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Increasing Confidence in Non-Targeted Metabolite Identification with Library Comparison and Simplified Unknown Analysis Workflow with Novel Software Solution

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Introduction

Untargeted Metabolomics on the Revident LC/Q-TOF with Data Analysis Supported by New Software: MassHunter Explorer 2.0

Common workflows for untargeted metabolomics by HRMS utilize multiple acquisition types. Data dependent acquisition provides rich fragment information important for identification and iterative injections give deeper metabolite identification utilizing exclusion lists. MS1 affords sensitive and comprehensive surveys of individual samples. This combination workflow gives analysts a wealth of information for observing changes in known metabolites, while also identifying metabolites or other analytes less frequently present in biologically curated metabolomic libraries. However, this heavy data load can create a barrier for some investigators. Herein is novel software that combines complex analysis into a streamlined workflow, untangling the interpretation of multiple data file types and giving researchers clear and confident interpretation of metabolomic alterations.

Figure 1. Revident LC/Q-TOF with 1290 Infinity III LC.

All data was acquired on the Revident LC/Q-TOF. Key performance elements of the Revident are a new detector, giving better mass accuracies even at saturation, as well as an increased dynamic range compared to previous instrument generations. In combination with the temperature-inert flight tube, contributing extended duration of mass stability, the overall mass accuracy has improved. These features enable Revident LC/Q-TOF to be principal hardware for untargeted metabolomic analysis.

Experimental

Robust HILIC Chromatography for Polar Metabolites

Metabolites extracted from plasma (20 µL) using Captiva Lipid EMR plates were separated by HILIC chromatography, using established and standardized methodology.[1] Mouse samples (20M/20F) were analyzed. A bioinert 1290 LC combined with LC/Q-TOF was used for the acquisition of both MS1 and iterative MS/MS data files.

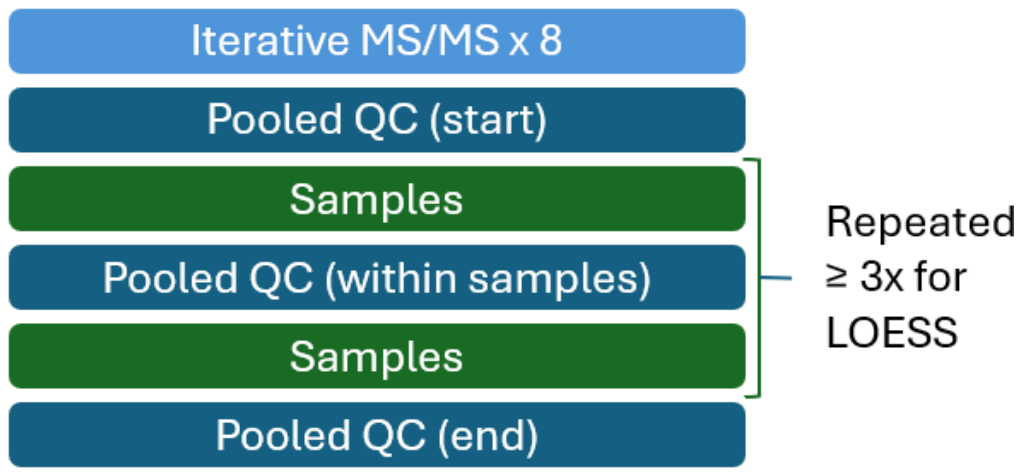

Figure 2. Experimental worklist setup format.

Worklist design included iterative MS/MS analysis on a pooled QC sample followed by MS1 analysis of all samples including evenly distributed QCs (Figure 2). Appropriate QC distribution allows for use of LOESS normalization. Iterative analysis allows for a deeper look into the samples leveraging AutoMSMS decision making with an accumulating exclusion list upon each injection (Figure 3). Both MS1 and AutoMSMS data files were processed in the same software, MassHunter Explorer 2.0, this update allows the combination of the two data file types into a succinct identification workflow.

