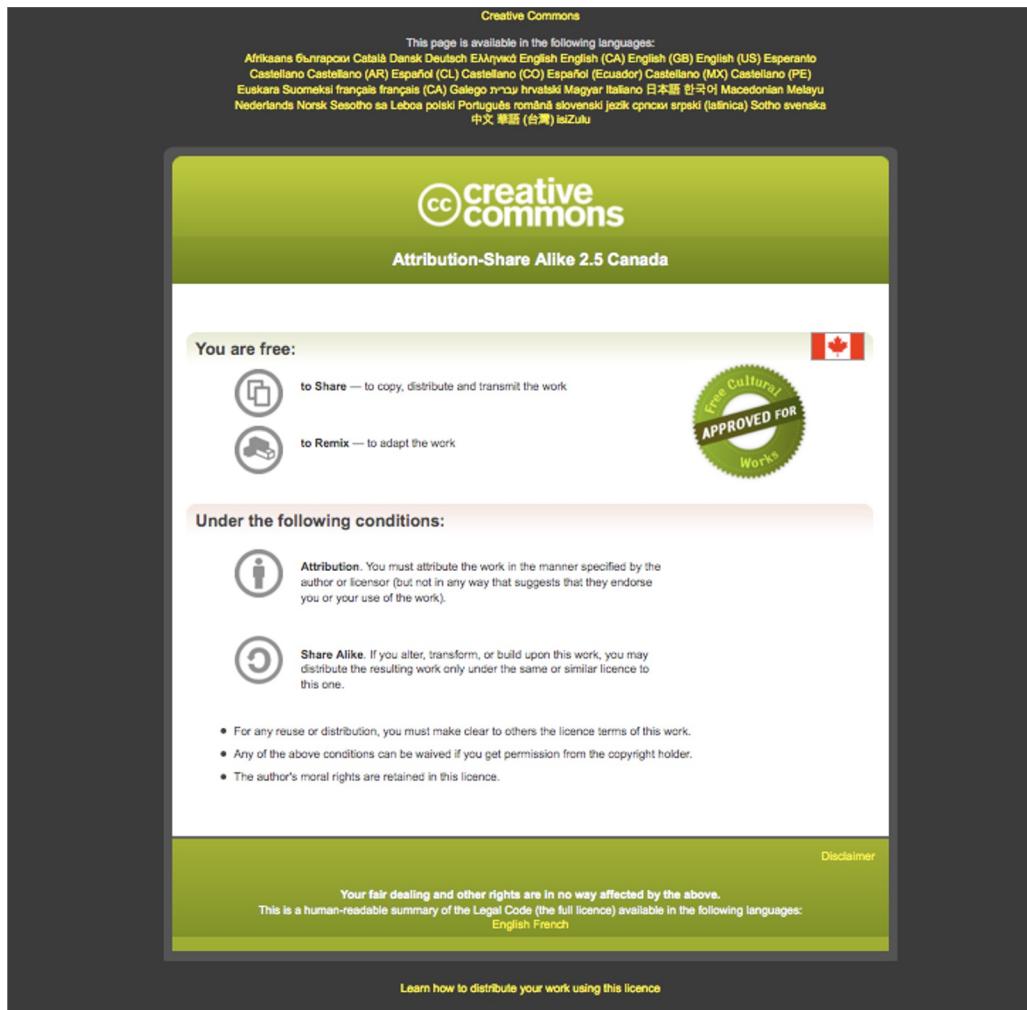




Canadian Bioinformatics Workshops

www.bioinformatics.ca

bioinformaticsdotca.github.io



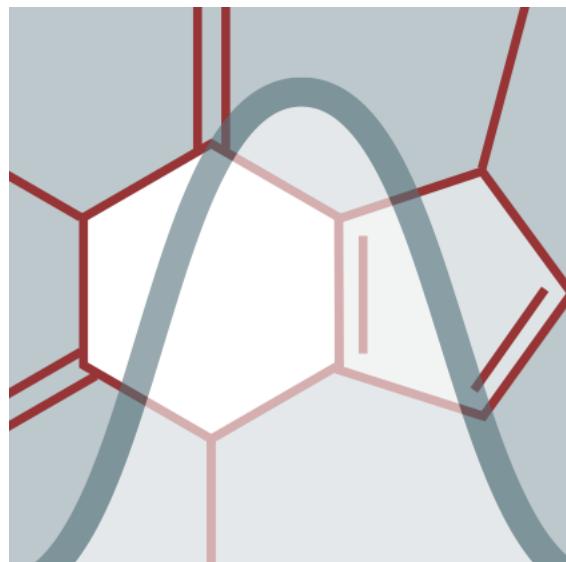
Quantitative Metabolomics (Lab)



David Wishart

Informatics and Statistics for Metabolomics

July 6-7, 2023



Schedule For July 6, 2023

Time	Module
8:00 (MST)/10:00 (EST)	Arrival & Check-in
8:30 (MST)/10:30 (EST)	Welcome (Nia Hughes)
9:00 (MST)/11:00 (EST)	Module 1: Introduction to Metabolomics (David Wishart)
10:30 (MST)/12:30 (EST)	Break/Lunch (45 min)
11:15 (MST)/13:15 (EST)	Module 2: Targeted, Quantitative Metabolomics (David Wishart)
12:15 (MST)/14:15 (EST)	Lunch/Break (45 min)
13:00 (MST)/15:00 (EST)	Module 3 (Lab): Quantitative Metabolomics (David Wishart)
15:00 (MST)/17:00 (EST)	Break (30 min)
15:30 (MST)/17:30 (EST)	Module 4: Databases for Biological Interpretation (David Wishart)
17:00 (MST)/19:00 (EST)	Finish

Learning Objectives

- Gain familiarity with processing targeted, quantitative metabolomics data from multiple platforms
- Learn how quantitative NMR metabolomics can be done using MagMet
- Learn how quantitative GC-MS metabolomics can be done using GC-AutoFit
- Learn how quantitative LC-MS metabolomics can be done using LC-AutoFit

How The Lab Will Be Conducted

- A total of three different analytical platforms will be tried (NMR, GC-MS, LC-MS)
- We will run MagMet (NMR), GC-AutoFit (GC-MS) and LC-AutoFit (LC-MS)
- The web servers have limited capacity (can't take 40+ simultaneous queries)
- Class will be divided into several groups and we will do a “round robin” test of each server to give a max load of 13-14 users each (2 files each for GC-MS and LC-MS, 1 file for NMR)
- Switch to a new server/platform after 30 minutes – if done early, explore the server, run some examples

How The Lab Will Be Conducted

- Check the sheets or CBW GitHub page to see your name and which technology platform (NMR, GC-MS, LC-MS) and software tool (MagMet, GC-AutoFit, LC-Autofit) you should be using
- Download your files (should have your name) onto your computer, start your designated webserver and upload your files to the webserver to begin the analysis
- After your analysis is complete, download the CSV files, take a look at them, save them for future work
- Upload your CSV files to the CBW “DropBox” page so that you can use the compiled data for the MetaboAnalyst lab tomorrow

The Spectral Files You Will Use

- **GC-MS Files – 84 urine samples from patients with and without sleep apnea analyzed on an Agilent 7890 GC-MS instrument (75 metabolites)**
- **NMR Files – 42 serum samples from sheep that are pregnant or non-pregnant analyzed on a 700 MHz NMR spectrometer (55 metabolites)**
- **LC-MS Files – 84 serum samples from patients with and without early-stage lung cancer analyzed on a Sciex Qtrap 4000 LC-MS/MS system (145 metabolites)**

First Group (30 minutes)

MagMet

Omolola Fatokun
Fatma Koc
Maryam Alyari
Michelle Asbury
Pavan Sai Kumar Attaluri
Fatma Elessawy
Amber Fedynak
Emma Finch
Vasuk Gautam
Hamza Jawad
Erica Kim
Prashanthi Kovur
Yaogeng Lei
Michael Lowings

GC-AutoFit

Scott MacKay
Ameneh Mohammadnezhad
Eponine Oler
Jenna Poelzer
Bernadette Quemerais
Ata Rafiee
Alyaa Selim
Gina Sykes
Siyang Tian
Claudia Torres
Fei Wang
Dorsa Yahya Rayat
Mahi Zakir
Yuli Zhao

LC-AutoFit

Xin Zhao
Ana Carolina Alves dos Santos
Karen Cristine Goncalves dos Santos
Fiona Hui
Fanfan Li
Alyssa Nouar
Meredith Sherrill
Hauna Sheyholislami
Roshan Timsina
Charles Viau
Lei Xu
Kai Ye
Olufemi Osonowo
Lise Cougnaud

Second Group (30 minutes)

MagMet

Scott MacKay
Ameneh Mohammadnezhad
Eponine Oler
Jenna Poelzer
Bernadette Quemerais
Ata Rafiee
Alyaa Selim
Gina Sykes
Siyang Tian
Claudia Torres
Fei Wang
Dorsa Yahya Rayat
Mahi Zakir
Yuli Zhao

GC-AutoFit

Xin Zhao
Ana Carolina Alves dos Santos
Karen Cristine Goncalves dos Santos
Fiona Hui
Fanfan Li
Alyssa Nouar
Meredith Sherrill
Hauna Sheyholislami
Roshan Timsina
Charles Viau
Lei Xu
Kai Ye
Olufemi Osonowo
Lise Cougnaud

LC-AutoFit

Omolola Fatokun
Fatma Koc
Maryam Alyari
Michelle Asbury
Pavan Sai Kumar Attaluri
Fatma Elessawy
Amber Fedynak
Emma Finch
Vasuk Gautam
Hamza Jawad
Erica Kim
Prashanthi Kovur
Yaogeng Lei
Michael Lowings

Third Group (30 minutes)

MagMet

Xin Zhao
Ana Carolina Alves dos Santos
Karen Cristine Goncalves dos Santos
Fiona Hui
Fanfan Li
Alyssa Nouar
Meredith Sherrill
Hauna Sheyholislami
Roshan Timsina
Charles Viau
Lei Xu
Kai Ye
Olufemi Osonowo
Lise Cougnaud

GC-AutoFit

Omolola Fatokun
Fatma Koc
Maryam Alyari
Michelle Asbury
Pavan Sai Kumar Attaluri
Fatma Elessawy
Amber Fedynak
Emma Finch
Vasuk Gautam
Hamza Jawad
Erica Kim
Prashanthi Kovur
Yaogeng Lei
Michael Lowings

LC-AutoFit

Scott MacKay
Ameneh Mohammadnezhad
Eponine Oler
Jenna Poelzer
Bernadette Quemerais
Ata Rafiee
Alyaa Selim
Gina Sykes
Siyang Tian
Claudia Torres
Fei Wang
Dorsa Yahya Rayat
Mahi Zakir
Yuli Zhao

Webserver Assignments (Part 1)

First Name	Last Name	GC_Autofit	MagMet	LC-Autofit
Omolola	Fatokun	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Fatma	Koc	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Maryam	Alyari	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Michelle	Asbury	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Pavan Sai Kumar	Attaluri	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Fatma	Elessawy	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Amber	Fedynak	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Emma	Finch	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Vasuk	Gautam	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Hamza	Jawad	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Erica	Kim	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Prashanthi	Kovur	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Yaogeng	Lei	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Michael	Lowings	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com

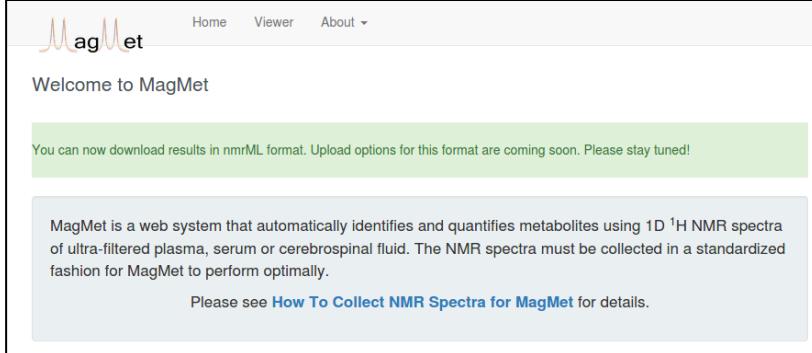
Webserver Assignments (Part 2)

First Name	Last Name	GC_Autofit	MagMet	LC-Autofit
Scott	MacKay	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Ameneh	Mohammadnezhad	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Eponine	Oler	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Jenna	Poelzer	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Bernadette	Quemerais	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Ata	Rafiee	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Alyaa	Selim	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Gina	Sykes	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Siyang	Tian	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Claudia	Torres	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Fei	Wang	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Dorsa	Yahya Rayat	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Mahi	Zakir	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Yuli	Zhao	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com

Webserver Assignments (part 3)

First Name	Last Name	GC_Autofit	MagMet	LC-Autofit
Xin	Zhao	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Ana Carolina	Alves dos Santos	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Karen Cristine	Goncalves dos Santos	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Fiona	Hui	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Fanfan	Li	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Alyssa	Nouar	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Meredith	Sherrill	gc-autofit1.wishartlab.com	teaching1.magmet.ca	dev-lcautofit.wishartlab.com
Hauna	Sheyholislami	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Roshan	Timsina	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Charles	Viau	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Lei	Xu	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Kai	Ye	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Olufemi	Osonowo	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com
Lise	Cougnaud	gc-autofit2.wishartlab.com	teaching2.magmet.ca	dev-lcautofit.wishartlab.com

MagMet - For NMR Spectral Deconvolution

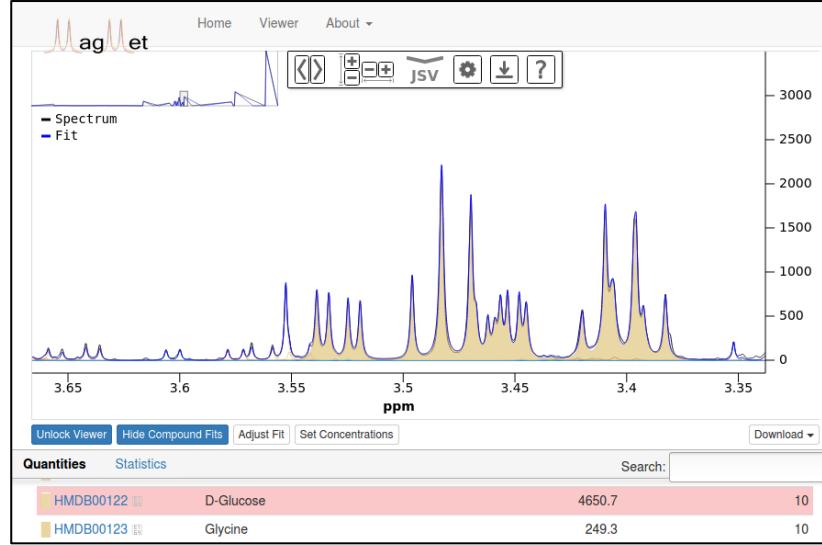


Welcome to MagMet

You can now download results in nmrML format. Upload options for this format are coming soon. Please stay tuned!

MagMet is a web system that automatically identifies and quantifies metabolites using 1D ^1H NMR spectra of ultra-filtered plasma, serum or cerebrospinal fluid. The NMR spectra must be collected in a standardized fashion for MagMet to perform optimally.

Please see [How To Collect NMR Spectra for MagMet](#) for details.



- MagMet – Magnetic Resonance for Metabolomics – Replaces Bayesil
- Offers more flexibility than Bayesil (which was limited to analyzing blood and CSF)
- Uses a combination of machine learning and expert-based rules to perform fitting and pattern finding
- Fully automated phasing, referencing, water removal, baseline correction, peak convolution, ID and quantification
- Detects & quantifies 55-60 serum metabolites in ~10 minutes
- Has been adopted to wine, beer, juice, milk, fermentation products

<https://teaching1.magmet.ca>

OR

<https://teaching2.magmet.ca>

GC-AutoFit (Automated GC-MS)

The screenshot shows the 'GC-AutoFit Analysis' page. At the top, there's a brief introduction about the tool's purpose: 'GC-AutoFit is a web application that automatically identifies and quantifies metabolites using Gas Chromatography Mass Spectrometry (GC-MS) spectra. For optimal GC-AutoFit performance, the query GC-MS spectra should be prepared according to the instructions (How to collect GC-MS Spectra for GC-AutoFit). GC-AutoFit currently accepts .CDF and .mzXML file formats. It uses alkane standards to calculate the retention index (RI) of each peak in the sample. The extracted EI-MS spectra from each peak, along with the RIs, are then compared to reference spectra (RIs and EI-MS) in the specified library to identify and quantify the compounds. The inclusion of blank spectra is optional, however, it is useful for removing noise effects from the query spectra. Extensive testing shows that GC-AutoFit meets or exceeds the performance of highly trained human experts.'

Instructions
To analyze GC-MS spectra with GC-AutoFit you must provide information on the biofluid being analyzed and the spectral files in .CDF or .mzXML format. Up to 30 spectra are recommended to analyze in one submission.

Submit your own mixture:

- Select Biofluid:** Serum (radio button), Urine (radio button, selected), Saliva (radio button), Upload Your Library (checkbox).
- Select internal standard:** Ribitol (radio button), Cholesterol (radio button), Succinate-D4 (radio button, selected), Tropic acid (radio button), Other (radio button).
- Upload Spectra:** Separate Files (checkbox), OR Single ZIP File (checkbox). All spectral files must be .CDF or .mzXML. Up to 30 samples can be submitted.
Alkane standards*: Choose File (button), No file chosen.
Blank: Choose File (button), No file chosen.
Samples*: Choose Files (button), No file chosen.
- Provide a MF(Match Factor) Score cutoff:** An integer between 0 - 999 but somewhere between 400 - 600 is recommended. (Input field: 400)

Submit

OR, run one of our examples:
Serum

latest update: March 11th, 2016

- Requires 3 spectra (sample, blank, alkane standards)
- Performs auto-alignment, peak ID, peak integration and concentration calculation
- Accepts NetCDF or mzXML files
- 60 sec per spectrum
- 40-116 cmpds ID'd and quantified, 96% accuracy
- Optimized for blood, urine, saliva and CSF
- Still requires careful sample preparation & derivatization

<http://gc-autofit1.wishartlab.com>

OR <http://gc-autofit2.wishartlab.com>

LC-AutoFit (Automated LC-MS)

Welcome to LC-AutoFit

LC-AutoFit is a web application that is tailored for processing the raw Liquid Chromatography-Mass Spectrometry(LC-MS) spectra collected by the TMC Prime and TMC Mega metabolomics kits. LC-AutoFit automatically identifies and quantifies the medically important metabolites targeted by the TMC Prime and TMC Mega kits.

LC-AutoFit currently accepts .wiff or .mzXML format that is collected by AB Sciex instruments. Users can download the quantitated results in csv file format after data processing. For optimal performance, the query LC-MS spectra should be collected following the manuals provided with the TMC Prime and TMC Mega kits. Please upload your raw files according to the instructions on this page and start your journey with LC-AutoFit!

LC-AutoFit Analysis

Instructions

To analyze LC-MS spectra with LC-AutoFit you must provide information on the biofluid being analyzed and the spectral files in .wiff or .mzXML format. Up to 100 spectra are recommended to analyze in one submission. Submit your own mixture.

1. Select Biofluid* - LC-AutoFit works with Urine, Blood, and Fecal Samples.
 Blood Urine Fecal Matter

2. Upload Spectra* - Separate Files OR Single ZIP File
All spectral files must be .wiff or .mzXML. Up to 100 samples can be submitted.

Calibration samples* 7 files
Quality control samples* 3 files
Experimental samples* 15 files
Blank samples* 2 files
Double blank samples 20220304_ueleBlank.wiff

3. Assay* - TMC MEGA Panel B

4. Upload Retention Time* - Download Blank Retention Time Table
The retention time file must be a .csv file. You can find template retention tables here for your specific biofluid and assay. You must use this template or LC-AutoFit will not be able to run its analysis.
 Choose file RT_TMC M... - B - Blood.csv

collected following the manuals provided with the TMC Prime and TMC Mega kits. Please upload your raw files according to the instructions on this page and start your journey with LC-AutoFit!

LC-AutoFit Analysis

Instructions

To analyze LC-MS spectra with LC-AutoFit you must provide information on the biofluid being analyzed and the spectral files in .wiff or .mzXML format. Up to 100 spectra are recommended to analyze in one submission. Submit your own mixture.

