Agilent MassHunter Profinder: Solving the Challenge of Isotopologue Extraction for Qualitative Flux Analysis

Technical Overview

Introduction

Metabolomics studies measure the relative abundance of metabolites to better understand biological systems. However, this does not provide insight into the dynamics of metabolism since significant changes in flux through a pathway may not result in altered abundance of metabolite intermediates. Qualitative flux analysis addresses this issue, and allows researchers to obtain information about selected pathways and fluxes. In these types of studies, a stable isotope-labeled tracer (typically containing ^{13}C, ^{15}N, or ^{2}H) is introduced into the biological system and results in changes in the natural isotopic pattern of metabolites over time. This generates characteristic patterns, whose mass signatures can be measured using a mass spectrometer (MS). Agilent TOF and Q-TOF LC/MS systems provide the high level of mass accuracy and isotopic fidelity required for qualitative flux analysis.

One of the key challenges in qualitative flux analysis is to effectively extract isotopically labeled metabolites from the analysis of complex samples. Isotopologues, metabolite ions differing only in isotopic composition, must be determined for each target metabolite and differentiated from background or matrix ions. Furthermore, raw isotopologue abundance must be corrected for the isotopic purity of the tracer and contributions of all naturally occurring isotopes.

To address this challenge, a batch isotopologue extraction algorithm has been implemented in Agilent MassHunter Profinder[1], a widely-used mass spectrometry-based batch feature extraction software. This algorithm is designed specifically for qualitative flux data acquired on Agilent LC/TOF and LC/Q-TOF systems. This Technical Overview describes the isotopologue extraction workflow in Profinder, a module in Agilent MassHunter VistaFlux[2].

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Workflow Overview: Batch Isotopologue Extraction

Profinder is a fast, batch-processing software for mass spectrometric data. It provides many useful data analysis tools, such as grouping of replicate data files, compound alignment, multiple chromatogram overlay, color by sample group, manual re-integration of peaks, and results export. Profinder B.08.00 features a new batch extraction algorithm for flux analysis that allows for confident extraction and visualization of isotopologue incorporation.

The batch isotopologue extraction algorithm in Profinder uses a file containing the target list of metabolites (.CDB or .CSV format) to extract all possible isotopologues based on the metabolite chemical formulas. The target list must contain chemical formula, retention time (RT), compound name, and compound IDs (CAS, KEGG, and so forth). Using the chemical formulas for the targeted metabolites, RTs can be determined using the Batch Targeted Feature Extraction workflow in Profinder[1].

The algorithm is designed for targeted data mining of ^{13}C, ^{15}N, or ^{2}H stable isotope-labeled ions acquired on an Agilent LC/TOF or LC/Q-TOF. Raw isotopologue abundance is determined for extracted metabolites. Corrected isotopologue abundance is also calculated using the natural isotope abundance and the purity of the isotope tracer.

The Profinder isotopologue extraction algorithm uses a multistage ion extraction process. First, the individual isotopologue extracted ion chromatograms (EICs) are extracted over the RT region specified in the target file at the m/z of each possible isotopologue, with a wide m/z tolerance window. The sum of those EICs is integrated to establish narrower RT regions in which there may be evidence for the target compound. The data are evaluated independently for each of those regions. Mass spectra are extracted from each region and used to disqualify ions that do not meet a more stringent m/z match criterion. Ions may also be disqualified if their individual EICs do not show similar elution profiles. A new, combined EIC is created that represents the sum of only those ions that were qualified in the first stage. If that new EIC has more than one peak, the most likely peak is selected with a scoring system that considers the number of qualified isotopologue ions under a peak, the relative abundance of the peak, and

the deviation of the peak from the target RT. Finally, the isotopologue abundance values are measured for the selected EIC peak (according to a user-selected abundance metric), and those abundance values are corrected for the natural abundance of the label element, and for the isotope purity of the tracer.

