Comprehensive relative quantitative of the cytochromes P450 by micro-LC and SWATH®

INTRODUCTION

Metabolites of drugs by the Cytochrome P450 enzyme superfamily is pivotal in determining their disposition, efficacy and safety. Since drugs may induce expression of several isoforms of Cytochrome P450s, they may enhance their own turnover, increasing the risk of toxic metabolite formation or altering interactions with co-administered compounds. P450 profiling is a fundamental aspect of drug safety evaluation. The Cytochrome P450s enzymes exhibit sequence homology, so that antibodies are incapable of discriminating between isoforms, and these values do not always correlate with protein level changes. Thus a method for being able to discriminate among these highly homologous family members is important in understanding drug disposition and metabolism.

SWATH acquisition is a data-independent MS acquisition method for robust quantification which enables discovery-oriented proteomics. The P450s vary among species, and are composed of different specific peptides and their fragment ions, and such, is perfectly suited to P450 profiling. Because SWATH acquisition will facilitate more highly homologous proteins to be discriminated, it may enhance our understanding of enzyme function. Here we report the results from SWATH acquisition with micro-LC MS/MS to increase the speed of analysis and improve the robustness of analysis while maintaining sensitivity.

MATERIALS AND METHODS

Sample Preparation:

Micro-LC-MS/MS Conditions:

Micro-LC-MS/MS analysis was performed on all samples on an Exactive® system using a micro-10 L/min HPLC. For MS-MS analysis, the samples were separated on a YMC-Pack C18 column (2.7 μm, 100 mm × 2.1 mm, 5 μm) at a flow rate of 0.3 μL/min using a linear gradient from 20% to 40% ACN, 0.2% formic acid in H2O for 60 min. On the YMC Thomas-C18 column (150 μm × 0.3 mm, 3 μm) to elute the peptides. The library was created from the samples using an isocratic method (DAI) applying 50% MS/MS per cycle. Subsequently, MS/MS data were then acquired in a variable window SWATH acquisition consisting of 100 variables and accumulation times of 50 ms which were then used as post-acquisition for relative quantification of proteins.

Data Processing:

Identification and quantification (proteins) was performed by processing the MS data with ProteomeFlex™ software. This library aligns with the included SWATH Acquisition database to be uploaded to the Broadleaf database environment using the Broadleaf CloudConnect environment and the data were processed using OneCroma™ software (Figure 1).

The ProteomeFlex™ library and SWATH are extracted using the Protein Extractor prior to processing using the Experiment Manager where settings can be uploaded for the sample type, number of biological replicates and number of technical replicates. Data can then be pushed through Analytica to determine statistical differences. FTMS metrics, Retention time metrics, ATA Cytosol Metrics, and Delta Cytosol Metrics can then be created to find and regulate the proteins to be viewed in the Broadleaf or through the Pathway guide to review metabolic pathways that are affected by the change in protein expression levels.

RESULTS

Figure 1. The OneCroma™ Project in Exploratory Cloud Environment. After SWATH data is acquired, it can be uploaded into the cloud to create annotated and peak group finding files that can be used for downstream analysis. To evaluate the MS/MS data, analysts should choose the best possible protein identification results, for deeper validation can be achieved by searching the experiment database and the parameters have been set.

Pathway analysis can be used to evaluate the results from a biological perspective. Finally, for deeper analysis, users can either perform directed analysis, an MS-MS method, or a single level analysis.

Figure 2. Determining Indication of Cytochrome P450’s by IOM and PB. Using the Enzyme Function database tools, the data can be analyzed to determine if a particular P450 is involved in a given metabolic pathway. This can help to discriminate the enzyme expression profiles of Cyp2c11 (Cyp102), Cyp2d6 (Cyp113), Cyp2f2 (Cyp114) and Cyp2j2 (Cyp123), even though the high levels of sequence similarity between these isoforms.

As expected, there was a dramatic induction of Cyp2c11 (CYP3A) expression in mouse liver exposed to PB (Figure 3). The induction of Cyp2c11 was marked in the mouse liver and brain but changing using SWATH acquisition was able to determine the differential expression profiles of Cyp2c11 (Cyp102), Cyp2d6 (Cyp113), Cyp2f2 (Cyp114), and Cyp2j2 (Cyp123), even though the high levels of sequence similarity between these isoforms.

REFERENCES


2. TRADMARKS/LICENSING

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