1. Select Biofluid* - LC-AutoFit works with Urine, Blood, and Fecal Samples.
 Blood Urine Fecal Matter

2. Upload Spectra* - Separate Files OR Single ZIP File
All spectral files must be .wiff or .mzXML. Up to 100 samples can be submitted.

Calibration samples* No file chosen
Quality control samples* No file chosen
Experimental samples* No file chosen
Blank samples* No file chosen
Double blank samples No file chosen

3. Assay* - TMC MEGA Panel A

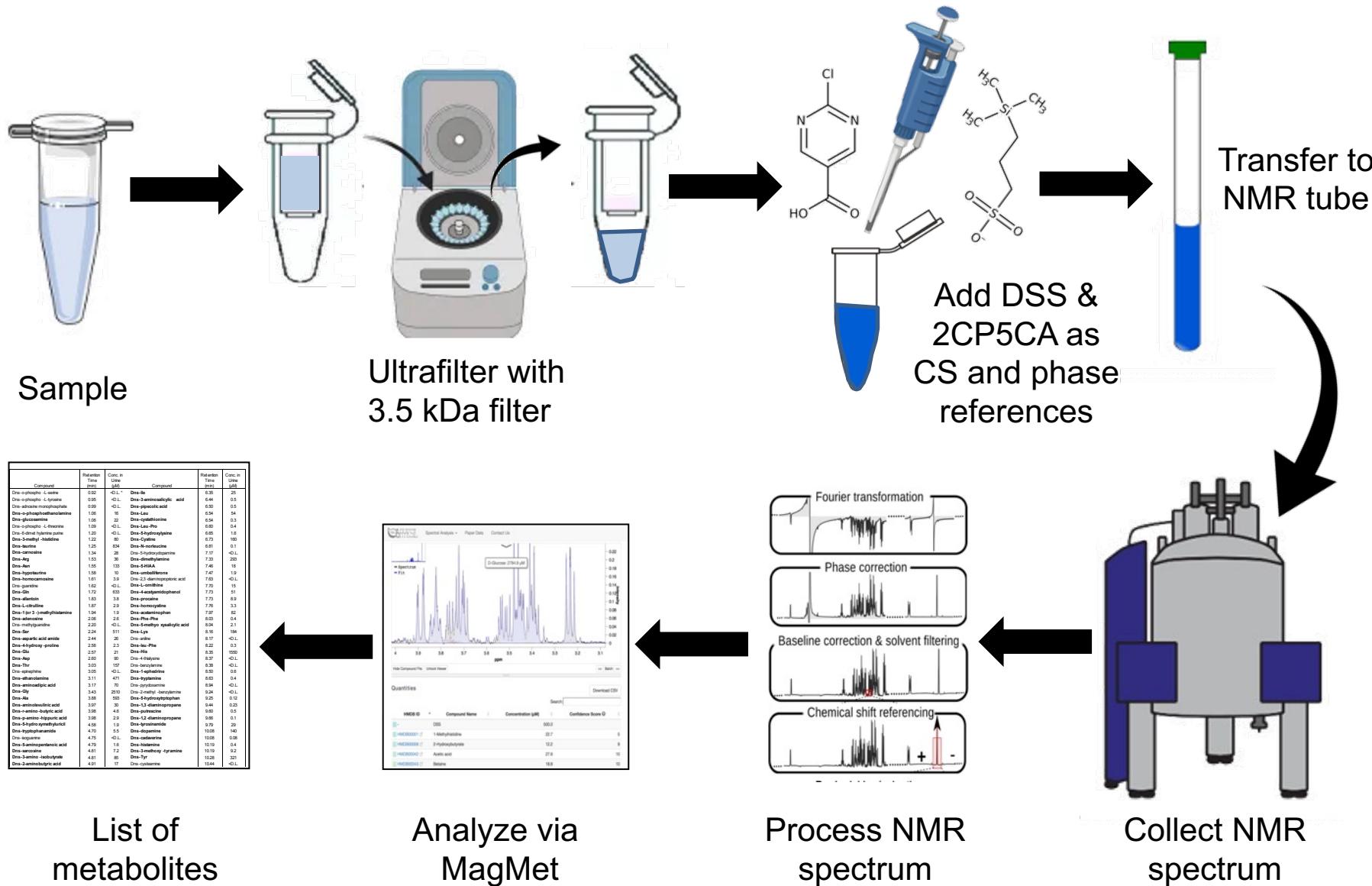
4. Upload Retention Time* - Download Blank Retention Time Table
The retention time file must be a .csv file. You can find template retention tables here for your specific biofluid and assay. You must use this template or LC-AutoFit will not be able to run its analysis.
 Choose file No file chosen

The screenshot shows a file explorer window displaying numerous .wiff and .csv files related to the TMC MEGA Panel A assay, including files for calibration, quality control, experimental samples, and retention time tables.

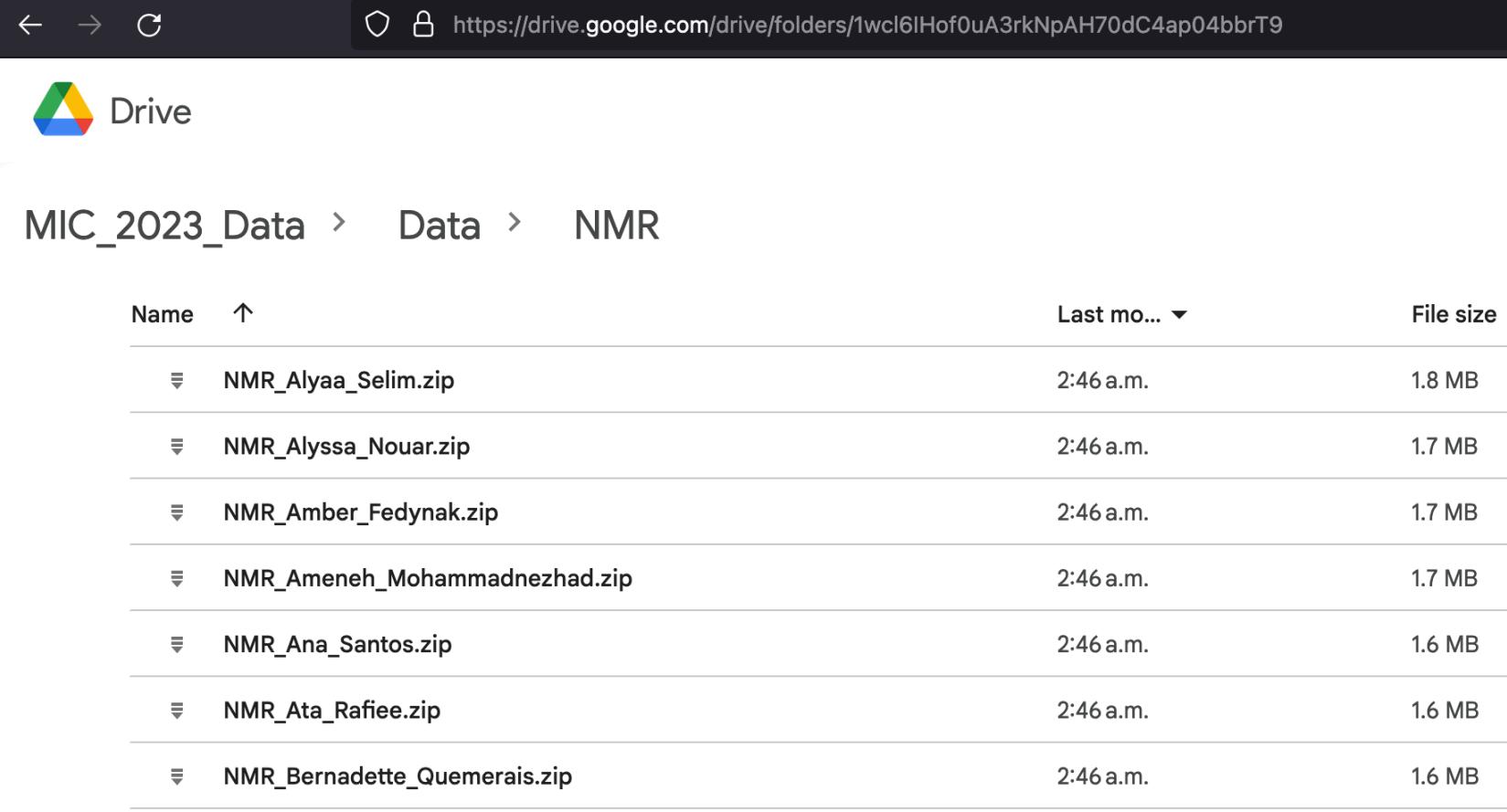
- Requires user to select biofluid
- Requires user to provide MS/MS spectral files and to indicate calibration files, sample files, QC sample files & blank sample files
- Retention time file and retention time list is also required
- Files can be dragged and dropped into LC-AutoFit browser to simplify file uploads
- Processes 96 samples in just 120-150 sec
- 180-640 compounds can be identified and quantified
- Optimized for blood, urine, stool samples for Qtrap instruments

<http://dev-lcautofit.wishartlab.com/>

NMR Metabolomics Overview



Download Your NMR Zip File From The CBW Team Drive



The screenshot shows a Google Drive interface. At the top, the URL https://drive.google.com/drive/folders/1wcl6IHof0uA3rkNpAH70dC4ap04bbrT9 is displayed. Below the URL, the Google Drive logo is visible. The navigation bar shows the path: MIC_2023_Data > Data > NMR. The main area is a table listing seven files:

Name	Last modified	File size
NMR_Alyaa_Selim.zip	2:46 a.m.	1.8 MB
NMR_Alyssa_Nouar.zip	2:46 a.m.	1.7 MB
NMR_Amber_Fedynak.zip	2:46 a.m.	1.7 MB
NMR_Ameneh_Mohammadnezhad.zip	2:46 a.m.	1.7 MB
NMR_Anna_Santos.zip	2:46 a.m.	1.6 MB
NMR_Atta_Rafiee.zip	2:46 a.m.	1.6 MB
NMR_Bernadette_Quemerais.zip	2:46 a.m.	1.6 MB

<https://drive.google.com/drive/folders/1wcl6IHof0uA3rkNpAH70dC4ap04bbrT9>

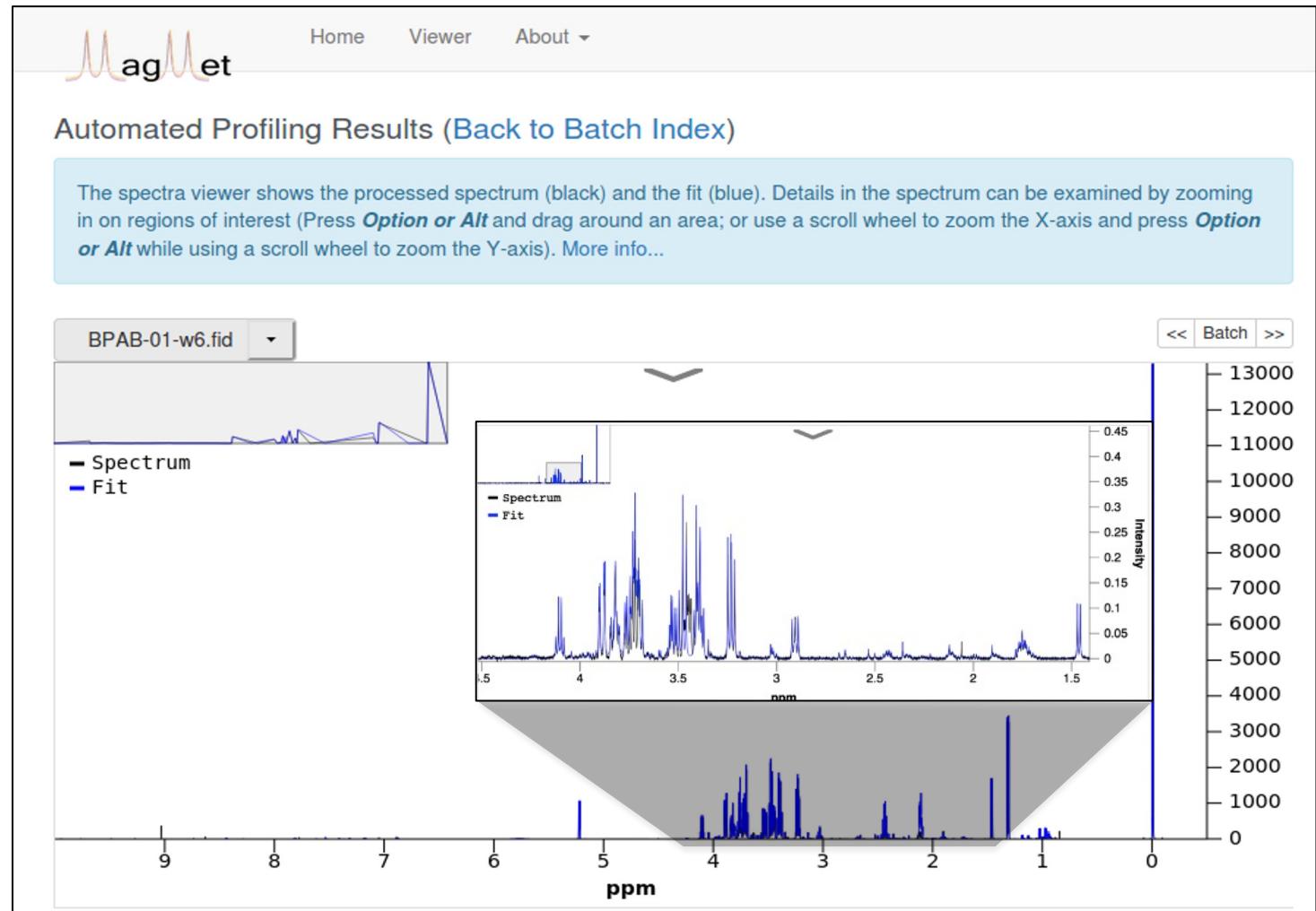
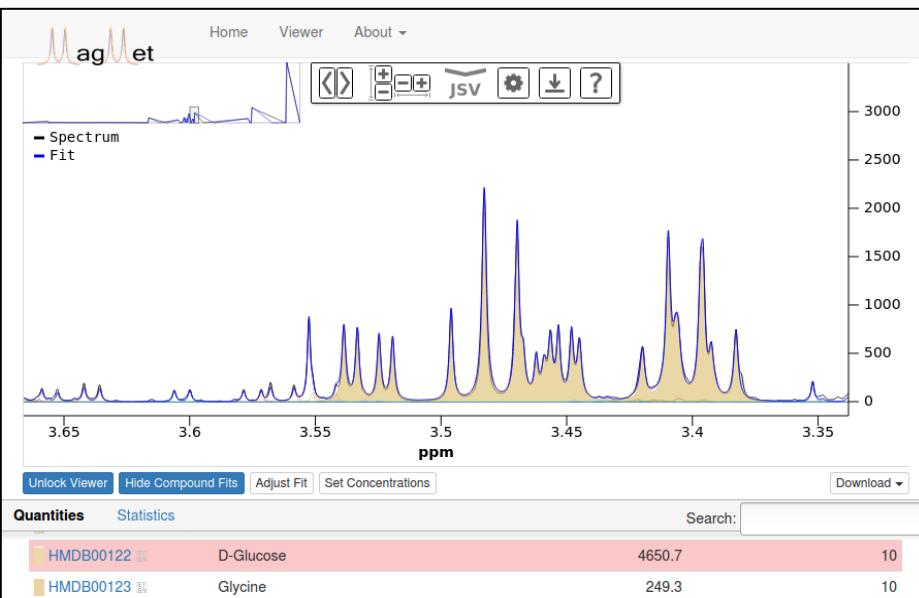
Go To The MagMet Server

Welcome to MagMet

You can now download results in nmrML format. Upload options for this format are coming soon. Please stay tuned!

MagMet is a web system that automatically identifies and quantifies metabolites using 1D ^1H NMR spectra of ultra-filtered plasma, serum or cerebrospinal fluid. The NMR spectra must be collected in a standardized fashion for MagMet to perform optimally.

Please see [How To Collect NMR Spectra for MagMet](#) for details.



<https://teaching1.magmet.ca>

OR <https://teaching2.magmet.ca>

Detailed Sample Instructions

The image shows two screenshots of the MagMet web application. The top screenshot displays the 'About' page, which includes a welcome message, a brief description of the system, and a link to 'How To Collect NMR Spectra for MagMet'. The bottom screenshot shows a detailed guide titled 'How To Collect NMR Spectra for Optimal Performance with MagMet', which provides instructions for sample preparation and acquisition conditions.

Welcome to MagMet

Thank you for using MagMet!

MagMet is a web system that automatically identifies and quantifies metabolites using 1D ^1H NMR spectra of ultra-filtered plasma, serum or cerebrospinal fluid. The NMR spectra must be collected in a standardized fashion for MagMet to perform optimally.

Please see [How To Collect NMR Spectra for MagMet](#)

MagMet Spectral Analysis

Instructions

To analyze a 1D ^1H NMR spectrum with MagMet you must provide information about the sample, the concentration of the reference standard, the spectrometer frequency and the spectral file (FID). A **credit** is required for each FID in the uploaded ZIP file. Only one credit per reason will count.

How To Collect NMR Spectra for Optimal Performance with MagMet

In order for MagMet to perform optimally, it is best if the sample preparation and the NMR data acquisition are performed in a manner similar to that used to prepare and collect the reference NMR spectra used in MagMet's spectral library. Using different spectral preparation and collection conditions will compromise the performance, particularly the quantification accuracy. Here we describe the recommended methods that MagMet users should follow when preparing biological samples and collecting NMR spectra of those samples.

Important points for best results:

- Filter sample through 3.5 kDa filters
- Adjust pH to 7.0 with 150 mM K_2HPO_4
- Add d_6 -DSS for chemical shift referencing
- For optimal automated phase correction, add 2-chloropyrimidine-5-carboxylic acid
- Collect using optimized 1D noesy at 25 °C as outlined

<https://teaching1.magmet.ca>

OR

<https://teaching2.magmet.ca>

File Submission

The screenshot shows the MagMet web application. At the top, there is a navigation bar with links for Home, Viewer, and About. The main content area has a header "Welcome to MagMet". Below it, a green box contains the message "Thank you for using MagMet!". A central gray box provides information about the system: "MagMet is a web system that automatically identifies and quantifies metabolites using 1D ^1H NMR spectra of ultra-filtered plasma, serum or cerebrospinal fluid. The NMR spectra must be collected in a standardized fashion for MagMet to perform optimally." It also includes a link "Please see [How To Collect NMR Spectra for MagMet](#) for details." Below this, another gray box is titled "MagMet Spectral Analysis" and contains instructions: "To analyze a 1D ^1H NMR spectrum with MagMet you must provide information on the biofluid being analyzed, the concentration of the reference standard, the spectrometer frequency and the 1D NMR spectral file (FID). **A credit is required for each FID in the uploaded ZIP file. FIDs that fail for whatever reason will not count.**" At the bottom of this box, there is a placeholder text "Submit your files:".

<https://teaching.magmet.ca>

Upload Your Spectrum File

 MagMet

Home Viewer About ▾

MagMet Spectral Analysis

Instructions

To analyze a 1D ^1H NMR spectrum with MagMet you must provide information on the biofluid being analyzed, the concentration of the reference standard, the spectrometer frequency and the 1D NMR spectral file (FID). A **credit** is required for each FID in the uploaded ZIP file. FIDs that fail for whatever reason will not count.

Submit your files:

1. **Upload Spectrum:***

No file selected. Example 1 Example 2

Please ensure your spectrum has been collected using the protocol described [here](#). The uploaded file must be a ZIP-compressed file containing one or more folders with raw NMR experimental data. Bruker folders must contain a fid and acqus file. Agilent/Variian folders must contain a fid and procpar file. The maximum upload file size is 200 MB.

2. **Submission Name:**

Provide a name for the submission for future reference. If no name is provided the uploaded zip file name will be used.

3. **Preprocessed (Optional)**

Need to add description here.

4. **Select Biofluid:***

MagMet can analyze spectra of human serum or plasma.