The benefit of this multistage extraction process is to minimize false positive results by discriminating target metabolite isotopologues from background and matrix ions. By using EICs and not mass spectra, the algorithm provides more accurate isotopologue abundances, which yields more precise isotopologue incorporation results.

The batch isotopologue extraction process is guided by a workflow wizard, which provides an easy way to set up the necessary parameters. The process starts with the import of data files and group assignment (Figure 1).

Acquisition of high quality data

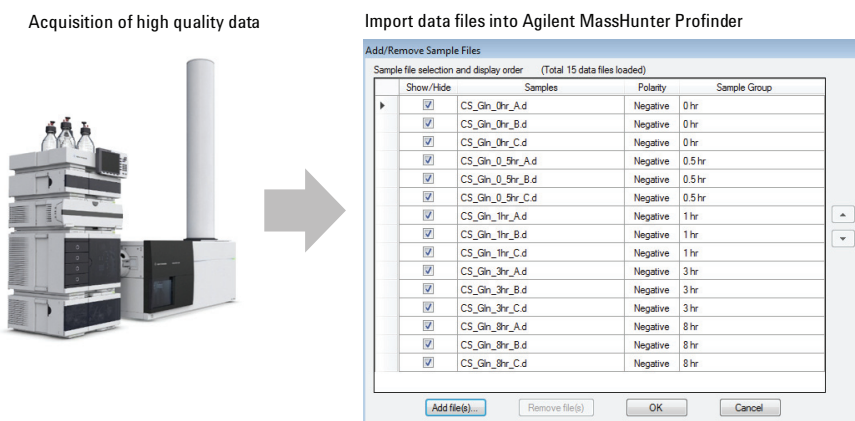Import data files into Agilent MassHunter Profinder

Figure 1. Samples are run on an Agilent LC/TOF or LC/Q-TOF to obtain high quality data. The data files are imported into Agilent MassHunter Profinder, and assigned a Sample Group.

An input file containing a target metabolite list is used as the target source, and the isotope label is selected (Figure 2). The method, which contains all the selected parameters and settings, can be saved. After choosing settings and selecting **Finish**, the batch isotopologue extraction is automatically performed. All results from data processing can be saved as a Profinder project (.Profinder file).

Figure 3 shows the processed results for ^{13}C-glutamine flux data acquired on an Agilent 6230 TOF LC/MS in negative ion mode, using the target list of metabolites created for the TCA pathway from the BioCyc database. The Profinder interface consists of five linked navigation windows, which enables easy review of extraction results. The Compound Group Table (3A) displays the compound-level

information grouped and summarized across multiple data files. The individual file details of a selected compound group are shown in the Compound Details Table (3B), the summed Chromatogram Results for all isotopologues (3C), the MS Spectrum Results (3D), and the Isotopologue Results (3E).
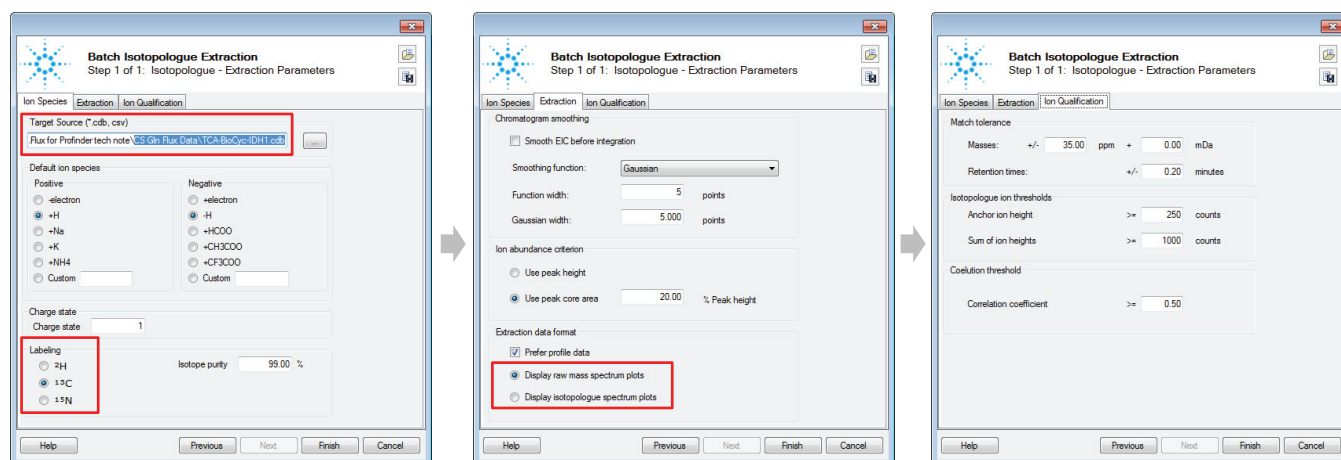