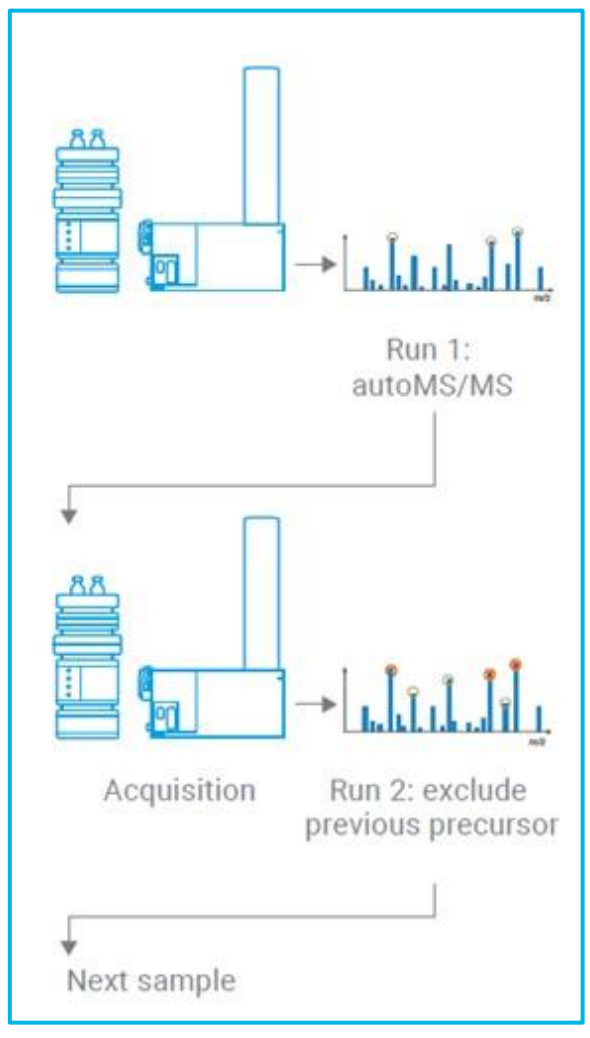

Figure 3. Workflow for iterative MS/MS analysis with accumulating exclusion list. One blank and eight sample iterations were used.

Results and Discussion

New Software to Combine MS Only and DDA Data into a Simple Identification Workflow

MassHunter Explorer 2.0 simplifies the data analysis intensive step of identification for untargeted metabolomics studies. Combining the specific and sensitive information from individual MS1 data files with the exclusive fragmentation of pooled samples that undergo iterative AutoMSMS analysis.

MH Explorer breaks the analysis process down into six basic steps: setup, find and align, normalization, filtering, statistics, and identification. Data files are imported directly from acquisition. Next, files are batched and sorted using custom groupings and easily selected types. Feature extraction settings are selected, including mass extraction limitations, adduct possibilities, and height filters, among others. Compound groups are normalized and filtered by abundance, variability, and frequency. A range of statistical analysis can be performed including HCA, volcano plots, PCA, ANOVA, unique features, and fold change analysis. Lastly, identification leveraging mass accuracy and isotope analysis, available retention times, and library fragmentation spectra is completed.

All steps described happen in a single user interface, making the laborious process of comparing MS1 and MS/MS data from untargeted metabolomics experiments simple and straightforward, as displayed in Figure 4. After feature extraction, identification is carried out using spectral libraries and databases curated in ChemVista.[2]

