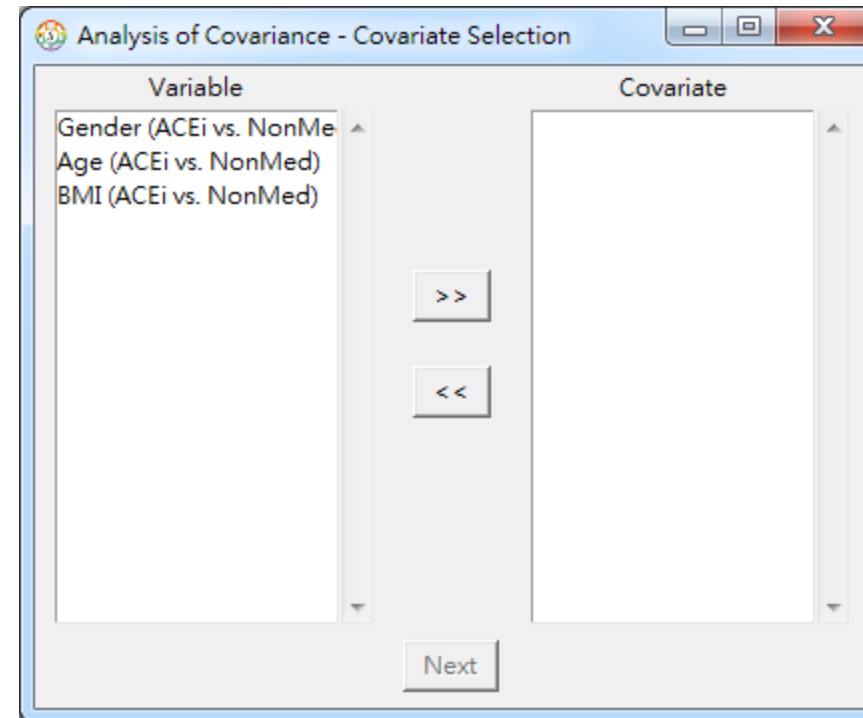
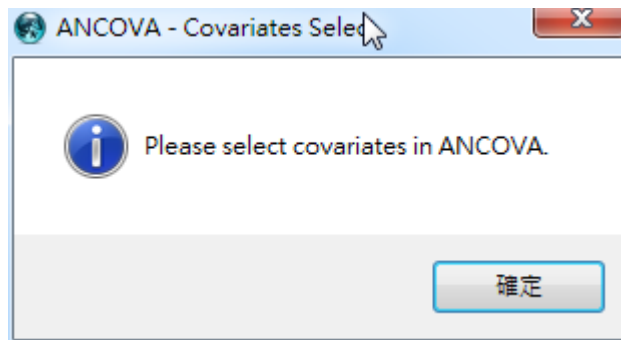
- Association analysis
 - ANCOVA
 - VIF



Statistical analysis (ANCOVA)

Latent group

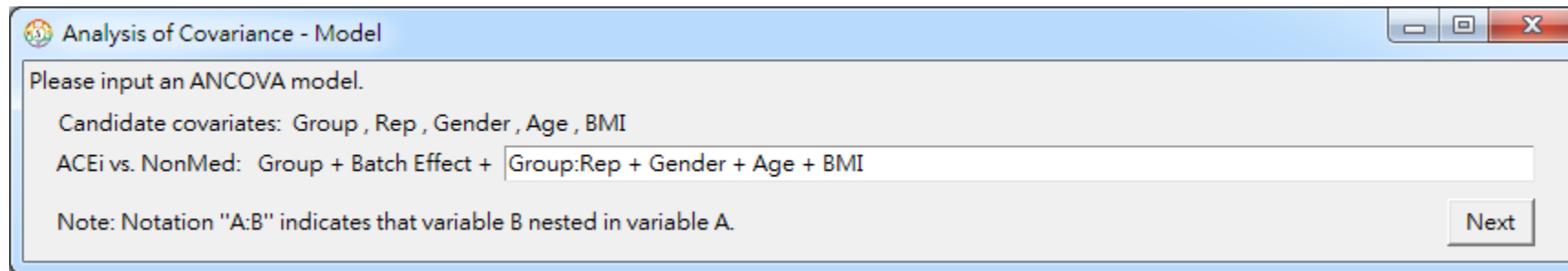
- Association analysis
 - ANCOVA
 - Covariate selection



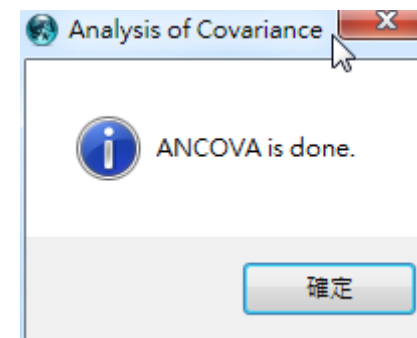
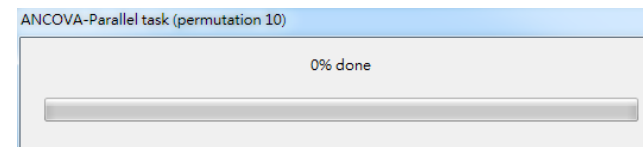
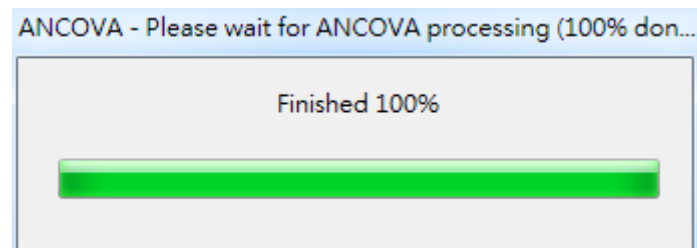
Statistical analysis (ANCOVA)

Latent group

- Association analysis
 - ANCOVA
 - Model setting : Group:Rep + Gender + Age + BMI



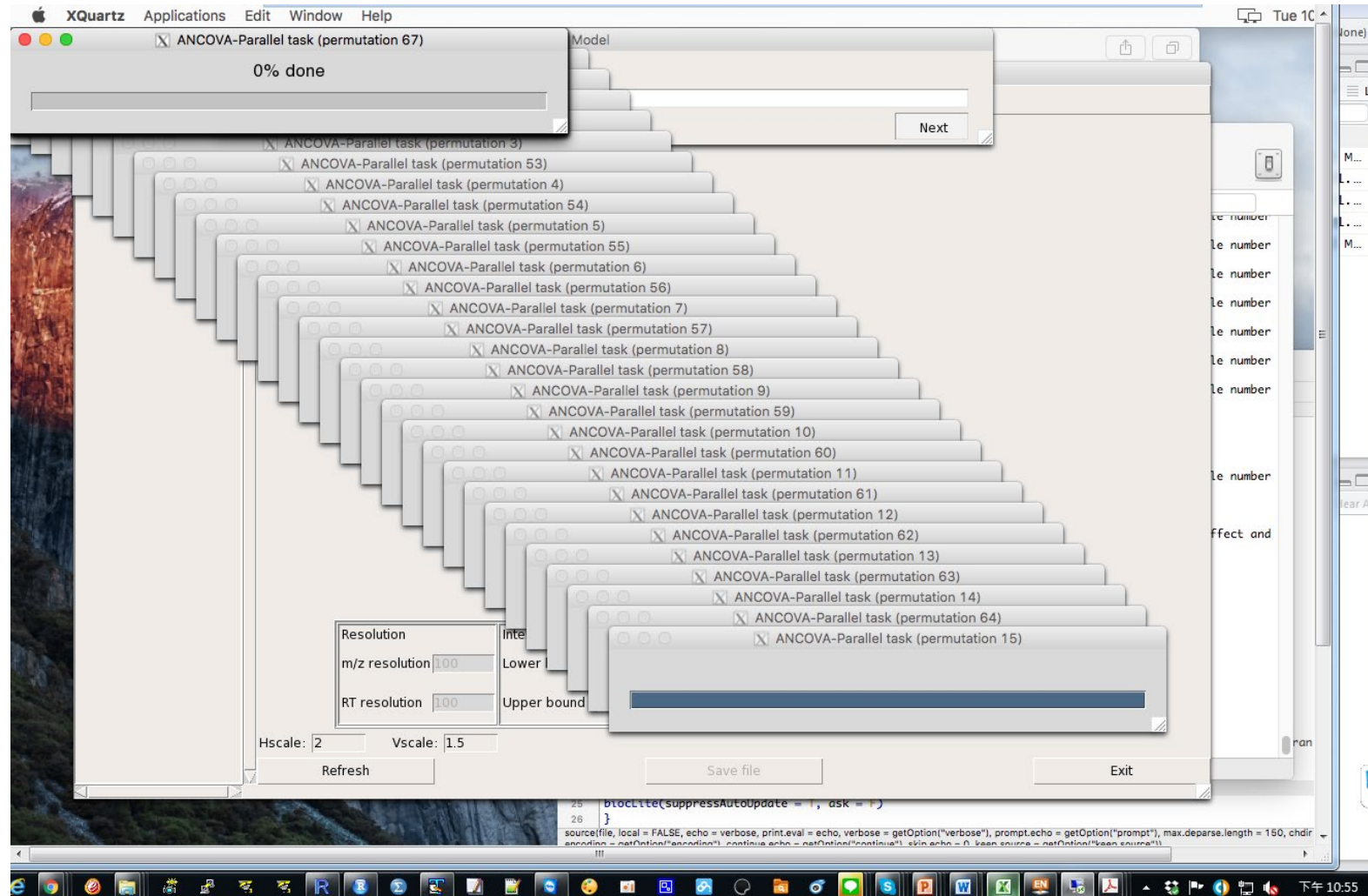
124



Statistical analysis (ANCOVA)

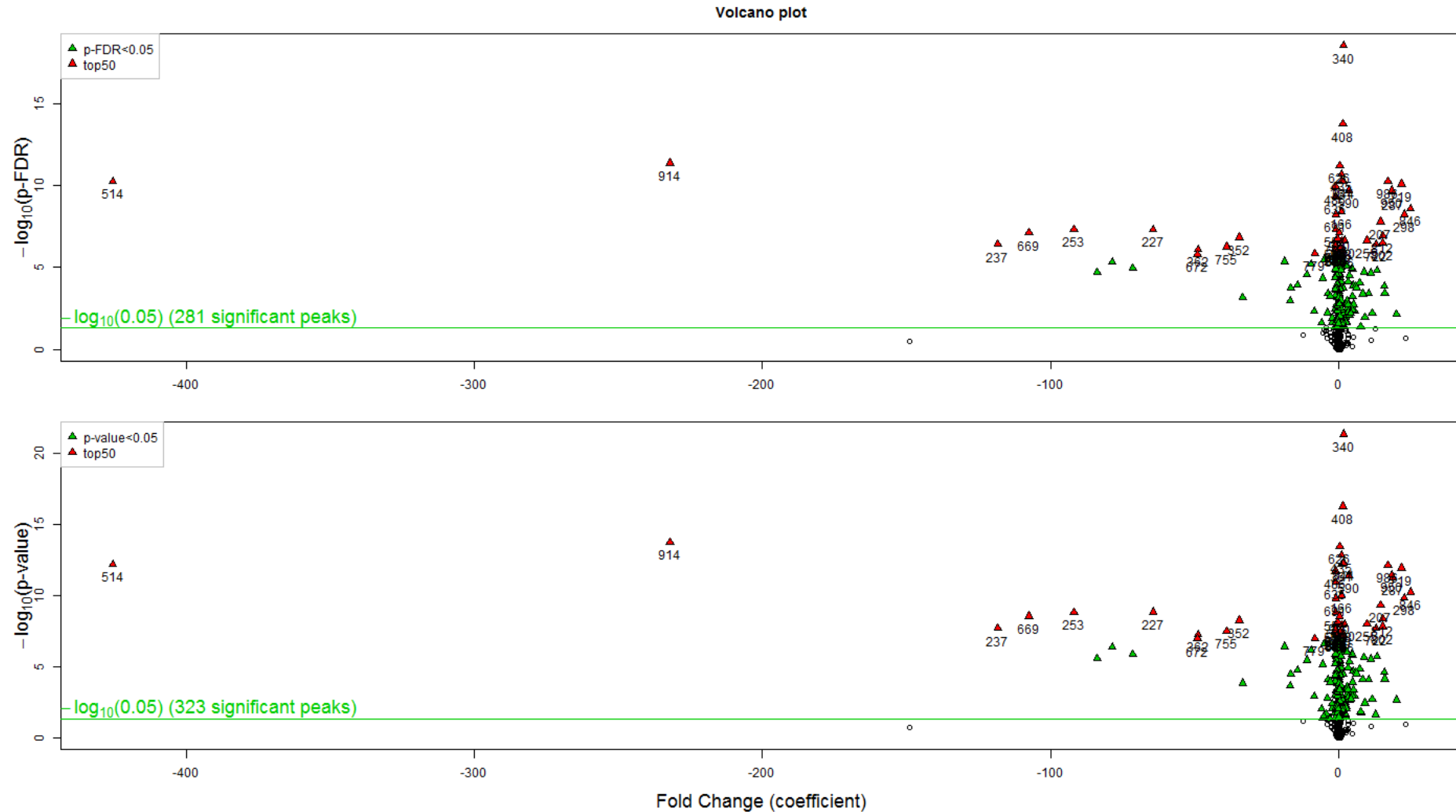
Latent group

- Association analysis
 - ANCOVA
 - Permutation



Statistical analysis (ANCOVA)

- Association analysis
 - ANCOVA output (Volcano plot)



outpath\ANCOVA\Volcanoplot_ACEi vs. NonMed.png

Statistical analysis (ANCOVA)

- Association analysis
 - ANCOVA output (numerical results)

outpath\ANCOVA\

VIF_threshold_10.csv

Group_Comparison	ACEivs.NonMed	ACEivs.NonMed	ACEivs.NonMed	ACEivs.NonMed
Variable	ACEi-NonMed(var)	gender	age	bmi
cluster_idx	1.1	1.1	1	1

covariate_select.csv

	ACEi
Dataset	0
gender	1
age	1
bmi	1
date	0

coef_ACEi vs. NonMed.csv

Peak_Index	mz	Ret_time.sec	ACEi_NonMed
4	60.07346	29.3075	0.193368544
5	68.97672	24.1263	-0.275392306
6	70.05911	32.3293	-13.3419297
7	72.07476	37.8294	-0.220116074
8	72.07494	64.0451	-6.089761406

p-value_ACEi vs. NonMed_details.csv

Peak_Index	mz	Ret_time.sec	(Intercept)	ACEi_NonMed	Gender	Age	BMI	cluster_idx	ACEi_NonMed:Rep
6	60.07116129	29.2696	1.07E-11	0.964754576	0.004593074	0.10313761	0.102492534	0.000784103	0.894761782
8	69.06404612	52.8168	0.000887385	0.001643264	0.002529177	0.007070659	0.000141511	NA	0.374987713
9	70.05927741	32.3226	0.01042616	0.005719337	0.013706893	0.000968562	NA	NA	0.024744527
20	84.0381544	48.7662	1.65E-05	0.44412925	0.048497137	0.482746084	0.953953795	4.00E-05	0.979548774
24	84.95377894	24.1371	4.03E-33	0.001005549	3.23E-11	4.07E-12	5.22E-19	4.04E-28	0.99475372

pFDR_ACEi vs. NonMed.csv

Peak_Index	mz	Ret_time.sec	ACEi_NonMed(pFDR)
4	60.07346317	29.3075	0.881473536
5	68.97671978	24.1263	0.14360528
6	70.05911424	32.3293	0.77584518
7	72.0747612	37.8294	0.020267411
8	72.07493563	64.0451	0.745393188

Empirical p-value_ACEi vs. NonMed

Peak_Index	mz	Ret_time.sec	ACEi_NonMed(epv)	ACEi_NonMed(epFDR)
8	69.06404612	52.8168	0.00990099	0.022442244
9	70.05927741	32.3226	0.02970297	0.059020188
24	84.95377894	24.1371	0.00990099	0.022442244
31	88.94712657	24.1306	0.00990099	0.022442244
51	104.1014407	29.0027	0.00990099	0.022442244

SMART

File Viewer Peak Analysis Statistical Methods

Resolution

m/z resolution 100

RT resolution 100

Intensity limit

Lower bound -Inf

Upper bound Inf

Retention time

Begin 20

Range 50

M/z

Begin 100

Range 100

Rotation angle

V angle 15

H angle 15

Hscale: 2 Vscale: 1.75

Refresh Save file Exit

ANCOVA(latent group)

PLS/PLS-DA

STATISTICAL ANALYSIS (2)

Statistical analysis (PLS/PLS-DA)

- Breast cancer data

Gene expression data

Peak_index	MZ	RT	GeneS	LHC3265	LHC3630	LHC4662	LHC5838	LHC6065
1	1	1	OR4F17	3.7453	5.3542	3.9132	3.9697	4.1630
2	2	2	14-Sep	8.1687	8.9836	8.6854	8.8927	8.9830
3	3	3	OR4F16	5.7183	7.7406	7.7985	7.0605	7.0725
4	4	4	GPAM	10.8207	8.1991	10.6819	8.3634	10.9824
5	5	5	LOC100287934	7.9763	8.1870	7.9481	8.3095	8.1858
6	6	6	FAM87A	6.0228	5.7201	5.7764	5.7067	5.9063
7	7	7	LOC643837	7.7452	6.6167	8.9223	7.5027	8.1036
8	8	8	SAMD11	7.0537	6.6805	6.9922	6.6381	6.8894
9	9	9	KLHL17	7.6677	7.2229	7.4896	7.0800	7.3386
10	10	10	PLEKHN1	7.5829	7.2255	7.6950	7.3089	7.3336