Figure 2. The Workflow Wizard provides guided steps to set up Batch Isotopologue Extraction. The red boxes highlight parameters that are most often adjusted.
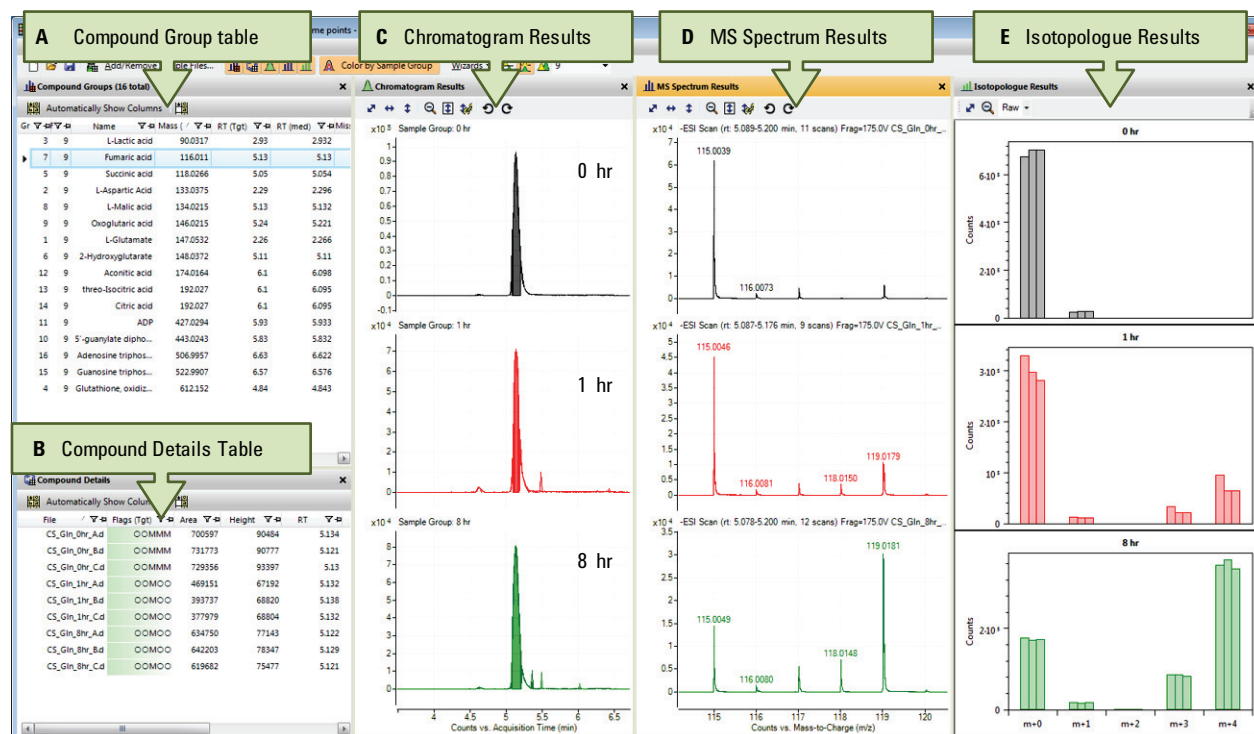

Figure 3. Compound-centric visualization provides batch isotopologue extraction results from three representative groups.

