INTRODUCTION

Common metabolic pathways often give rise to multiple instances of isobaric metabolites such as multiple mono-hydroxylations and di-hydroxylations and common phase II conjugations, such as glucuronidation. LC–MS analysis of metabolic pathways for drugs requires accurate detection and identification of species with similar RTs and spectral profiles. Modern chromatography (and mass spectrometry) is sufficient in many cases to track and resolve these peaks, however tracking and identifying isobaric species remain problematic. Ensuring closely eluting species have not chromatographically shifted requires standard addition or co-mixing of matrix and true isobaric coelutions may go undetected with conventional LC–MS approaches as they rely solely on discrimination using precursor mass. Furthermore, the MS/MS patterns from related compounds are often extremely similar.

Ion mobility can...

1) Provide a secondary identifier, CCS (Collision Cross Section) for multiple precursors with the same mass. Robust CCS measurement is key.
2) Improve DIA (Data Independent Acquisition) quality of fragmentation data (remove more matrix interference) to provide cleaner spectra.
3) Aid structural elucidation. Change in shape is an indication of where the metabolite is transformed, which can provide additional structural clues to the metabolism chemist.

Herein we describe use cases for ion mobility using the metabolism of nefazodone and buspirone spiked in urine as classic examples.

RESULTS

Common nefazodone metabolites increase in physical size from 209 Å (Parent compound) up to 259 Å (glucuronidative species). Figure 2 shows the CCS/drift time spread of commonly detected isobaric metabolites (+O, +2O, and +O +gluc, +2O+gluc). The spread in size per isobaric metabolite varies from 1 Å for +O about 16 Å for +O glucuronides (or 0.5% to 6.6%).

Figure 2. Nefazodone commonly observed metabolites m/z (x-axis) vs ion mobility (y-axis). Vertical direction shows the measured spread of size for each isobaric metabolite.

An important characteristic of CCS is that it needs to be reproducible across repeated measurements and also at varying concentrations. Across this time course and three different injection volumes, the concentration varies over 3 orders while producing robust measurement over 250 hours (%RSD = 0.2%).

Figure 3. Nefazodone CCS measurement at many concentrations and injection levels spiked 1:10 into urine matrix. %RSD was measured at 0.2%.

The structure of a key buspirone metabolite reported by BMS is shown in figure 4. Buspirone incubation (80 or 120 min) spiked in urine is shown in figures 5 (+O), figure 6 (+2O) and figure 7 (+O +glucuronidation). Figure 5 shows that the largest +O metabolite detected corresponds to the reported 5-OH-Bu and is confirmed by the (HDMS)5 fragmentation pattern. Similarly, the 2 largest (CCS) metabolites for the +O2 series also contain the 5-OH-Bu diagnostic ions. Finally, the largest (CCS) glucuronide also corresponds to the 5-OH-Bu pathway. In this example, the 4-para pyrimidinyl hydroxylation positively correlates to the largest CCS shift in all metabolites that proceed via this pathway that were detected.

Figure 4. Major hydroxyl metabolite, para substituted on the pyrimidinyl ring.

Figure 5. 5 major +O metabolites for buspirone and their corresponding CCS resolved XICs (extracted ion chromatograms) and CCS filtered product ion spectra.

Figures 5 through 7 also show the absolute spectra cleanliness obtained through use of HDMS5. This is enables high quality DIA spectra without prespecification of defined acquisition mass ranges. This holds true even for the spectral quality of all fragment ion spectra even in trace metabolites, as exemplified in figure 8.

Figure 8. HDMS5 spectra for a trace (10th least abundant +2O) metabolite with CCS enabled (top) spectrum and CCS unfiltered (bottom). Usable peaks are readily observed.

CONCLUSIONS

- Accurate and robust measurement of CCS (0.2%) enables confident detection of molecules in matrix down to low levels
- The HDMS5 DIA approach enables a broad coverage of high quality spectra and the ability to generate CCS resolved extracted ion chromatograms which improves analytical detection and elucidation of complex metabolism
- Isobaric metabolites were observed to have a CCS range of between 0.5% and 6.5% better enabling differentiation and tracking of similar metabolites across samples
- CCS provides a measurement of molecular size that is useful in inferring structural changes and tracking the presence of certain modifications across metabolic pathways.

References
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2. Zhu et al. Drug Metabolism and Disposition April 2005, 33 (4) 500-507