Cov file

SampleID	CAGE	DRACE/ETHNICITY
LHC3265	51	W
LHC3630	39	B
LHC3660	42	W
LHC4662	91	B
LHC5838	42	W
LHC6065	39	B
LHC6075	81	B

Factor file

SampleID	DGroup
LHC3265	NORMAL
LHC3630	NORMAL
LHC3660	NORMAL
LHC4662	NORMAL
LHC3264	TUMOR
LHC3631	TUMOR
LHC3661	TUMOR
LHC4333	TUMOR

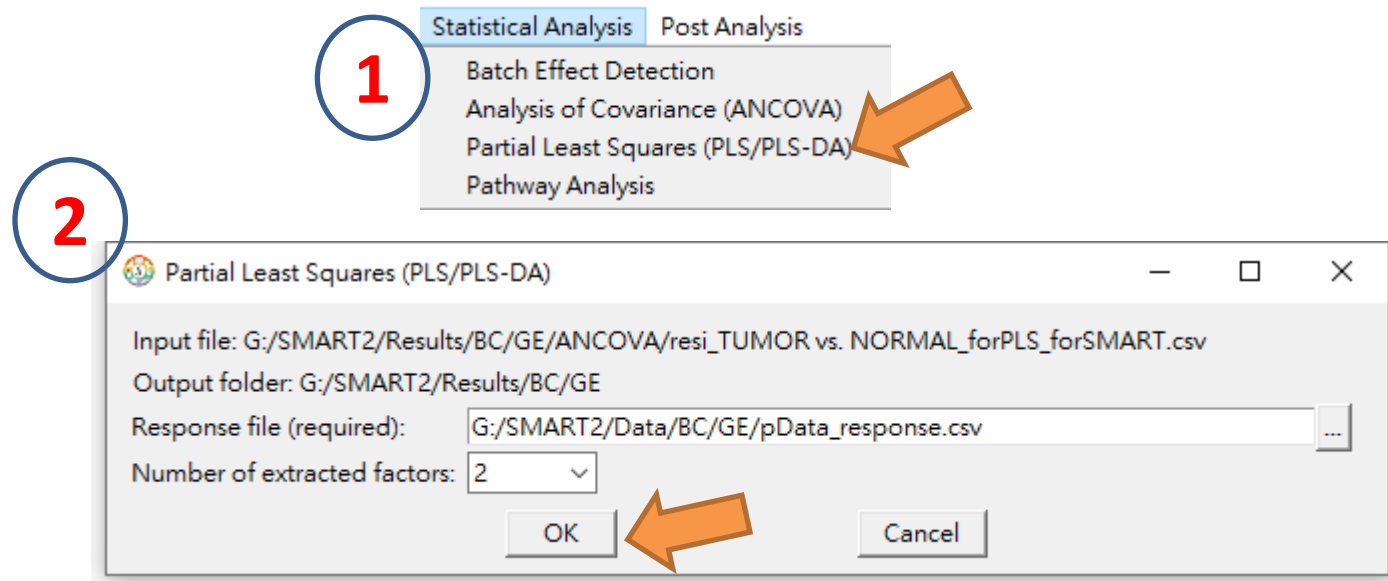
Metabolomics data

Peak_index	MZ	RT	Metabolite	LHC3265	LHC3630	LHC3660	LHC4662	LHC5838
1	1	1	1-arachidonoylglycerophosphoethanolamine*	-1.4956	-0.8435	-0.4623	-1.7410	0.3248
2	2	2	1-arachidonoylglycerophosphoinositol*	-1.2812	-1.2812	-0.7580	-1.2812	0.1697
3	3	3	1-linoleoylglycerophosphocholine	-2.7585	-2.7585	-0.1364	-2.7585	-2.7585
4	4	4	1-methylimidazoleacetate	-0.8876	-0.8876	-0.8876	-0.8876	-0.8876
5	5	5	1-methylnicotinamide	-1.2717	-2.1301	-0.2701	-2.5847	0.7415
6	6	6	1-myristoylglycerophosphocholine	-1.9470	-1.9470	-1.9470	-1.9470	-1.9470
7	7	7	1-oleoylglycerol (1-monoolein)	-4.8191	-4.8191	-4.8191	-4.8191	-4.8191
8	8	8	1-oleoylglycerophosphocholine	-3.3124	-3.3124	-0.4852	-3.3124	-3.3124
9	9	9	1-oleoylglycerophosphoethanolamine	-3.3418	-3.3418	-3.3418	-3.3418	-3.3418
10	10	10	1-palmitoylglycerol (1-monopalmitin)	-3.2395	-3.2395	-1.6936	-3.2395	-3.2395

Statistical analysis (PLS/PLS-DA)

- PLS-DA

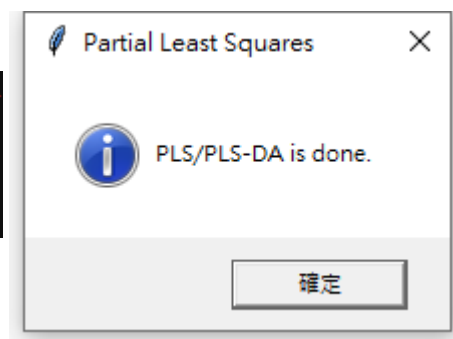
– **Input:** Peak abundance table **or** Residuals from ANCOVA are covariate-adjusted.



PA / Residual file

Peak_In	mz	Ret	LHC3265	LHC3630	LHC4662	LHC5838	LHC6065
1	1	1	-0.4827	1.1505	-0.1989	-0.2741	-0.0407
2	2	2	-0.3548	0.2817	-0.2449	0.4087	0.2811
3	3	3	-0.8935	0.9525	1.1760	0.4201	0.2843
4	4	4	1.9564	0.0220	3.1320	-0.6095	2.8053
5	5	5	-0.3283	-0.1210	-0.4228	0.0158	-0.1222
6	6	6	0.1040	-0.1262	0.1050	-0.2424	0.0600
7	7	7	0.1356	-0.7696	1.5441	-0.1083	0.7173
8	8	8	0.1454	-0.1397	0.1416	-0.2649	0.0692
9	9	9	0.3133	-0.1251	0.1743	-0.2801	-0.0094

```
PLS/PLS-DA - Please wait for PLS/PLS-DA processing 0 PLS-DA
108 samples x 20254 variables and 1 response
standard scaling of predictors and response(s)
      R2X(cum) R2Y(cum) Q2(cum) RMSEE pre ort pR2Y pQ2
Total 0.237    0.685    0.539 0.282  2  0 0.05 0.05
```

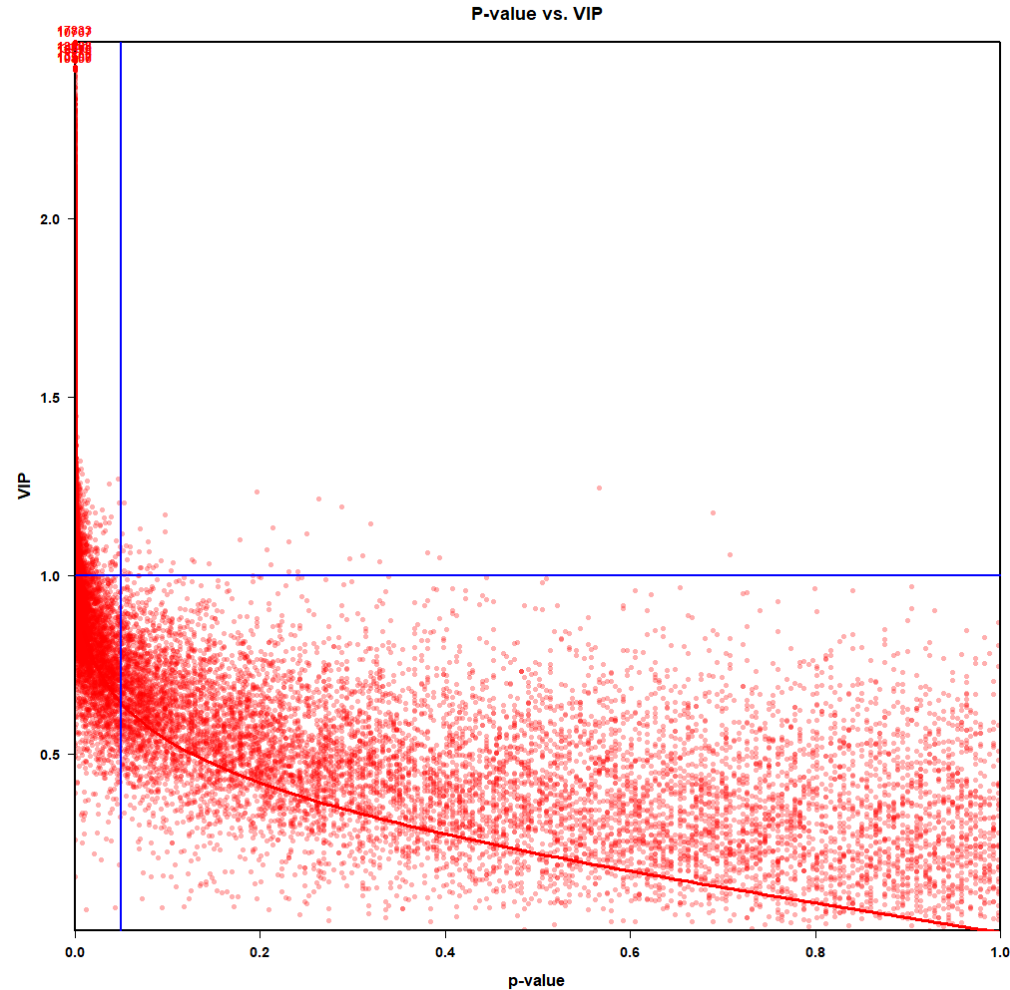


Response file

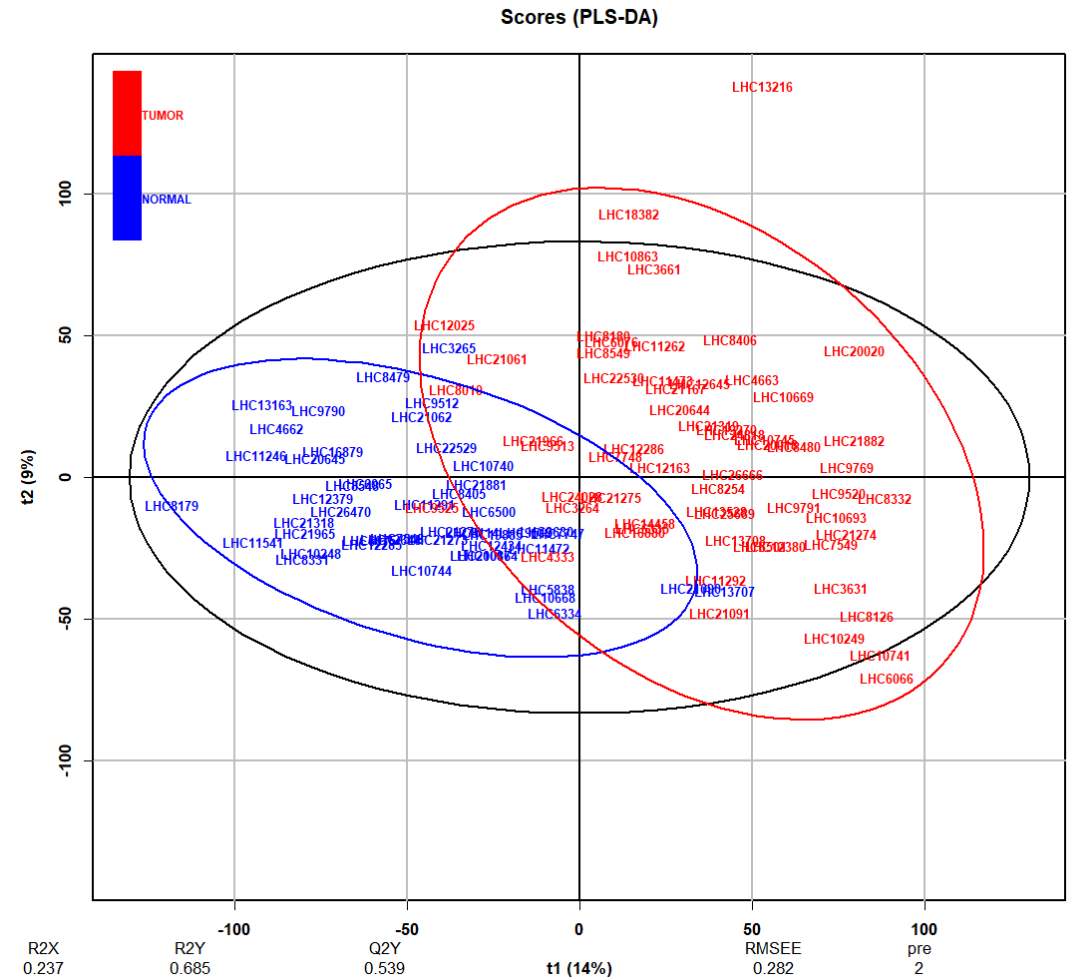
SampleID	DGroup
LHC3265	NORMAL
LHC3630	NORMAL
LHC3660	NORMAL
LHC4662	NORMAL
LHC3264	TUMOR
LHC3631	TUMOR
LHC3661	TUMOR
LHC4333	TUMOR

- PLS/PLS-DA output (figures)

outpath\PLS-DA\VIPvalues.png



outpath\PLS-DA\x_score.png



Statistical analysis (PLS/PLS-DA)

- PLS/PLS-DA output (numerical results)

outpath\PLS-DA\

summary.csv

Variation explanation										
R2X	R2X(cum)	R2Y	R2Y(cum)	Q2	Q2(cum)	Signif.	Iter.	RMSEE	pR2Y	pQ2
0.146	0.146	0.589	0.589	0.549	0.549	R1	1			
0.1	0.246	0.0905	0.679	0.0153	0.556	R1	1	0.285	0.05	0.05

VIPvalues.csv

Peak	mz	Ret_t	VIP	P-value
1	1	1	0.848680576	0.051310251
2	2	2	0.957861769	0.01262452
3	3	3	0.250738031	0.9629312
4	4	4	2.224399175	3.21E-12
5	5	5	0.60991584	0.062585973

x_score.csv

Sample	p1	p2
LHC10248	-79.65393937	-9.205999313
LHC10249	69.64932109	-22.63991244
LHC10668	-19.95370836	-34.39035425
LHC10669	48.6971083	45.06335989
LHC10693	83.12152012	-30.24721724
LHC10740	-41.28603181	12.68094071
LHC10741	71.66903023	-45.54072944

summary.csv
VIPvalues.csv
x_coefficient.csv
x_loading.csv
x_score.csv
x_weight.csv
VIPvalues.png
x_score.png

Resolution

m/z resolution

RT resolution

Intensity limit

Lower bound

Upper bound

Retention time

Begin

Range

M/z

Begin

Range

Rotation angle

V angle

H angle

Hscale: Vscale:

*Integrative **omics** pathway analysis (IOPA)*

STATISTICAL ANALYSIS (3)

Integrative *omics* pathway analysis (IOPA)

- Multi-omics data

- Gene expression
- Metabolomics

- Association analysis

- ANCOVA
 - Fold-change(beta value) and p-value

- Pathway database

- KEGG

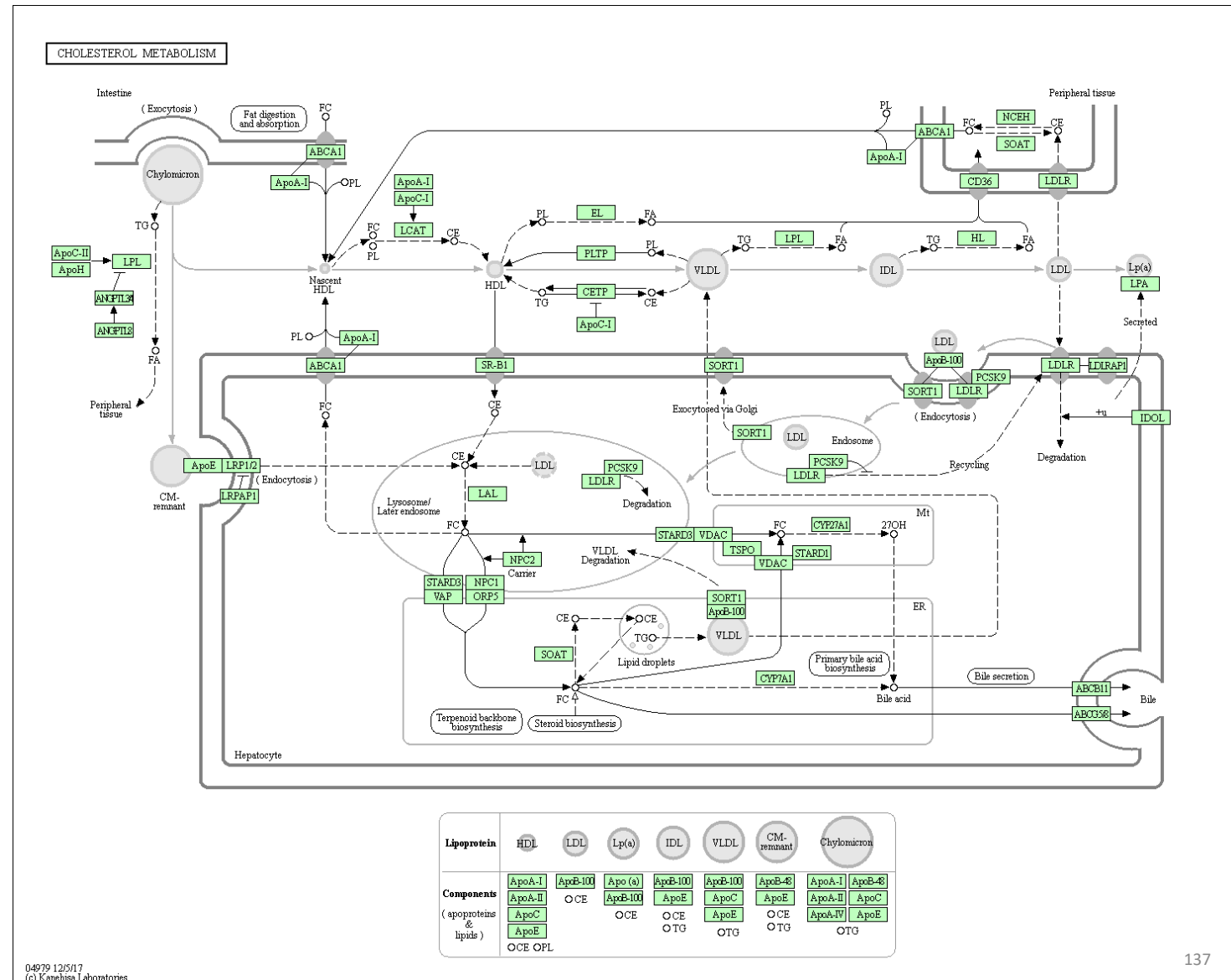
- Statistical methods

- ORA (Over-representation Analysis)
- SPIA (Signaling Pathway Impact Analysis)

$$PF(g_i) = \Delta E(g_i) + \sum_{j=1}^n \beta_{ij} \cdot \frac{PF(g_j)}{N_{ds}(g_j)}$$