5. **NMR Frequency:***

Provide the frequency of the NMR spectrometer used to collect the spectrum.

Monitor MagMet Progress

magMet

Home Viewer About ▾

MagMet Submission: BPAB-01-w6.fid

Settings

Uploaded File: [BPAB-01-w6.fid.zip](#)
Biofluid: Serum (Filtered)
DSS Concentration: 1000.0 μ M
Date: 2023-04-24 17:09:53 UTC

NMR Frequency: 700 MHz
Fast Profile: No
FIDs: 1

Status

⌚ Processing...

You will be redirected to your results after the analysis is complete. A typical analysis takes 5-10 minutes.
You can also bookmark this page and check back later.

magMet

Home Viewer About ▾

Automated Profiling Results

The spectra viewer shows the processed spectrum (black) and the fit (blue). Details in the spectrum can be examined by zooming in on regions of interest (Press **Option** or **Alt** and drag around an area; or use a scroll wheel to zoom the X-axis and press **Option** or **Alt** while using a scroll wheel to zoom the Y-axis). More info...

BPAB-01-w6.fid

Spectrum Fit

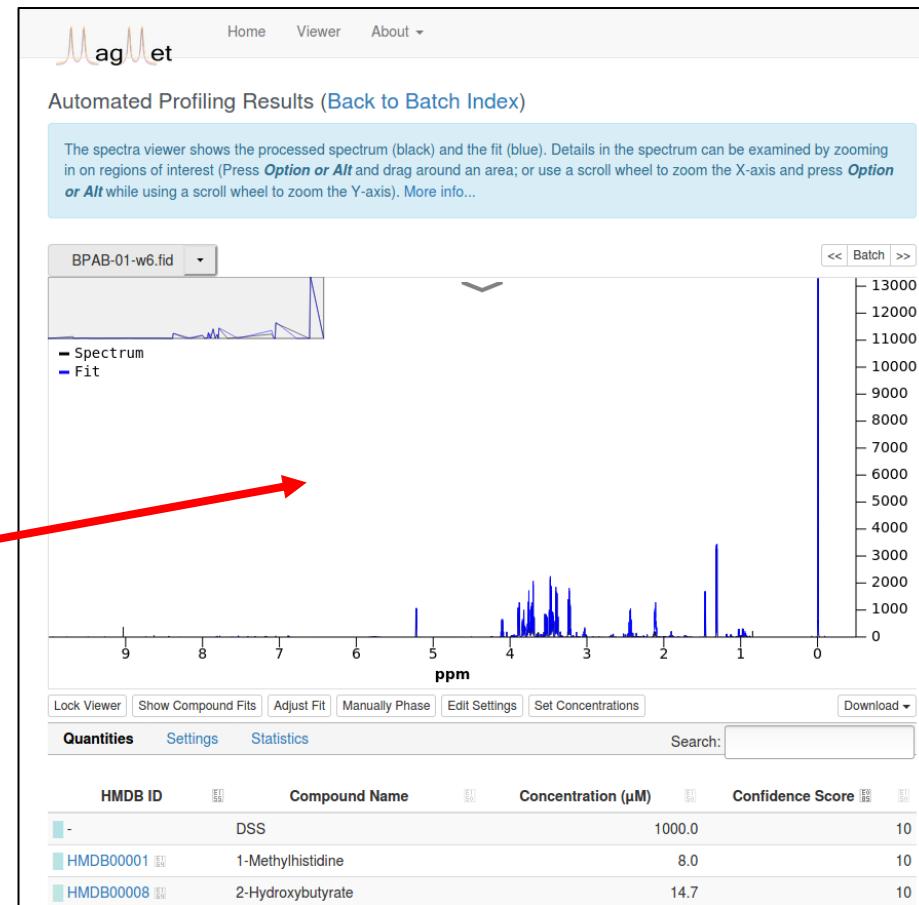
ppm

Lock Viewer Show Compound Fits Adjust Fit Manually Phase Edit Settings Set Concentrations Download ▾

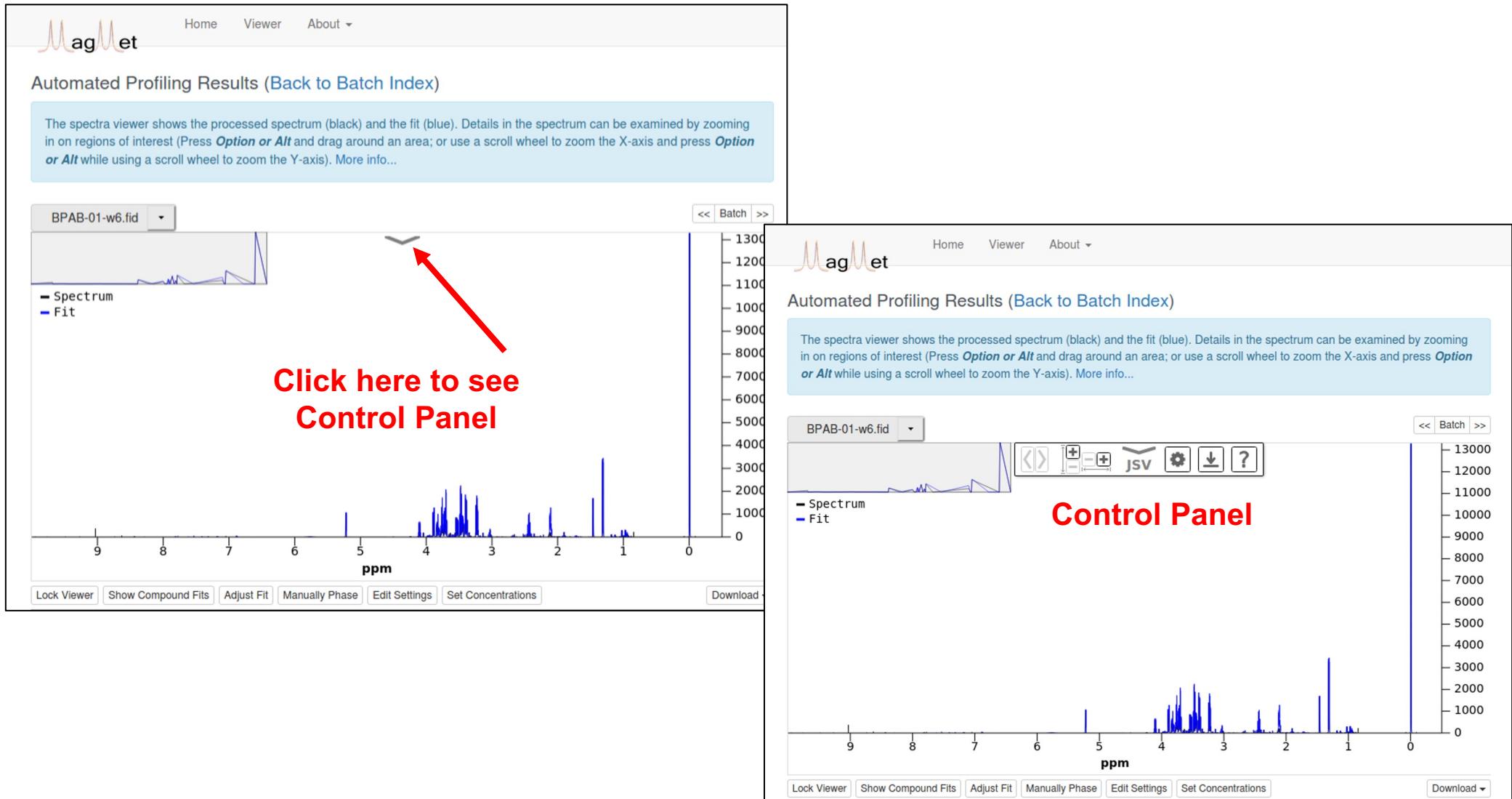
View NMR Spectra

The screenshot shows the MagMet submission interface. At the top, there's a navigation bar with 'Home', 'Viewer', and 'About'. A red box highlights the 'Home' button. Below it, the text 'MagMet Submission: BPAB-01-w6.fid' is displayed. Under 'Settings', it shows 'Uploaded File: BPAB-01-w6.fid.zip', 'Biofluid: Serum (Filtered)', 'DSS Concentration: 1000.0 μ M', 'Date: 2023-04-24 17:09:53 UTC', 'NMR Frequency: 700 MHz', 'Fast Profile: No', and 'FIDs: 1'. In the 'Status' section, a message says 'Processing...' with a circular progress icon. It continues: 'You will be redirected to your results after the analysis is complete. A typical analysis takes 5-10 minutes. You can also bookmark this page and check back later.'

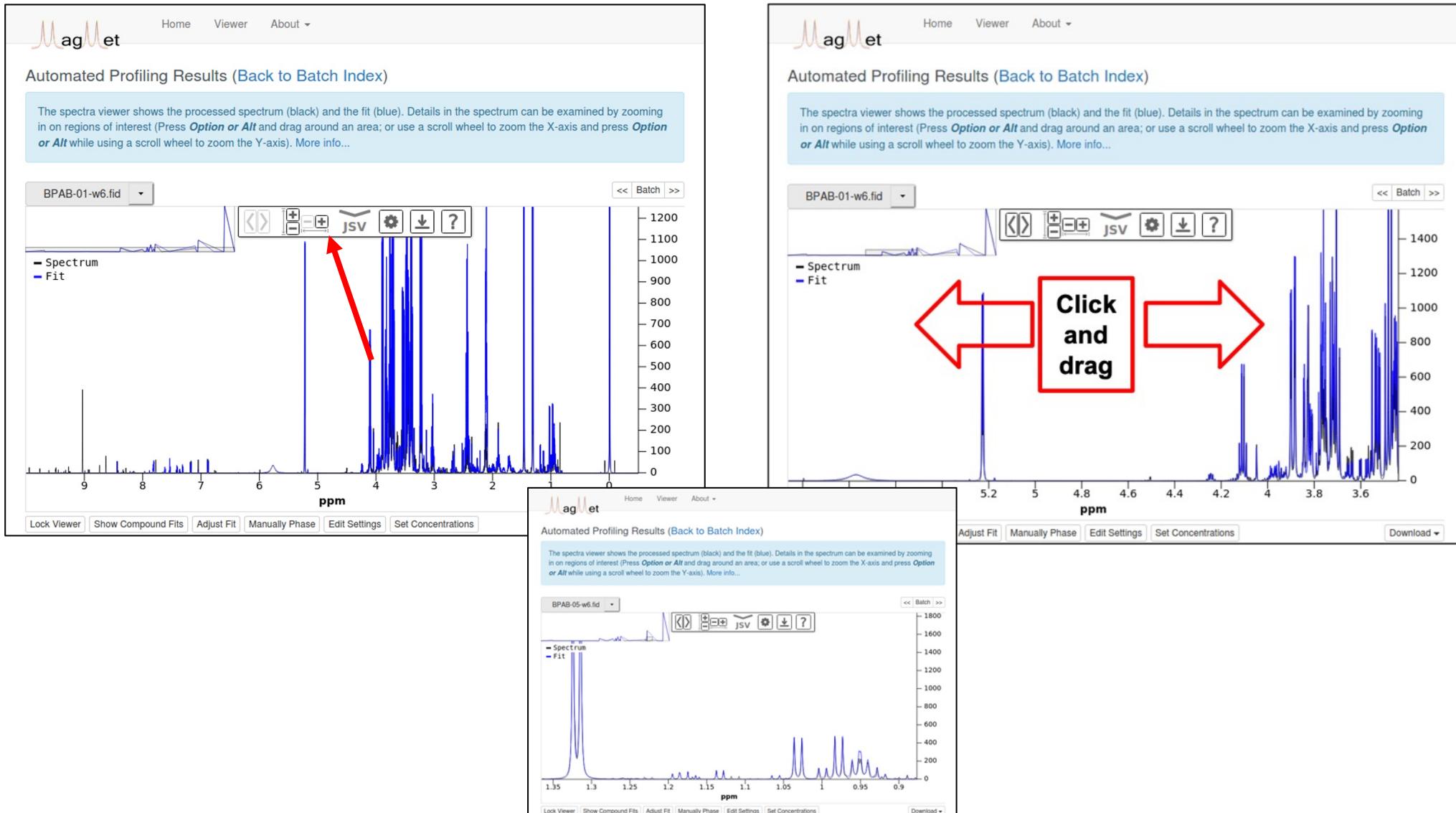
- To save time, click on completed spectrum



