METABOLITE PROFILING AND IDENTIFICATION IN CRYOPRESERVED HUMAN HEPATOCYTES USING THERMO SCIENTIFIC COMPOUND DISCOVERER SOFTWARE

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ABSTRACT

A consistent challenge of the drug discovery project team is to make rapid impactful decisions on limited data sets to move a program forward. Traditional methods of understanding metabolic clearance issues (include screening half-lives in in vitro systems and trying to understand structure activity relationships) are rapid although imprecise. Alternately, determining metabolic soft-spots empirically can be time consuming with spectral interpretation being rate limiting. The combination of several different technologies: cryopreserved hepatocytes, high resolution mass spectrometry and data analysis and interpretation software, can provide more rapid and accurate results to help teams make the right decisions. Cryopreserved hepatocytes provide in vivo-like enzyme expression levels, proper cell morphology, and increased ability to draw in vitro/in vivo correlations to make sound decisions regarding a compound's fate. High resolution mass spectrometry itself has improved the quality of metabolite identification, but also has enabled the advent of software that can take advantage of the precision of accurate mass instruments and combine that with computational power and techniques to further enhance the value of this data. This poster will share the results of a series of marketed drugs that were incubated with cryopreserved human hepatocytes, profiled by high resolution mass spectrometry and analyzed using Compound Discoverer software (Thermo Scientific).

INTRODUCTION

Historically, the rate limiting step in providing metabolic profiling and structure elucidation results has been data interpretation. Due diligence required an extensive process of manually interrogating data for analytes of unknown variety and quantity, then comparing those results to control samples. The convergence of UPLC, High Resolution Mass Spectrometry (HRMS) and structure elucidation software are allowing the bottleneck of data interpretation to be significantly reduced. UPLC provides ~5-10 fold decrease in traditional analysis time without losing any of the chromatographic resolution. HRMS spectral accuracy provides the precision for high confidence fragment interpretation and structure elucidation. Compound Discoverer software, takes advantage of the high level of mass accuracy, the algorithmic nature of mass accurate metabolite predictions in addition to providing tools to organize, compare, contrast and summarize results.

INTRODUCTION

Compound Discoverer enables the scientist to customize the level of analysis through the use of 'nodes' to meet the needs of the issue at hand (Figures 1 & 2). In this example, Compound Discoverer is being used to screen compounds for metabolic clearance pathways in cryopreserved human hepatocytes against control incubations using several detection techniques (Mass Spectrum, UV, Isotope Ratio, Fragment Traces).

METHODS

Compounds (10 µM) were incubated with cryopreserved isolated human hepatocytes (50 µL, 96-well plate). Incubate extracts were injected onto Accela UPLC system running a wide 12 minute gradient (0.1% formic acid (mobile phase A) and ACN(mobile phase B)). Chromatographic separations were performed with an ACQUITY C18 BEH (2.1x100mm, 1.7µm) column with UV spectral data collected from 200-450 nm. Mass spectral data was collected using a Obitrap Elite Mass Spectrometer in FTMS mode (150-1000 amu, 15,000 resolution, MS^2 data dependent). Data analysis performed using Compound Discoverer (Thermo Scientific).