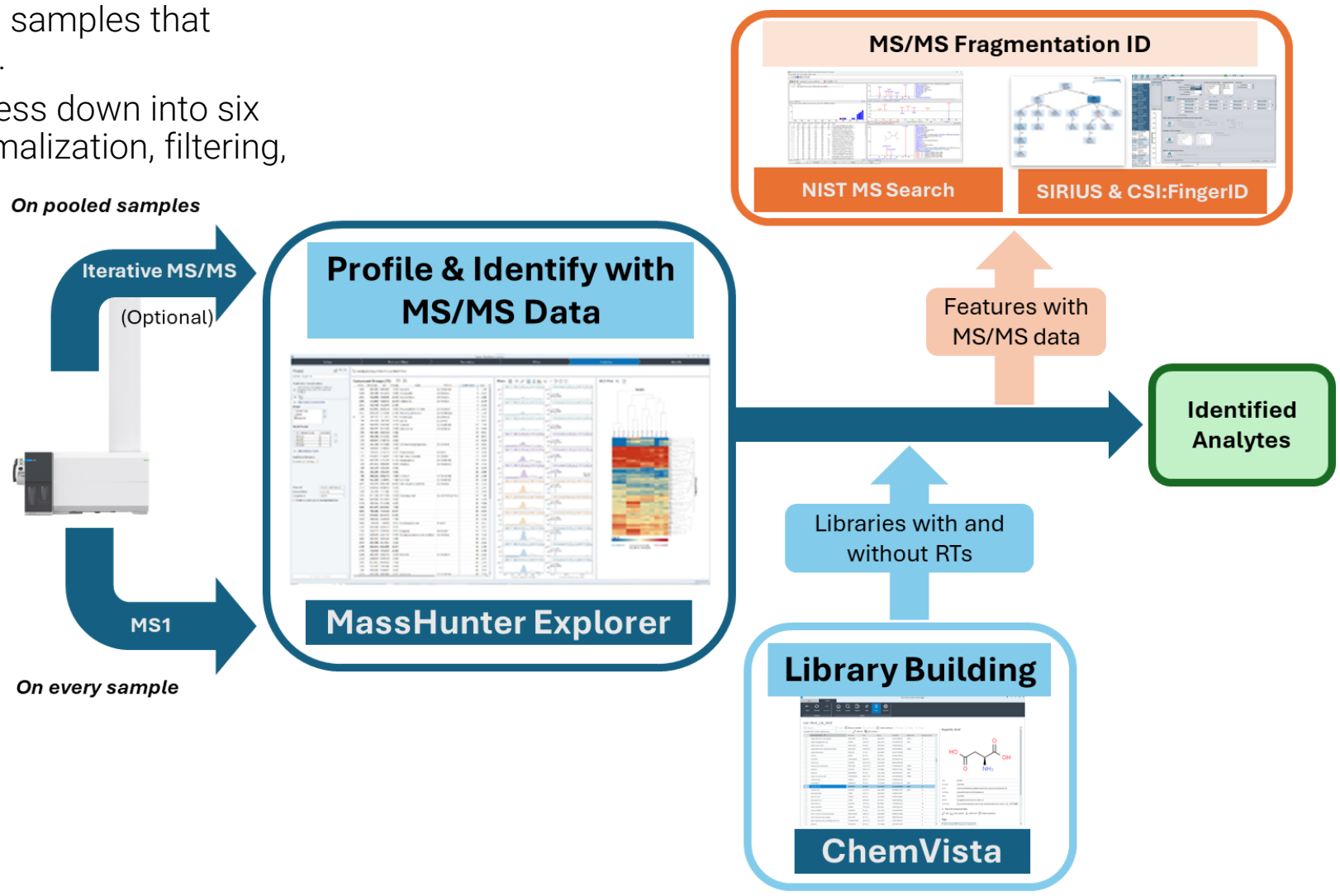

Figure 4. MH Explorer 2.0 workflow from MS1 and MS/MS data files to identification of significant features.