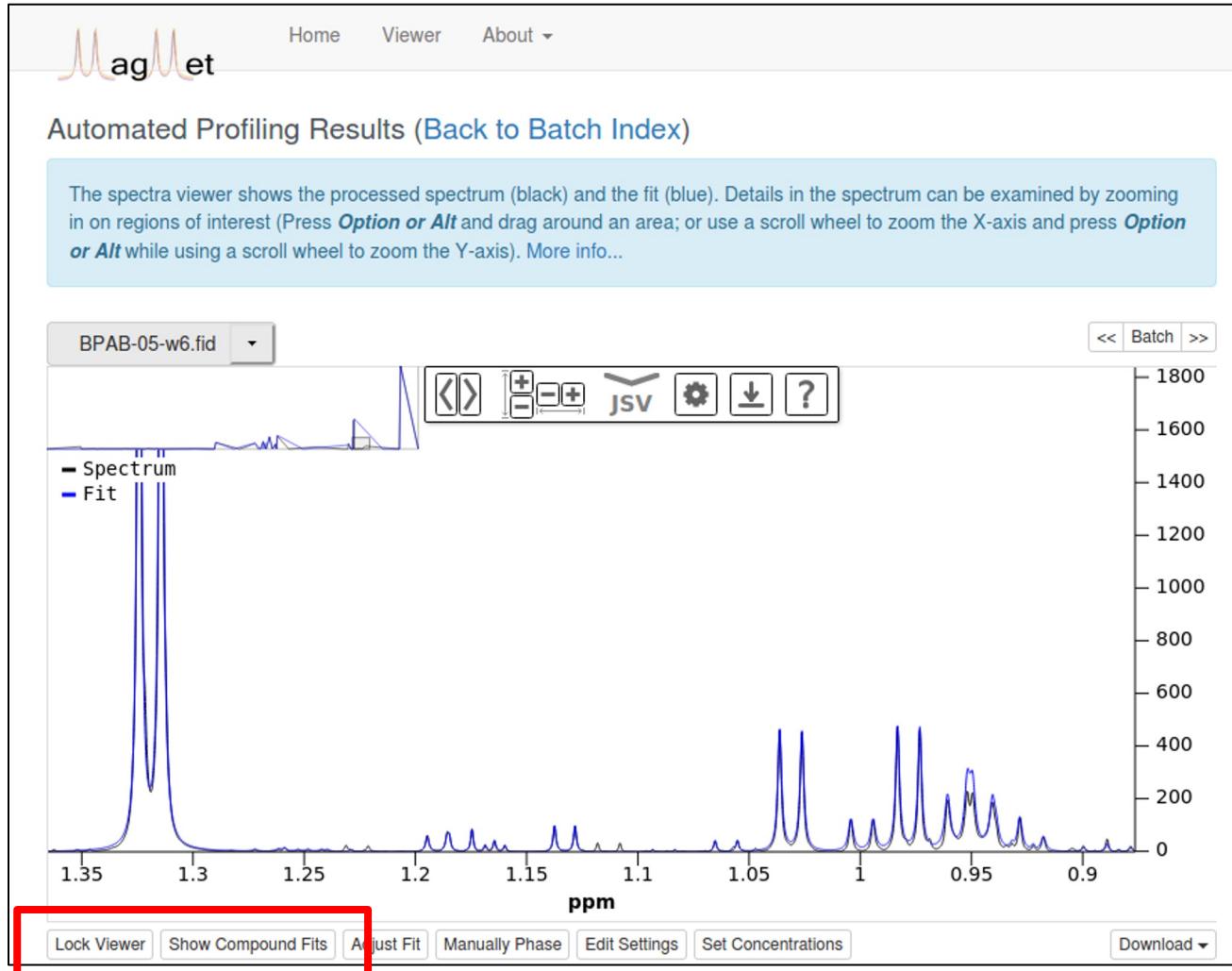
Browse NMR Spectra



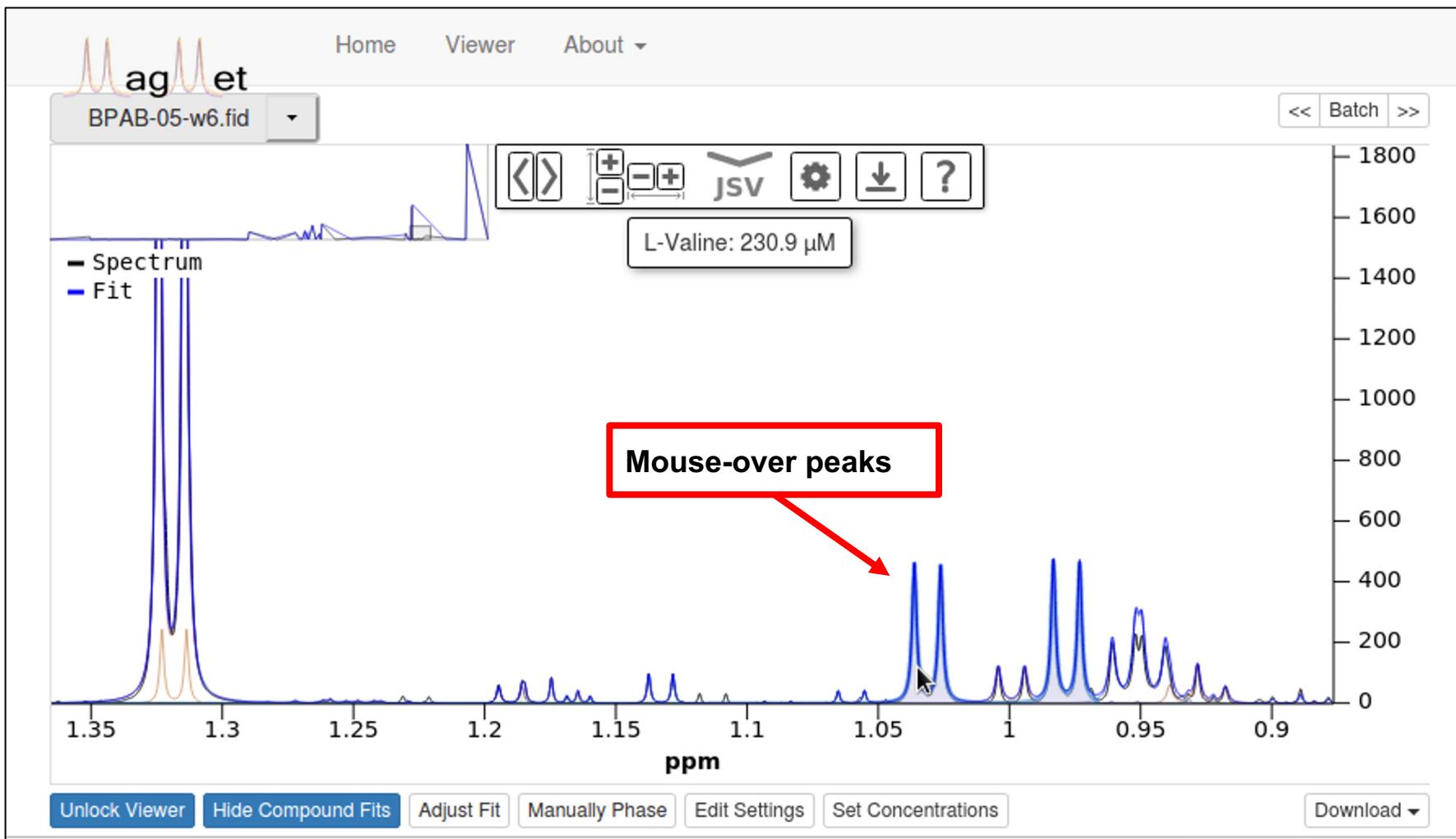
Interactive Zoom



View Spectra



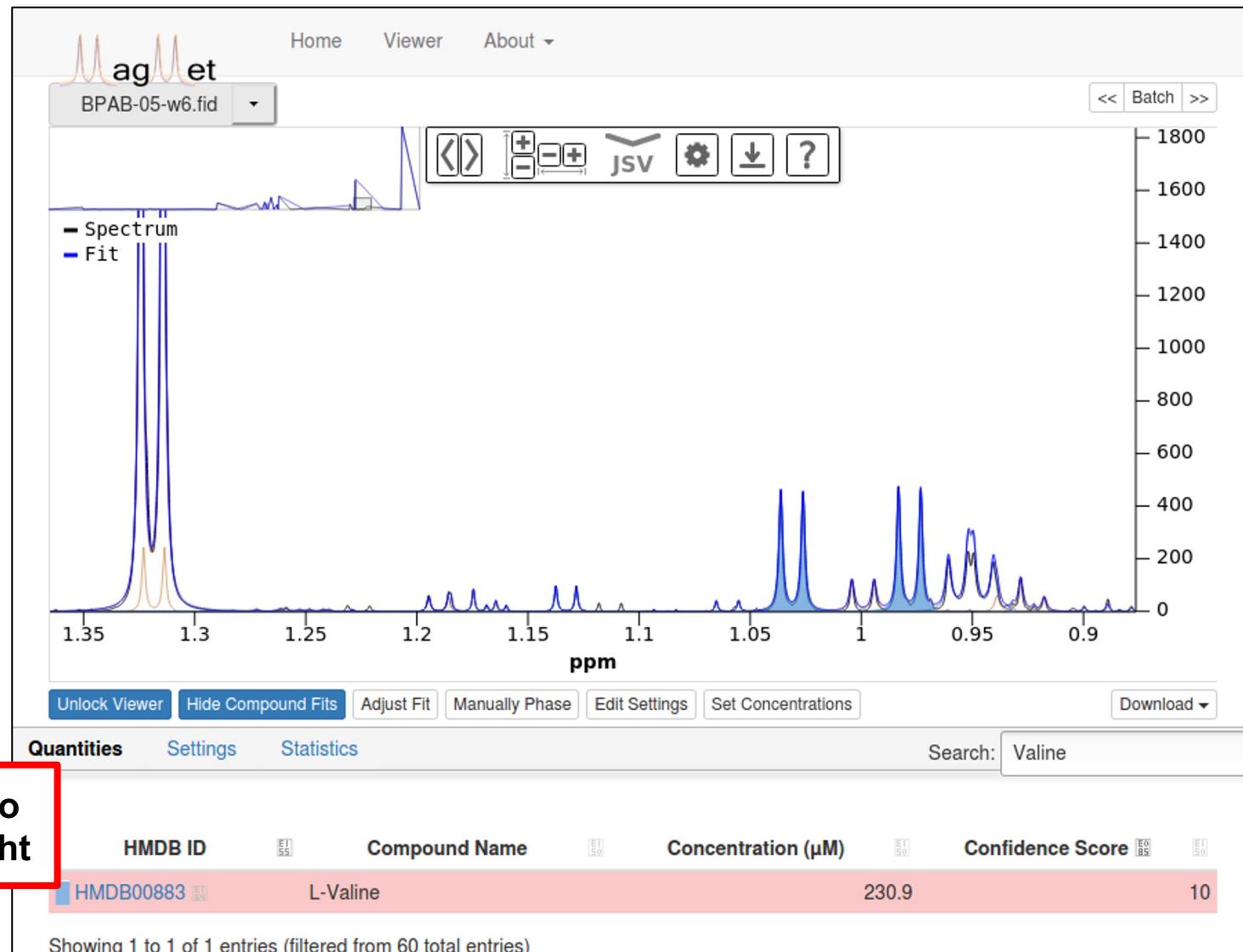
View Spectra



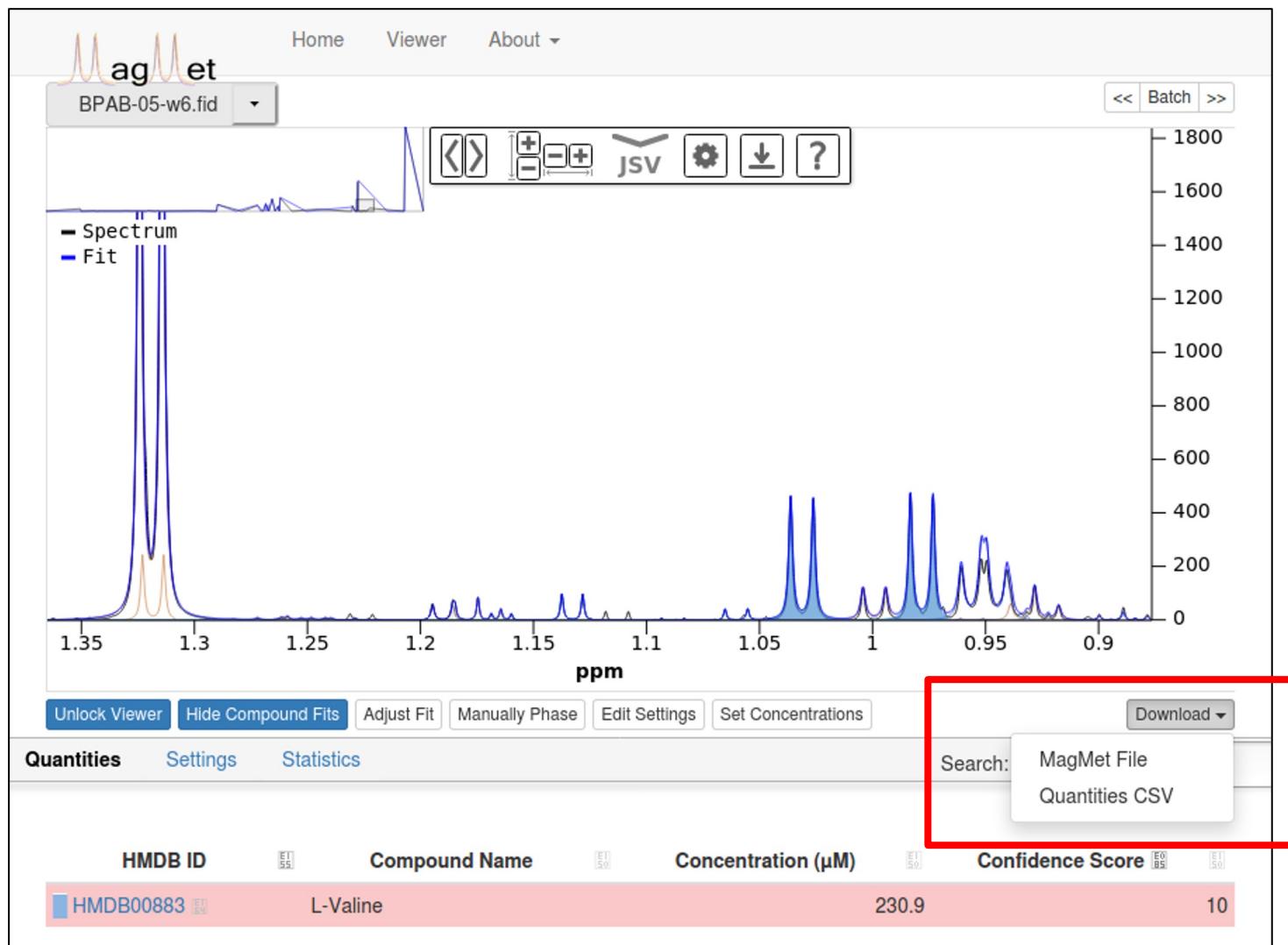
Concentration Table



Concentration Table



Download Spectra or Data



Concentration Data or Spectra

“magmet_results.csv”

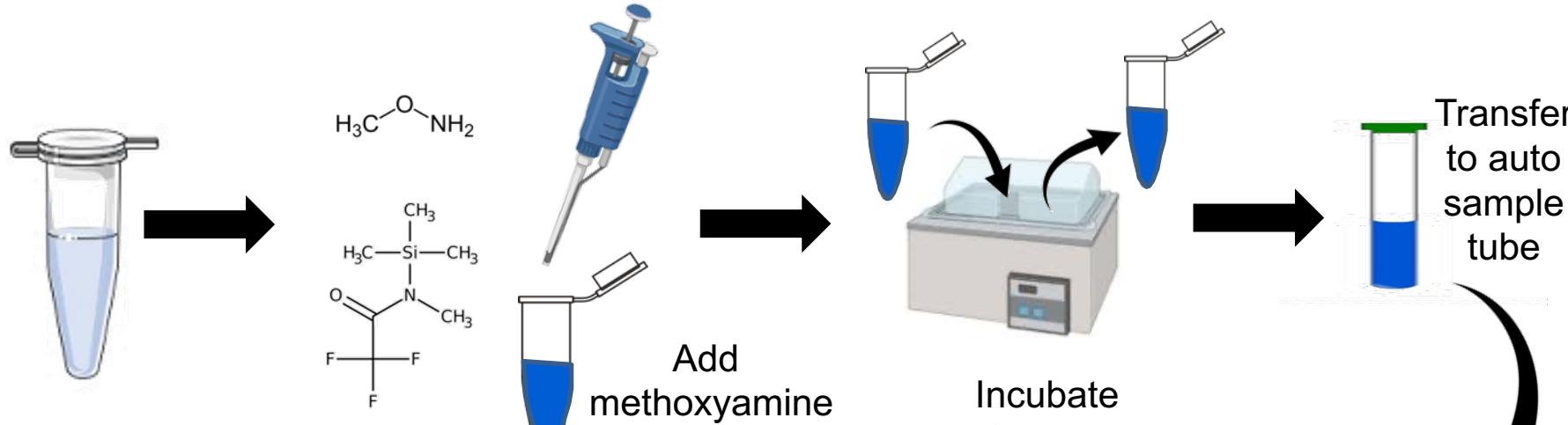
	A	B	C
	HMDB ID	Compound Name	Concentration (μ M)
10	-	DSS	1000
11	HMDB00001	1-Methylhistidine	4.7
12	HMDB00008	2-Hydroxybutyrate	15.8
13	HMDB00042	Acetic acid	28.5
14	HMDB00043	Betaine	28.4
15	HMDB00060	Acetoacetate	17.5
16	HMDB00062	L-Carnitine	48.8
17	HMDB00064	Creatine	18.2
18	HMDB00087	Dimethylamine	2.1
19	HMDB00092	Dimethylglycine	1.9
20	HMDB00094	Citric acid	49.8
21	HMDB00097	Choline	6.7
22	HMDB00108	Ethanol	40
23	HMDB00122	D-Glucose	4794.4
24	HMDB00123	Glycine	232.4
25	HMDB00131	Glycerol	108.7
26	HMDB00142	Formic Acid	44.7
27	HMDB00148	L-Glutamic acid	94
28	HMDB00157	Hypoxanthine	5.8

JSON Raw Data Headers
Save Copy Collapse All Expand All (slow) Filter JSON
dss_conc: 1000
metabolites:
0:
name: "DSS"
id: "HMBD00000"
concentration: 1000
dss_ratio: 1
score: 10
threshold: 0.1
1:
2:
3:
4:
Viewer About ▾
Drag MAGMET JSON file here...
Intensity
ppm
Lock Viewer Show Compound Fits Adjust Fit Set Concentrations Download ▾

“magmet_data.json”

Drag-and-drop file into standalone viewer

GC-MS Metabolomics Overview

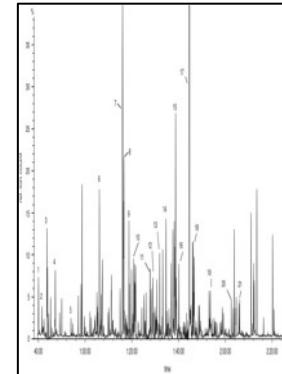


Compound	Retention time (min)	Conc. in sample (µM)	Compound	Retention time (min)	Conc. in sample (µM)
Dna-amine	0.00	0.00	Dna-2-aminoacrylic acid	0.39	29
Dna-2-phospho-L-lysine	0.56	0.1	Dna-3-hydroxyproline	0.41	23
Dna-adenosine monophosphate	0.59	0.1	Dna-3-phosphoglycolic acid	0.50	0.5
Dna-2,3-dihydroxyacetonephosphate	0.59	0.1	Dna-4-hydroxyproline	0.50	0.5
Dna-glucosamine	1.06	22	Dna-glycolaldehyde	0.54	34
Dna-D-glucosamine	1.09	0.1	Dna-L-Glu-Pro	0.60	0.4
Dna-D-glucosamine	1.10	0.1	Dna-Lysine	0.60	0.4
Dna-3-hydroxy-L-alanine	1.22	30	Dna-Oxoproline	0.73	180
Dna-3-hydroxy-L-alanine	1.23	20	Dna-2-hydroxyproline	0.73	180
Dna-3-hydroxy-L-alanine	1.34	28	Dna-3-hydroxyproline	0.77	180
Dna-3-hydroxy-L-alanine	1.35	20	Dna-4-hydroxyproline	0.77	180
Dna-Arg	1.53	36	Dna-4-hydroxyproline	0.77	180
Dna-Asn	1.55	10	Dna-5-hydroxyproline	0.77	180
Dna-Hypothreonine	1.58	10	Dna-6-hydroxyproline	0.77	180
Dna-2-hydroxyproline	1.61	2.9	Dna-2-hydroxyproline	7.47	1.9
Dna-2-hydroxyproline	1.62	2.9	Dna-3-hydroxyproline	7.47	1.9
Dna-Gln	1.72	633	Dna-4-hydroxydiphenylalanine	7.75	51
Dna-Gln	1.73	2.9	Dna-5-hydroxyproline	7.75	51
Dna-Glutamate	1.87	2.9	Dna-6-hydroxyproline	7.76	33
Dna-1,3-(2-methylbutyryl)alanine	1.94	1.9	Dna-Arginine	7.97	82
Dna-1,3-(2-methylbutyryl)alanine	1.95	2.9	Dna-Asp-Pro	8.02	24
Dna-2-hydroxyproline	2.20	0.1	Dna-5-hydroxy-sarcophagine	8.04	2.1
Dna-2-hydroxyproline	2.21	0.1	Dna-6-hydroxy-sarcophagine	8.04	2.1
Dna-2-hydroxyproline	2.44	26	Dna-Asp	8.17	0.1
Dna-2-hydroxyproline	2.56	2.3	Dna-Asn	8.22	0.3
Dna-2-hydroxyproline	2.57	2.9	Dna-Asp-Pro	8.22	0.3
Dna-Asp	2.60	30	Dna-4-hydroxyproline	8.37	0.1
Dna-Asp	2.67	0.1	Dna-5-hydroxyproline	8.37	0.1
Dna-Asp	3.05	0.1	Dna-6-hydroxyproline	8.39	0.8
Dna-2-hydroxyproline	3.11	471	Dna-Histidine	8.43	0.4
Dna-2-hydroxyproline	3.17	73	Dna-Arginine	8.43	0.4
Dna-Gly	3.43	20.0	Dna-2-hydroxy-sarcophagine	0.24	0.1
Dna-Gly	3.47	30	Dna-5-hydroxy-sarcophagine	0.24	0.1
Dna-2-hydroxyproline	3.47	30	Dna-6-hydroxy-sarcophagine	0.24	0.1
Dna-2-hydroxyproline	3.47	30	Dna-Asp-Pro	0.25	0.2
Dna-2-hydroxyproline	3.48	4.6	Dna-Asp	0.30	0.5
Dna-2-hydroxyproline	3.48	4.6	Dna-Asn	0.30	0.5
Dna-2-hydroxyproline	3.48	4.6	Dna-Asp-Pro	0.30	0.5
Dna-2-hydroxyproline	3.48	5.5	Dna-Glycine	0.79	29
Dna-2-hydroxyproline	3.48	5.5	Dna-Glutamate	10.08	140
Dna-2-hydroxyproline	3.48	5.5	Dna-Glutamine	10.10	120
Dna-2-hydroxyproline	3.48	5.5	Dna-Histidine	10.19	0.4
Dna-2-hydroxyproline	3.48	5.5	Dna-Histidine	10.21	0.2
Dna-2-hydroxyproline	3.48	5.5	Dna-TFA	10.28	201
Dna-2-hydroxyproline	3.48	5.5	Dna-cysteine	10.44	401

List of metabolites



Analyze via GC-AutoFit

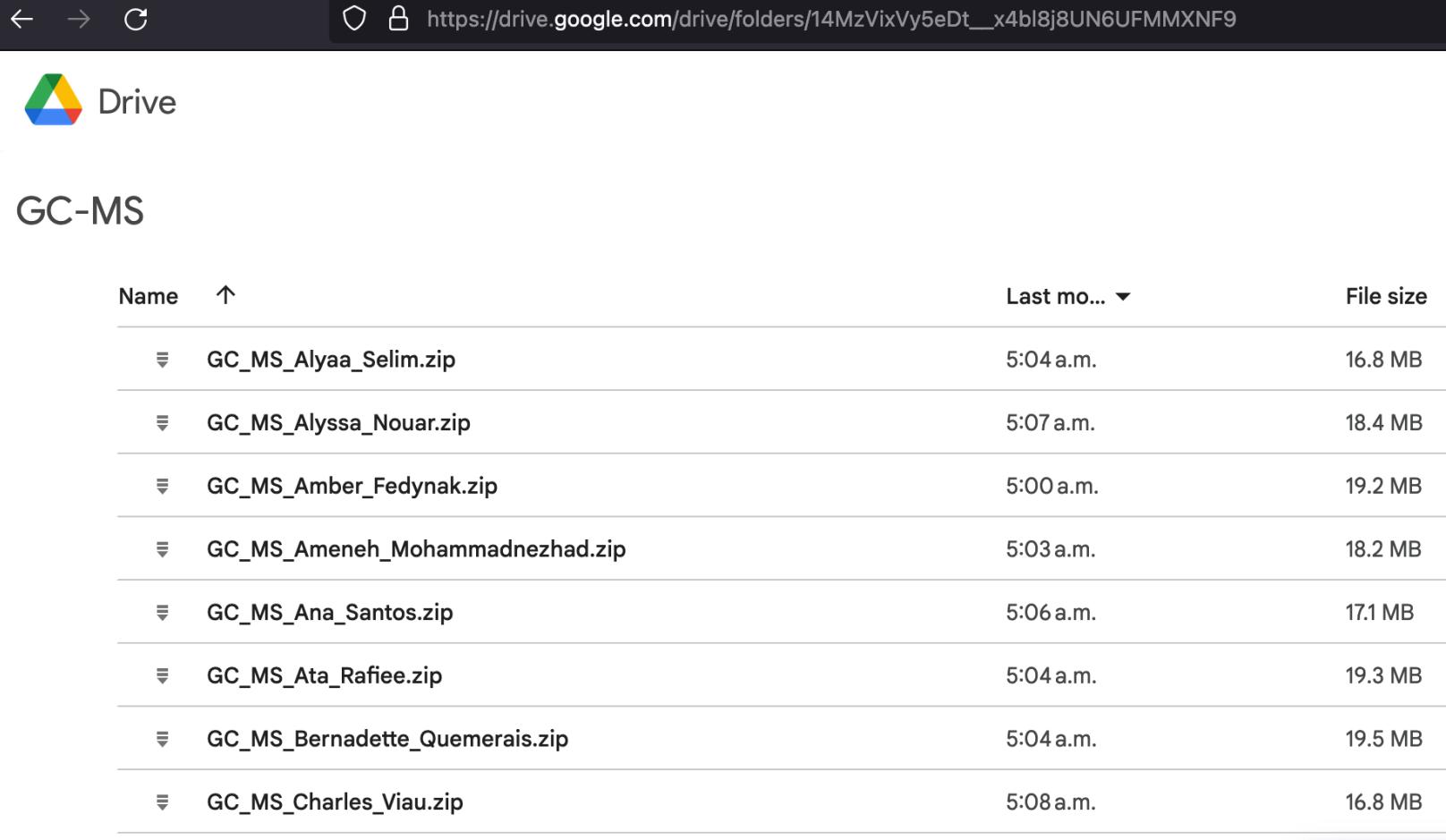


Process GC-MS chromatogram



Collect GC-MS data

Download Your GC-MS Files From The CBW Team Drive



The screenshot shows a Google Drive interface. At the top, there's a navigation bar with back, forward, and refresh buttons, followed by the URL https://drive.google.com/drive/folders/14MzVixVy5eDt__x4bl8j8UN6UFMMXNF9. Below the URL is the Google Drive logo and the word "Drive". The main area is titled "GC-MS". A table lists eight files:

Name	Last modified	File size
GC_MS_Alyaa_Selim.zip	5:04 a.m.	16.8 MB
GC_MS_Alyssa_Nouar.zip	5:07 a.m.	18.4 MB
GC_MS_Amber_Fedynak.zip	5:00 a.m.	19.2 MB
GC_MS_Ameneh_Mohammadnezhad.zip	5:03 a.m.	18.2 MB
GC_MS_Anna_Santos.zip	5:06 a.m.	17.1 MB
GC_MS_Ata_Rafiee.zip	5:04 a.m.	19.3 MB
GC_MS_Bernadette_Quemerais.zip	5:04 a.m.	19.5 MB
GC_MS_Charles_Viau.zip	5:08 a.m.	16.8 MB

https://drive.google.com/drive/folders/14MzVixVy5eDt__x4bl8j8UN6UFMMXNF9

Go To The GC-AutoFit Server

Welcome to GC-AutoFit

GC-AutoFit is a web application that automatically identifies and quantifies metabolites using Gas Chromatography Mass Spectrometry (GC-MS) spectra. For optimal GC-AutoFit performance, the query GC-MS spectra should be prepared according to the instructions (How to collect GC-MS Spectra for GC-AutoFit). GC-AutoFit currently accepts .CDF and .mzXML file formats. It uses alkane standards to calculate the retention index (RI) of each peak in the sample. The extracted EI-MS spectra from each peak, along with the RIs, are then compared to reference spectra (RIs and EI-MS) in the specified library to identify and quantify the compounds. The inclusion of blank spectra is optional, however, it is useful for removing noise effects from the query spectra. Extensive testing shows that GC-AutoFit meets or exceeds the performance of highly trained human experts.