- P-value combination

- Fisher
- Pbine (Yin-Chun Lin et. al., 2022)



Statistical analysis (IOPA)

- IOPA
 - Input: Fold-changes and p-values from ANCOVA

1

Statistical Analysis Post Analysis

Batch Effect Detection

Analysis of Covariance (ANCOVA)

Partial Least Squares (PLS/PLS-DA)

Pathway Analysis

2

Integrative Omics Pathway Analysis

Input file: G:/SMART2/Data/BC/BC_GEandMT_FC_PV_forIOPA.csv

Output folder: G:/SMART2/Results/BC

Parameter setting:

Inclusion criteria: Yes (p-value < 0.05 (0~1) and -0.005 <= logFC <= 0.005)

No

Pbine: Relative accuracy <= 1e-5

Run

Cancel

Input file		beta value	raw p-value
	ID	TUMOR_NORMAL_logFC	TUMOR_NORMAL_pv
Gene ID	1	-0.00110327	0.071059437
	2	-0.000916425	7.18E-05
	9	0.015210523	0.000932944
	10	0.000653423	0.630181778
	12	-0.012109371	0.012604048
	13	-0.003937972	2.75E-08
	14	-0.002344647	0.864390741
	15	-8.70E-06	0.609908795
	16	-0.000819847	0.006943451
	18	0.008067477	0.05162788
	C00064	0.000881739	0.031417287
	C00065	0.006986954	3.65E-11
	C00072	0.013958216	1.24E-07
	C00073	0.001264568	1.42E-07
	C00074	0.013968516	1.72E-06
	C00077	-0.000517312	2.16E-07
and ID	C00078	0.0037256	2.34E-10
	C00079	0.004466391	4.24E-07
	C00082	0.004255505	2.10E-06
	C00086	0.01605101	6.20E-08

Done pathway 301 : Diabetic cardiomyopathy..
Done pathway 302 : Viral myocarditis..
Done pathway 303 : Lipid and atherosclerosis..
Done pathway 304 : Fluid shear stress and atheros..
Post analysis and graph plotting
Progress 10.2
Progress 20.39

Peak Alignment and Annotation

Statistical Analysis - Integrative Omics Pathway Analysis is done.

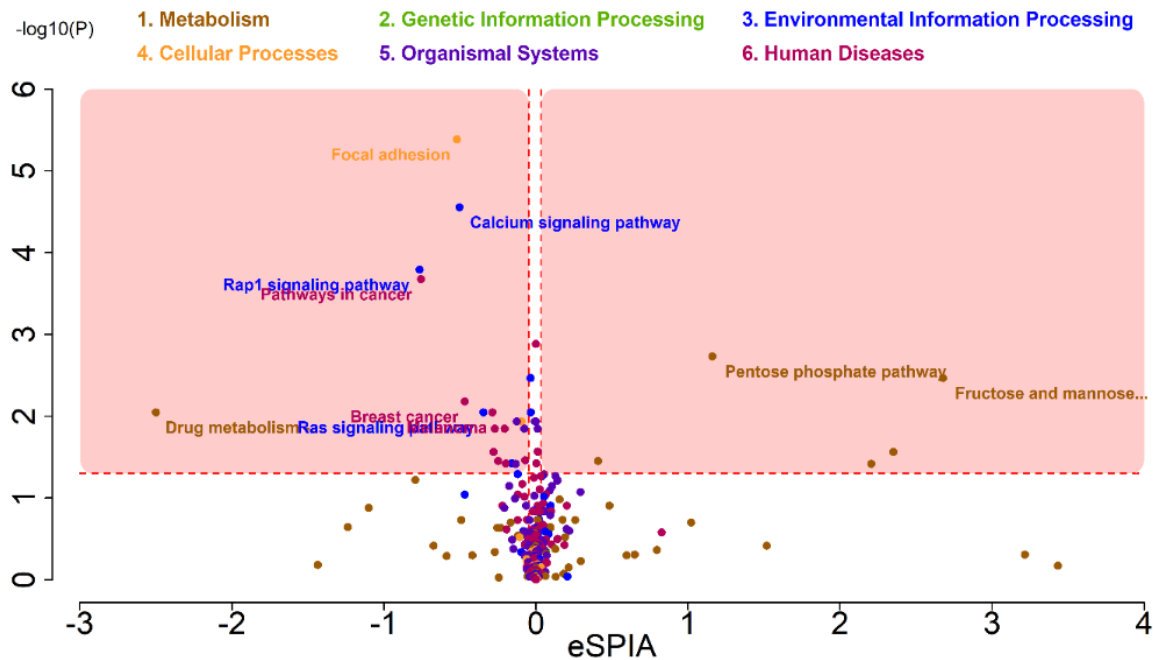
確定

Compound ID

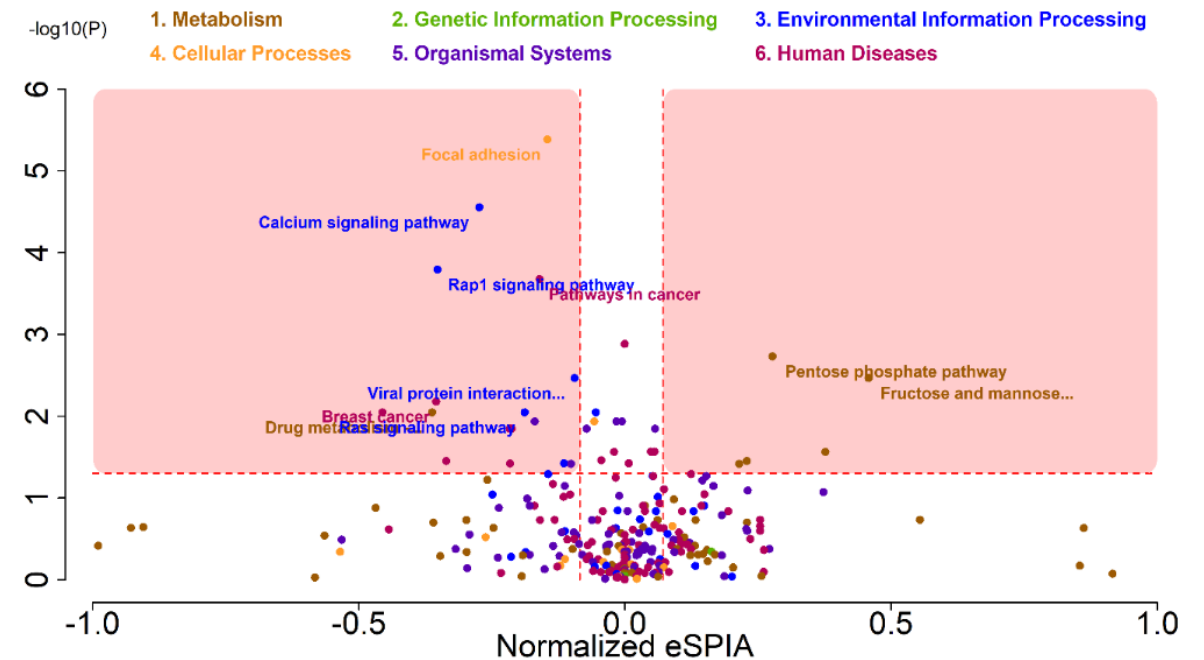
Statistical analysis (IOPA)

- IOPA output (figures)

outpath\IOPA\Pathway analysis_volcano.tiff



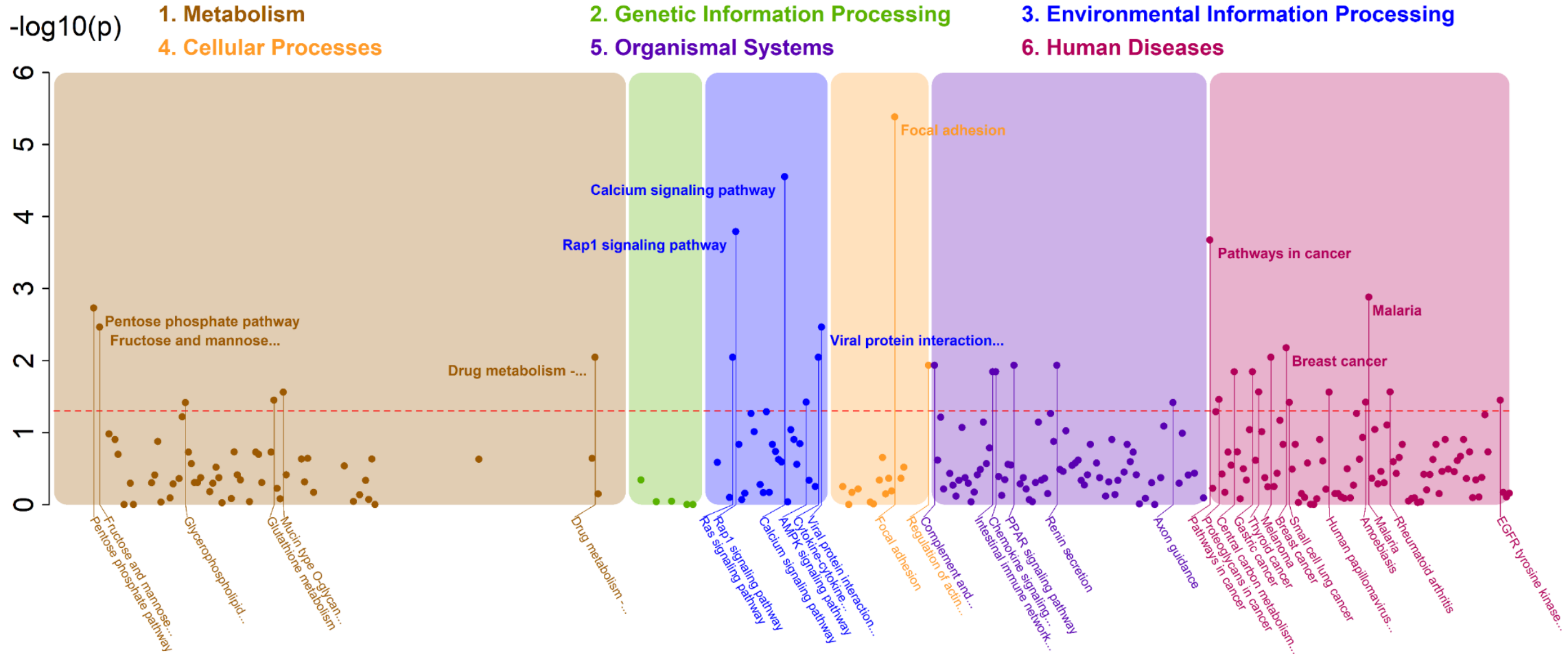
outpath\IOPA\Pathway analysis_volcano_normalized.tiff



Statistical analysis (IOPA)

- IOPA output (figures)

outpath\IOPA\Pathway analysis_manhattan.tiff



Statistical analysis (IOPA)

- IOPA output (numerical results)

outpath\IOPA\

IOPA_fcl_fcu_pverr1e-05.csv



Name	ID	G_size	deG_n	G_pORA	C_size	deC_n	C_pORA	P_size	deP_n	pORA	pORA_Bc	pORA_FI	raw_eSPI	eSPIA	eSPIA_nc	peSPIA	peSPIA_F	peSPIA_Fp	pFisher	pFisher_F	pFisher_Fp	pPbine	pPbine_B	pPbine_F	Status	KEGGLink
Focal adhesion	4510	199	33	2.51E-08	0	0	1	199	33	9.64E-08	2.62E-05	1.31E-05	-0.52457	-0.51835	-0.14581	0.002	0.542	0.1355	4.51E-09	1.23E-06	6.13E-07	2.22E-08	6.00E-06	6.00E-06	Inhibited	http://www
Calcium signaling pathway	4020	239	28	0.000216	0	0	1	239	28	0.000539	0.146561	0.006979	-0.50532	-0.50228	-0.27379	5.00E-06	0.001355	0.000678	5.59E-08	1.52E-05	5.06E-06	1.71E-07	4.62E-05	2.31E-05	Inhibited	http://www
Rap1 signaling pathway	4015	209	30	2.52E-06	0	0	1	209	30	7.81E-06	0.002123	0.000425	-0.77625	-0.7646	-0.35228	0.004	1	0.180667	5.71E-07	0.000155	3.88E-05	1.78E-06	0.00048	0.00016	Inhibited	http://www
Pathways in cancer	5200	529	55	1.01E-05	4	0	1	533	55	6.00E-05	0.016323	0.001814	-0.7608	-0.75518	-0.16013	0.001	0.271	0.090333	1.06E-06	0.000288	5.76E-05	3.13E-06	0.000845	0.000211	Inhibited	http://www
Malaria	5144	49	14	3.35E-07	0	0	1	49	14	6.67E-07	0.000181	6.05E-05	0	0	0	1	1	1	1.02E-05	0.002762	0.00046	2.43E-05	0.006569	0.001314	Inhibited	http://www
Pentose phosphate pathway	30	29	5	0.022018	8	6	0.020092	37	11	6.89E-06	0.001874	0.000425	1.132867	1.160247	0.277502	0.182	1	0.679356	1.83E-05	0.004977	0.000711	4.15E-05	0.011198	0.001866	Activated	http://www
Fructose and mannose metab	51	33	4	0.114111	7	6	0.006934	40	10	9.35E-05	0.025419	0.002118	2.637291	2.677882	0.457923	0.039	1	0.529095	4.93E-05	0.013405	0.001489	0.000101	0.027249	0.003406	Activated	http://www
Viral protein interaction with	4061	96	18	6.36E-06	0	0	1	96	18	1.38E-05	0.003767	0.000628	-0.03463	-0.03399	-0.09449	0.251	1	0.738832	4.72E-05	0.012831	0.001489	9.69E-05	0.02617	0.003406	Inhibited	http://www
Breast cancer	5224	147	18	0.001661	0	0	1	147	18	0.003078	0.837214	0.024223	-0.46963	-0.46674	-0.35471	0.003	0.813	0.1626	0.000116	0.031628	0.003163	0.000222	0.059848	0.00665	Inhibited	http://www
Drug metabolism - cytochrome	982	59	12	9.63E-05	0	0	1	59	12	0.000165	0.044868	0.003205	-2.50511	-2.49872	-0.36212	0.12	1	0.616306	0.000234	0.063695	0.004714	0.000419	0.113101	0.008986	Inhibited	http://www
Ras signaling pathway	4014	232	25	0.001543	0	0	1	232	25	0.003295	0.896239	0.024223	-0.35004	-0.34545	-0.18837	0.006	1	0.20325	0.000234	0.063622	0.004714	0.000418	0.112984	0.008986	Inhibited	http://www
Cytokine-cytokine receptor i	4060	287	35	1.60E-05	0	0	1	287	35	5.18E-05	0.0141	0.001762	-0.03616	-0.03346	-0.05458	0.397	1	0.879675	0.000243	0.066002	0.004714	0.000433	0.116817	0.008986	Inhibited	http://www
Melanoma	5218	72	11	0.002296	0	0	1	72	11	0.003563	0.969157	0.02485	-0.29144	-0.28558	-0.45544	0.005	1	0.193571	0.000213	0.057837	0.004714	0.000384	0.103609	0.008986	Inhibited	http://www
PPAR signaling pathway	3320	72	14	4.38E-05	0	0	1	72	14	8.10E-05	0.022041	0.002118	-0.00688	-0.00688	-0.01593	0.44	1	0.889851	0.000401	0.109023	0.00647	0.000683	0.184344	0.011614	Inhibited	http://www
Regulation of actin cytoskeleton	4810	215	28	3.39E-05	0	0	1	215	28	9.12E-05	0.024815	0.002118	-0.10275	-0.09474	-0.05758	0.377	1	0.879675	0.000388	0.105507	0.00647	0.000663	0.178929	0.011614	Inhibited	http://www
Renin secretion	4924	69	12	0.00045	2	1	0.566055	71	13	0.000271	0.073626	0.004331	-0.12699	-0.12576	-0.1694	0.133	1	0.616306	0.000404	0.109986	0.00647	0.000688	0.185824	0.011614	Inhibited	http://www
Complement and coagulation	4610	85	16	1.95E-05	0	0	1	85	16	3.90E-05	0.010619	0.001517	-0.00525	0.000728	-0.00529	0.996	1	1	0.000434	0.117978	0.006554	0.000734	0.198064	0.011651	Activated	http://www
Chemokine signaling pathway	4062	188	25	6.20E-05	0	0	1	188	25	0.000152	0.041463	0.003189	-0.08032	-0.0769	-0.07157	0.389	1	0.879675	0.000636	0.173113	0.008459	0.00104	0.280745	0.014279	Inhibited	http://www
Intestinal immune network for	4672	45	10	0.000168	0	0	1	45	10	0.000268	0.072787	0.004331	0.011799	0.011799	0.056716	0.24	1	0.738832	0.000684	0.186098	0.008459	0.001111	0.299849	0.014279	Activated	http://www