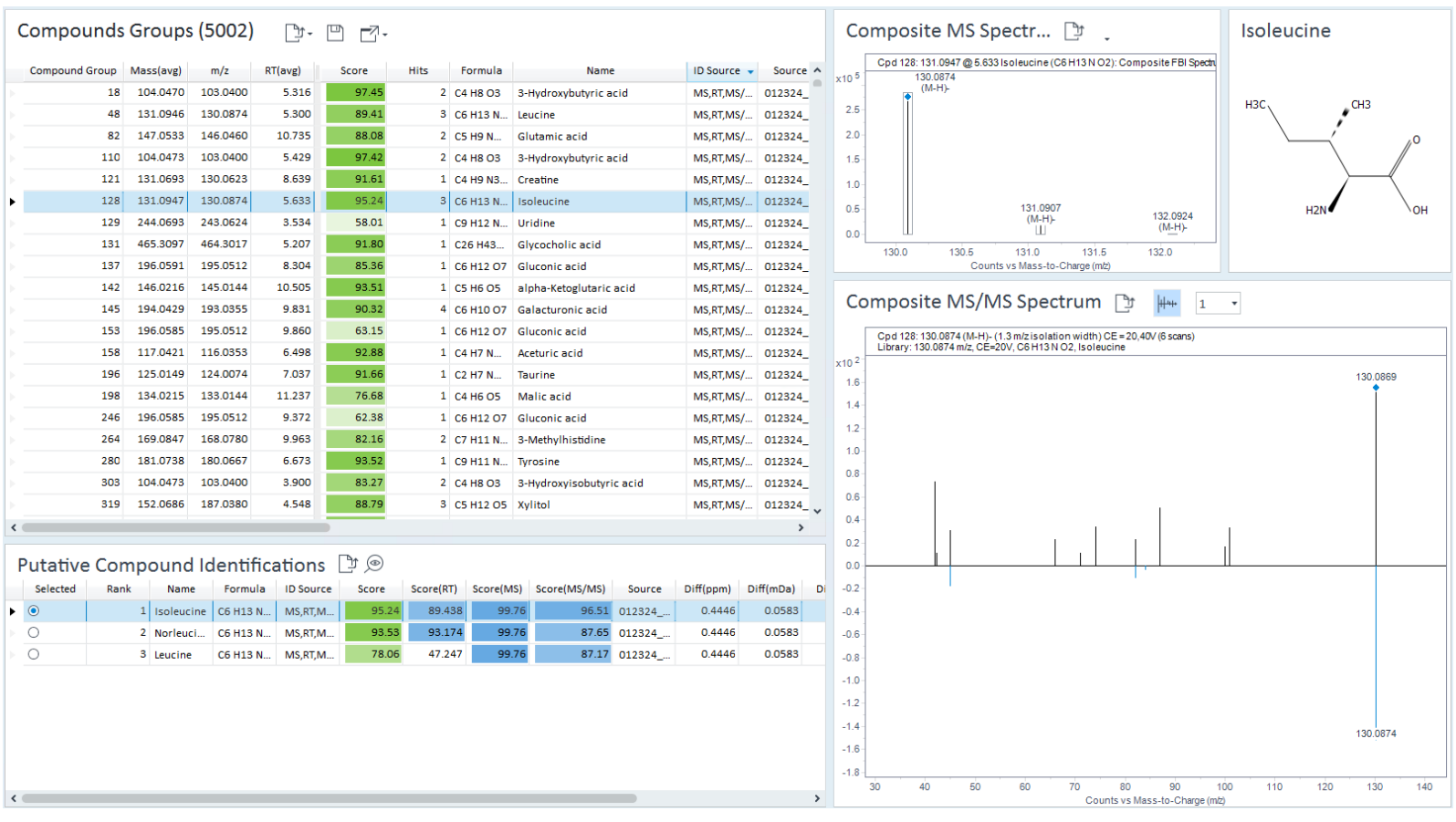

Figure 5. MH Explorer 2.0, identification, displaying isotope matching, putative compound identifications, and fragment spectra mirror plot.

All of the figures are easily exportable as high-quality images ready for publication. At any stage compound information can be exported in various formats.

The identification step has been updated to include the evaluation of fragmentation information from MS/MS data. As shown in Figure 5, identifications are scored, easily displayed with gradient highlighting. Molecular ion and isotopologue matching (Fig. 5, top right) is overlayed for comparison and (Fig. 5, bottom right) the mirror plot compares the experimental fragmentation pattern to available library spectra.