GC-AutoFit Analysis

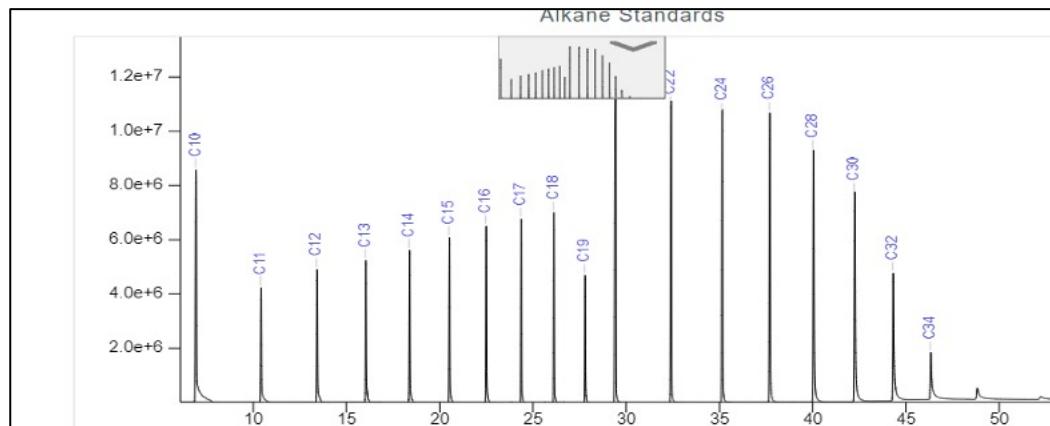
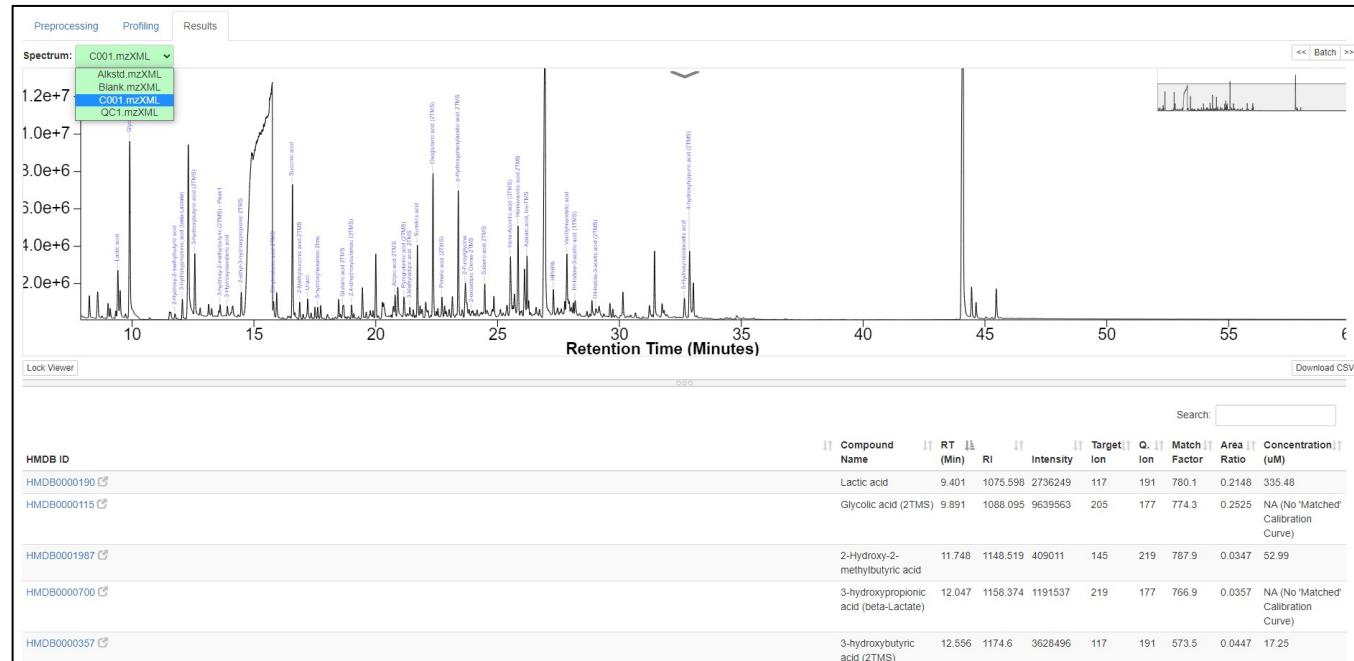
Instructions
To analyze GC-MS spectra with GC-AutoFit you must provide information on the biofluid being analyzed and the spectral files in .CDF or .mzXML format. Up to 30 spectra are recommended to analyze in one submission.

Submit your own mixture:

- Select Biofluid:*** GC-AutoFit works with urine and your own custom libraries.
 Urine Tropic acid
- Select internal standard:*** Compound must be present in selected library.
 Tropic acid
- Upload Spectra:*** OR
All spectral files must be .CDF or .mzXML. Up to 30 samples can be submitted.

Alkane standards* No file chosen
Blank* No file chosen
Samples* No file chosen

- Provide a MF(Match Factor) Score cutoff:*** An integer between 0 - 999 but somewhere between 400 - 600 is recommended.



<http://gc-autofit1.wishartlab.com>
<http://gc-autofit2.wishartlab.com>

Upload Your GC-MS Spectral Files

- Option: Individual files (A) or a zip file (B) or (C) an example file

(A)

3. Upload Spectra:
 OR

All spectral files must be .CDF or .mzXML. Up to 30 samples can be submitted.

Alkane standards* No file selected.

Blank No file selected.

Samples* No files selected.

(B)

3. Upload Spectra:
 OR

The ZIP file must contain an alkane standards spectrum (e.g. Alkane.mzXML, ALKstd.mzXML), an optional blank spectrum (e.g. Blank.mzXML, Blk.mzXML) and at least one sample spectrum. All the spectral files must be .CDF or .mzXML. Up to 30 samples can be submitted.

Zip file* No file selected.

(C)

OR, run one of our examples:

Spectral Processing

The screenshot shows the GC-AutoFit preprocessing interface. At the top, there is a navigation bar with links for "New Submission", "Instructions", "Library", and tabs for "Preprocessing" (which is selected), "Profiling", and "Results". Below the navigation bar, the title "GC-AutoFit Preprocessing" is displayed, followed by the heading "Settings". Under "Settings", there are several configuration parameters: "Biofluid: Urine", "Number of Samples: 2", "MF Score Cut Off: 400", "Date: 2023-04-24 18:04:11 UTC", "Internal Standard: Cholesterol", and "Blank Provided: true". In the "Status" section, a green oval highlights the text "Processing". Below this, a message states "Your spectra are being preprocessed. You can bookmark this page and check back later." A blue arrow points from the right side of the slide towards this status message, with the red text "Each spectrum takes ~ 1 min" placed near the arrow.

GC-AutoFit

New Submission Instructions Library

Preprocessing Profiling Results

GC-AutoFit Preprocessing

Settings

Biofluid: Urine
Number of Samples: 2
MF Score Cut Off: 400
Date: 2023-04-24 18:04:11 UTC

Internal Standard: Cholesterol
Blank Provided: true

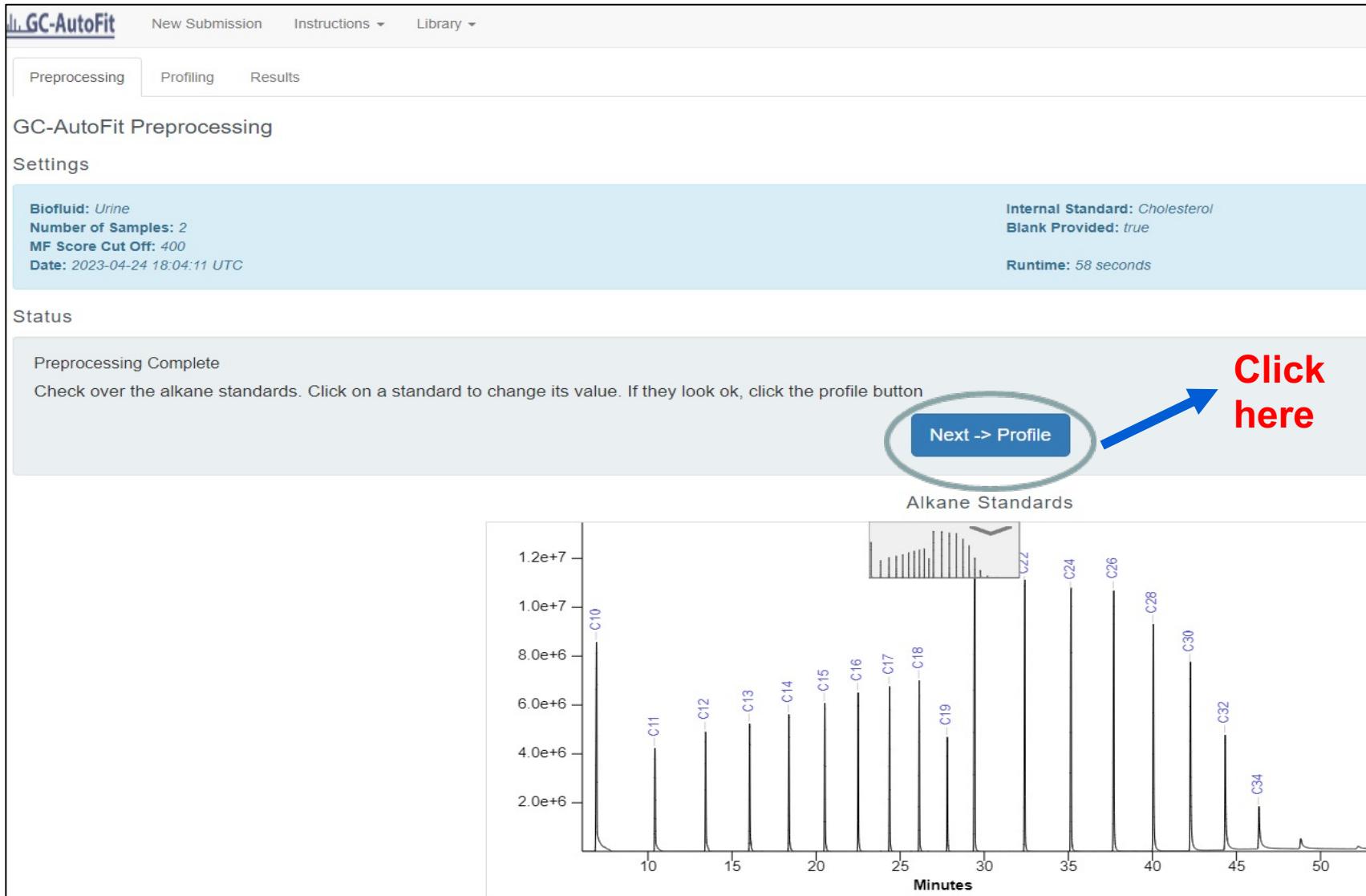
Status

Processing

Your spectra are being preprocessed. You can bookmark this page and check back later.

Each spectrum takes ~ 1 min

Spectral Processing



Spectral Profiling

GC-AutoFit

New Submission Instructions Library

Preprocessing Profiling Results

Must wait until the profiling is done

Samples

Name	Run Time	Status
C001.mzXML	NA	Profiling...
QC1.mzXML	NA	Queued (1 submission ahead of yours)...

GC-AutoFit

New Submission Instructions Library

Preprocessing Profiling Results

Profiling is completed!!

Click here to download the results in Excel format

Samples (Download Results)

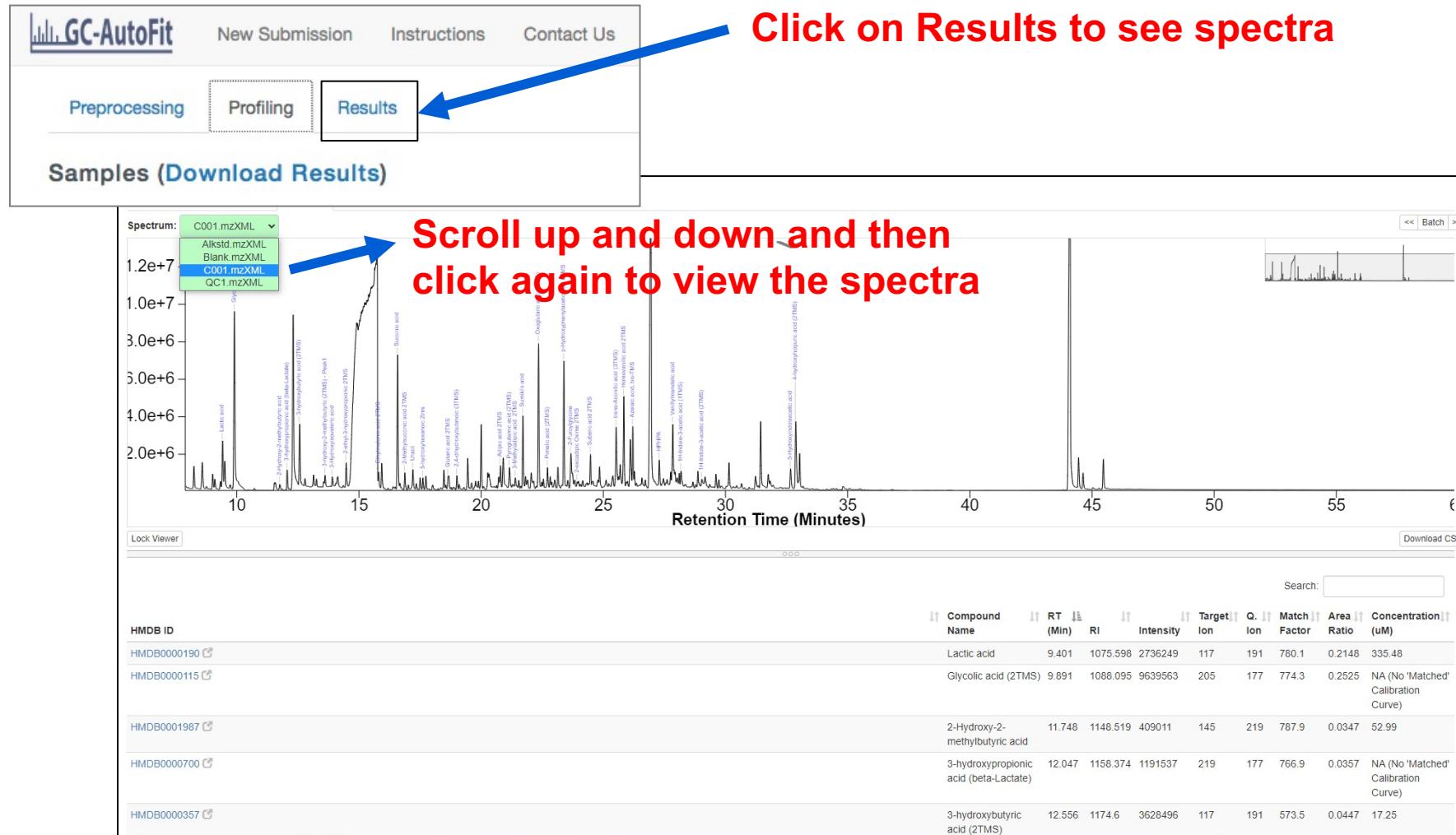
Name	Run Time	Status
C001.mzXML	1 minutes 32 seconds	Complete
QC1.mzXML	45 seconds	Complete

GC-AutoFit Results

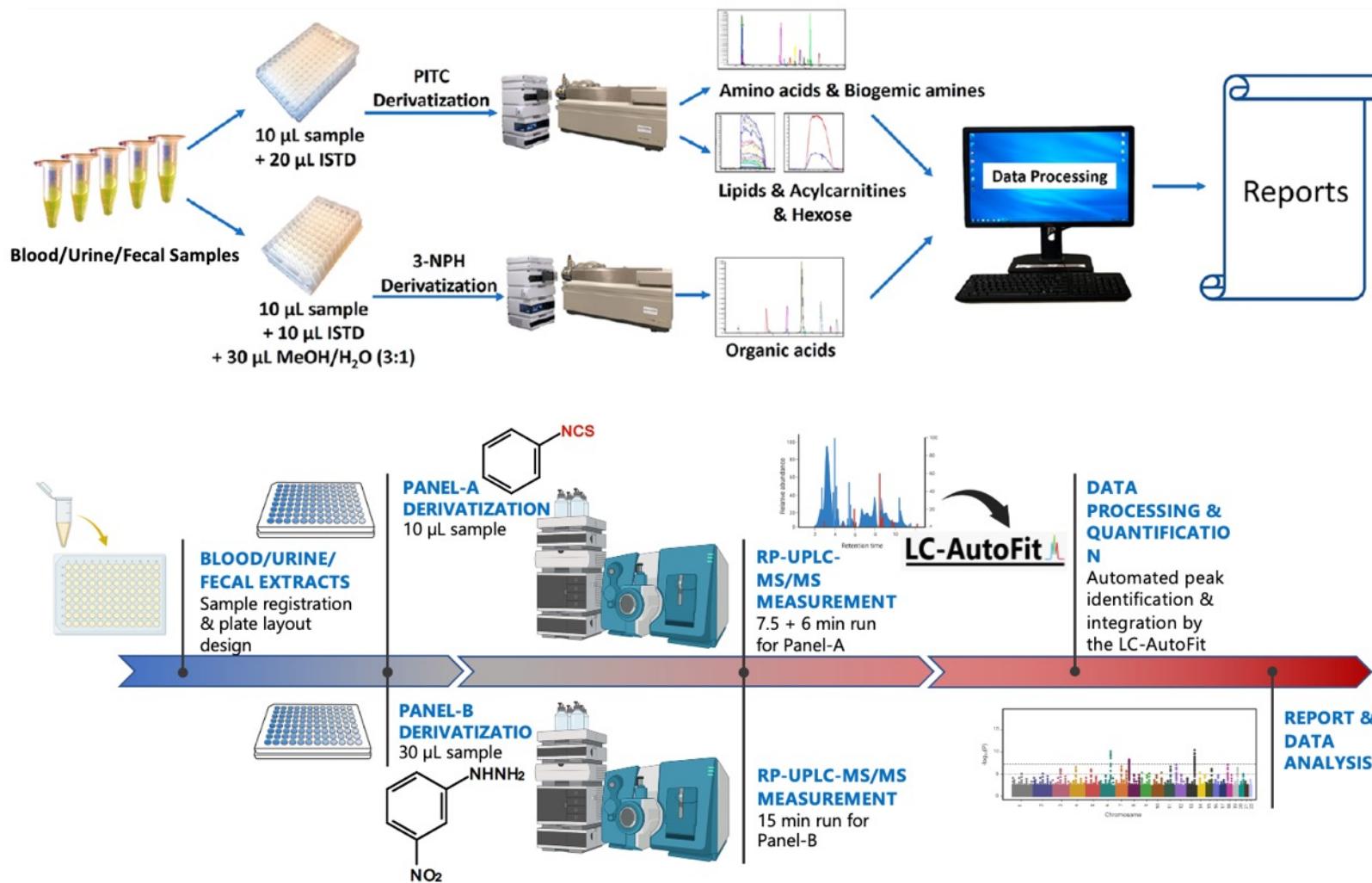
- **Table (CSV format file)**
 - Table for each sample
 - Merged concentration for all samples
 - **Spectrum Viewer**
 - Spectra with assigned compound names