Source on Save

SMART

File Viewer Peak Analysis Statistical Analysis Post Analysis

```

1 rm(list = ls())
2 gc()
3 library(tcltk)
4 setwd("C:/forDemo/code/SMART2.0")
5
6 getwd()
7
8 load("dbMetabo.RData")
9 load("function_ls.RData")
10 load("hsaSPIA_reversible(cumulative_sum).R")
11 load("KEGG_pathway_class.Rdata")
12
13 file <- list.files(pattern = ".R")
14 file <- file[!grep("SMART|Rdata|RData", file)]
15
16 file
17
18 sapply(file, function(x)source(x))
19
20
21 #msconvert C:/Users/yujen/AppData/Local/Temp/SMART2.0/SMART2.0.ms
22
23

```

23:1 (Top Level)

Console

Terminal

Background Jobs

R 4.4.1 C:/forDemo/code/SMART2.0/

```

Done pathway 295 : Systemic lupus erythematosus
Done pathway 296 : Rheumatoid arthritis..
Done pathway 297 : Allograft rejection..
Done pathway 298 : Graft-versus-host disease..
Done pathway 299 : Arrhythmogenic right ventric
Done pathway 300 : Dilated cardiomyopathy..
Done pathway 301 : Diabetic cardiomyopathy..
Done pathway 302 : Viral myocarditis..
Done pathway 303 : Lipid and atherosclerosis..
Done pathway 304 : Fluid shear stress and atheros..
Post analysis and graph plotting
r = 0.105640860400678
>

```

Resolution

m/z resolution 100

RT resolution 100

Intensity limit

Lower bound -Inf

Upper bound Inf

Retention time

Begin 20

Range 50

M/z

Begin 100

Range 100

Rotation angle

V angle 15

H angle 15

Hscale: 2

Vscale: 1.75

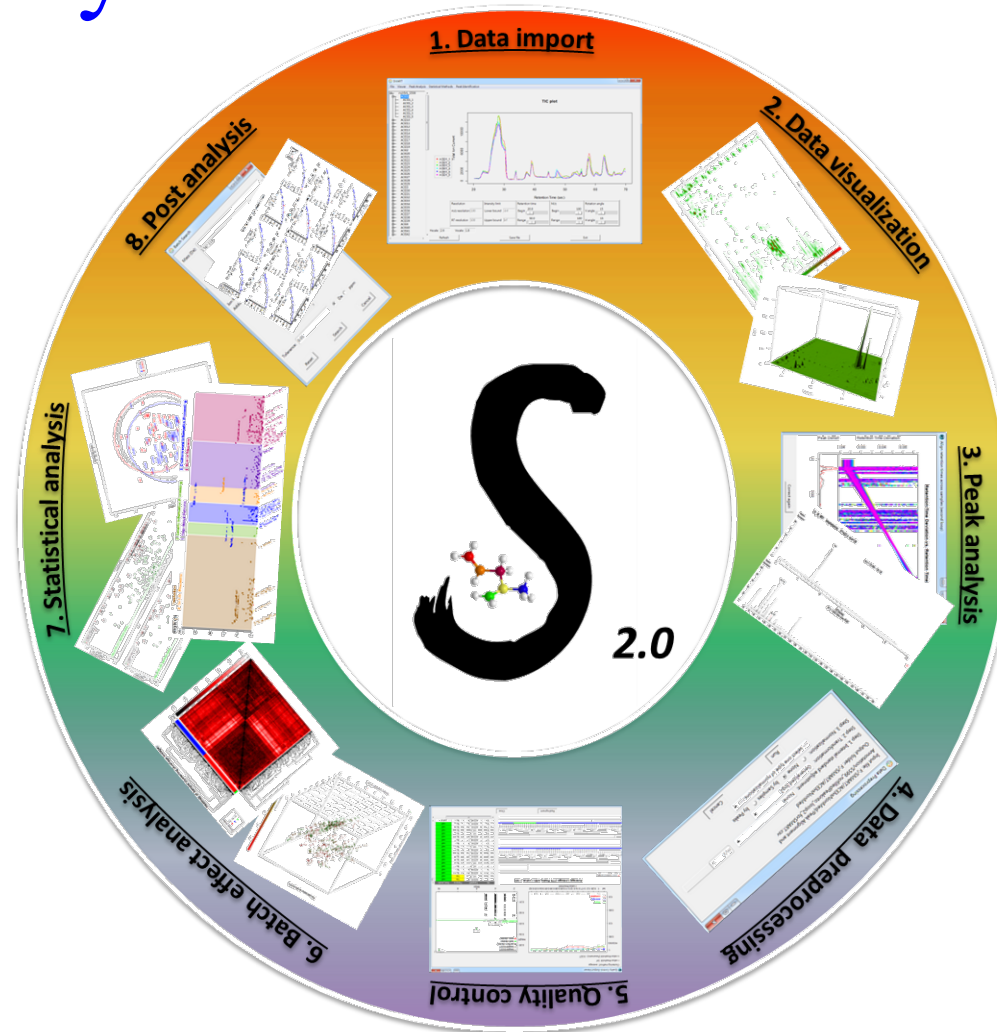
Refresh

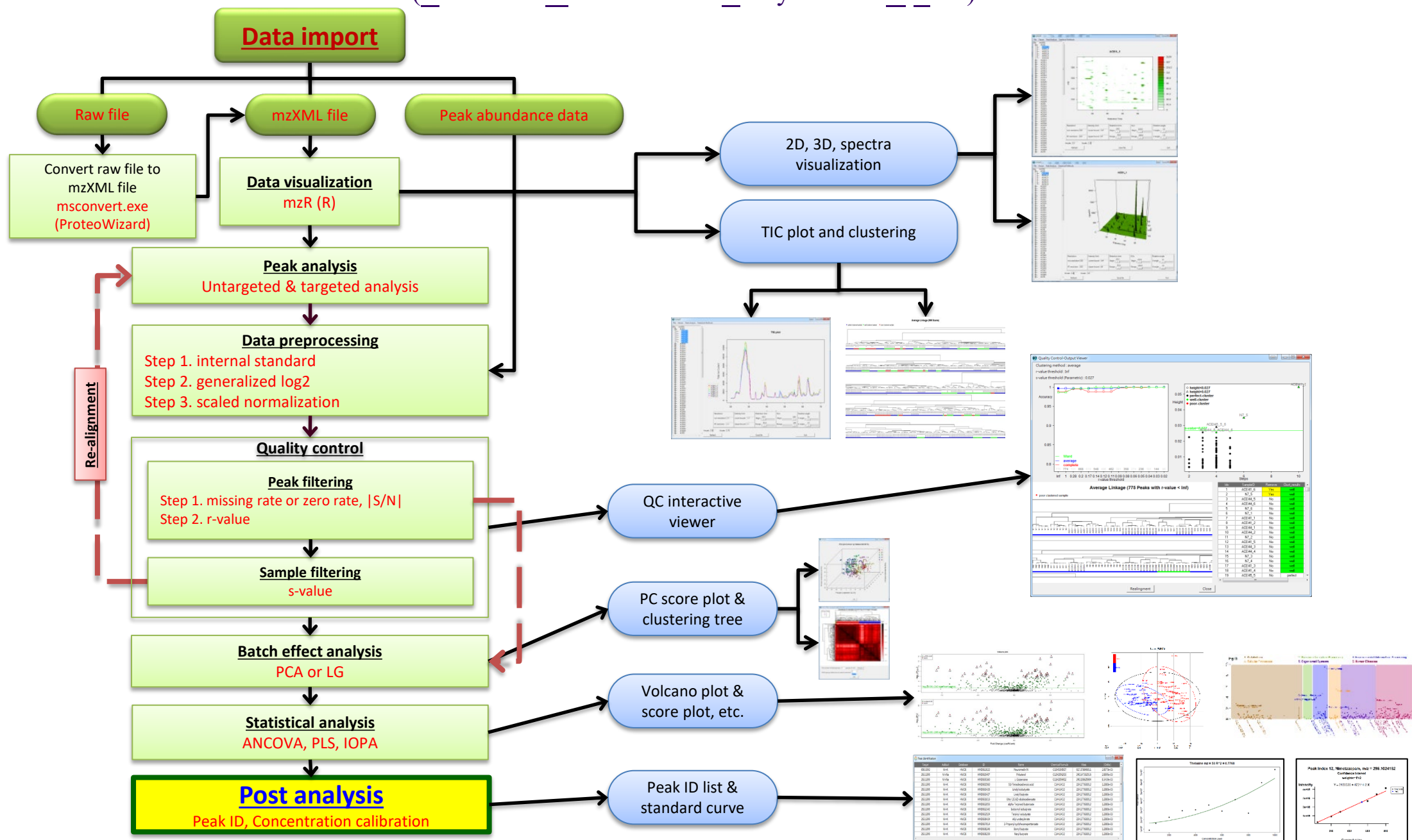
Save file

Exit

Statistical Metabolomics Analysis – an R Tool

8. Post analysis





Peak identification

POST ANALYSIS (1)

Post analysis (Peak identification)

- Input m/z

Batch Search

Output folder: G:/SMART2/Results/Drug

Mass (Da):

370.1642727
286.1431455
304.1538273
312.1587545
315.2302273
136.0803727
150.1020727
194.1172545
180.0865727
238.0988455

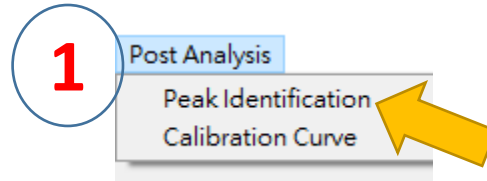
Ion Mode: ☐ Neutral ☒ Positive ☐ Negative

Adduct:

M+NH4
M+Na
M+K
M+IsoProp+Na+H
M+IsoProp+H
M+H+NH4
M+H+Na
M+H+K
M+H+2Na
M+H

Tolerance: 50 ☐ Da ☒ ppm

Reset Search Cancel



ANCOVA

Peak_Index	mz	Ret_time.sec	ACEi_NonMed
724	512.3515	116.3137	1.67E-09
425	321.3204	189.6585	5.66E-06
162	173.1252	60.0519	6.44E-06
179	183.111	52.2998	6.44E-06
308	261.1474	53.2244	6.44E-06
532	377.1709	155.9171	6.44E-06
864	619.3877	70.1135	6.44E-06
375	304.2394	155.9289	7.49E-06
427	322.3229	189.6408	7.49E-06

3

Peak Identification

Target	Adduct	Database	ID	Name	Chemical Formula	Mass	Delta
656.3392	M+K	HMDB	HMDB13022	Neuromedin N	C32H51N5O7	617.378849011	2.8070e-03
263.1395	M+Na	HMDB	HMDB15407	Pirbuterol	C12H20N2O3	240.147392516	2.8895e-03
263.1395	M+Na	HMDB	HMDB39160	L-Gizzerosine	C11H20N4O2	240.158625904	8.3439e-03
263.1395	M+K	HMDB	HMDB00560	5,8-Tetradecadienoic acid	C14H24O2	224.177630012	1.2880e-03
263.1395	M+K	HMDB	HMDB30426	Linalyl isobutyrate	C14H24O2	224.177630012	1.2880e-03
263.1395	M+K	HMDB	HMDB30427	Linalyl butyrate	C14H24O2	224.177630012	1.2880e-03
263.1395	M+K	HMDB	HMDB31015	Ethyl (2E,6Z)-dodecadienoate	C14H24O2	224.177630012	1.2880e-03
263.1395	M+K	HMDB	HMDB32053	alpha-Terpineol butanoate	C14H24O2	224.177630012	1.2880e-03
263.1395	M+K	HMDB	HMDB32342	Isobornyl isobutyrate	C14H24O2	224.177630012	1.2880e-03
263.1395	M+K	HMDB	HMDB32524	Terpinyl isobutyrate	C14H24O2	224.177630012	1.2880e-03
263.1395	M+K	HMDB	HMDB36424	Allyl undecylenate	C14H24O2	224.177630012	1.2880e-03
263.1395	M+K	HMDB	HMDB37814	2-Propenyl cyclohexanepentanoate	C14H24O2	224.177630012	1.2880e-03
263.1395	M+K	HMDB	HMDB38246	Bornyl butyrate	C14H24O2	224.177630012	1.2880e-03
263.1395	M+K	HMDB	HMDB38259	Neryl butyrate	C14H24O2	224.177630012	1.2880e-03

HMDB & MassBank

Post analysis (Peak identification)

- Peak ID output (numerical results)

outpath\Peak Identification\

peakID_search_results_2023-03-31 18-09-07.csv



Target	Adduct.Model	DB	ID	Name	Chem.Formu	Mass	abs.delta
512.3515 M		HMDB	HMDB31879	11a,12a-Epoxy-3b-hydroxy-28,13-oleananolid 3-acetate	C32H48O5	512.3501746	0.001325354
512.3515 M		HMDB	HMDB34646	3beta-Acetoxy-12-oxo-28,13beta-oleananolid	C32H48O5	512.3501746	0.001325354
512.3515 M		HMDB	HMDB35313	Ganoderic acid S	C32H48O5	512.3501746	0.001325354
512.3515 M		HMDB	HMDB35330	Ganoderic acid Mf	C32H48O5	512.3501746	0.001325354
512.3515 M		HMDB	HMDB35332	Ganoderic acid X	C32H48O5	512.3501746	0.001325354
512.3515 M		HMDB	HMDB36672	3alpha-Acetoxy-11-keto-beta-boswellic acid	C32H48O5	512.3501746	0.001325354
173.1252 M		HMDB	HMDB59607	apo-[3-methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)]	C7H15N3O2	173.1164267	0.008773261
183.111 M		HMDB	HMDB40067	4-Butyl-5-propylthiazole	C10H17NS	183.1081702	0.002829761
183.111 M		HMDB	HMDB40083	2,4-Dimethyl-5-pentylthiazole	C10H17NS	183.1081702	0.002829761
183.111 M		HMDB	HMDB40084	4,5-Dimethyl-2-pentylthiazole	C10H17NS	183.1081702	0.002829761

SMART

File Viewer Peak Analysis Statistical Analysis Post Analysis

Test

Resolution	Intensity limit	Retention time	M/z	Rotation angle
m/z resolution 100	Lower bound -Inf	Begin 20	Begin 100	V angle 15
RT resolution 100	Upper bound Inf	Range 50	Range 100	H angle 15

Hscale: 2 Vscale: 1.75

Refresh Save file Exit

Peak identification

Concentration calibration

- *Calibration curve construction*
- *Concentration calculation*

POST ANALYSIS (2)

Calibration curve construction

Calibration curve construction

- Input file : peak abundance data
 - Compound name and peak abundance in different concentration levels (required)
 - Sample name: D1_50_MS1 ...
 - Rule: **samplID_conc_MSx**
- Method :
 - Regression (linear or quadratic)
 - Weight (1, 1/x, 1/x²)
 - Outlier detection
 - None, Cook's D, 95% CI, Deviation
- Output file :
 - Per drug : PID1_Heroin_mz370.1642727_lm.tiff
 - Summarized files
 - Calibration-curve_info.csv
 - Peak_abundance_S10_P12.csv

Input file: peak abundance data (from Peak analysis)
-- standard compounds

Peak_Index	Name	mz_est	Ret_Time_est.sec	D1_50_MS1	D10_900_MS1	D11_1000_MS1	D2_100_MS1	D3_200_MS1	D4_300_MS1	D5_400_MS1
1	Heroin	370.1643	685.3724	20454233	4.85E+08	5.37E+08	33246830	65461545	1.08E+08	1.59E+08
2	Morphine	286.1431	146.2696	41215408	5.53E+08	6.23E+08	55632394	1.11E+08	1.59E+08	1.59E+08
3	Cocaine	304.1538	701.136	67391965	1.91E+09	2.16E+09	1.09E+08	2.8E+08	4.54E+08	4.54E+08
4	Thebaine	312.1588	678.8176	13918057	6.54E+08	7.9E+08	22156818	57057964	1.12E+08	1.12E+08
5	delta9-THC	315.2302	1513.319	584689	12324894	10750614	1040859	3017275	4419165	4419165
6	Amphetamine	136.0804	419.1944	28233349	38237498	37707935	30552141	36745807	37802678	37802678
7	MA	150.1021	496.8365	88366857	4.84E+08	5.19E+08	1.14E+08	1.84E+08	2.48E+08	2.48E+08
8	MDMA	194.1173	538.9991	57005641	1.36E+09	1.61E+09	77917415	1.73E+08	3.32E+08	3.32E+08
9	Love Drug	180.0866	505.6407	2977060	32927321	40623589	3508615	5215888	7552811	7552811
10	Ketamine	238.0988	599.9313	65361457	1.9E+09	2.12E+09	1.1E+08	3.04E+08	5.13E+08	5.13E+08
11	FM2	314.093	1044.274	23235201	4.47E+08	5.37E+08	37148568	83573478	1.28E+08	1.28E+08
12	Nimetazepam	296.1024	1040.522	27111684	5.03E+08	5.88E+08	41037216	91247724	1.44E+08	1.44E+08

Output file: Calibration-curve_info.csv

Peak_Index	Name	mz	Ret_time.sec	model	weights	Original	concentration	theta1
1	Heroin	370.1643	685.3724	linear	unweighted		50;100;200;300;400;600;700;800;900;1000	-4.9E+
1	Heroin	370.1643	685.3724	linear	1/x		50;100;200;300;400;600;700;800;900;1000	-1.5E+
1	Heroin	370.1643	685.3724	linear	1/x ²		50;100;200;300;400;600;700;800;900;1000	-3676
1	Heroin	370.1643	685.3724	quadratic	unweighted		50;100;200;300;400;600;700;800;900;1000	19931
1	Heroin	370.1643	685.3724	quadratic	1/x		50;100;200;300;400;600;700;800;900;1000	14796
1	Heroin	370.1643	685.3724	quadratic	1/x ²		50;100;200;300;400;600;700;800;900;1000	11317
2	Morphine	286.1431	146.2696	linear	unweighted		50;100;200;300;400;600;700;800;900;1000	-2.2E+
2	Morphine	286.1431	146.2696	linear	1/x		50;100;200;300;400;600;700;800;900;1000	2718
2	Morphine	286.1431	146.2696	linear	1/x ²		50;100;200;300;400;600;700;800;900;1000	12145
2	Morphine	286.1431	146.2696	quadratic	unweighted		50;100;200;300;400;600;700;800;900;1000	27509
2	Morphine	286.1431	146.2696	quadratic	1/x		50;100;200;300;400;600;700;800;900;1000	25624
2	Morphine	286.1431	146.2696	quadratic	1/x ²		50;100;200;300;400;600;700;800;900;1000	24114

Post analysis (Concentration calibration)

- Calibration curve construction

— Input: Peak abundance table

1

Post Analysis

Peak Identification

Concentration Calibration

Calibration Curve Construction

Concentration Calculation

2

Calibration Curve Construction

Step1: Import Data -> Step2: Quality Control -> Step3: Parameter Setting

Input file:

Output folder:

Missing data:

Units of Concentration:

Column Information

Please provide column information:

Column index of peak index:	1
Column index of name:	2
Column index of mass:	3
Column index of retention time:	4
Column index of first sample:	5

unit:

Data Import

 Data import is done.
0 out of 10 non-informative replicate sample(s) are excluded.
0 out of 12 non-informative peak(s) are excluded.