Results and Discussion

Direct Export to SIRIUS and NIST MS Search Start Identification of Features Not Present in Available Libraries

Despite the availability of extensive and curated libraries for comparison, there will still be situations where an analyte of interest is not present, and identification may be limited to chemical formula. The option to directly link to SIRIUS CSI:FingerID or NIST MS Search can initiate a deeper search into putative identifications (Figure 6).

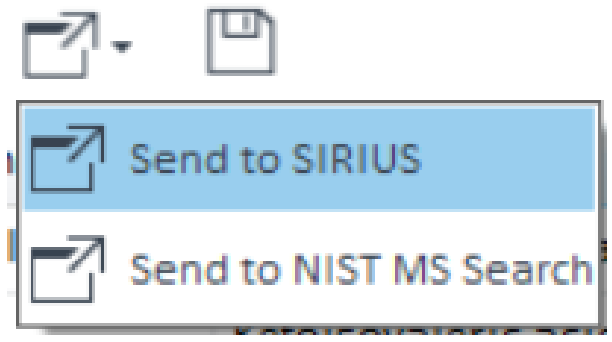

Figure 6. Direct export of features to SIRIUS or NIST MS Search for further identification.

Upon import to SIRIUS CSI:FingerID or NIST MS Search a wider search associated with the acquired fragmentation spectra can be used for structure elucidation and scoring (Figure 7).

