

Evaluation of a New Prototype Accurate Mass System for Simultaneous Quantitative and Qualitative Bioanalysis and Metabolite Profiling

Henrianna Pang¹; Hesham Ghobarah²; Tanya Gamble²; Yingbo Yang¹; Sophie Pan¹; Brad Gien¹; Douglas J. Turk¹
¹NoAb BioDiscoveries, Inc., Mississauga, Canada; ²AB SCIEX, Concord, Canada

Novel Aspect

Use of new prototype accurate mass analyzer with enhanced sensitivity, speed and quantitative linearity for PK studies under UHPLC conditions

Introduction

Combining quantitative analysis on the parent drug and qualitative analysis on the metabolites profile for early ADME studies has shown significant promise for improving the efficiency of drug discovery. Additionally, earlier information on the metabolite profile of lead compounds has gained increasing importance due to recent MIST guidelines. A number of different types of mass analyzers have been evaluated for these studies. Most of these studies have been focused on in vitro samples where the analytical requirements are generally less demanding than in vivo PK studies. In this study, we evaluate the sensitivity, linearity and speed of a prototype accurate mass system for Quant / Qual analysis of in vivo samples derived from rat blood/plasma under UHPLC conditions.

Methods

Methoxyfenoterol was administered to SD rats (IV 5 mg/Kg, PO 25 mg/Kg). Blood samples were serially collected pre-dose and at 0.05, 0.25, 0.5, 1, 2, 4, 6 and 8 hours post dose. Samples were protein precipitated and injected without pre-concentration. Samples were analyzed under UHPLC conditions on a Shimadzu Prominence XR system coupled to a prototype accurate mass system operated in ESI mode. Data acquisition was performed in the dedicated TOF MS mode and TOF MS with information dependant acquisition (IDA) and real time multiple mass defect triggering of product ion scans. Dedicated looped TOF MS and product ion scans were also performed to compare quantitative performance of the two scan modes.

Preliminary Data

Linearity, sensitivity, and scanning speed performance were evaluated for the dedicated TOF MS mode as well as TOF MS with IDA. System was operated at a resolution of 30,000. Cycle times comparable to those used during traditional QqQ analysis were achieved, allowing for acquisition of 5-10 spectra per second for quantitation under UHPLC conditions. System performance demonstrated quantitative correlation in TOF MS mode of 3 orders of magnitude or more for methoxyfenoterol and fenoterol. The lower limits of quantitation in extracted whole blood were 0.5 and 2.5 ng/mL for methoxyfenoterol and fenoterol, respectively. The width of the extracted ion chromatogram (XIC) window was evaluated for optimal selectivity and sensitivity for parent compound in this in vivo matrix. In addition to quantitation of the parent, the in vivo data was interrogated for metabolites using mass defect filtering and sample / control comparisons. The major metabolites were the glucuronidation and demethylation products. The novel approach of applying multiple mass defect filters in real time to select parent ions for MS/MS during IDA significantly increased the efficiency of MS/MS data acquisition on relevant metabolites. Finally, estimation of metabolite quantity was performed by comparing the relative area counts in the accurate mass extracted ion chromatograms from the TOF MS data to that of the parent compound.