3

Calibration Curve Construction

Step1: Import Data -> Step2: Quality Control -> Step3: Parameter Setting

Input file: G:/SMART2/Results/Drug/Concentration Calibration/S11_peak_abundance_MS1_forSMART_no500.csv

Output folder: G:/SMART2/Results/Drug

Sample Selection:

Sample	Sample Selection
	D1_50_MS1
	D10_900_MS1
	D11_1000_MS1
	D2_100_MS1
	D3_200_MS1
	D4_300_MS1
	D5_400_MS1
	D7_600_MS1
	D8_700_MS1
	D9_800_MS1

>> <<

Retention time (RT) and m/z: ☐ Yes ☒ RT: From to (sec) and ☐ m/z: From to

☐ No

Post analysis (Concentration calibration)

- Calibration curve construction
 - Input: Peak abundance table

4

Calibration Curve Construction

Step1: Import Data -> Step2: Quality Control -> Step3: Parameter Setting

Input file: G:/SMART2/Results/Drug/Concentration Calibration/S10_P12_202405122033/Peak_abundance_S10_P12.csv

Output folder: G:/SMART2/Results/Drug

of sample: 10

of peak: 12

Parameter setting:

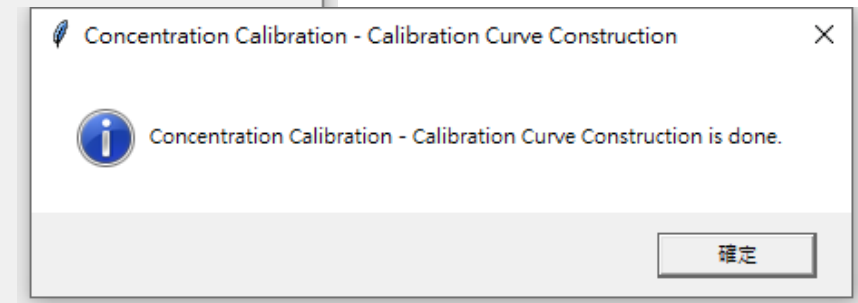
Model: ☒ Linear ☒ Quadratic

Weighting: ☒ unweighted ☒ 1/x ☒ 1/x^2

Outlier Detection: ☐ No

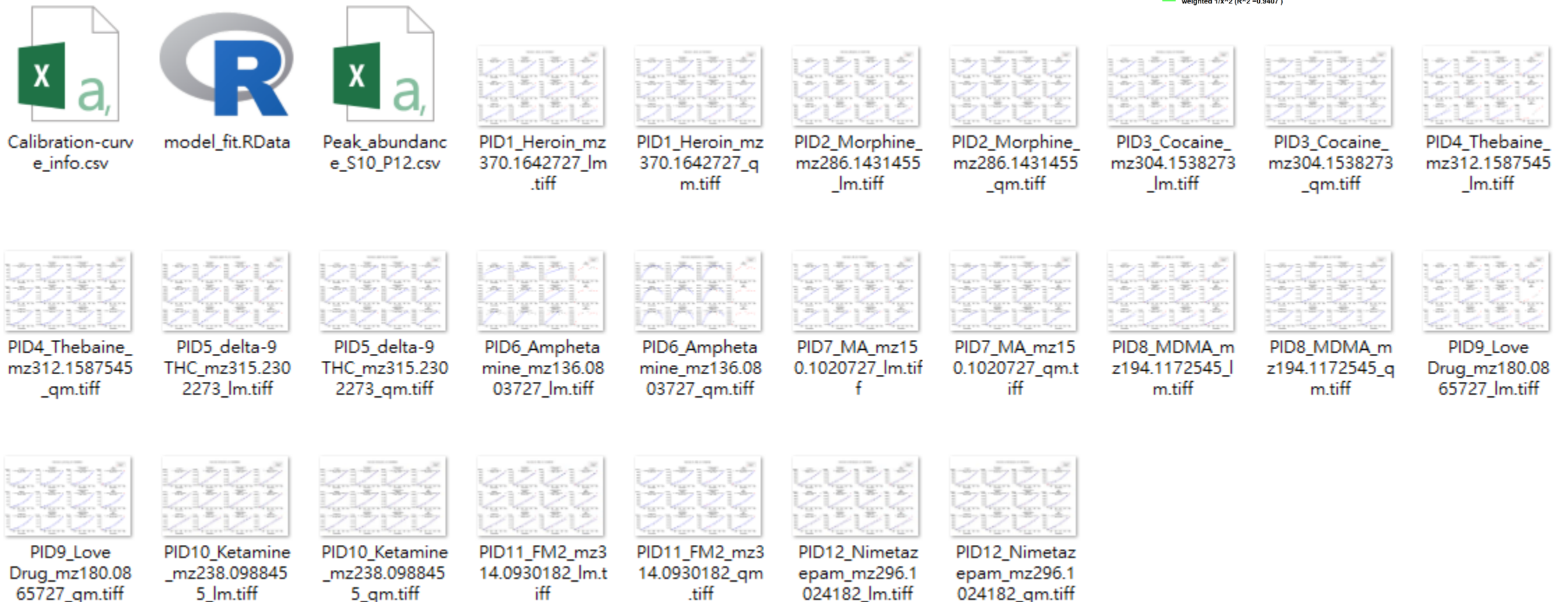
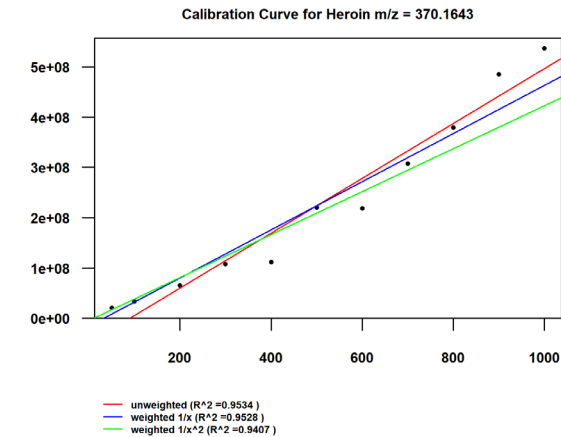
☒ Yes (☒ Cook's Distance, ☒ Confidence Interval (0~1), ☒ Deviation % (0~100))

Run



Calibration curve construction

- Output file :
 - Calibration curve
 - Summarized files
 - Calibration-curve_info.csv
 - Peak_abundance_S10_P12.csv



Calibration curve construction

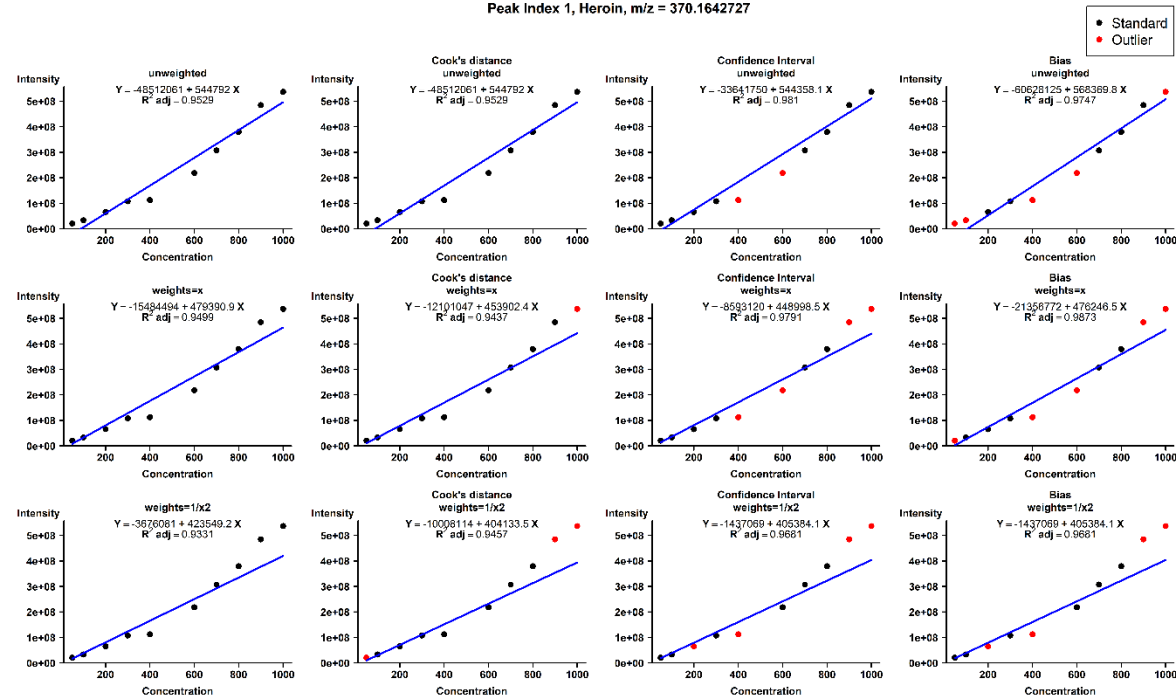
Heroin

Output file :

- Calibration curve
- PID1_Heroin_mz370.1642727_lm.tiff
- PID1_Heroin_mz370.1642727_qm.tiff

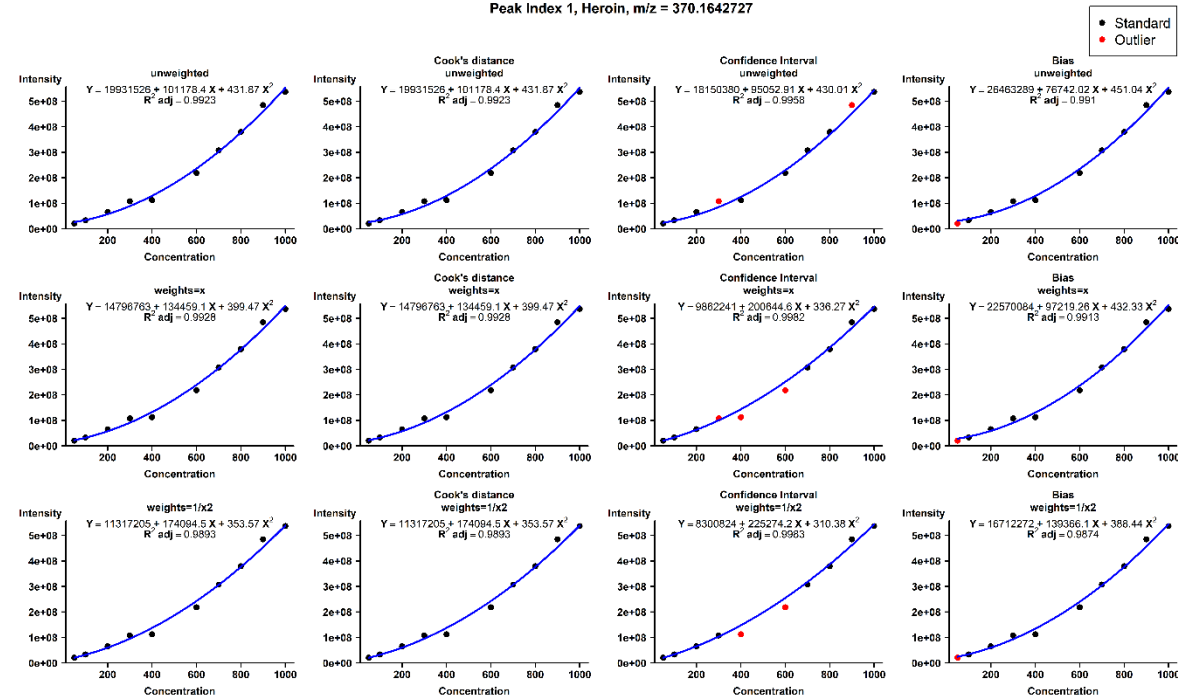
Linear

Peak Index 1, Heroin, m/z = 370.1642727



Quadratic

Peak Index 1, Heroin, m/z = 370.1642727



Calibration curve construction

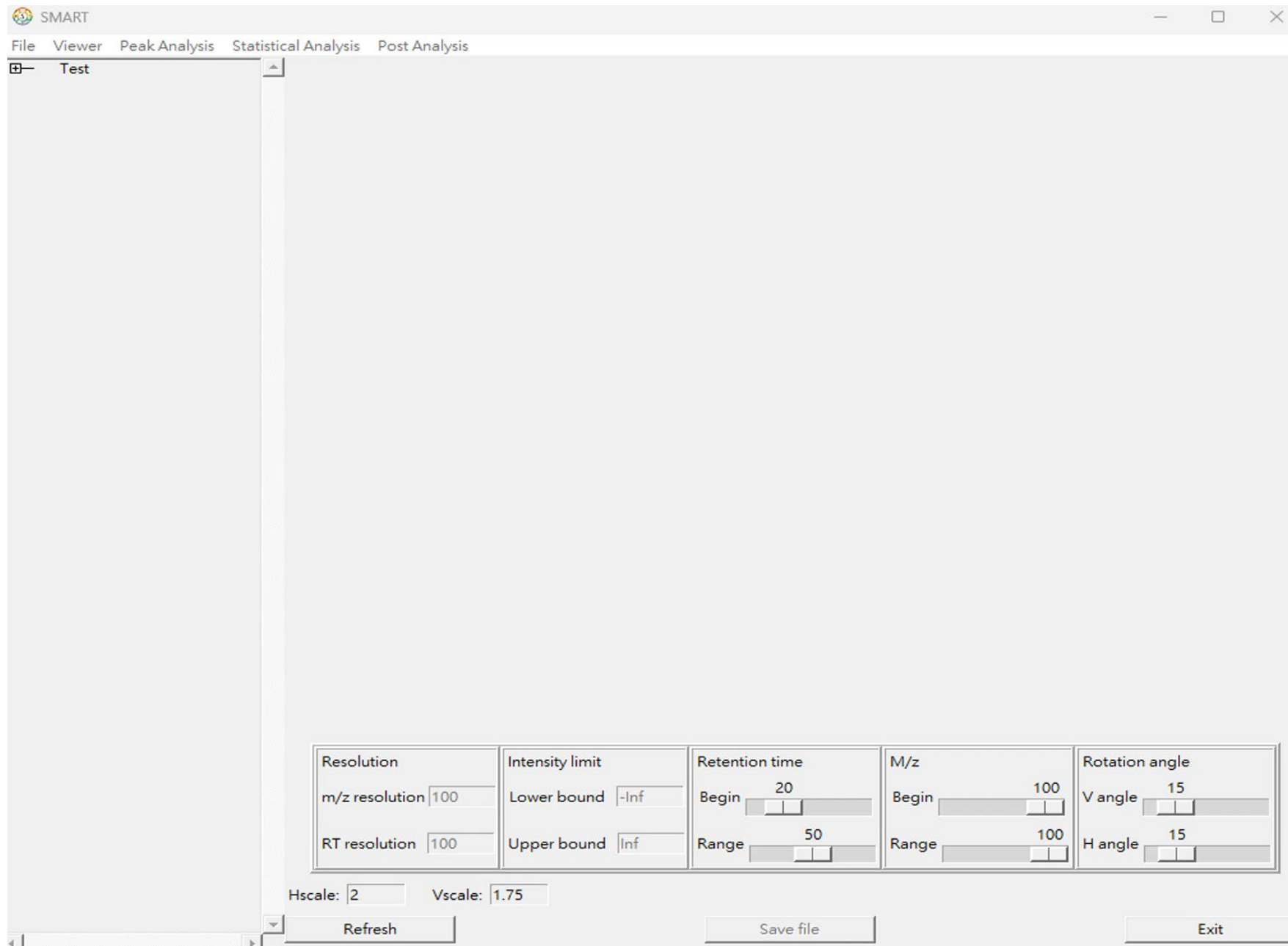
- Output file :
 - Per drug : PID1_Heroin_mz370.1642727_lm.tiff
 - Summarized files
 - Calibration-curve_info.csv
 - Peak_abundance_S10_P12.csv

