Addressing the challenge of rapid drug metabolite identification using Cyclic Ion Mobility Mass Spectrometry

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Introduction

Rapid, accurate characterization of drug metabolism is critical in drug discovery. Early ADME studies use liver microsomes or hepatocytes with LC–MS but must balance throughput and resolution. Faster LC reduces separation, causing coelution of metabolites, especially isomeric glucuronides. Here, we combine rapid UHPLC with cyclic IMS-QToF to improve resolution and enable better identification and quantification of parent compounds and isomeric glucuronides in human hepatocyte incubations. We demonstrate sensitivity gains using Wideband Enhancement (WBE) and show the utility of multi-pass IMS experiments to resolve isomers by extending the ion mobility path length.

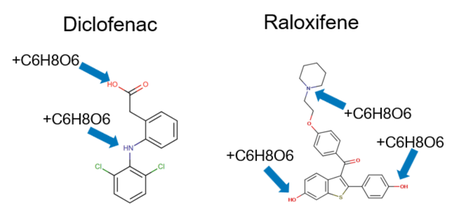

Figure 1 :Drugs can contain multiple potential conjugation sites e.g. Glucuronidation (C6H8O6)

Experimental

Diclofenac and raloxifene (1 µM) were incubated separately in human hepatocytes (1 × 10^6 cells/mL). Aliquots were collected at 0–60 min, quenched with methanol, vortexed, centrifuged, and analyzed using a 10 min reversed-phase UHPLC–Cyclic IMS-MS (HDMSE, +ESI) acquisition. Ion mobility path length (1–5 m) was varied to assess isomer separation. Data acquired in MassLynx™ software were processed in waters_connect™ software and MassMetaSite. Predicted CCS values (waters_connect CCS on Demand) were used as an orthogonal ID metric with ≤5% deviation acceptance. Standards were used to demonstrate WBE for improved sensitivity and multi-pass ion mobility for enhanced separation of isomeric metabolites.

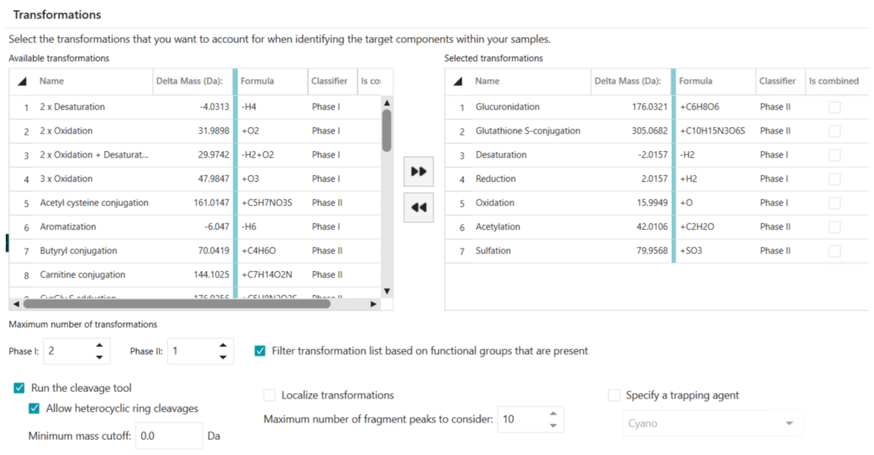

Figure 2. waters_connect software enables searches for numerous potential transformation of the drugs as they metabolized.

