Use of Different High-Resolution Mass Spectrometry Platforms for Drug Metabolite Characterisation and Quantitation: A Case Study

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Introduction
Over the last decade, high-resolution mass spectrometry (HRMS) has evolved to be the standard platform for metabolite profiling and identification. More recently, modern combined qualitative and quantitative workflows have added another dimension to the performance characteristics required from instruments and associated software. Two technologies routinely employed on these studies are Orbitrap and Time of Flight (ToF) based mass spectrometers. While many studies are performed on a single platform, each has unique strengths that can complement one another. A series of experiments using model compounds were performed in order to demonstrate and evaluate these two platforms.

Sample Preparation
Nefazodone and glibenclamide (1 and 10µM) were incubated with cryopreserved human hepatocytes for 0, 5, 10, 15, 30, 60, 120 and 240 minutes. The incubations were terminated by the addition of an equal volume of ice cold acetone, centrifuged and the supernatant submitted for analysis.

Sample Analysis
Analysis was performed using Waters Xevo G2-S Q-ToF and Thermo Q-Exactive mass spectrometers equipped with suitable UPLC systems.

UPLC-MS Method
Mobile phase A: 0.1% formic acid in water
Mobile phase B: 0.1% formic acid in acetonitrile
Gradient
Time (min) %A %B
0 98 2
5.0 35 65
5.5 0 100
6.4 0 100
6.5 98 2
8.0 98 2
Flow rate: 650 µL min
Column: Waters HSS T3 C18, 2.1 x 100 mm 1.7µm

Data Acquisition
Samples were analysed using both positive and negative electrospray. Initial experiments were conducted using simple polarity methods and data-independent MS/MS acquisition. The Xevo Q-ToF was run in sensitivity mode to maximise the detection of metabolites. The Q-Exactive was run at 17,500 resolution to give maximum scan speed. Glibenclamide samples were also analysed using combined positive/negative ion switching on the Q-Exactive and at extra-high resolution to investigate the effects of experimental design on duty cycle and the ability to interrogate isotope ion switching on the Q-Exactive and at extra-high resolution to investigate the Glibenclamide samples were also analysed using combined positive/negative ion electrospray. Initial experiments were conducted using simple polarity methods and data-independent MS/MS acquisition. The Xevo Q-ToF was run in sensitivity mode to maximise the detection of metabolites. The Q-Exactive was run at 17,500 resolution to give maximum scan speed. Glibenclamide samples were also analysed using combined positive/negative ion switching on the Q-Exactive and at extra-high resolution to investigate the effects of experimental design on duty cycle and the ability to interrogate isotope ion switching on the Q-Exactive and at extra-high resolution to investigate the Glibenclamide samples were also analysed using combined positive/negative ion switching on the Q-Exactive and at extra-high resolution to investigate the effects of experimental design on duty cycle and the ability to interrogate isotope ion switching on the Q-Exactive and at extra-high resolution to investigate the Glibenclamide samples were also analysed using combined positive/negative ion switching on the Q-Exactive and at extra-high resolution to investigate the effects of experimental design on duty cycle and the ability to interrogate isotope ion switching on the Q-Exactive and at extra-high resolution to investigate the Glibenclamide samples were also analysed using combined positive/negative ion switching on the Q-Exactive and at extra-high resolution to investigate the effects of experimental design on duty cycle and the ability to interrogate isotope ion switching on the Q-Exactive and at extra-high resolution to investigate the Glibenclamide samples were also analysed using combined positive/negative ion switching on the Q-Exactive and at extra-high resolution to investigate the effects of experimental design on duty cycle and the ability to interrogate isotope ion switching on the Q-Exactive and at extra-high resolution to investigate the