Peak_Index	Name	mz	Ret_time.sec	model	weights	Original	concentration	theta1	theta2	theta3	R2	adj.R2	Cook's Distance	concentration	theta1	theta2	theta3	R2	adj.R2	Confidence Interval	concentration	theta1	theta2	theta3	R2	adj.R2	Bias	Bias_n	concentration	theta1	theta2	theta3	R2	adj.R2		
1	Heroin	370.1643	685.3724	linear	unweighted		50;100;200;300;400;600;700;800;900;1000	-4.9E+07	544792		0.958136	0.952903		50;100;200;300;400;600;700;800;900;1000	-4.9E+07	544792		0.958136	0.952903		50;100;200;300;700;800;900;1000	-3.4E+07	544358.1		0.98374	0.98103		1000	200;300;700;800;900	-6.1E+07	568369.8		0.981002	0.974669		
1	Heroin	370.1643	685.3724	linear	1/x		50;100;200;300;400;600;700;800;900;1000	-1.5E+07	479390.9		0.955433	0.949862		50;100;200;300;400;600;700;800;900	-1.2E+07	453902.4		0.950724	0.943685		50;100;200;300;700;800	-	8593120	448998.5		0.983258	0.979073		900;1000	100;200;300;700;800	-2.1E+07	476246.5		0.990442	0.987257	
1	Heroin	370.1643	685.3724	linear	1/x2		50;100;200;300;400;600;700;800;900;1000	-	423549.2		0.940529	0.933096		100;200;300;400;600;700;800	-1E+07	404133.5		0.954751	0.945701		50;100;300;600;700;800	-	1437069	405384.1		0.974506	0.968132		900;1000	50;100;300;600;700;800	-	1437069	405384.1		0.974506	0.968132
1	Heroin	370.1643	685.3724	quadratic	unweighted		50;100;200;300;400;600;700;800;900;1000	19931526	101178.4	431.8703	0.994044	0.992342		50;100;200;300;400;600;700;800;900;1000	19931526	101178.4	431.8703	0.994044	0.992342		50;100;200;400;600;700;800;1000	18150380	95052.91	430.0097	0.997018	0.995826		50	100;200;300;400;600;700;800;900;1000	26463289	76742.02	451.0403	0.993256	0.991008		
1	Heroin	370.1643	685.3724	quadratic	1/x		50;100;200;300;400;600;700;800;900;1000	14796763	134459.1	399.4706	0.994369	0.99276		50;100;200;300;400;600;700;800;900;1000	14796763	134459.1	399.4706	0.994369	0.99276		50;100;200;700;800;900;1000	9862241	200644.6	336.2698	0.9988	0.9982		50	100;200;300;400;600;700;800;900;1000	22570084	97219.26	432.3327	0.993467	0.991289		
1	Heroin	370.1643	685.3724	quadratic	1/x2		50;100;200;300;400;600;700;800;900;1000	11317205	174094.5	353.5682	0.991672	0.989293		50;100;200;300;400;600;700;800;900;1000	11317205	174094.5	353.5682	0.991672	0.989293		50;100;200;300;700;800;900;1000	8300824	225274.2	310.381	0.998758	0.998261		50	100;200;300;400;600;700;800;900;1000	16712272	139366.1	388.4399	0.990517	0.987355		
2	Morphine	286.1431	146.2696	linear	unweighted		50;100;200;300;400;600;700;800;900;1000	-2.2E+07	614982.2		0.980222	0.97775		50;100;200;300;400;600;700;800;900;1000	-2.2E+07	614982.2		0.980222	0.97775		50;100;200;300;700;800;900;1000	-	8656085	612829.3		0.995813	0.995116		1000	200;300;600;700;800;900;1000	-2.9E+07	620594.2		0.984702	0.980877	
2	Morphine	286.1431	146.2696	linear	1/x		50;100;200;300;400;600;700;800;900;1000	2718972	565511.4		0.978751	0.976095		100;200;300;400;600;700;800;900	-	574439.3		0.976138	0.972161		50;100;200;300;700;800;900;1000	3511241	588795		0.99382	0.99279		1000	100;200;300;600;700;800;900	-	6890489	583750.9		0.991752	0.990102	
2	Morphine	286.1431	146.2696	linear	1/x2		50;100;200;300;400;600;700;800;900;1000	12145662	520932.9		0.96943	0.965609		100;200;300;400;600;700;800	2524560	522646.5		0.974253	0.969104		50;200;300;600;700;800	14920579	514716.7		0.992002	0.990002		900;1000	50;100;200;300;600;700;800	13432841	508416.2		0.982305	0.978766		
2	Morphine	286.1431	146.2696	quadratic	unweighted		50;100;200;300;400;600;700;800;900;1000	27509410	292379.9	314.0624	0.995468	0.994173		50;100;200;300;400;600;700;800;900;1000	27509410	292379.9	314.0624	0.995468	0.994173		50;100;200;800;900;1000	24312931	346814.2	257.3256	0.998147	0.99753		50	100;200;300;800;900;1000	29484511	284990.8	319.8591	0.994575	0.992767		
2	Morphine	286.1431	146.2696	quadratic	1/x		50;100;200;300;400;600;700;800;900;1000	25624342	304597.9	302.1678	0.995151	0.993766		50;100;200;300;400;600;700;800;900;1000	25624342	304597.9	302.1678	0.995151	0.993766		50;100;200;300;600;700;800;900;1000	21499894	366836.4	237.6349	0.99833	0.997773		50	100;200;300;400;600;700;800;900;1000	27109026	297485.2	308.4444	0.993657	0.991542		
2	Morphine	286.1431	146.2696	quadratic	1/x2		50;100;200;300;400;600;700;800;900;1000	24114845	321792.5	282.2545	0.991638	0.989249		50;100;200;300;400;600;700;800;900;1000	24114845	321792.5	282.2545	0.991638	0.989249		50;100;200;300;600;700;800;900;1000	21580848	365864.8	238.7356	0.996388	0.995184			50;100;200;300;400;600;700;800;900;1000	24114845	321792.5	282.2545	0.991638	0.989249		

Calibration curve construction

- Output file :
 - Per drug : PID1_Heroin_mz370.1642727_lm.tiff
 - Summarized files
 - Calibration-curve_info.csv
 - Peak_abundance_S10_P12.csv

Peak_Index	Name	mz	Ret_time.sec	D1_50_MS1	D10_900_MS1	D11_1000_MS1	D2_100_MS1	D3_200_MS1	D4_300_MS1	D5_400_MS1	D7_600_MS1	D8_700_MS1	D9_800_MS1
1	Heroin	370.1643	685.3724	20454233	4.85E+08	5.37E+08	33246830	65461545	1.08E+08	1.12E+08	2.18E+08	3.07E+08	3.79E+08
2	Morphine	286.1431	146.2696	41215408	5.53E+08	6.23E+08	55632394	1.11E+08	1.59E+08	1.66E+08	3.05E+08	4.01E+08	4.69E+08
3	Cocaine	304.1538	701.136	67391965	1.91E+09	2.16E+09	1.09E+08	2.8E+08	4.54E+08	4.79E+08	9.95E+08	1.32E+09	1.57E+09
4	Thebaine	312.1588	678.8176	13918057	6.54E+08	7.9E+08	22156818	57057964	1.12E+08	1.14E+08	3.04E+08	4.41E+08	5.71E+08
5	delta-9 THC	315.2302	1513.319	584689	12324894	10750614	1040859	3017275	4419165	4255491	8052278	9572914	11385840
6	Amphetamine	136.0804	419.1944	28233349	38237498	37707935	30552141	36745807	37802678	38425018	39861533	37695091	38418264
7	MA	150.1021	496.8365	88366857	4.84E+08	5.19E+08	1.14E+08	1.84E+08	2.48E+08	2.57E+08	3.78E+08	4.43E+08	4.61E+08
8	MDMA	194.1173	538.9991	57005641	1.36E+09	1.61E+09	77917415	1.73E+08	3.32E+08	3.11E+08	7.22E+08	9.57E+08	1.14E+09
9	Love Drug	180.0866	505.6407	2977060	32927321	40623589	3508615	5215888	7552811	8060482	15982588	21387478	24410233
10	Ketamine	238.0988	599.9313	65361457	1.9E+09	2.12E+09	1.1E+08	3.04E+08	5.13E+08	5.38E+08	1.13E+09	1.41E+09	1.64E+09
11	FM2	314.093	1044.274	23235201	4.47E+08	5.37E+08	37148568	83573478	1.28E+08	1.31E+08	2.43E+08	3.1E+08	3.84E+08
12	Nimetazepam	296.1024	1040.522	27111684	5.03E+08	5.88E+08	41037216	91247724	1.44E+08	1.48E+08	2.67E+08	3.45E+08	4.14E+08



Concentration calibration (calibration curve)

Concentration calculation

Concentration calculation

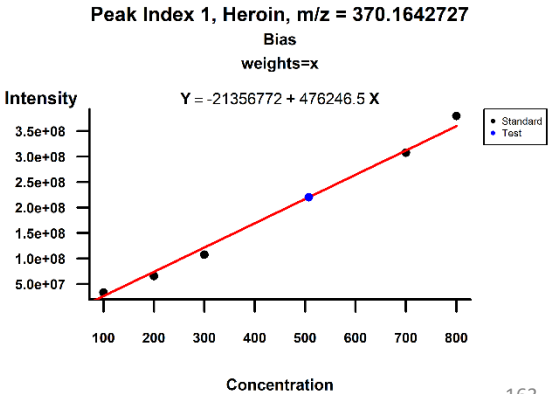
- Input: **peak abundance data** of test samples
 - Compound name and peak abundance
 - Test sample name: T1_500_MS1
 - Rule: **sampledID_MSx**
- Input: **Model Info.** of *Calibration curve construction*
 - Regression (linear or quadratic)
 - Weight (1, 1/x, 1/x²)
 - Outlier detection
 - Cook's D, 95% CI, Deviation
 - **Rdata from Calibration curve construction**
- Output file :
 - per drug : Conc.Calculation_PID1_S1_P12.tiff
 - Summarized files
 - Conc.Calculation_S1_P12.csv
 - Conc.Calculation_CI_S1_P12.csv
 - best_model.csv

Input file: peak abundance data of test samples
Model info: **model_fit.RData**

Peak_Index	Name	mz_est	Ret_Time_est.sec	T1_500_MS1
1	Heroin	370.1643	685.3724	2.2E+08
2	Morphine	286.1431	146.2696	2.97E+08
3	Cocaine	304.1538	701.136	9.74E+08
4	Thebaine	312.1588	678.8176	2.89E+08
5	delta9-THC	315.2302	1513.319	6385888
6	Amphetamine	136.0804	419.1944	38130787
7	MA	150.1021	496.8365	3.68E+08
8	MDMA	194.1173	538.9991	6.96E+08
9	Love Drug	180.0866	505.6407	14862760
10	Ketamine	238.0988	599.9313	1.07E+09
11	FM2	314.093	1044.274	2.39E+08
12	Nimetazepam	296.1024	1040.522	2.57E+08

Output file

Peak_Index	Name	mz	Ret_time.se	T1_500_M
1	Heroin	370.1643	685.3724	507.4039
2	Morphine	286.1431	146.2696	499.5544
3	Cocaine	304.1538	701.136	501.2351
4	Thebaine	312.1588	678.8176	450.4228
5	delta9-THC	315.2302	1513.319	467.1095
6	Amphetam	136.0804	419.1944	773.8217
7	MA	150.1021	496.8365	595.2049
8	MDMA	194.1173	538.9991	520.7164
9	Love Drug	180.0866	505.6407	492.0814
10	Ketamine	238.0988	599.9313	547.7417
11	FM2	314.093	1044.274	512.0763
12	Nimetazepa	296.1024	1040.522	537.2955



Post analysis (Concentration calibration)

T1_500_MS1

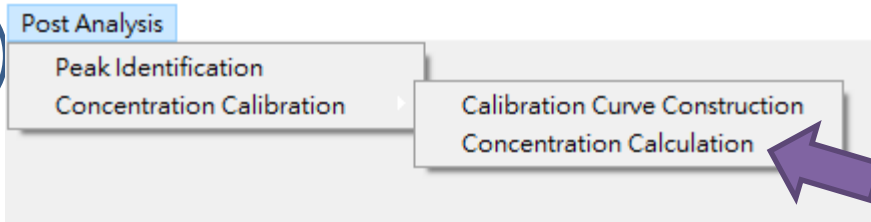
- Concentration calculation

- Input:

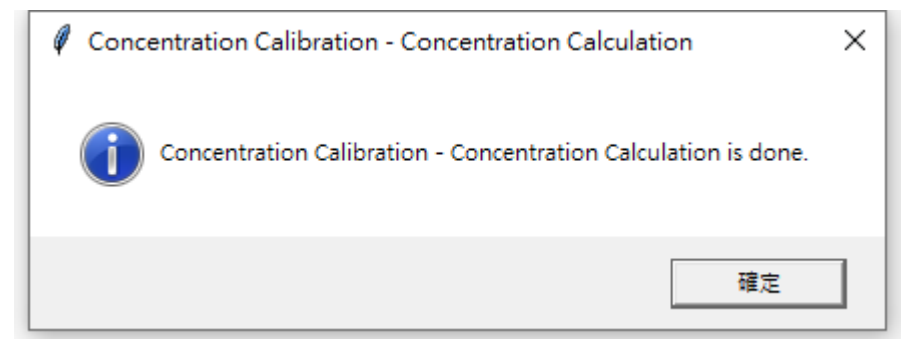
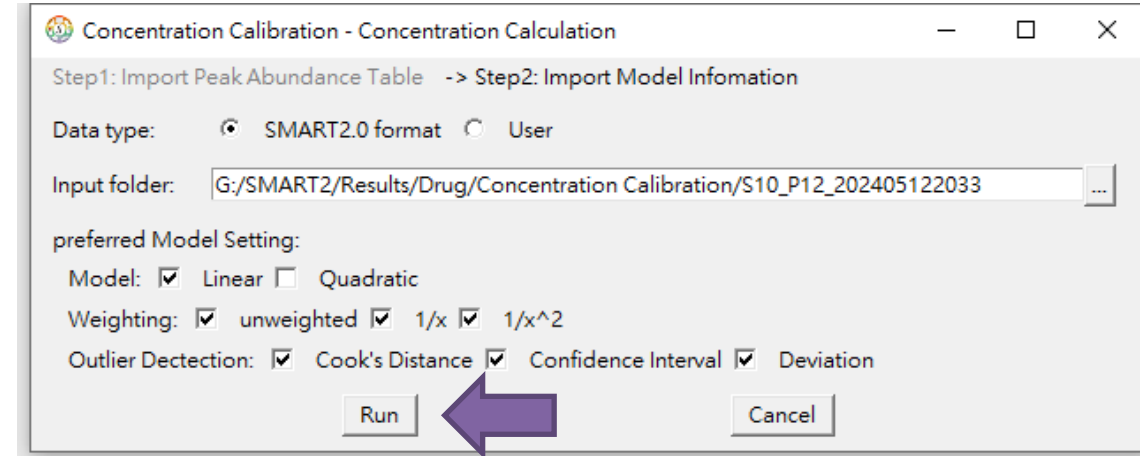
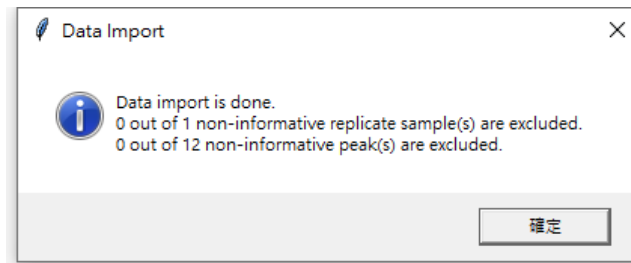
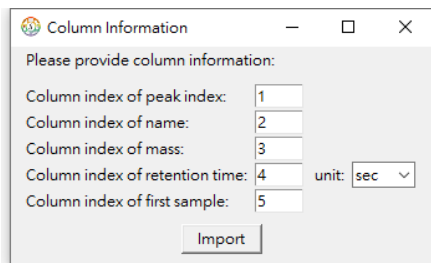
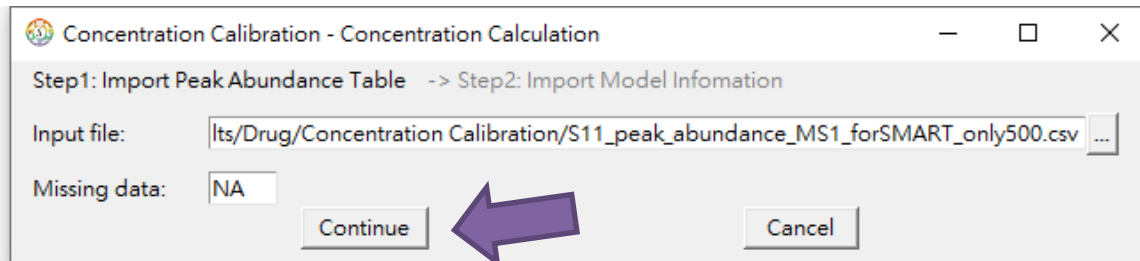
- Peak abundance table of test samples
 - Model Info. of calibration curve construction

3

1



2



Concentration calculation

T1_500_MS1

- Output file :
 - Concentration of test samples and figures
 - Conc.Calculation_S1_P12.csv
 - Conc.Calculation_CI_S1_P12.csv
 - best_model.csv