Results

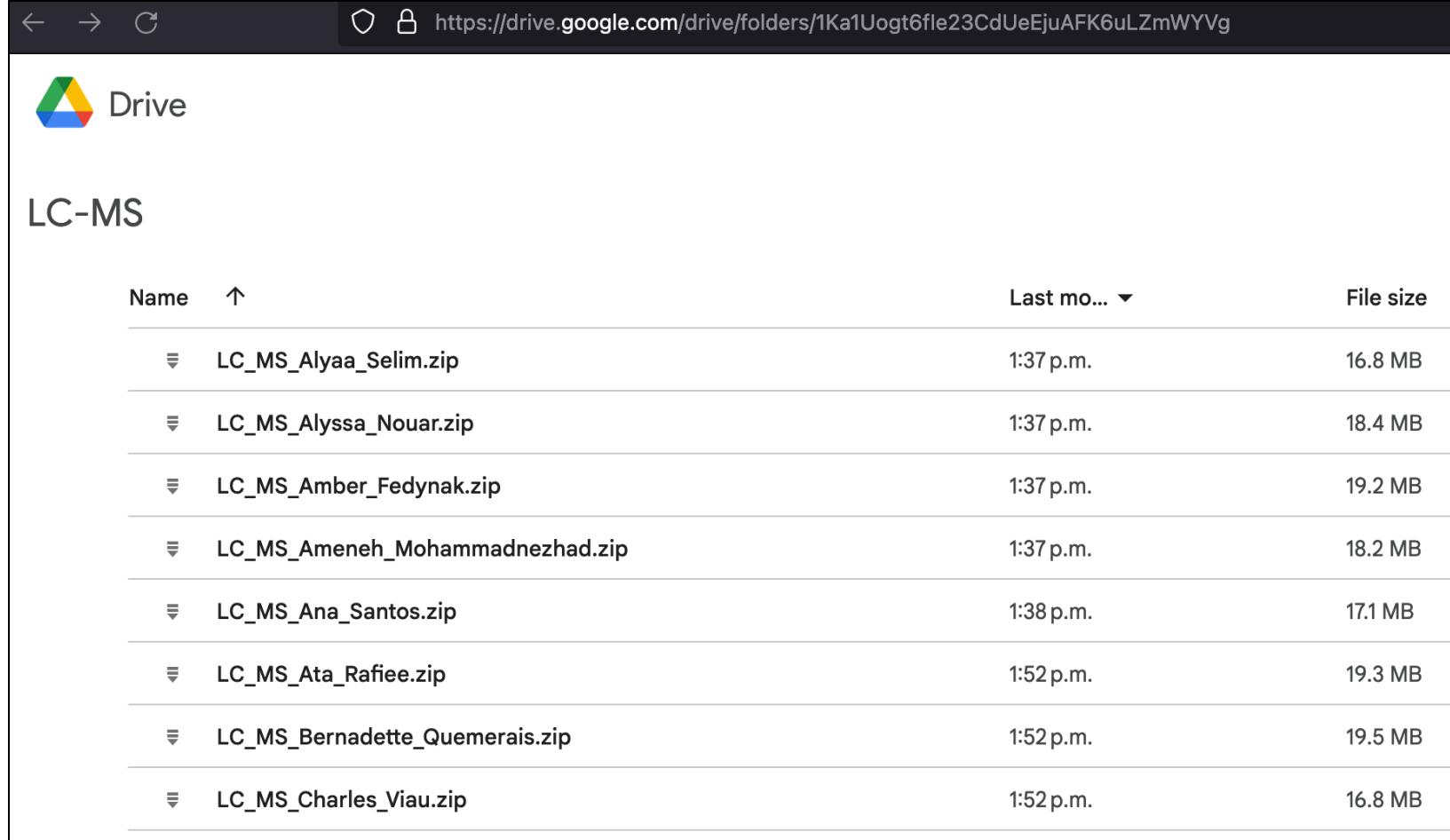
(View Annotated Spectra)



LC-MS Metabolomics Overview



Download Your LC-MS Zip File From The CBW Team Drive



The screenshot shows a Google Drive interface with the URL <https://drive.google.com/drive/folders/1Ka1Uogt6fle23CdUeEjuAFK6uLZmWYVg> in the address bar. The folder is titled "LC-MS". The table lists eight zip files:

Name	Last modified	File size
LC_MS_Alyaa_Selim.zip	1:37 p.m.	16.8 MB
LC_MS_Alyssa_Nouar.zip	1:37 p.m.	18.4 MB
LC_MS_Amber_Fedynak.zip	1:37 p.m.	19.2 MB
LC_MS_Ameneh_Mohammadnezhad.zip	1:37 p.m.	18.2 MB
LC_MS_Anna_Santos.zip	1:38 p.m.	17.1 MB
LC_MS_Ata_Rafiee.zip	1:52 p.m.	19.3 MB
LC_MS_Bernadette_Quemerais.zip	1:52 p.m.	19.5 MB
LC_MS_Charles_Viau.zip	1:52 p.m.	16.8 MB

<https://drive.google.com/drive/folders/1Ka1Uogt6fle23CdUeEjuAFK6uLZmWYVg>

Go To The LC-AutoFit Server

Welcome to LC-AutoFit

LC-AutoFit Analysis

Instructions

To analyze LC-MS spectra with LC-AutoFit you must provide information on the biofluid being analyzed and the spectral files in .wif or .mzML format. Up to 100 spectra are recommended to analyze in one submission.

1. Select Biofluid: LC-AutoFit works with Urine, Blood, and Fecal Samples.
 Blood Urine Fecal Matter

2. Upload Spectra: Separate Files OR Single ZIP File
 All spectral files must be .wif or .mzML. Up to 100 samples can be submitted.

Calibration samples: Choose Files [7 files]
 Quality control samples: Choose Files [3 files]
 Experimental samples: Choose Files [15 files]
 Blank samples: Choose Files [2 files]
 Double blank samples: Choose Files [2022004 - udefBlank.wif]

3. Assay: TMIC MEGA Panel B

4. Upload Retention Time: Download Blank Retention Time Table
 The retention time file must be a .csv file. You can find template retention tables here for your specific biofluid and assay. You must use this template or LC-AutoFit will not be able to run its analysis.
 Retention time file: Choose file [RT_TMIC M... - Blood.csv]

Submit **Clear**

collected following the manuals provided with the TMIC Prime and TMIC Mega kits. Please upload your raw files according to the instructions on this page and start your journey with LC-AutoFit!

LC-AutoFit Analysis

Instructions

To analyze LC-MS spectra with LC-AutoFit you must provide information on the biofluid being analyzed and the spectral files in .wif or .mzML format. Up to 100 spectra are recommended to analyze in one submission.

Submit your own mixture:
 1. Select Biofluid: LC-AutoFit works with Urine, Blood, and Fecal Samples.
 Blood Urine Fecal Matter

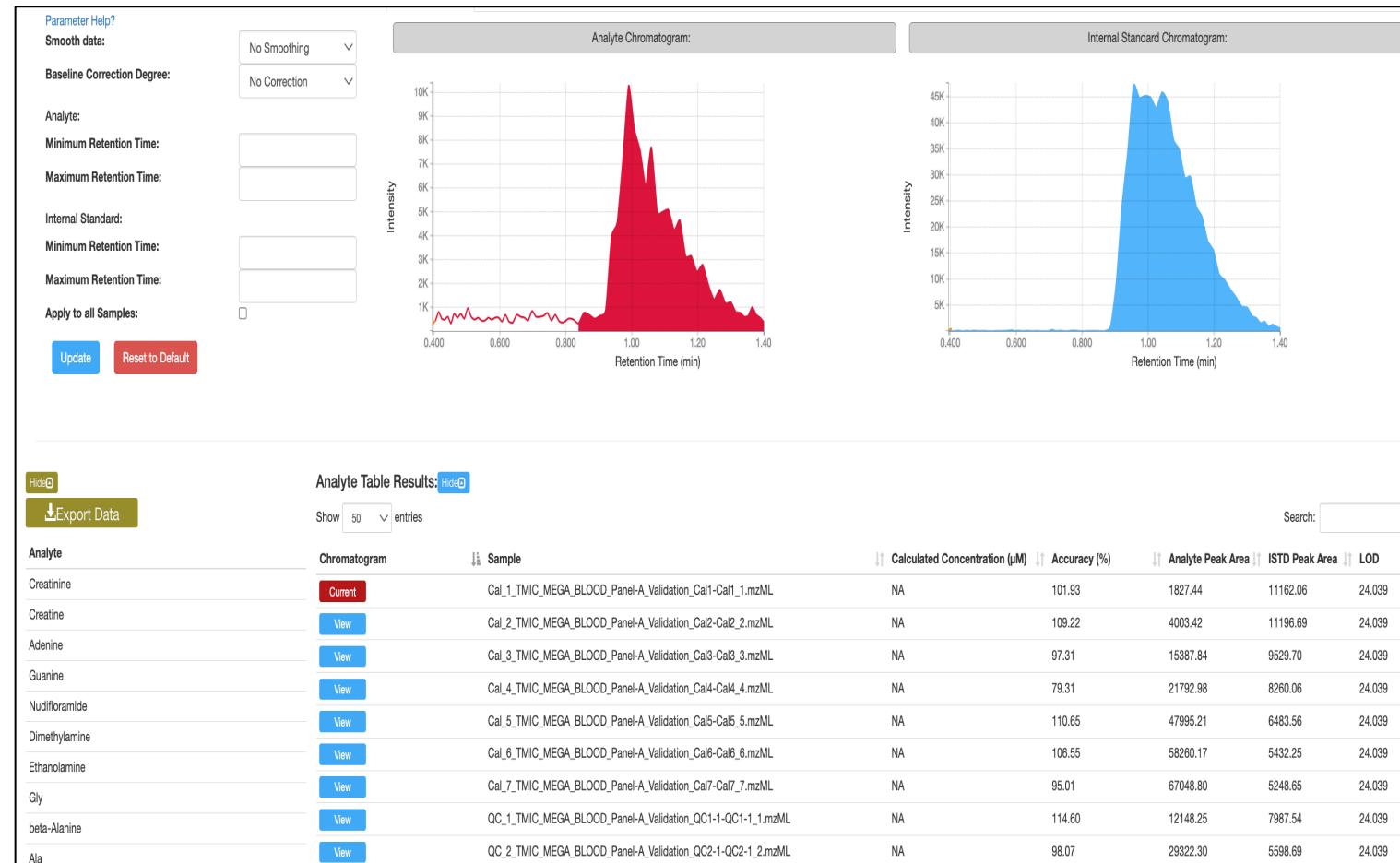
2. Upload Spectra: Separate Files OR Single ZIP File
 All spectral files must be .wif or .mzML. Up to 100 samples can be submitted.

Calibration samples: Choose Files [No file chosen]
 Quality control samples: Choose Files [Cal_1_MEGA 1.0 Blood Panel A Validation_Cal1...]
 Experimental samples: Choose Files [Cal_1_MEGA 1.0 Blood Panel A Validation_Cal1...]
 Blank samples: Choose Files [No file chosen]
 Double blank samples: Choose Files [No file chosen]

3. Assay: TMIC MEGA Panel A

4. Upload Retention Time: Download Blank Retention Time Table
 The retention time file must be a .csv file. You can find template retention tables here for your specific biofluid and assay. You must use this template or LC-AutoFit will not be able to run its analysis.
 Retention time file: Choose file [No file chosen]

Submit **Clear**



<http://dev-lcautofit.wishartlab.com/>

Step 1: Assay Selection

Step 1: Drop down menu for Assay Selection

Submit your own mixture:

1. Select Biofluid*: LC-AutoFit works with Urine, Blood, and Fecal Samples.

Blood

TMIC MEGA Panel A
TMIC MEGA Panel B

2. Assay*: ✓ TMIC PRIME Panel A
TMIC PRIME Panel B
PRIME FIA Positive
PRIME FIA Negative

3. Upload Samples*

All spectra

zML. Up to 100 samples can be submitted.

Choose Files No file chosen

Quality control samples*

Choose Files No file chosen

Calibration samples*

Choose Files No file chosen

Quality control samples*

Choose Files No file chosen

Calibration samples*

Choose Files No file chosen

Quality control samples*

Choose Files No file chosen

Submit your own mixture:

1. Select Biofluid*: LC-AutoFit works with Urine, Blood, and Fecal Samples.

Blood

TMIC MEGA Panel A
TMIC MEGA Panel B
TMIC PRIME Panel A

2. Assay*: ✓ TMIC PRIME Panel B
PRIME FIA Positive
PRIME FIA Negative

3. Upload Samples*

All spectra

zML. Up to 100 samples can be submitted.

Choose Files No file chosen

Quality control samples*

Choose Files No file chosen

Calibration samples*

Choose Files No file chosen

Quality control samples*

Choose Files No file chosen

Calibration samples*

Choose Files No file chosen

Quality control samples*

Choose Files No file chosen

LC-AutoFit New Submission Contact Us

To analyze LC-MS spectra with LC-AutoFit you must provide information on the biofluid being recommended to analyze in one submission.

Submit your own mixture:

1. Select Biofluid*: LC-AutoFit works with Urine, Blood, and Fecal Samples.

Blood

TMIC MEGA Panel A
TMIC MEGA Panel B
TMIC PRIME Panel A

2. Assay*: ✓ TMIC PRIME Panel B
PRIME FIA Positive
PRIME FIA Negative

3. Upload Samples*

All spectra

zML. Up to 100 samples can be submitted.

Choose Files No file chosen

Quality control samples*

Choose Files No file chosen

Calibration samples*

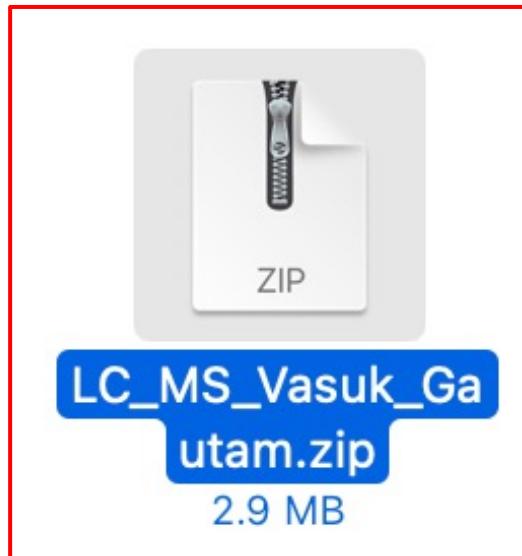
Choose Files No file chosen

Quality control samples*

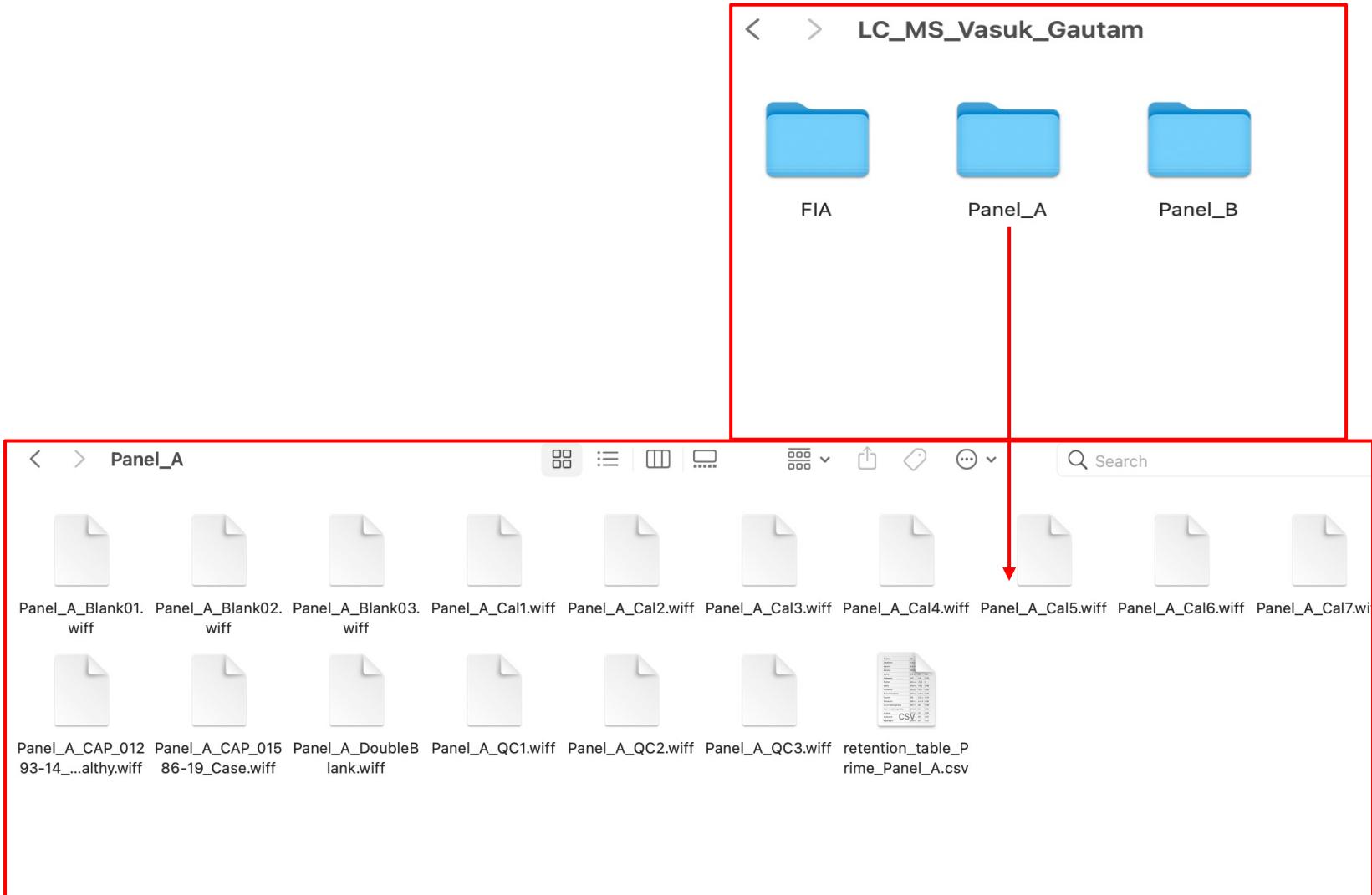
Choose Files No file chosen

<http://dev-lcautofit.wishartlab.com/>

Step 2 & 3: Download and Unzip Files



Step2:
Download the
Zipped folder
on local
machine.



Step3: Unzip the LC-MS zipped folder.

Load your Files for PRIME Panel A

LC-AutoFit New Submission Contact Us

Welcome to LC-AutoFit

LC-AutoFit is a web application that is tailored for processing the raw Liquid Chromatography-Mass Spectrometry(LC-MS) spectra collected by the TMIC Prime and TMIC Mega metabolomics kits. LC-AutoFit automatically identifies and quantifies the medically important metabolites targeted by the TMIC Prime and TMIC Mega kits.

LC-AutoFit currently accepts .wiff file format that is collected by AB Sciex instruments. Users can download the quantitated results in.csv file format after data processing. For optimal performance, the query LC-MS spectra should be collected following the manuals provided with the TMIC Prime and TMIC Mega kits. Please upload your raw files according to the instructions on this page and start your journey with LC-AutoFit!