FIGURE 1. SIMPLE WORKFLOWS

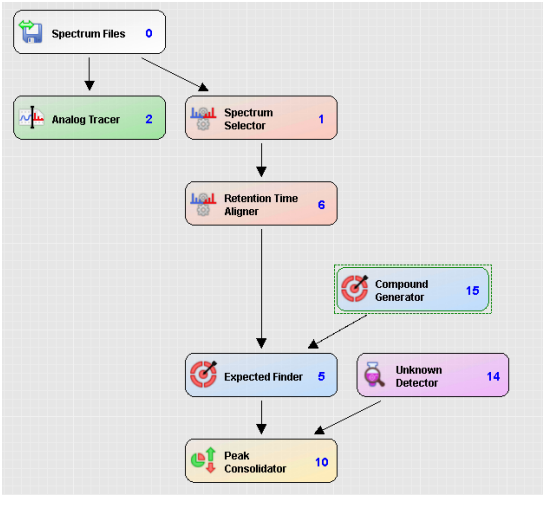


FIGURE 2. SIMPLE ANALYSIS

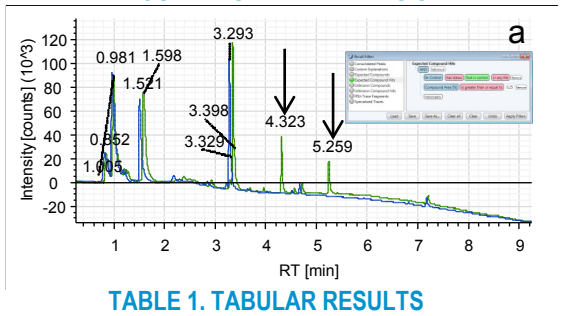


TABLE 1. TABULAR RESULTS

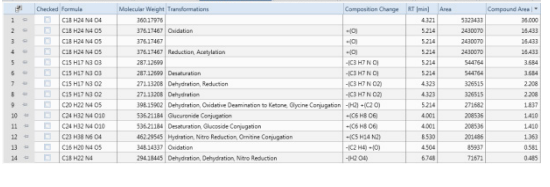


FIGURE 3. ADVANCED WORKFLOW

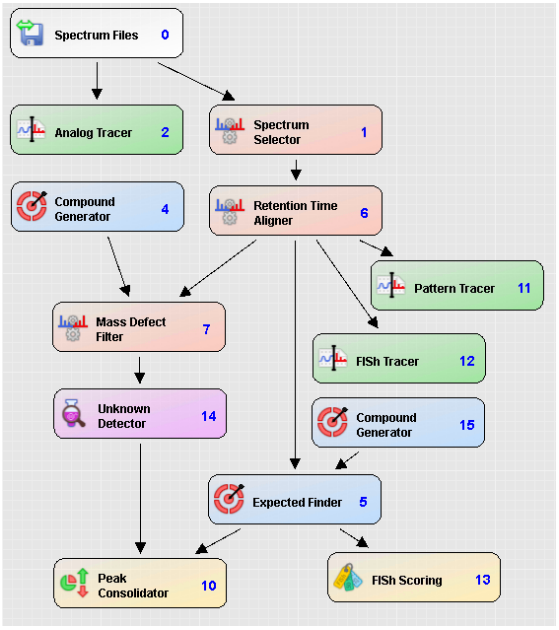
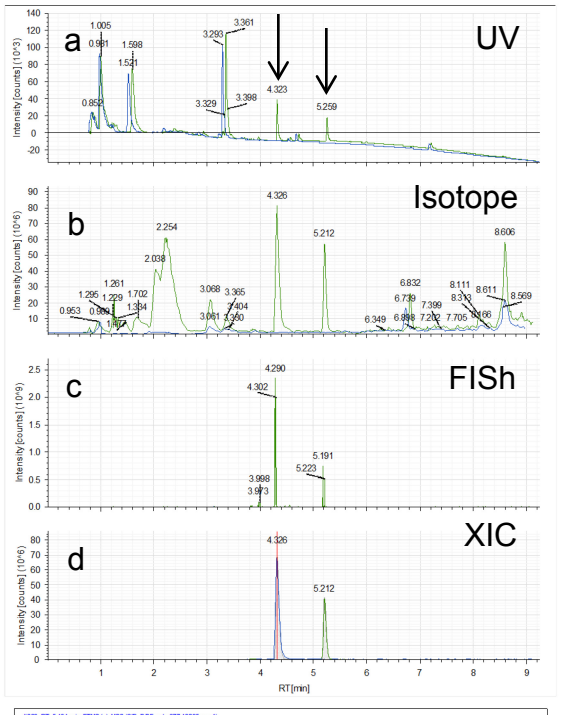


FIGURE 4. ORTHOGONAL ANALYSIS



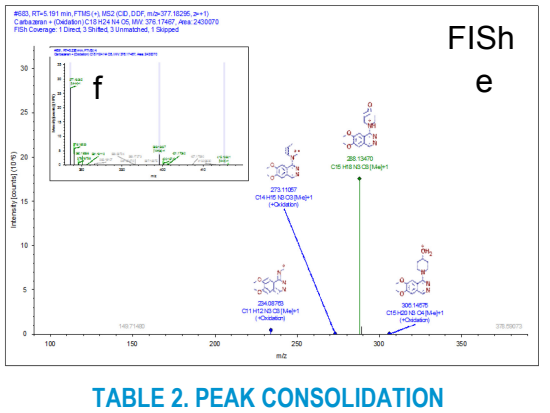
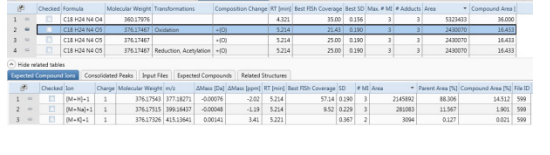


TABLE 2. PEAK CONSOLIDATION



RESULTS

In a simple workflow to identify the major metabolic clearance pathways, dosed and control samples can be processed and visualized together (expected, unexpected peaks all compared with control, Figure 2).

Compound Discoverer provides the ability to customize the views of chromatograms, spectra and analyte tables, allowing a rapid initial semi-quantitative assessment of the abundance (UV) of compound related peaks. Filters can then be used to quickly prioritize tabular data for further analysis (Table 1) in under 30 sec.

In an advanced workflow (Figure 3), additional nodes can be added (Isotope Trace, FISh Tracers, Mass Defect and FISh Scoring) to provide orthogonal detection techniques to build confidence and focus on compound derived products.

Employing the Pattern Tracer node, a trace is extracted based on the parent molecule elemental composition (Figure 4b & f). The FISh Tracer node compares the predicted parent fragments to all MS^2 fragments to create a trace of peaks that share fragments with the parent molecule (Figure 4c).

FISh scoring (Figure 4e) provides fragment assignment based on Mass Frontier literature based in silico fragmentation rules and comparisons to the parent molecule for metabolite assignment.

Peak consolidator (Table 2) combines all of the defined data from the parent nodes consolidates the data for an assigned peak (e.g. $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$).

CONCLUSIONS

Compound Discoverer is a powerful software tool that provides significant speed and organizational benefits to aid the biotransformation scientist interpret metabolic profiling and structure elucidation data when compared to traditional techniques.

Taking full advantage of HRMS data, Compound Discoverer consolidates several complimentary metabolite detection strategies, makes them easy to visualize and places them in one customizable piece of software.

ACKNOWLEDGMENTS

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