The best model

Peak_Index	Name	mz	Ret_time.s	model	weights	outlier_det	theta1	theta2
1	Heroin	370.1643	685.3724	linear	weights=x	Bias	-2.1E+07	476246.5
2	Morphine	286.1431	146.2696	linear	unweighted	Confidence	-8656085	612829.3
3	Cocaine	304.1538	701.136	linear	unweighted	Confidence	-1.4E+08	2212685
4	Thebaine	312.1588	678.8176	linear	weights=x	Bias	-5.5E+07	763859.4
5	delta9-THC	315.2302	1513.319	linear	weights=1/	Confidence	-111095	13908.91
6	Amphetam	136.0804	419.1944	linear	weights=x	Confidence	28228757	12796.27
7	MA	150.1021	496.8365	linear	weights=1/	Confidence	62618904	513772.2
8	MDMA	194.1173	538.9991	linear	weights=x	Bias	-8.6E+07	1500882
9	Love Drug	180.0866	505.6407	linear	weights=1/	Bias	1629805	26891.8
10	Ketamine	238.0988	599.9313	linear	weights=x	Bias	-1.2E+08	2183668
11	FM2	314.093	1044.274	linear	unweighted	Confidence	-1.2E+07	491873
12	Nimetazep	296.1024	1040.522	linear	weights=1/	Confidence	3150656	472114.2

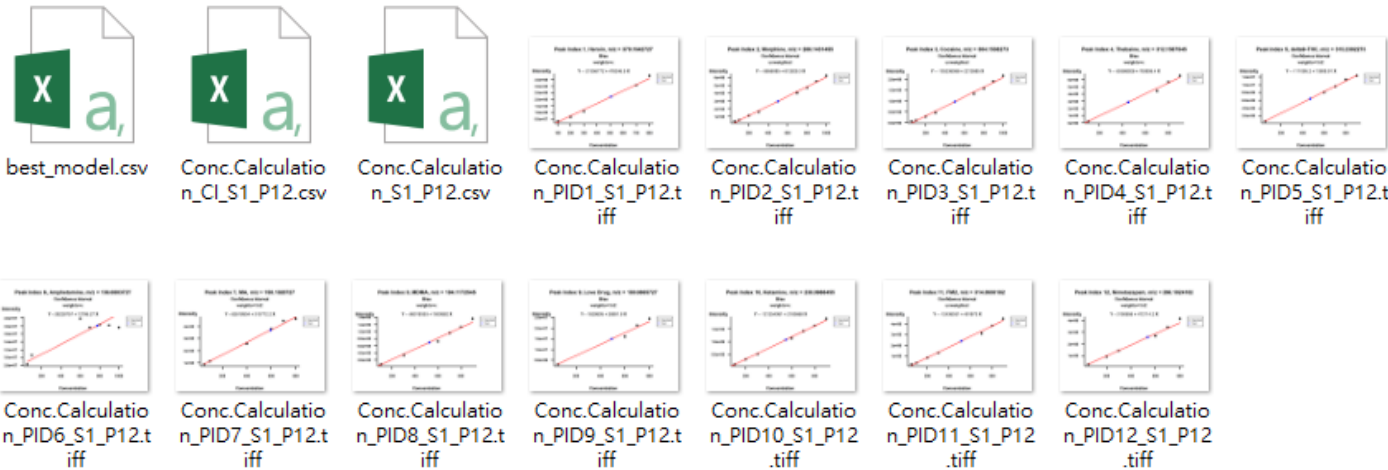
95% CI of Concentration

Peak_Index	Name	mz	Ret_time.s	model	weights	outlier_det	T1_500_M	T1_500_M	T1_500_M
							estimate	lower	upper
1	Heroin	370.1643	685.3724	linear	weights=x	Bias	507.4039	453.1017	579.853
2	Morphine	286.1431	146.2696	linear	unweighted	Confidence	499.5544	429.5806	569.4717
3	Cocaine	304.1538	701.136	linear	unweighted	Confidence	501.2351	407.8891	594.506
4	Thebaine	312.1588	678.8176	linear	weights=x	Bias	450.4228	388.4139	524.2525
5	delta9-THC	315.2302	1513.319	linear	weights=1/	Confidence	467.1095	450.9682	484.5766
6	Amphetam	136.0804	419.1944	linear	weights=x	Confidence	773.8217	577.1688	1208.008
7	MA	150.1021	496.8365	linear	weights=1/	Confidence	595.2049	562.0866	632.9944
8	MDMA	194.1173	538.9991	linear	weights=x	Bias	520.7164	477.562	571.1082
9	Love Drug	180.0866	505.6407	linear	weights=1/	Bias	492.0814	401.8802	643.4005
10	Ketamine	238.0988	599.9313	linear	weights=x	Bias	547.7417	530.4445	566.2845
11	FM2	314.093	1044.274	linear	unweighted	Confidence	512.0763	435.9247	589.2633
12	Nimetazep	296.1024	1040.522	linear	weights=1/	Confidence	537.2955	494.561	589.2658

Concentration

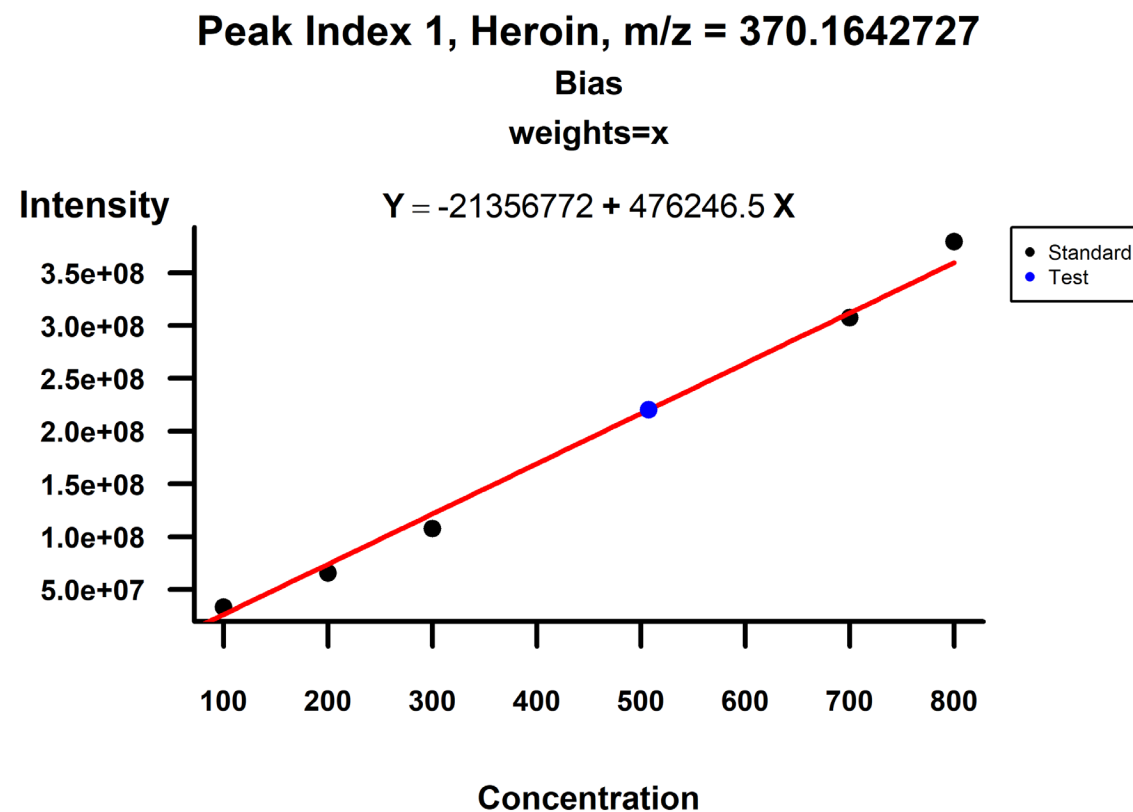
Peak_Index	Name	mz	Ret_time.s	T1_500_M
1	Heroin	370.1643	685.3724	507.4039
2	Morphine	286.1431	146.2696	499.5544
3	Cocaine	304.1538	701.136	501.2351
4	Thebaine	312.1588	678.8176	450.4228
5	delta9-THC	315.2302	1513.319	467.1095
6	Amphetam	136.0804	419.1944	773.8217
7	MA	150.1021	496.8365	595.2049
8	MDMA	194.1173	538.9991	520.7164
9	Love Drug	180.0866	505.6407	492.0814
10	Ketamine	238.0988	599.9313	547.7417
11	FM2	314.093	1044.274	512.0763
12	Nimetazep	296.1024	1040.522	537.2955

Figures



Concentration calculation

- Output file :
 - Figures (per drug)
 - **Conc.Calculation_PID1_S1_P12.tiff**
 - Files
 - Conc.Calculation_S1_P12.csv
 - Conc.Calculation_CI_S1_P12.csv
 - best_model.csv



Concentration calculation

- Output file :
 - Figures (per drug)
 - Conc.Calculation_PID1_S1_P12.tiff
 - Files
 - **Conc.Calculation_S1_P12.csv**
 - Conc.Calculation_CI_S1_P12.csv
 - best_model.csv

Peak_Index	Name	mz	Ret_time.sec	T1_500_MS1
1	Heroin	370.1642727	685.3723636	507.4039481
2	Morphine	286.1431455	146.2696364	499.5543803
3	Cocaine	304.1538273	701.136	501.2351081
4	Thebaine	312.1587545	678.8176364	450.4228135
5	delta9-THC	315.2302273	1513.319455	467.1095339
6	Amphetamine	136.0803727	419.1943636	773.8216709
7	MA	150.1020727	496.8365455	595.2048664
8	MDMA	194.1172545	538.9990909	520.7163928
9	Love Drug	180.0865727	505.6407273	492.0814322
10	Ketamine	238.0988455	599.9312727	547.7417022
11	FM2	314.0930182	1044.273818	512.0763418
12	Nimetazepam	296.1024182	1040.522182	537.2955304

Concentration calculation

- Output file :
 - Figures (per drug)
 - Conc.Calculation_PID1_S1_P12.tiff
 - Files
 - Conc.Calculation_S1_P12.csv
 - Conc.Calculation_CI_S1_P12.csv
 - best_model.csv

Peak_Index	Name	mz	Ret_time.sec	model	weights	outlier_detection	T1_500_MS1	T1_500_MS1	T1_500_MS1
							estimate	lower	upper
1	Heroin	370.1642727	685.3723636	linear	weights=x	Bias	507.4039481	453.1017284	579.852959
2	Morphine	286.1431455	146.2696364	linear	unweighted	Confidence Interval	499.5543803	429.5806458	569.471698
3	Cocaine	304.1538273	701.136	linear	unweighted	Confidence Interval	501.2351081	407.8890646	594.5059527
4	Thebaine	312.1587545	678.8176364	linear	weights=x	Bias	450.4228135	388.4139437	524.2524615
5	delta9-THC	315.2302273	1513.319455	linear	weights=1/x2	Confidence Interval	467.1095339	450.9681808	484.5765898
6	Amphetamine	136.0803727	419.1943636	linear	weights=x	Confidence Interval	773.8216709	577.1687899	1208.00774
7	MA	150.1020727	496.8365455	linear	weights=1/x2	Confidence Interval	595.2048664	562.0866302	632.9944493
8	MDMA	194.1172545	538.9990909	linear	weights=x	Bias	520.7163928	477.5620114	571.1081788
9	Love Drug	180.0865727	505.6407273	linear	weights=1/x2	Bias	492.0814322	401.8801958	643.4004515
10	Ketamine	238.0988455	599.9312727	linear	weights=x	Bias	547.7417022	530.4445343	566.2844961
11	FM2	314.0930182	1044.273818	linear	unweighted	Confidence Interval	512.0763418	435.9246784	589.2633238
12	Nimetazepam	296.1024182	1040.522182	linear	weights=1/x2	Confidence Interval	537.2955304	494.5609818	589.2657992

Concentration calculation

- Output file :
 - Figures (per drug)
 - Conc.Calculation_PID1_S1_P12.tiff
 - Files
 - Conc.Calculation_S1_P12.csv
 - Conc.Calculation_CI_S1_P12.csv
 - **best_model.csv**

Peak_Index	Name	mz	Ret_time.sec	model	weights	outlier_detection	theta1	theta2
1	Heroin	370.1642727	685.3723636	linear	weights=x	Bias	-21356771.92	476246.4938
2	Morphine	286.1431455	146.2696364	linear	unweighted	Confidence Interval	-8656085.301	612829.2678
3	Cocaine	304.1538273	701.136	linear	unweighted	Confidence Interval	-135236596.4	2212685.355
4	Thebaine	312.1587545	678.8176364	linear	weights=x	Bias	-55368926.41	763859.4158
5	delta9-THC	315.2302273	1513.319455	linear	weights=1/x2	Confidence Interval	-111095.1716	13908.90747
6	Amphetamine	136.0803727	419.1943636	linear	weights=x	Confidence Interval	28228756.57	12796.26888
7	MA	150.1020727	496.8365455	linear	weights=1/x2	Confidence Interval	62618903.73	513772.2091
8	MDMA	194.1172545	538.9990909	linear	weights=x	Bias	-86018352.72	1500881.862
9	Love Drug	180.0865727	505.6407273	linear	weights=1/x2	Bias	1629804.848	26891.79976
10	Ketamine	238.0988455	599.9312727	linear	weights=x	Bias	-121254060.5	2183668.181
11	FM2	314.0930182	1044.273818	linear	unweighted	Confidence Interval	-12436531.12	491872.9913
12	Nimetazepam	296.1024182	1040.522182	linear	weights=1/x2	Confidence Interval	3150656.25	472114.1824


```

1 rm(list = ls())
2 gc()
3 library(tcltk)
4 setwd("C:/ForDemo/code/SMART2.0")
5
6 getwd()
7
8 load("dbMetabo.RData")
9 load("function_ls.RData")
10 load("heaSPIA_reversible(cumula
11 load("KEGG_pathway_class.Rdata"
12
13 file <- list.files(pattern = ".
14 file <- file[!grepl("SMART|Rdat
15
16 file
17
18 sapply(file, function(x)source(
19
20
21 #msconvert C:/Users/yujen/AppDa
22
23

```

File
Viewer
Peak Analysis
Statistical Analysis
Post Analysis

Test

Resolution m/z resolution 100 RT resolution 100	Intensity limit Lower bound -Inf Upper bound Inf	Retention time Begin 20 Range 50	M/z Begin 100 Range 100	Rotation angle V angle 15 H angle 15
--	---	---	--	---

Hscale:
Vscale:

23:1 (Top Level)

Console
Terminal
Background Jobs

R 4.4.1 C:/forDemo/code/SMART2.0/

Data Import - Output: C:/forDemo.

Data Import-Data import is done.

Peak Analysis (Targeted) - Please wait and step 1 ... OK

100

Provided scanrange was adjusted to 1

Peak Analysis (Targeted) is done.

Data import is done.

0 out of 5 non-informative replicates

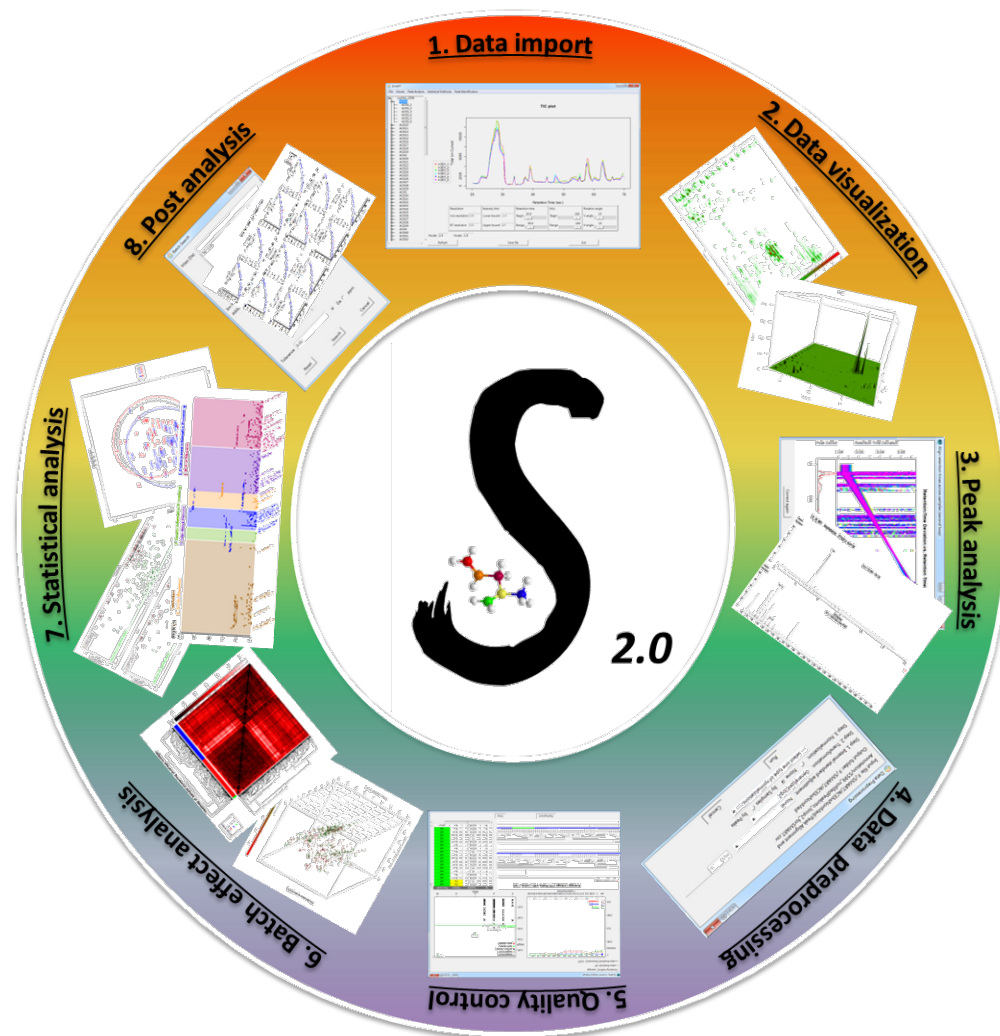
0 out of 12 non-informative peak(s) are excluded.