This compound-centric graphical user interface provides simplified data review, manual curation capabilities, and visualization of metabolite isotopologue incorporation. The images can be colored by individual sample file (Figure 4A), or by sample group (4B) for easy viewing based on the user's preference. They can also be stacked by individual sample file (4A-B), overlaid by sample group (4C), or all the files can be overlaid (4D). These options allow for a more effective way to compare isotopologues across sample files or sample groups. In addition, they provide the ability to visualize missing peaks, as well as enable manual editing of chromatograms. Correcting for the natural isotope abundances and the purity of the isotope tracer is a critical step to ensure accuracy in qualitative flux analysis. In MassHunter Profinder, the isotopologue results can be viewed as either absolute or normalized abundance with or without natural isotope abundance correction. Profinder offers different options for viewing isotopologue results including raw, corrected, normalized, and averaged (Figure 5).
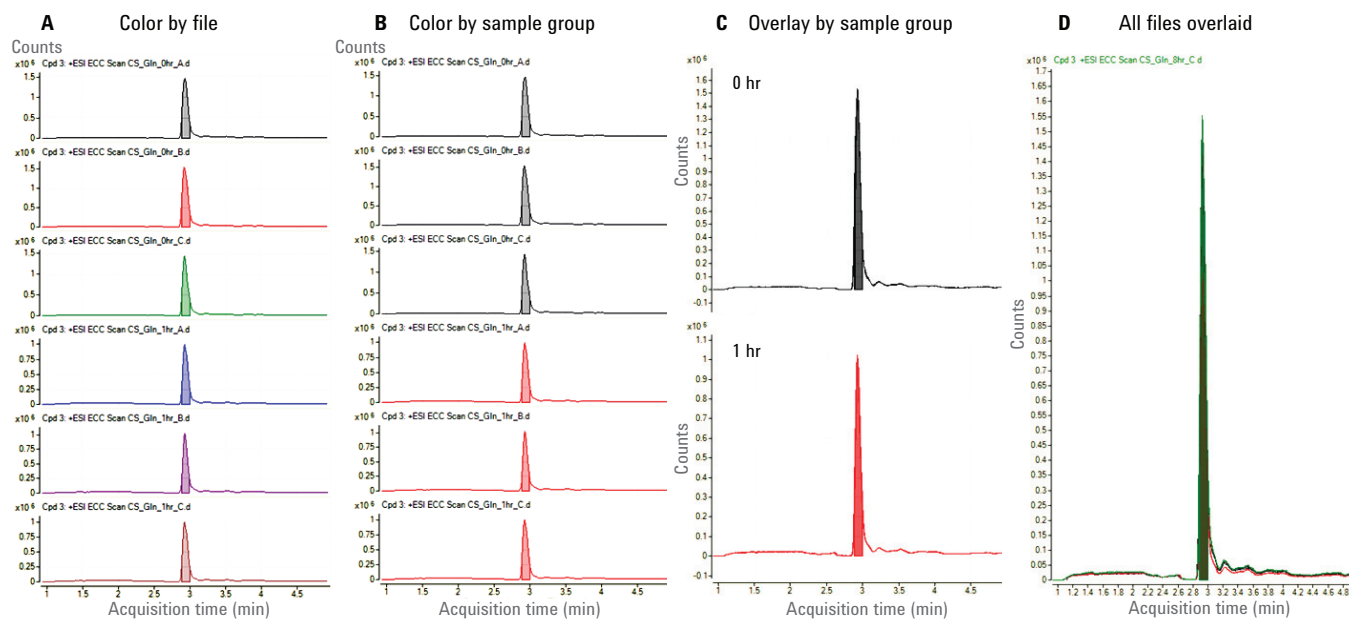