LC-AutoFit Analysis

Instructions

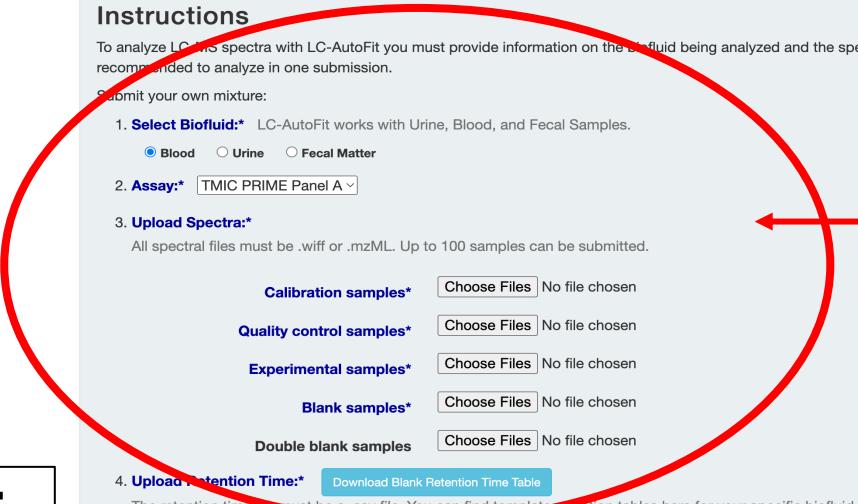
To analyze LC-MS spectra with LC-AutoFit you must provide information on the biofluid being analyzed and the spectral files in .wiff or .mzML format. Up to 100 spectra are recommended to analyze in one submission.

Submit your own mixture:

- Select Biofluid:*** LC-AutoFit works with Urine, Blood, and Fecal Samples.
 Blood Urine Fecal Matter
- Assay:*** TMIC PRIME Panel A
- Upload Spectra:*** All spectral files must be .wiff or .mzML. Up to 100 samples can be submitted.
Calibration samples* Choose Files No file chosen
Quality control samples* Choose Files No file chosen
Experimental samples* Choose Files No file chosen
Blank samples* Choose Files No file chosen
Double blank samples Choose Files No file chosen
- Upload Retention Time:*** Download Blank Retention Time Table
The retention time file must be a .csv file. You can find template retention tables here for your specific biofluid and assay. You must use this template or LC-AutoFit will not be able to run its analysis.
Retention time file* Choose File No file chosen

Submit Clear

Latest update: June 15, 2022



Drop your RT
File

Drag n Drop
your files one
by one here
for PRIME A

Panel_A 17 items		
Name	Last modified	File size
Panel_A_Blank01.wiff	Jul 5, 2023	392 KB
Panel_A_Blank02.wiff	Jul 5, 2023	392 KB
Panel_A_Blank03.wiff	Jul 5, 2023	392 KB
Panel_A_Cal1.wiff	Jul 5, 2023	412 KB
Panel_A_Cal2.wiff	Jul 5, 2023	412 KB
Panel_A_Cal3.wiff	Jul 5, 2023	412 KB
Panel_A_Cal4.wiff	Jul 5, 2023	412 KB
Panel_A_Cal5.wiff	Jul 5, 2023	412 KB
Panel_A_Cal6.wiff	Jul 5, 2023	412 KB
Panel_A_Cal7.wiff	Jul 5, 2023	412 KB

Load Your files for PRIME Panel B

LC-AutoFit | New Submission | Contact Us

Welcome to LC-AutoFit

LC-AutoFit is a web application that is tailored for processing the raw Liquid Chromatography-Mass Spectrometry(LC-MS) spectra collected by the TMIC Prime and TMIC Mega metabolomics kits. LC-AutoFit automatically identifies and quantifies the medically important metabolites targeted by the TMIC Prime and TMIC Mega kits.

LC-AutoFit currently accepts .wiff file format that is collected by AB Sciex instruments. Users can download the quantitated results in.csv file format after data processing. For optimal performance, the query LC-MS spectra should be collected following the manuals provided with the TMIC Prime and TMIC Mega kits. Please upload your raw files according to the instructions on this page and start your journey with LC-AutoFit!

LC-AutoFit Analysis

Instructions

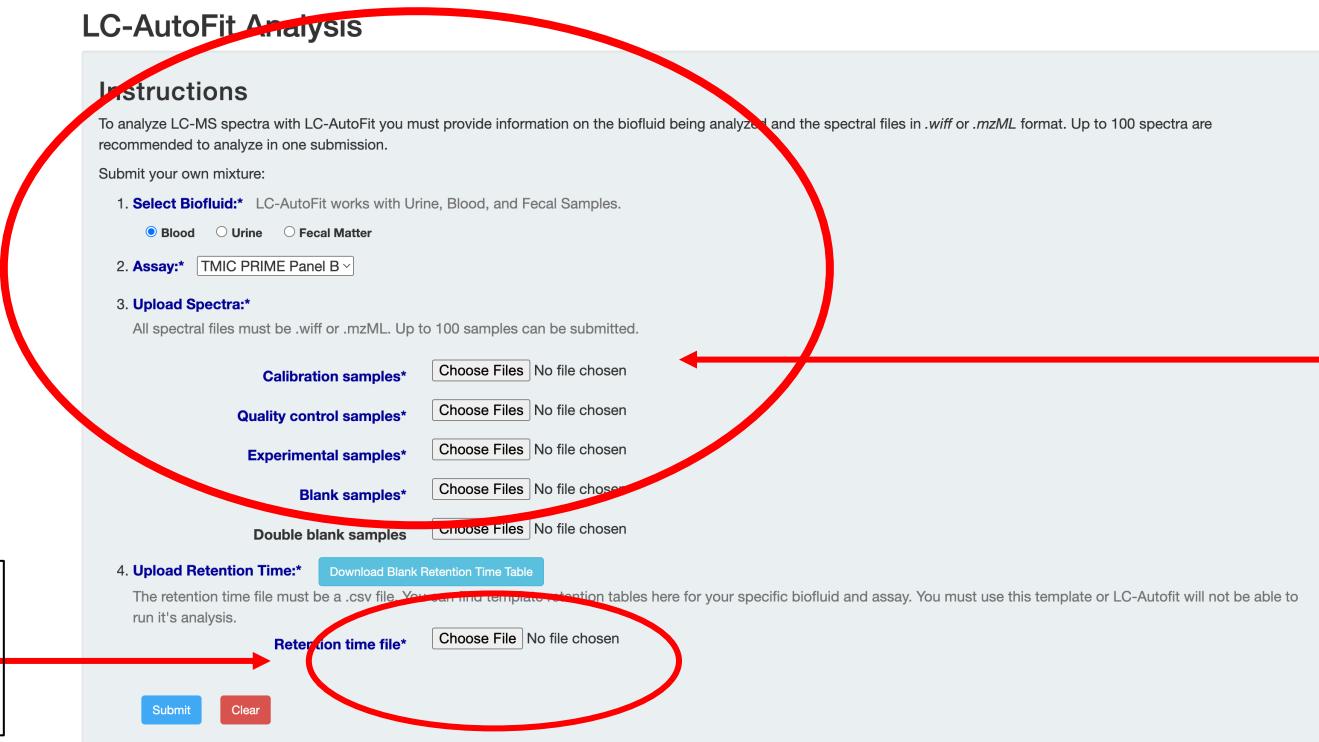
To analyze LC-MS spectra with LC-AutoFit you must provide information on the biofluid being analyzed and the spectral files in .wiff or .mzML format. Up to 100 spectra are recommended to analyze in one submission.

Submit your own mixture:

- Select Biofluid:*** LC-AutoFit works with Urine, Blood, and Fecal Samples.
 Blood Urine Fecal Matter
- Assay:*** TMIC PRIME Panel B
- Upload Spectra:***
All spectral files must be .wiff or .mzML. Up to 100 samples can be submitted.
Calibration samples* No file chosen
Quality control samples* No file chosen
Experimental samples* No file chosen
Blank samples* No file chosen
Double blank samples No file chosen
- Upload Retention Time:***
The retention time file must be a .csv file. You can find template retention tables here for your specific biofluid and assay. You must use this template or LC-AutoFit will not be able to run its analysis.
Retention time file:* No file chosen

Submit Clear

Latest update: June 15, 2022



**Drag n Drop
your files one
by one here
PRIME B**

Panel_B 17 items		
Name	Last modified	File size
panel_B_Blank01.wiff	Jul 5, 2023	452 KB
panel_B_Blank02.wiff	Jul 5, 2023	444 KB
panel_B_Blank03.wiff	Jul 5, 2023	440 KB
panel_B_Cal1.wiff	Jul 5, 2023	460 KB
panel_B_Cal2.wiff	Jul 5, 2023	460 KB
panel_B_Cal3.wiff	Jul 5, 2023	460 KB
panel_B_Cal4.wiff	Jul 5, 2023	460 KB
panel_B_Cal5.wiff	Jul 5, 2023	464 KB
panel_B_Cal6.wiff	Jul 5, 2023	464 KB
panel_B_Cal7.wiff	Jul 5, 2023	460 KB

**Drop your RT
File**

<http://dev-lcautofit.wishartlab.com/>

Load Your Files for FIA Positive

LC-AutoFit | New Submission | Contact Us

Welcome to LC-AutoFit

LC-AutoFit is a web application that is tailored for processing the raw Liquid Chromatography-Mass Spectrometry(LC-MS) spectra collected by the TMIC Prime and TMIC Mega metabolomics kits. LC-AutoFit automatically identifies and quantifies the medically important metabolites targeted by the TMIC Prime and TMIC Mega kits.

LC-AutoFit currently accepts .wiff file format that is collected by AB Sciex instruments. Users can download the quantitated results in.csv file format after data processing. For optimal performance, the query LC-MS spectra should be collected following the manuals provided with the TMIC Prime and TMIC Mega kits. Please upload your raw files according to the instructions on this page and start your journey with LC-AutoFit!

LC-AutoFit Analysis

Instructions

To analyze LC-MS spectra with LC-AutoFit you must provide information on the biofluid being analyzed and the spectral files in .wiff or .mzML format. Up to 100 spectra are recommended to analyze in one submission.

Submit your own mixture:

- Select Biofluid:*** LC-AutoFit works with Urine, Blood, and Fecal Samples.
 Blood Urine Fecal Matter
- Assay:*** PRIME FIA Positive
- Upload Spectra:***
All spectral files must be .wiff or .mzML up to 100 samples can be submitted.
Calibration samples* No file chosen
Quality control samples* No file chosen
Experimental samples* No file chosen
Blank samples* No file chosen
Double blank samples No file chosen
- Please enter a minimum and maximum value for the FIA retention times.
Minimum retention time:* 0.5
Maximum retention time:* 2.7

Submit Clear

Latest update: June 15, 2022

Enter default RT Time(s)
Min.RT: 0.5
Max RT: 2.7

<http://dev-lcautofit.wishartlab.com/>

Drag n Drop your files one by one here for FIA Positive

FIA 16 items		
Name	Last modified	File size
FIA_Bank01.wiff	Jul 5, 2023	148 KB
FIA_Bank02.wiff	Jul 5, 2023	148 KB
FIA_Bank03.wiff	Jul 5, 2023	148 KB
FIA_Cal1.wiff	Jul 5, 2023	572 KB
FIA_Cal2.wiff	Jul 5, 2023	572 KB
FIA_Cal3.wiff	Jul 5, 2023	572 KB
FIA_Cal4.wiff	Jul 5, 2023	572 KB
FIA_Cal5.wiff	Jul 5, 2023	572 KB
FIA_Cal6.wiff	Jul 5, 2023	572 KB
FIA_Cal7.wiff	Jul 5, 2023	572 KB

Preprocess & Select QC Level

LC-AutoFit  New Submission Contact Us

Preprocessing Calibration Profiling Calibration Optimization Profiling Results

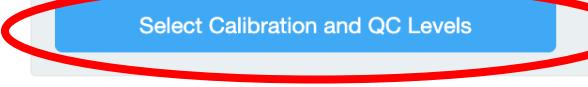
LC-AutoFit Preprocessing

Settings

Biofluid: Blood
Number of Samples: 20
Assay: TMIC MEGA Panel A
Date: 2023-04-24 20:10:50 UTC

Status

Preprocessing Complete

Select Calibration and QC Levels 

Click Here

LC-AutoFit Calibration and Quality Control Level Selection

To continue with the preprocessing, please select the level associated with each sample. There should be 7 calibration samples and 3 quality control samples, unless your panel is MEGA FIA 2, in which case there should be 4 calibration samples and 4 quality control samples. In order to automatically select levels, please make sure that each file name ends with ".x" where x is the level. For example: "Cal_Sample_4.wifff", "Cal_Sample_4.mzML".

File Name	Level
Cal_1_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal1-Cal1_1.mzML	1
Cal_2_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal2-Cal2_2.mzML	2
Cal_3_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal3-Cal3_3.mzML	3
Cal_4_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal4-Cal4_4.mzML	4
Cal_5_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal5-Cal5_5.mzML	5
Cal_6_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal6-Cal6_6.mzML	6
Cal_7_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal7-Cal7_7.mzML	7

File Name	Level
QC_1_TMIC_MEGA_BLOOD_Panel-A_Validation_QC1-QC1-1_1.mzML	1
QC_2_TMIC_MEGA_BLOOD_Panel-A_Validation_QC2-QC2-1_2.mzML	2
QC_3_TMIC_MEGA_BLOOD_Panel-A_Validation_QC3-QC3-1_3.mzML	3

Submit 

Click Here

Preprocess & Calibrate

LC-AutoFit Preprocessing

Settings

Biofluid: Blood
Number of Samples: 20
Assay: TMIC MEGA Panel A
Date: 2023-04-24 20:10:50 UTC

Runtime: 1 seconds

Status

Preprocessing Complete

[Start Profiling](#)

Click Here

Preprocessing

Calibration Profiling

Calibration Optimization

Profiling

Results

Samples

Name

Cal_Cal_1_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal1-Cal1_1.mzML
Cal_Cal_2_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal2-Cal2_2.mzML
Cal_Cal_3_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal3-Cal3_3.mzML
Cal_Cal_4_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal4-Cal4_4.mzML
Cal_Cal_5_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal5-Cal5_5.mzML
Cal_Cal_6_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal6-Cal6_6.mzML
Cal_Cal_7_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal7-Cal7_7.mzML
Blank_Blank_TMIC_MEGA_BLOOD_Panel-A_Validation_Blank-1-Blank.mzML
Blank_Blank_TMIC_MEGA_BLOOD_Panel-A_Validation_Blank-2-Blank.mzML
Blank_Blank_TMIC_MEGA_BLOOD_Panel-A_Validation_Blank-3-Blank.mzML

Run Time

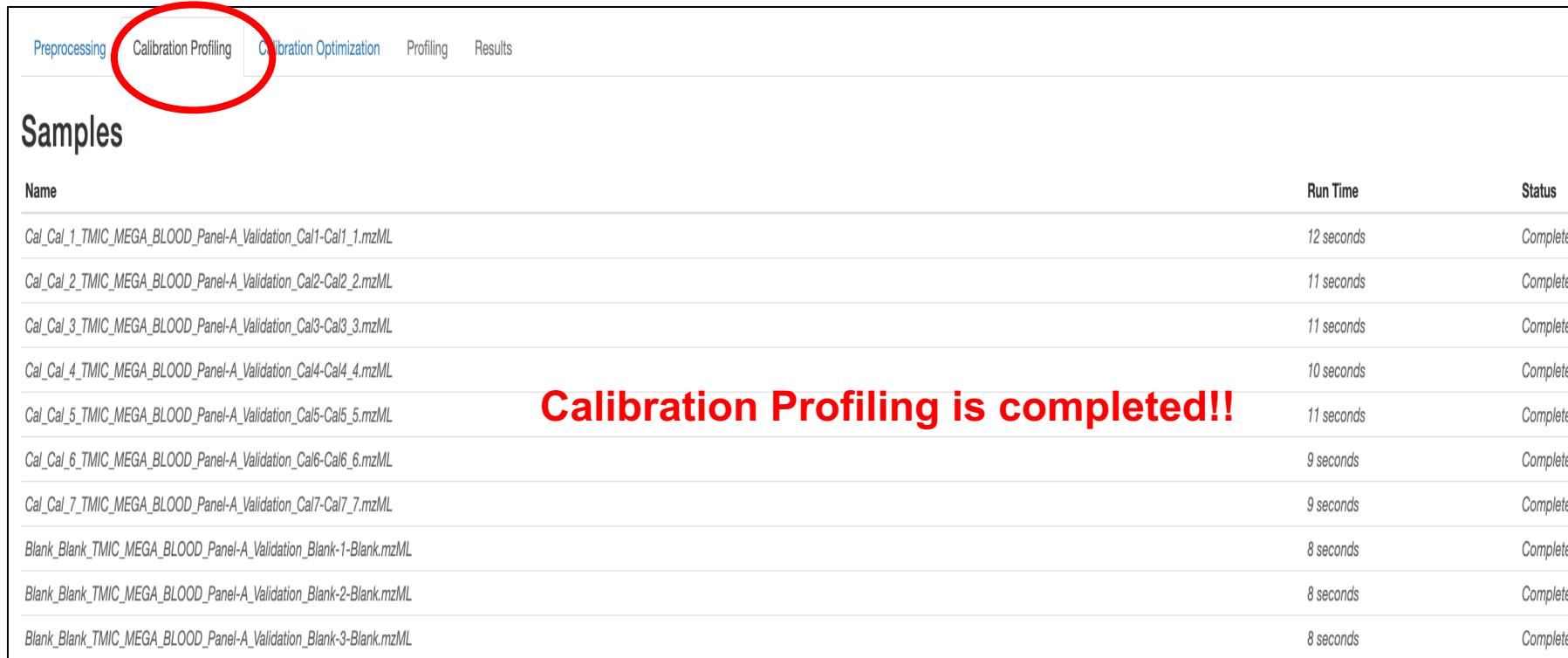
12 seconds	Complete
11 seconds	Complete
11 seconds	Complete
10 seconds	Complete
11 seconds	Complete
Running...	Queued (You're next!...)

Status

Click Here to start Calibration Profiling

Need to wait ~ 1 minute until the profiling is done

Calibration Profiling



Preprocessing Calibration Profiling Calibration Optimization Profiling Results

Samples

Name	Run Time	Status
Cal_Cal_1_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal1-Cal1_1.mzML	12 seconds	Complete
Cal_Cal_2_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal2-Cal2_2.mzML	11 seconds	Complete
Cal_Cal_3_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal3-Cal3_3.mzML	11 seconds	Complete
Cal_Cal_4_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal4-Cal4_4.mzML	10 seconds	Complete
Cal_Cal_5_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal5-Cal5_5.mzML	11 seconds	Complete
Cal_Cal_6_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal6-Cal6_6.mzML	9 seconds	Complete
Cal_Cal_7_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal7-Cal7_7.mzML	9 seconds	Complete
Blank_Blank_TMIC_MEGA_BLOOD_Panel-A_Validation_Blank-1-Blank.mzML	8 seconds	Complete
Blank_Blank_TMIC_MEGA_BLOOD_Panel-A_Validation_Blank-2-Blank.mzML	8 seconds	Complete
Blank_Blank_TMIC_MEGA_BLOOD_Panel-A_Validation_Blank-3-Blank.mzML	8 seconds	Complete

Calibration Profiling is completed!!

Calibration Optimization

Preprocessing Calibration Profiling **Calibration Optimization** Profiling Results

Click Here

Parameters:

Parameter Help?

Smooth data: No Smoothing

Baseline Correction Degree: No Correction

Analyte: Minimum Retention Time: Maximum Retention Time:

Internal Standard: Minimum Retention Time: Maximum Retention Time:

Apply to all Samples:

Chromatograms **Calibration Curve**

Calibration Curve Points

You are able to remove points on the calibration curve. From the points below, uncheck which calibration points to remove, then click Update. The choices under the parameters will be applied. Select reset all calibration points to rest the calibration curve to the default values.