Concentration Calibration - Calibration Curve Construction is done.

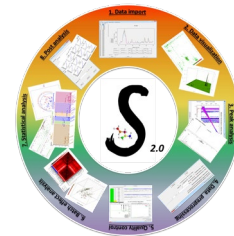
>

Concentration calibration (concentration calculation)

CONCLUSION AND DISCUSSION



Conclusion and Discussion



代謝組學研究面臨的挑戰有很多，以下是其中一些主要的困難：

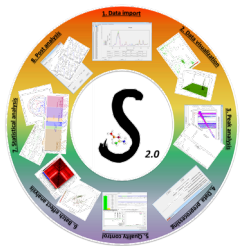
- **樣本準備和處理:** 樣本的準備和處理對代謝組學的結果影響很大。樣本的收集、保存、提取和前處理都需要非常謹慎，以確保結果的可靠性和可重複性。
- **代謝產物的識別和定量:** 代謝組學研究需要識別和定量大量的代謝產物，這可能需要使用質譜學、核磁共振等高分辨率的分析技術。識別未知的代謝物是一個挑戰，因為代謝物的結構和特性可能非常多樣，且存在許多未知的代謝物。
- **數據處理和分析:** 代謝組學產生的數據量通常非常大，需要複雜的統計和計算方法來處理和分析。這包括校正樣本間的差異、識別生物學上有意義的變化、進行多變量分析等。
- **生物學解釋:** 從代謝組學數據中獲得生物學上有意義的信息是一個挑戰。代謝組學研究通常需要與其他組學數據（如基因組學、蛋白質組學）結合，以更好地理解代謝途徑和生物學過程。
- **樣本異質性:** 生物體內的代謝異質性可能會導致代謝組學研究的難度增加。例如，不同組織、不同個體之間的代謝物水平可能存在差異，這需要在研究設計和分析中加以考慮。

總的來說，代謝組學研究需要多學科的結合，包括化學、生物學、數學和計算機科學等，以應對這些困難並取得有意義的結果。

Other info.

- Database
 - HMDB
 - <https://hmdb.ca/>
 - MassBank
 - <https://massbank.eu/MassBank/>
 - Metabolomics Workbench
 - <https://www.metabolomicsworkbench.org/>
 - MetaboLights
 - <https://www.ebi.ac.uk/metabolights>
 - LIPID MAPS
 - <https://www.lipidmaps.org/>
 - METLIN
 - https://metlin.scripps.edu/landing_page.php?pgcontent=mainPage
- Tools
 - MetaboAnalyst 6.0
 - <https://www.metaboanalyst.ca/>
 - MetDNA2
 - <http://metdna.zhulab.cn/>
 - MetaCore
 - <https://portal.genego.com/>
 - Ingenuity Pathway Analysis (IPA)
 - <https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-ipa/>





智慧型代謝體學解析系統

Intelligent Metabolomics Analytical System (iMAS)

Activities Toplevel Oct 7 10:11

iMAS System 產品首頁 系列分析平台 English

iMAS 智慧型代謝體學解析系統

全國首創 專為代謝體學設計的 **一站式** 自動化分析解決方案

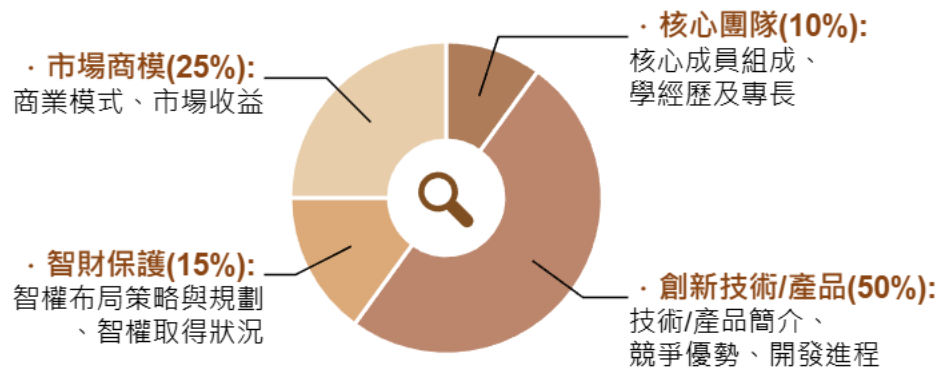
化繁為簡 → 專注生物意義
執簡馭繁 → 解析代謝全貌

iMAS 助您將龐雜的「原始數據」輕鬆轉化為清晰的「生物學解讀」，提供直覺式的圖形化介面 (GUI)。
研究人員無需撰寫程式碼，即可高效完成進階統計運算與專業的數據視覺化，大幅加速科研產出。

榮獲第 21 屆國家新創獎肯定： [查看獲獎資訊](#)

2024 國家新創獎 – 學研新創

• 評審指標



• 參賽流程

參賽申請	線上申請 • 填寫線上報名表	參賽資格與類別核定 • 企業新創獎/國際新創獎 • 初創企業獎/學研新創獎 • 臨床新創獎/新創育成獎	確認受理參賽 • 報名確認函	資料與費用繳交 【第一梯次】5/15 【第二梯次】8/15 • 線上繳交參賽同意書 • 線上繳交參賽計畫書/影片 • 繳交參賽費用	行政審查 【第一梯次】5月 【第二梯次】8月 • 審閱參賽文件完整性 • 通知線上資料補正
審查時程	[初審]書面審查 【第一梯次】6月 【第二梯次】9月 • 出具第一階段書審意見 • 通知參賽團隊回覆說明 • 通知參賽團隊簡報備審	[複審]簡報審查 【第一梯次】6月-7月 【第二梯次】9月-10月 • 參賽團隊現場簡報與說明 • 新創評審團即時詢答與建議 • 實體新創產品/原型機展示	[查核]關鍵資訊查閱 【第一梯次】7月 【第二梯次】10月 • 企業財務信用與風險評估 • 企業營運里程與紀錄評估 • 學研臨床團隊論文/專利調查 • 通知參賽團隊提出回覆說明	[決審]召集人決審會議 【第一梯次】8月 【第二梯次】11月 • 國家新創獎獲獎項目核定 • 年度亮點新創科技擇選	
獲獎公告	結果公告 【第一梯次】8月 【第二梯次】11月 • 正式函文通知審查結果以及新創審查委員建議	年度授獎榮耀與創新推廣 11月-12月 • 國家新創獎授獎典禮暨Demo Day • 台灣醫療科技展公告年度醫療創新突破科技 • 台灣醫療科技展InnoZone新創技術展秀 • 媒體報導推薦與安排	新創幫推薦與技術擴散 • 獲獎新創技術解析 • 獲獎新創團隊/人才推薦 • 獲獎新創焦點報導	創新創業資源挹注 • 募資架構與推廣 • 財務/商模/智權法務諮詢 • 產業鏈結與推薦	

• 參賽計畫書

(一)封面

1. 參賽項目中/英文名稱。
2. 參賽代表人姓名。
3. 所屬機構名稱與聯絡資訊。

(二)摘要

1. 參賽項目中/英文摘要重點說明 (各500字以內)。

(三)核心團隊

1. 研究團隊核心成員組成、學經歷及專長。

(四)創新技術/ 產品

1. 技術/產品簡介：說明技術/產品創新價值及市場競爭力。
2. 競爭優勢：技術/產品可解決之市場痛點、競品分析與關鍵驗證數據。
3. 開發進程：技術/產品之生產製造、取證進度與規劃。

(五)市場商模

1. 商業模式：技術/產品市場營運與獲利模式規劃。
2. 市場收益：技術/產品成本、售價、毛利率等規劃。

(六)智財保護

1. 智權布局策略與規劃：說明技術/產品包含專利、營業秘密或其他型態之智權布局策略規劃。
2. 智權取得現況：說明技術/產品專利與其他智財之取得狀況。

(七)計畫附件

1. 相關佐證資料(如：實證或臨床數據報告、期刊論文發表、專利等)。

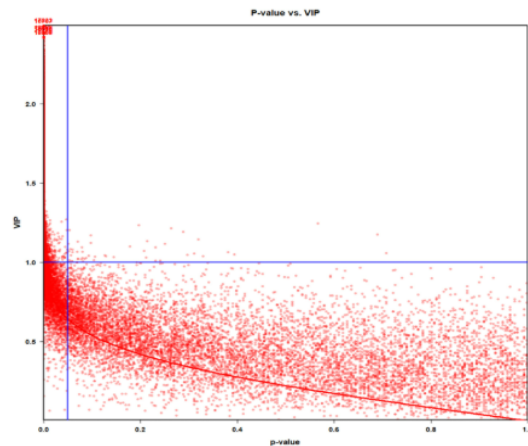
2024 國家新創獎 – 照片集錦



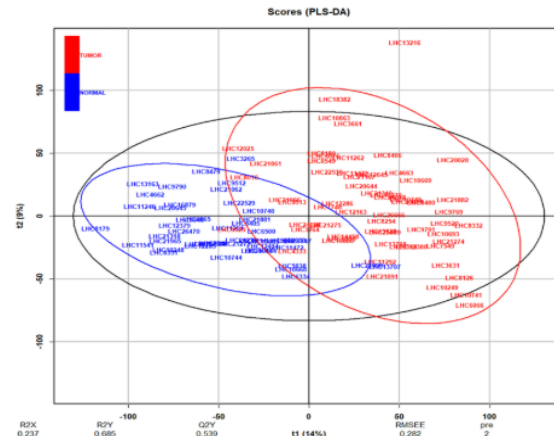
Statistical analysis (PLS/PLS-DA)

- PLS/PLS-DA output (figures)

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高階統計分析功能--PLS-DA

楊欣洲研究員兼所長團隊/中央研究院 統計科學研究所、中央研究院 基因體研究中心

楊欣洲、梁佑任、楊智婷、陳佳煒、林胤均、王亦生、林舒瑤

一站式整合多功能的代謝體資料解析工具：本團隊開發的智慧型代謝體學解析系統（iMAS），提供從上游至下游的全方位資料處理與分析，讓使用者能夠在單一平台完成所有代謝體分析任務。iMAS涵蓋八大核心功能：資料匯入、資料檢視、波峰分析、資料前處理、品質管控、批次效應校正、高階統計分析及事後分析，適用於標靶與非標靶代謝體研究。iMAS的技術優勢與特性：iMAS的分析優勢有：1) 兼容多種LC/GC-MS資料格式，方便各類儀器使用者。2) 高效資料處理：支援自動化波峰檢測，提升分析效率。3) 資料品質管控：提供內標校正、資料標準化及批次效應校正功能，確保資料可靠性。4) 先進統計分析：支援多變量分析、反應路徑分析及多體學整合分析，深入資料探勘。5) 代謝物鑑定與定量：自動化代謝物比對與檢量線建構，協助精準定量。6) 資料可視化：提供多樣化的視覺化工具，協助使用者解讀原始數據及分析結果。iMAS的成功應用：iMAS系統已展現於多領域的廣泛應用潛力，包括食品安全、藥物代謝、環境科學與個人化醫療。在實際案例中，iMAS成功應用於食用油代謝體、蔬菜飲食介入代謝體、三陰性乳癌代謝體、心血管疾病代謝體及高血壓藥物代謝體等研究，充分展現其在科學研究、臨床應用及多元市場中的價值與競爭力。

不可取代

- 【生成式AI醫級戰場5】長佳智能布局三領域 聚焦病歷生成與健康管理

MORE...

掌握・新創動態

- 《新創動態》佩圖思生醫獲得FDA pre-IND 正式回覆並於AACR發表 PLT012 研究成果
- 《新創動態》高效生產抗菌表面素 開啟無抗飼養新時代
- 《新創動態》仲恩公佈台日二期結果 臨床指標分數優異穩定

MORE...

看見・新創人物

- 鄭隆賓講座教授, Long-Bin Jeng, MD
- 莊曜宇教授, Eric Y. Chuang, Ph. D.
- 王仲祺部主任, Chen-Chi Wang, MD

MORE...

<https://youtu.be/lhUEY38yxJI>

Welcome



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中央研究院IMAS研發團隊

THE IMAS DEVELOPMENT TEAM AT ACADEMIA SINICA

廠商分類：[新創區](#)

攤位號碼：N915

公司網址：<https://staff.stat.sinica.edu.tw/hsinchou/>

廠商介紹

一站式整合多功能的代謝體資料解析工具：

本團隊開發的智慧型代謝體學解析系統 (iMAS)，提供上游至下游全方位資料處理與分析，讓使用者可在單一平台完成所有代謝體分析任務。iMAS涵蓋八大核心功能：資料匯入、資料檢視、波峰分析、資料前處理、品質管控、批次效應校正、高階統計分析與事後分析，適用於標靶與非標靶代謝體研究。

iMAS技術優勢與特性：

iMAS 的分析優勢包括：

- 1) 相容多種LC/GC-MS格式，方便各類儀器使用者。
- 2) 高效資料處理：支援自動化波峰偵測，提升效率。
- 3) 資料管控：提供內標校正、標準化與批次效應校正功能，確保資料可靠。
- 4) 進階統計分析：支援多變量分析、反應路徑分析與多體學整合分析，深入資料探勘。
- 5) 代謝物鑑定與定量：自動比對代謝物並建構檢量線，協助精準定量。
- 6) 資料可視化：提供多樣視覺化工具，輔助解讀原始數據與分析結果。

iMAS的成功應用：

iMAS系統已展現多領域的廣泛應用潛力，包括食品安全、藥物代謝、環境科學與個人化醫療。實例中，iMAS成功應用於食用油代謝體、蔬菜飲食介入代謝體、三陰性乳癌代謝體、心血管疾病代謝體與高血壓藥物代謝體研究，展現其於科研、臨床與市場的價值與競爭力。

The banner for the BIO Asia-Taiwan 2025 exhibition features a green and blue background with a stylized DNA helix. The text includes the event name "BIO Asia-Taiwan", the dates "July 23-27, 2025", and the location "台北南港展覽館1館 4F". It also mentions "Next Wave of Biotech Opportunities" and "近850家廠商 近2,200個攤位". A button at the bottom right says "立即登錄·免費參觀".



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台灣質譜學會
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智慧型代謝體學解析系統

Intelligent Metabolomics Analysis System (iMAS)

梁佑佐¹, iMAS團隊¹, 楊欣洲^{1*} ¹中央研究院 統計科學研究所



iMAS 全國首創整合代謝體學全流程的一站式智慧分析平台

技術簡介

「智慧型代謝體學解析系統」(Intelligent Metabolomics Analytical System, iMAS) 為本團隊自主開發，突破高維度代謝體資料處理瓶頸以及軟體功能難以整合之痛點，以R語言開發涵蓋上游多樣態資料讀取至下游多體學機轉探索之一站式大數據分析平台，成果刊登於國際頂尖期刊《Analytical Chemistry》，並榮獲「第21屆國家新創獎」肯定。團隊已於國網中心部署SaaS雲端服務平台，系統全面整合多廠牌質譜檔轉換、精準波峰偵測、獨創的穩健度品質管控 (r-value/s-value)，以及跨體學路徑整合 (IOPA) 等核心分析模組，為代謝體學研究提供嚴謹、高效且具高擴充性的全方位解決方案。

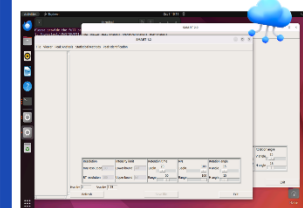


應用實例與國家級肯定

- 食用油脂代謝研究 - Wang PS, et al. (2018). J Agric Food Chem. 66, 4977-4984. (PMID: 297161922)
- 油脂攝取會顯著改變體內脂質與代謝物組成，影響心血管健康與代謝平衡。
- 癌症飲食介入代謝物研究 - Lynn KS, et al. (2019). J Proteome Res. 18, 159-168. (PMID: 30517004)
- 最著具有特異的「標化率」與「代謝特徵」，可應用於膳食監測、個人化營養與公共衛生營養研究。
- 三陰乳癌代謝物研究 - Apaya MK, et al. (2019). J Exp Clin Cancer Res. 38, 187. (PMID: 31072371)
- 癌症轉移伴隨特定時空與代謝特徵的活化，有助開發新診斷與治療工具。
- 心血管與代謝物研究 - Chiang KM, et al. (2022). Metabolites. 12, 495. (PMID: 35736428)
- 血液中的脂質 (oxylipins) 代謝特徵能區分冠狀動脈病發生或晚期早期病變活化，其中與發炎、氧化壓力及脂質代謝相關的特定脂質，可望成為預測冠狀動脈病風險的新生物標記。
- 高血壓與代謝物研究 - Liang YJ, et al. (2022). Comput Struct Biotechnol J. 20, 6458-6466. (PMID: 36467587)
- 能定與降血壓藥物及高血壓相關之關鍵代謝物，建立關鍵代謝物反應預測模型，為高血壓精準醫療與個人化營養提供代謝體學依據。



SaaS 雲端平台



系統展示

1. Liang YJ, Lin YT, Chen CW, Lin CW, Chao KM, Pan WH, Yang HC. SMART: Statistical Metabolomics Analysis-An R Tool. Anal Chem. 2016 Jun 21;88(12):6339-41. doi: 10.1021/acs.analchem.6b00603. PMID: 27298519.
2. Liang YJ, Yang CT, Chen CW, Lin YC, Lin SY, Wang YS, Yang HC. SMART 2.0 Statistical Metabolomics Analysis: An R Tool 2.0. Anal Chem. 2025 Nov 25;97(46):25953-25968. doi: 10.1021/acs.analchem.5c03225. PMID: 41171891.



THANK YOU!

e-mail: lyj626@stat.sinica.edu.tw