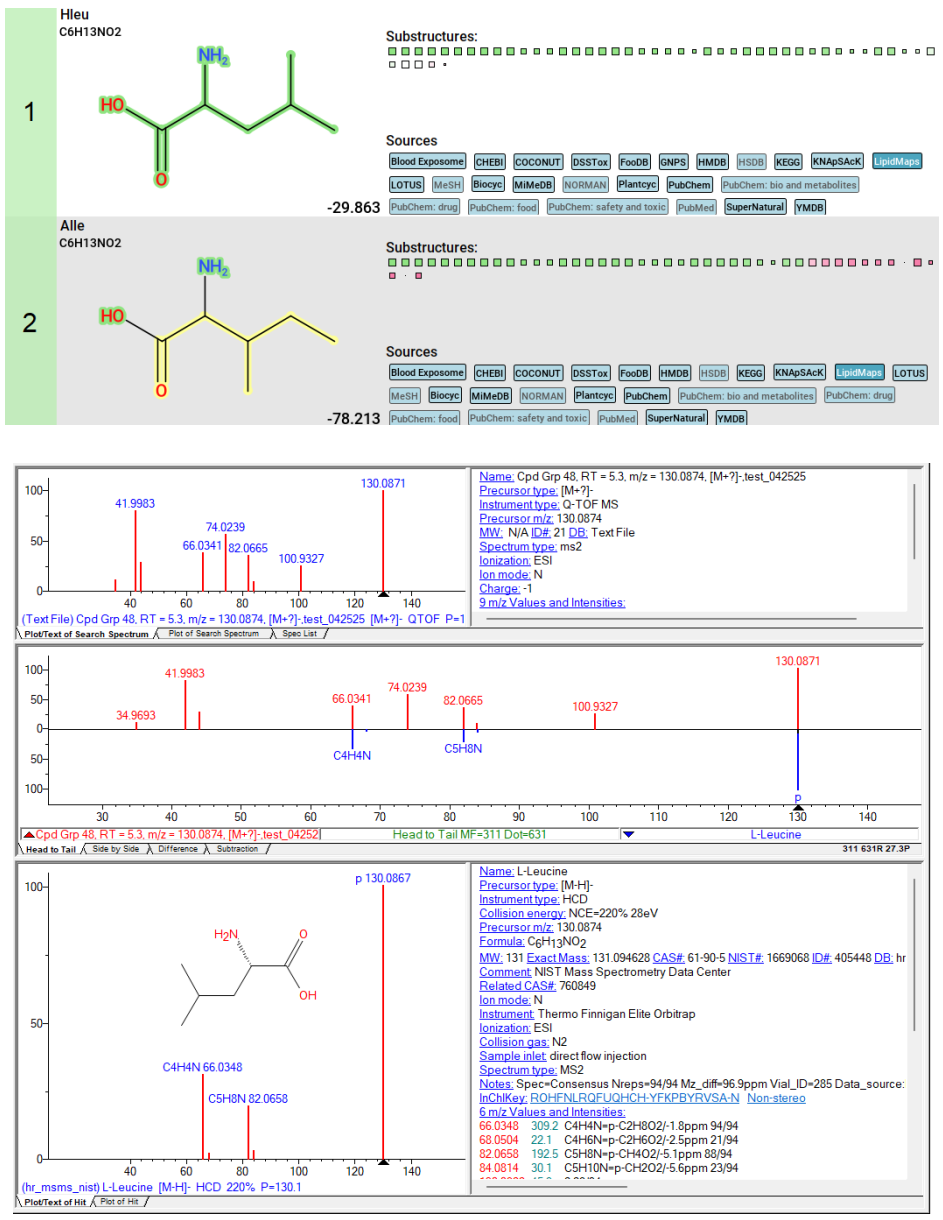

Figure 7. SIRIUS CSI:FingerID (top) and NIST MS Search (bottom) output from direct feature search with MH Explorer 2.0.

Profiling and Identification of Statistically Significant Analytes Between Mouse Plasma Samples

From the experimental samples 2705 and 1996 features were accompanied with MS/MS negative and positive data, respectively. For negative mode, of the significant features by 2-fold between the two sample groups 17 were assigned identification by the curated HILIC Metabolomics Database using MS and RT and 222 identified with METLIN and a score over 70. Of those 73 had associated MS/MS data and were exported to SIRIUS:CSI Finger ID to yield 58 tentative identifications, 2 with scores >-20 (Figure 8).

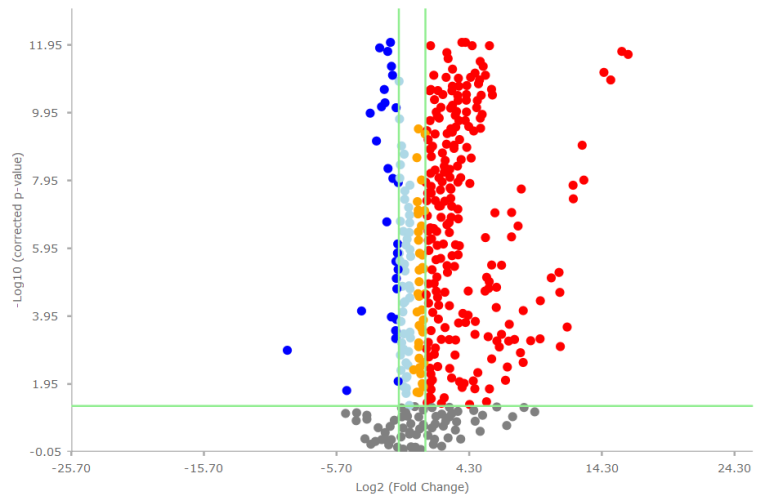

Figure 8. Volcano plot, male vs female, 2-fold difference and p value cutoff <0.05.

Conclusions

Novel Software Simplifies Untargeted Metabolomics Data Analysis

Profiling, Statistical Analysis and Identification in One Software Workflow Combining MS1 and MS/MS Data Files

- Combines Iterative MS/MS Analysis + MS1 Untargeted Metabolomics Workflow
- Direct Export to SIRIUS CSI:FingerID and NIST MS Search for Further Identification
- Identification of Statistically Different Compounds Between Mouse Plasma Samples
- Streamlined Data Analysis Workflow

References

[1]Yannell, KE et al. An End-to-End Targeted Metabolomics Workflow. Agilent Application Note 5994-5628EN. 2023.

[2]Agilent ChemVista Library Manager. Agilent Technical Overview 5994-5924EN. 2023.

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