Metabolic Stability Assay Using Human Hepatocyte Co-cultures and Integrated Qualitative/Quantitative High Resolution Mass Spectrometry

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Introduction

Traditional metabolic stability methodology using suspended hepatocyte for drug candidates screening are limiting in their ability to accurately predict clinical outcomes. Hepatocyte co-culture platform is a bioengineered, *in vitro* system with a defined cyto-architecture that provides sustained hepatic functions for at least four weeks. In this presentation, we investigated human hepatocyte co-cultures model using an integrated qualitative/quantitative high resolution mass spectrometry approach to assess metabolic stability using SWATH (Sequential Window acquisition of All Theoretical fragment ion spectra) for non-targeted MS/MS metabolite analysis. The assay was evaluated using propranolol, lorazepam, zoniporide, and ranitidine at clinically relevant concentration (1 µM) in 96-well format.

Methods

Propranolol, lorazepam, zoniporide, ranitidine, buspirone as IS, and 96-well human hepatocyte co-culture plates were purchased commercially. 60-µL aliquots of samples were collected serially at 0, 1, 4, 24, 48, 72, 120, and 168 hours. Once collected, each aliquot was added to 60-µL of acetonitrile containing IS. A generic 3.5 min LC-MS/MS qual/quan method was developed on a TripleTOF® 5600 system coupled with a Shimadzu Nexera UHPLC. Metabolic stability data were generated using peak-area-ratios of the analyte-to-IS using MultiQuant software. The same raw data files were also mined for preliminary metabolite information using MetabolitePilot software which utilizes post-acquisition data mining tools such as mass defect filter, isotope pattern filter, and background subtraction.

Preliminary Data

In the hepatocyte co-cultures, propranolol and zoniporide were close to completely turned over by 72 h, while lorazepam showed continuous turnover through 168 h, and ranitidine was slowly turned over, with a large percentage remaining even at 168 h. The estimated hepatic clearance values were 16.8, 5.5, 12.4, and 2.1 mL/min*kg for propranolol, lorazepam, zoniporide and ranitidine, respectively using the parallel tube model. Propranolol showed glucuronide metabolite, oxidation/sulfation metabolite, and parent as a major peak in the 24 h sample, but by 120 h the glucuronide metabolite became the major peak. Zoniporide showed mainly the parent, and a minor oxidation metabolite at 4 h, which progressed to two oxidation metabolites plus a minor parent peak at 24 h, at 120 h only two oxidation metabolites were left, and based on the SWATH data, the oxidation sites were narrowed to the pyridine ring. This initial evaluation using propranolol, lorazepam, zoniporide, and ranitidine as model compounds to acquire simultaneous metabolic stability and metabolite profile information at clinically relevant concentration shows that this approach is feasible when using high resolution TOF-MS in combination with data independent MS/MS scan.

Novel Aspect

Simultaneous Metabolic Stability of Pharmaceuticals and Metabolite Profiling Using Hepatocyte Co-culture Plate and UHPLC-HRMS Based Data Independent MS/MS.

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