Cal 1: (10, 0.164)
 Cal 2: (20, 0.358)
 Cal 3: (100, 1.61)
 Cal 4: (200, 2.64)
 Cal 5: (400, 7, 40)
 Cal 6: (600, 10.7)
 Cal 7: (800, 12.8)

Reset all calibration points

Update **Reset to Default**

R Squared Value: 0.9882

Peak Area Ratio

Calibration Concentration

Creatinine

Confirm Calibration

Click Here to apply all

Chromatograms **Calibration Curve**

Analyte Chromatogram:

Intensity

Retention Time (min)

Internal Standard Chromatogram:

Intensity

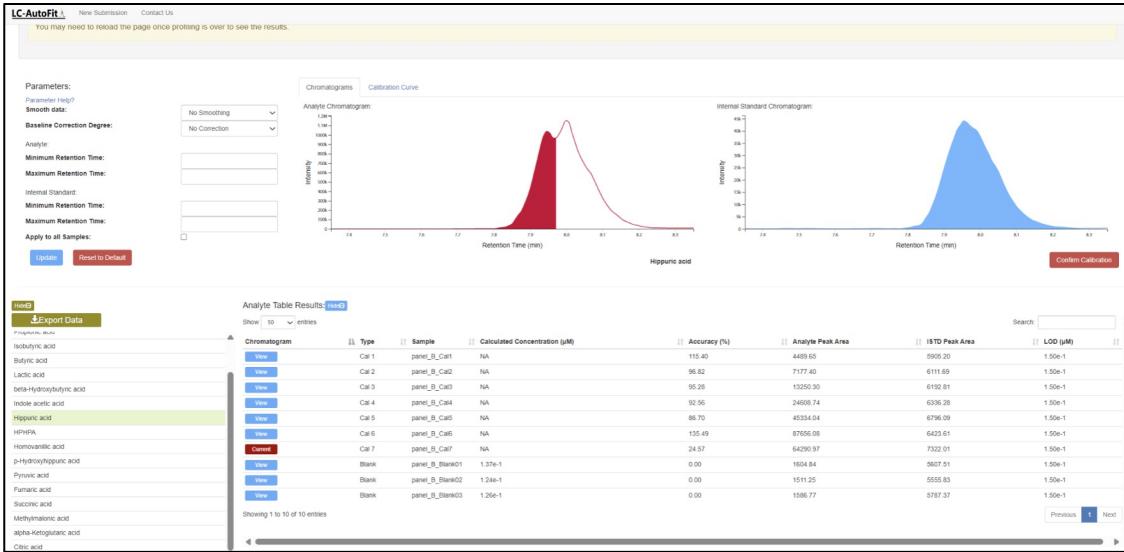
Retention Time (min)

Creatinine

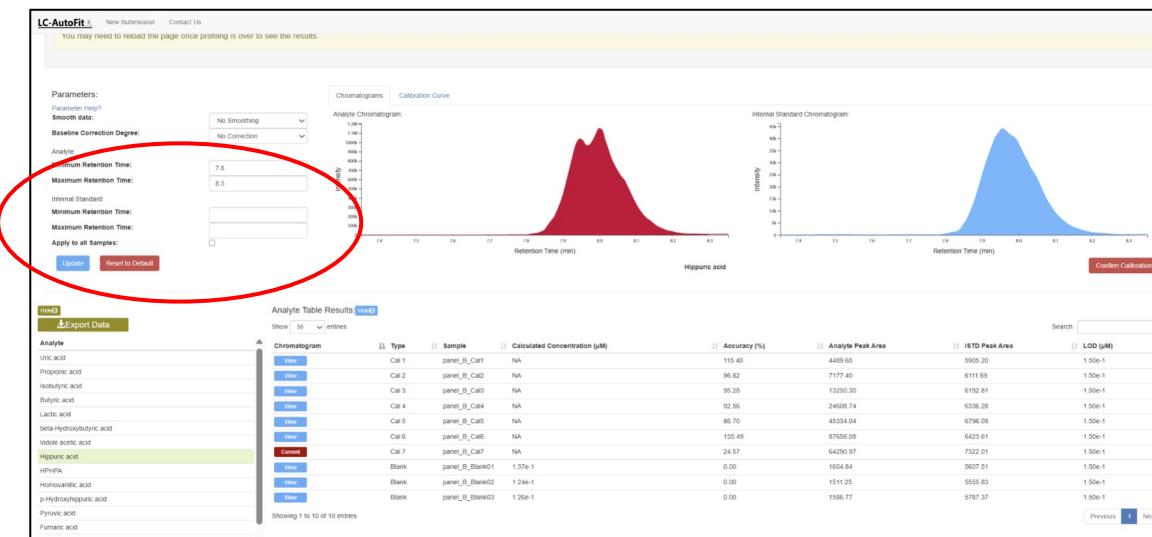
Confirm Calibration

Calibration Optimization/Correction

Before Parameter Adjustments



After Parameter Adjustments



- An example of “bad” or erroneous peak integration (happens sometimes due to peak drift or erroneous retention time)
- Users need to do manual parametric adjustments to ensure proper integration
- The parameter panel on the left can be used to adjust peak integration parameters
- Users can change the Retention Time (RT) on the left panel and update Max. RT & Min. RT
- After parametric update, click Submit to get proper peak integration (and concentration)

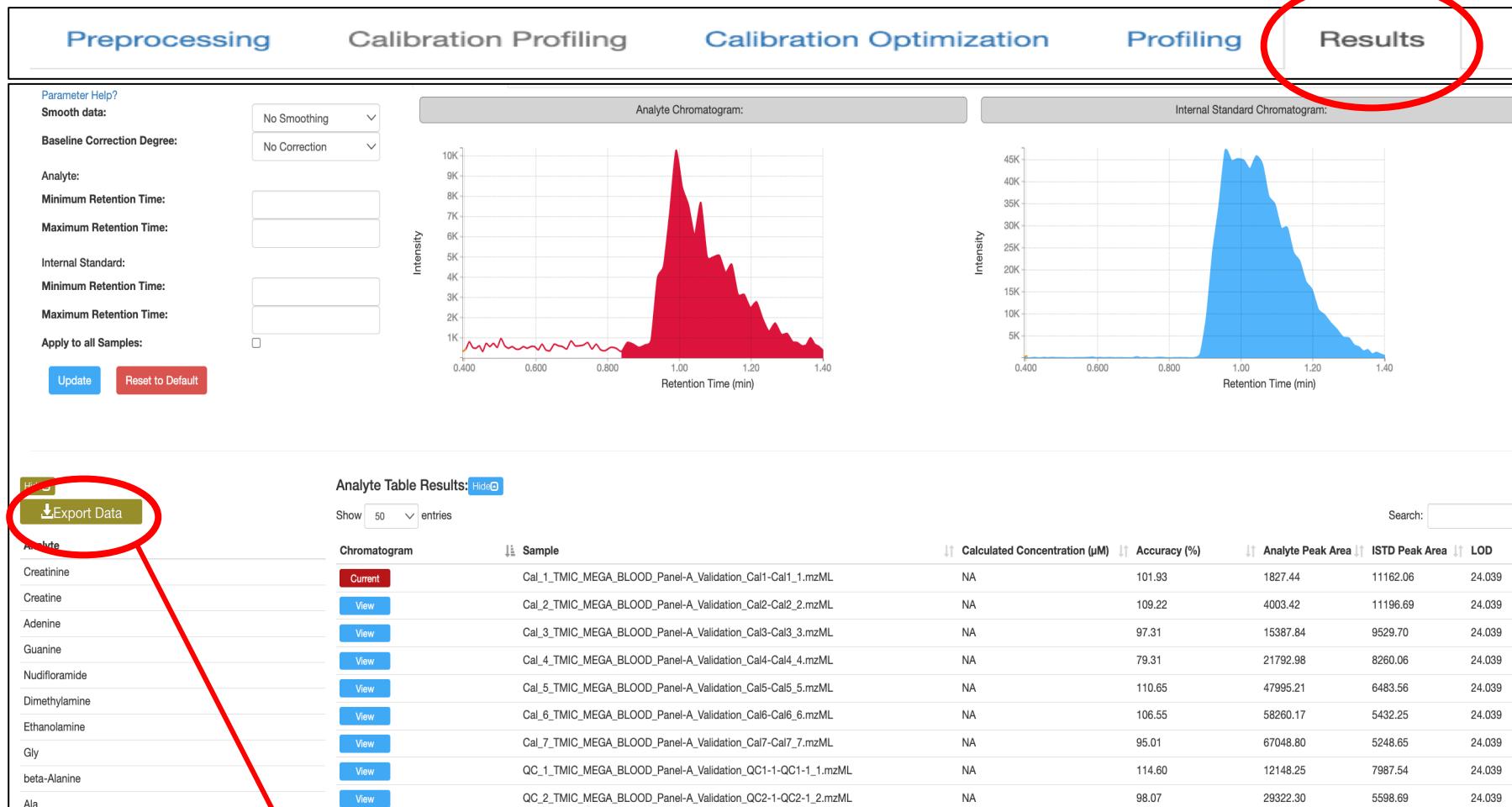
Profiling

Click Here



Samples			
Name	Run Time	Status	
Cal_Cal_1_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal1-Cal1_1.mzML	Running...	Complete	
Cal_Cal_2_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal2-Cal2_2.mzML	Running...	Complete	
Cal_Cal_3_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal3-Cal3_3.mzML	Running...	Complete	
Cal_Cal_4_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal4-Cal4_4.mzML	Running...	Complete	
Cal_Cal_5_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal5-Cal5_5.mzML	Running...	Complete	
Cal_Cal_6_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal6-Cal6_6.mzML	Running...	Complete	
Cal_Cal_7_TMIC_MEGA_BLOOD_Panel-A_Validation_Cal7-Cal7_7.mzML	Running...	Complete	
QC_QC_1_TMIC_MEGA_BLOOD_Panel-A_Validation_QC1-1-QC1-1_1.mzML	2 seconds	Complete	
QC_QC_2_TMIC_MEGA_BLOOD_Panel-A_Validation_QC2-1-QC2-1_2.mzML	2 seconds	Complete	
QC_QC_3_TMIC_MEGA_BLOOD_Panel-A_Validation_QC3-1-QC3-1_3.mzML	2 seconds	Complete	
Blank_Blank_TMIC_MEGA_BLOOD_Panel-A_Validation_Blank-1-Blank.mzML	2 seconds	Complete	
Blank_Blank_TMIC_MEGA_BLOOD_Panel-A_Validation_Blank-2-Blank.mzML	2 seconds	Complete	
Blank_Blank_TMIC_MEGA_BLOOD_Panel-A_Validation_Blank-3-Blank.mzML	4 seconds	Complete	
Double_Blank_Double_Blank_1_TMIC_MEGA_BLOOD_Panel-A_Validation_DoubleBlank-1-Double_Blank.mzML	4 seconds	Complete	
Double_Blank_Double_Blank_2_TMIC_MEGA_BLOOD_Panel-A_Validation_DoubleBlank-2-Double_Blank.mzML	4 seconds	Complete	
Exp_Exp_TMIC_MEGA_BLOOD_Panel-A_Validation_(35)-4S-1.mzML	2 seconds	Complete	
Exp_Exp_TMIC_MEGA_BLOOD_Panel-A_Validation_(36)-4S-2.mzML	2 seconds	Complete	
Exp_Exp_TMIC_MEGA_BLOOD_Panel-A_Validation_(40)-5S-2.mzML	3 seconds	Complete	
Exp_Exp_TMIC_MEGA_BLOOD_Panel-A_Validation_(46)-6S-4.mzML	3 seconds	Complete	
Exp_Exp_TMIC_MEGA_BLOOD_Panel-A_Validation_(50)-7S-4.mzML	3 seconds	Complete	

Results



Click Here to Download the
Result File (zip format)

Downloading Results

Hide

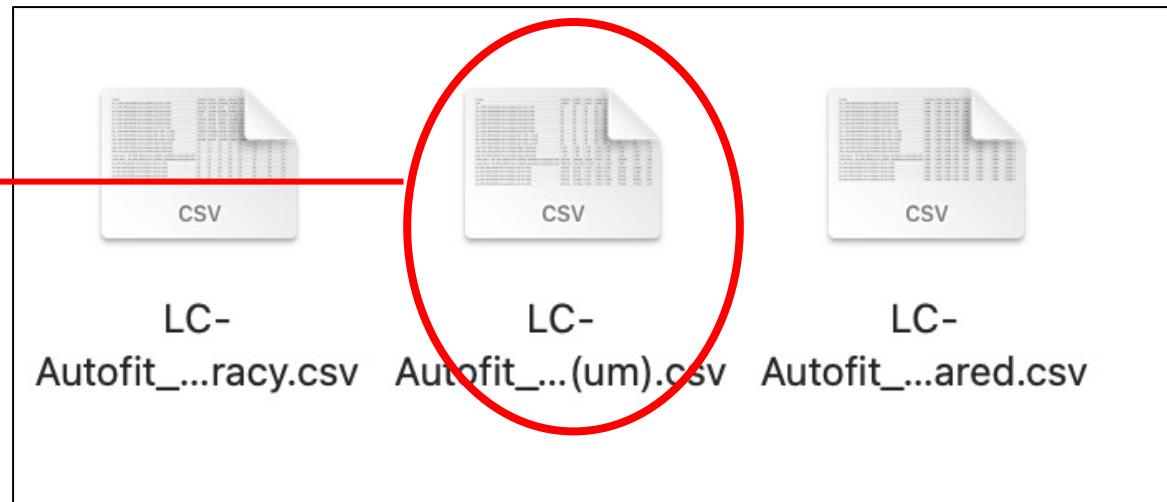
Export Data

Analyte

- Creatinine
- Creatine
- Adenine
- Guanine

Click Here to Download the Result File (zip format)

Final Metabolite Concentration



LC-AutoFit Final Results

- Table (CSV format file)
 - Table for each sample
 - Merged concentration for all samples
- Spectrum Viewer
 - Spectra with assigned compound names

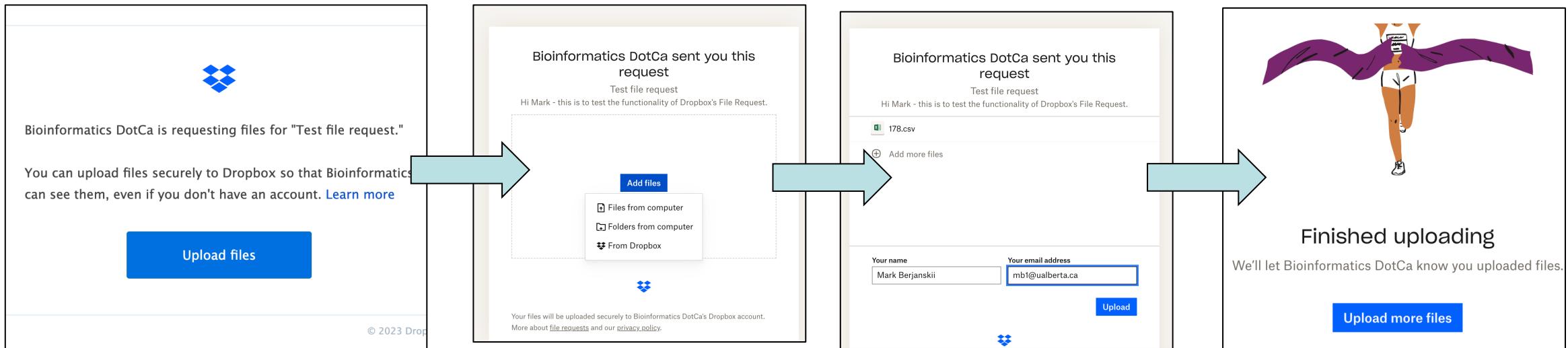
The screenshot shows the LC-AutoFit software interface. At the top, there is a navigation bar with links for 'New Submission' and 'Contact Us'. Below this is a main menu with tabs: 'Preprocessing', 'Calibration Profiling', 'Calibration Optimization', 'Profiling' (which is currently selected), and 'Results'. A red circle highlights the 'Results' tab. An arrow points from this circle down to a blue button labeled 'Export Data' with a download icon. Below the button is a table of analytical results.

Analytes	Creatinine	Creatine	Adenine	Guanine	Nudifloram	Dimethylar	Ethanolam	Gly	beta-Alanir	Ala
LOD	24.0387	4.3769	0.4476	0.0069	0.0768	3.1194	0.0008	0.0005	0.0648	0.0975
QC_1_TMIC_MEGA_BLOOD_Panel-A_Validation_QC1-1-QC1-1_1.mzML	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
QC_2_TMIC_MEGA_BLOOD_Panel-A_Validation_QC2-1-QC2-1_2.mzML	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
QC_3_TMIC_MEGA_BLOOD_Panel-A_Validation_QC3-1-QC3-1_3.mzML	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Blank_TMIC_MEGA_BLOOD_Panel-A_Validation_Blank-1-Blank.mzML	< LOD	< LOD	< LOD	< LOD	< LOD	0.137	< LOD	< LOD	< LOD	< LOD
Blank_TMIC_MEGA_BLOOD_Panel-A_Validation_Blank-2-Blank.mzML	< LOD	< LOD	< LOD	0.0226	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Blank_TMIC_MEGA_BLOOD_Panel-A_Validation_Blank-3-Blank.mzML	< LOD	< LOD	< LOD	0.0128	< LOD	< LOD	0.0789	< LOD	< LOD	< LOD
Double_Blank_1_TMIC_MEGA_BLOOD_Panel-A_Validation_DoubleBlank-1-Double_Blank.mzML	2420.283	148.4536	38.4674	6.7495	< LOD	105.8223	< LOD	152.6601	5.9532	11.003
Double_Blank_2_TMIC_MEGA_BLOOD_Panel-A_Validation_DoubleBlank-2-Double_Blank.mzML	33590.2211	1581.4543	197.2453	< LOD	16.5572	3.8657	< LOD	378.8703	< LOD	1187.3031
Exp_TMIC_MEGA_BLOOD_Panel-A_Validation_(35)-45-1.mzML	130.0631	47.7792	0.7214	0.5942	5.1469	17.9428	14.1964	383.2179	9.8286	393.2586
Exp_TMIC_MEGA_BLOOD_Panel-A_Validation_(36)-45-2.mzML	145.6214	66.8237	0.6664	0.5158	4.4727	20.2461	12.3066	405.4053	10.619	394.8352
Exp_TMIC_MEGA_BLOOD_Panel-A_Validation_(40)-55-2.mzML	244.0174	80.1153	1.3367	0.9888	9.2603	33.0987	18.439	597.2617	19.6383	618.7566
Exp_TMIC_MEGA_BLOOD_Panel-A_Validation_(46)-65-4.mzML	307.5205	102.9897	2.129	1.4933	12.9187	61.1304	28.6453	978.044	29.1924	849.9887
Exp_TMIC_MEGA_BLOOD_Panel-A_Validation_(50)-75-4.mzML	448.7512	121.9923	2.7969	2.1128	17.266	75.4464	37.3215	1139.79	33.6933	1076.5526

When You Are Done...

Upload your files to the CBW Dropbox folder using the link sent by CBW

Example of Dropbox upload interface



Upload link: <https://www.dropbox.com/request/ooHSrGu8Qnz4KnaSjTiO>

See also instructions "How to upload files to a Dropbox file request" at
<https://contentsnare.com/dropbox-file-request/>

Summary

- This lab has allowed you to analyze three types of targeted, quantitative metabolomics data sets from three types of metabolomics platforms (NMR, GC-MS and LC-MS)
- These are simplified examples due to class size and limited class time
- Normally you would analyze 10-1000 samples with this software, not a few samples
- Not everything is automated, normally you would do some interactive editing to “clean” things up – all three programs support interactive editing

Busting Some Myths About Quantitative Metabolomics

- More expensive? No (2-10X cheaper)
- More time-consuming? No (10-100X faster)
- Less sensitive? No (10-100X more sensitive)
- Less comprehensive No (1.5-10X more coverage)
- Less likely to lead to significant discoveries than untargeted metabolomics? No (see what you'll discover tomorrow)
- Less likely to translate to clinic? No (see what you'll discover tomorrow)



We are Now Working on The Lab (Module 3)

Workshop Sponsors:



Canadian Centre for
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HPC4Health



OICR
Ontario Institute
for Cancer Research



GenomeCanada



We are on a Coffee Break & Networking Session

Workshop Sponsors:



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Computational
Genomics



HPC4Health



OICR
Ontario Institute
for Cancer Research



GenomeCanada