Figure 4. Agilent MassHunter Profinder provides the user with the ability to easily view their data.
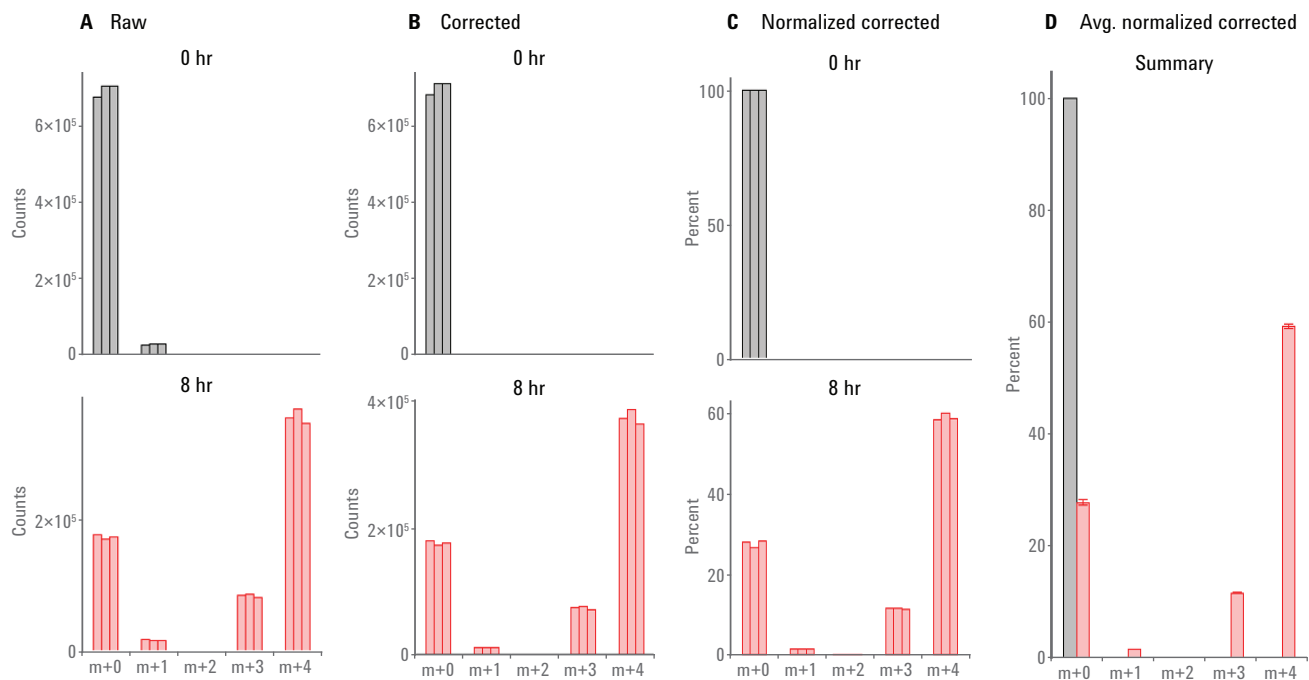
Figure 5. Agilent MassHunter Profinder provides multiple options to easily view the raw or corrected isotopologue abundances.

To visually inspect isotopologue results, the workflow wizard offers a choice of raw mass spectra or isotopologue spectra as an extraction data format. This enables quick identification of background or matrix ions from raw mass spectra, and allows assessment of the accuracy of the isotopologue extraction. For example, the raw mass spectrum for fumaric acid shows an apparent M+2 isotopologue (Figure 6). The isotopologue extraction algorithm, using a multistage ion extraction process, quickly determined that this ion was not an isotopologue of fumaric acid, and excluded it from the isotopologue results and isotopologue spectra.