Results

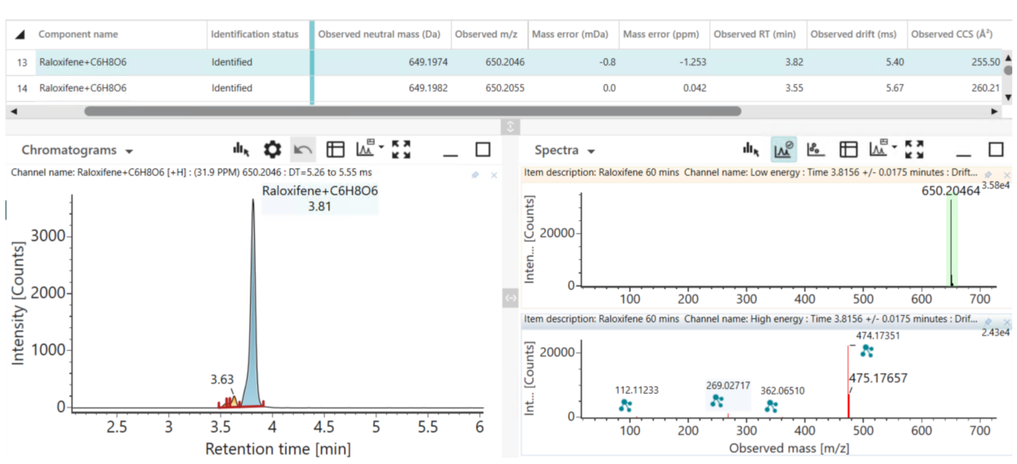

Figure 3. Rapid UHPLC–cyclic IMS–MS (HDMSE) identified potential glucuronide metabolites of diclofenac and raloxifene. Data processed in waters_connect showed <2 ppm mass error, with supporting fragment and CCS values. Two putative raloxifene glucuronides were observed at 3.55 and 3.82 min (60 min timepoint).

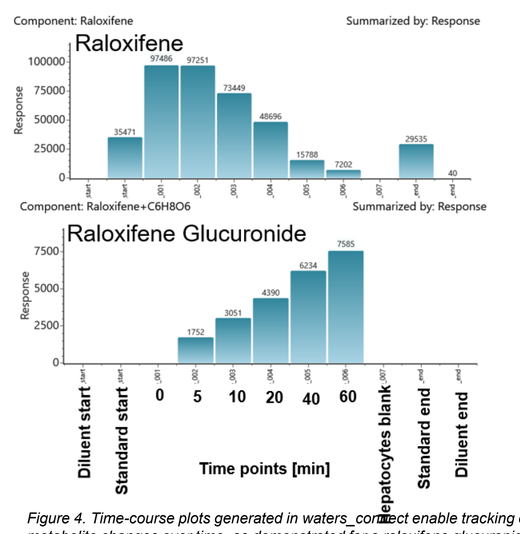

Figure 4. Time-course plots generated in waters_connect enable tracking of metabolite changes over time, as demonstrated for a raloxifene glucuronide in hepatocyte incubations

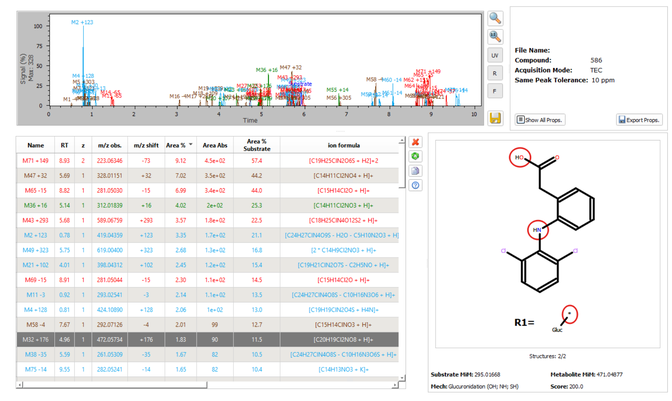

Figure 5. An example of data processing using MassMetaSite. The Diclofenac glucuronide reported at 4.96 mins is highlighted.

Technology

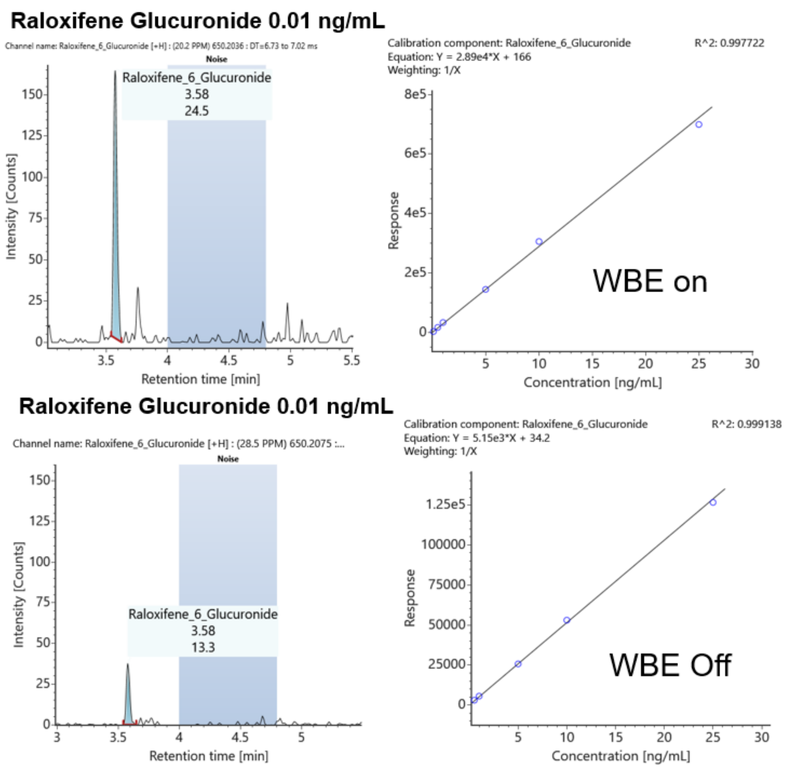

Figure 6. Raloxifene Glucuronide standards were used to assess linear response for quantification. Over a calibration range of 0.01–25 ng/mL, the peak-to-peak signal to noise at lowest concentrations, 0.01 ng/mL was 24.5 with WBE, compared to 13.3 without. The R^2 values were <0.99.