Once the review and curation is complete, the isotopologue extraction results can be exported from Profinder as a .PFA format file. The file contains information for sample grouping, compound name, identifiers, and isotopologue data. This file can be imported into Omix Premium, a module of VistaFlux[2], designed to seamlessly import isotopologue extraction results for visualization of qualitative flux results on pathways.
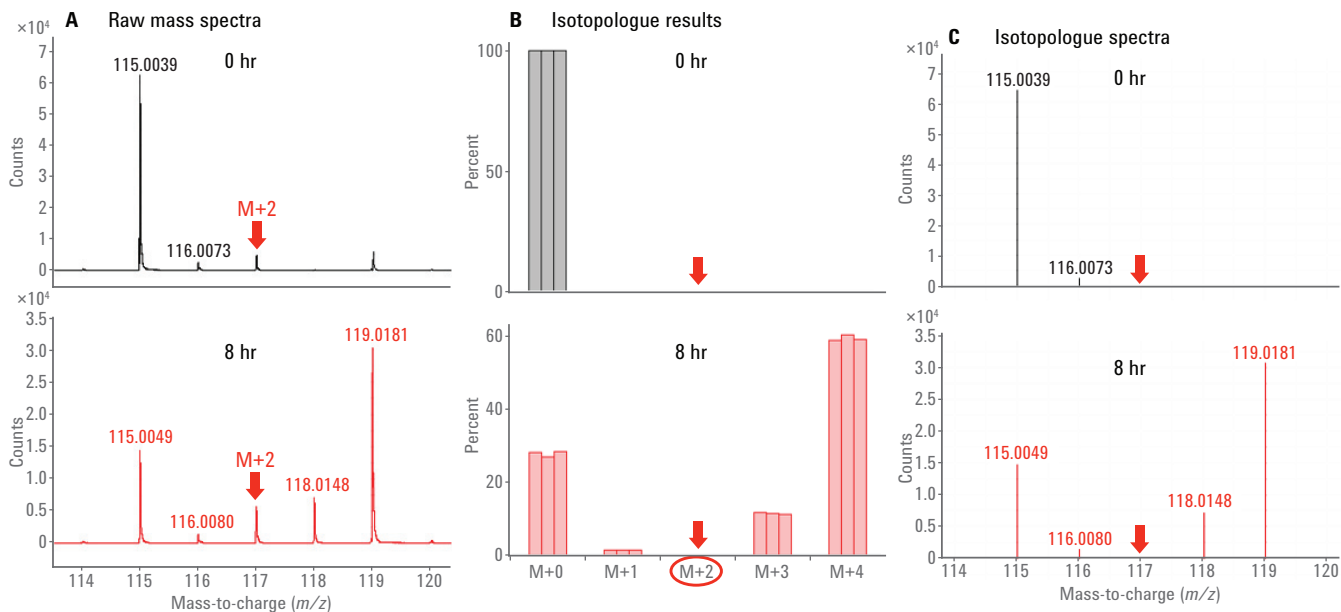
Figure 6. Results for fumaric acid show an apparent M+2 isotopologue in the raw mass spectra. However, the algorithm determined that this ion was not an isotopologue of fumaric acid, and excluded it from the isotopologue results and isotopologue spectra.

Conclusions

The Agilent MassHunter Profinder batch isotopologue extraction algorithm provides a powerful tool for targeted mining of stable isotope-labeled metabolites from data acquired on Agilent LC/TOF and LC/Q-TOF systems. The software is intuitive and easy-to-use, allowing researchers to get results quickly. The isotopologue extraction process is fully automated with multistage batch extraction. This provides high quality results for reliable, high-throughput qualitative flux analyses. The results can easily be exported to Omix Premium for interactive visualization of metabolic fluxes on pathways of interest.

References

1. MassHunter Profinder: Batch Processing Software for High Quality Feature Extraction of Mass Spectrometry Data, *Agilent Technologies Technical Overview*, publication number 5991-3947EN, **2014**.

2. MassHunter VistaFlux for Qualitative Flux Analysis, *Agilent Technologies Technical Overview*, publication number 5991-6756EN, **2016**.

www.agilent.com/chem/Profinder

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