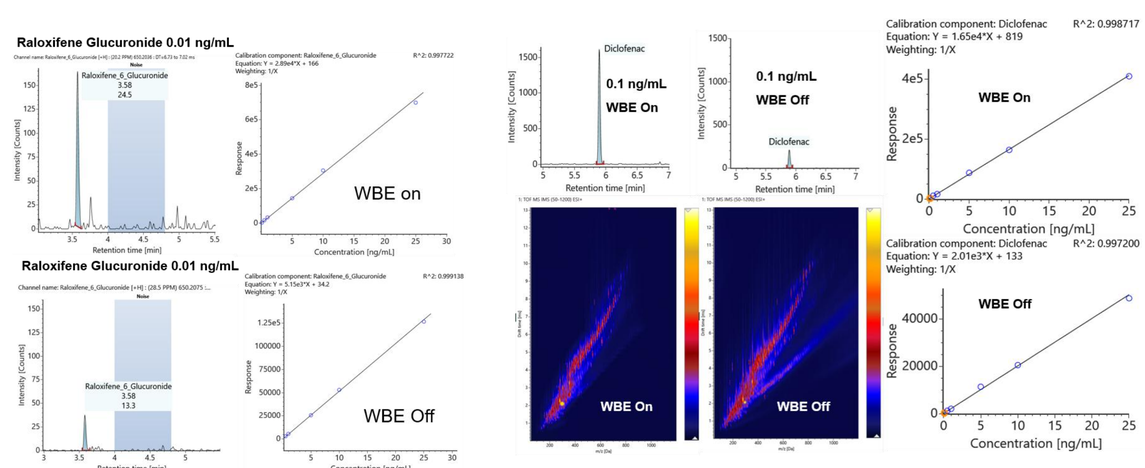

Figure 7. Extracted chromatograms of diclofenac at 0.1 ng/mL with m/z vs. drift time plots, showing ~10 × higher response with WBE enabled compared to disabled.

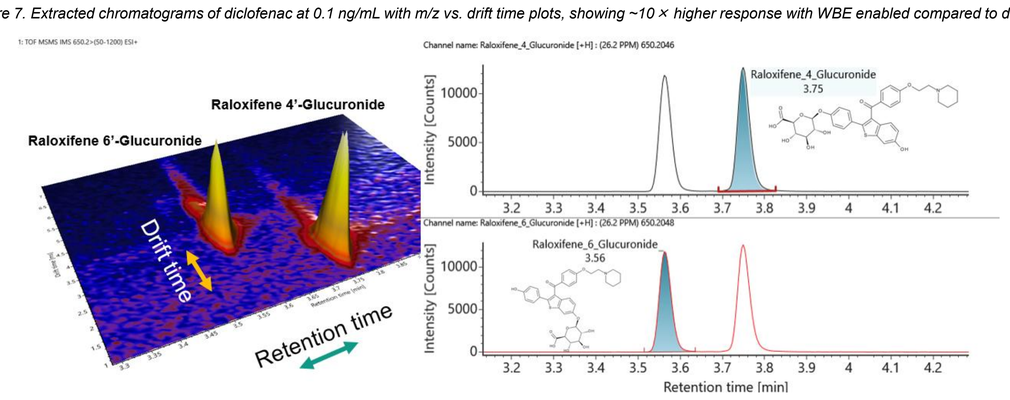

Figure 8. Raloxifene glucuronide species with transformations at the 4' and 6' positions are separated by LC retention time and drift time when IMS path length is extended via multipass experiments, enabling shorter LC gradients.

Conclusions

- **Enhanced confidence in metabolite ID**: Predicted CCS values with Cyclic IMS improve structural elucidation of complex metabolites.
- **Up to 10-time sensitivity gain when WBE is enabled**- Improved LOD and LOQ
- **Faster analysis without compromise**: Multipass IMS separates isomeric species in drift time, enabling shorter LC methods and rapid data turnaround.
- **Flexible, workflow-ready data**: High-quality outputs are fully compatible with 3rd party tools e.g. MassMetaSite

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