Structural elucidation of conjugation drug metabolites by utilizing novel electron-activated dissociation (EAD)

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Introduction

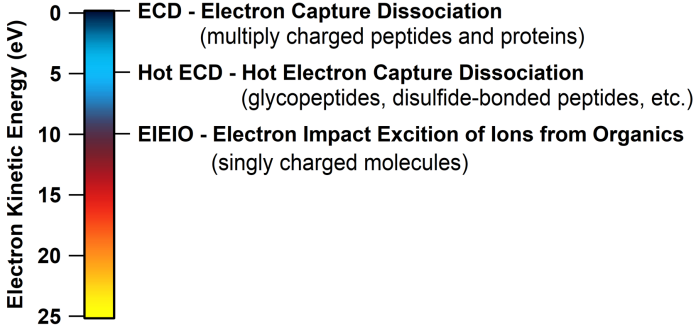
- Collision-induced dissociation (CID) is the fragmentation approach widely used in LC/MS in drug metabolite identification (MetID) studies. Many conjugation metabolites are found to have multiple bonding potentials to parent drugs. It is a great challenge to locate the bonding sites by CID due to the information lost by highly selective cleavage on these bonds.

- The electron-activated dissociation (EAD) technology on QTOF produces varied fragmentation patterns and helps to generate additional or different fragments to CID. These fragments can be crucial to locate the metabolic modification sites, especially for conjugations.

- In this study, the application of EAD in drug metabolite profiling is explored. The efficiency in the structure elucidation of conjugation metabolites is compared with that of CID.

Electron Activated Dissociation (EAD)

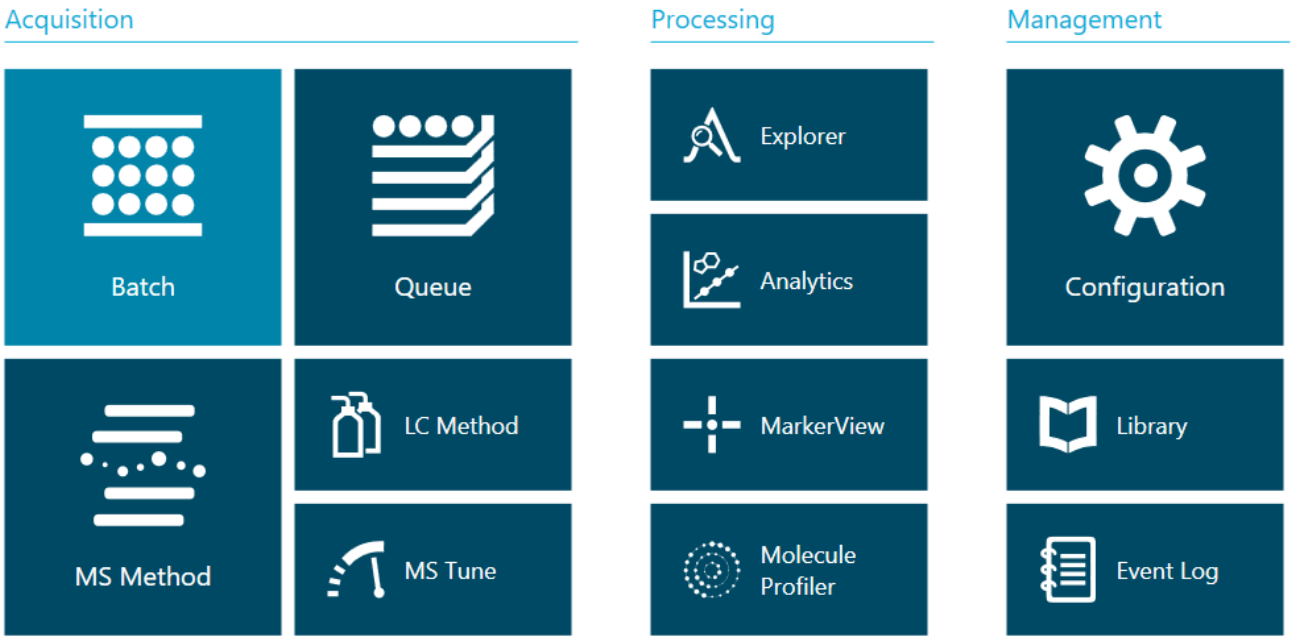
- **Free electrons captured by ions and form a radical state which then fragments**
  - Electrons introduced with different energies will induce fragmentation in different molecule types



- **EAD involves one-electron transfer to the analyte molecule causing multiple bond cleavages and rearrangements.**
- **EAD has the capability to provide more detailed structural information than CID.**

T. Baba *et al. Anal. Chem*. 2014

SCIEX OS Software



Experimental and Analytical Methods

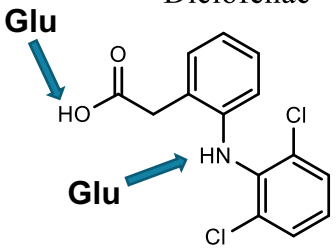
- ***Sample preparation:*** 10 µM 60-min incubation with rat liver microsomes in the presence of UDPGA, Glutathione (GSH) and NADPH at 37° C.
- ***Chromatography:*** Column: Phenomenex Kinetex Polar C18 (2.1 x 50 mm, 1.7 µm, 100 Å). Mobile phase A: 0.1% (v/v) formic acid in water; mobile phase B: 0.1% (v/v) formic acid in acetonitrile; Total run time: 12 min.
- ***Mass Spectrometry*:** Zeno 7600 TOF

CID	Setting	EAD	Setting
CE	40	CE	12
CES	15	CES	0
		Electron Beam Current(nA):	5000, 6000
		Electron KE (eV):	10, 12 and 14

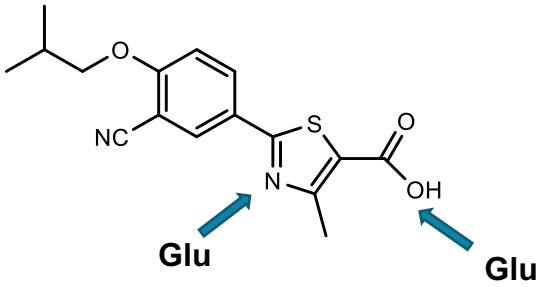
- ***Data Analysis:*** Molecule profiler

Selected Drugs and Potential Conjugation Sites

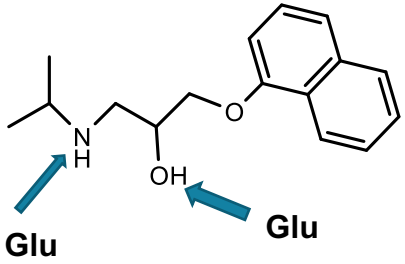
Diclofenac



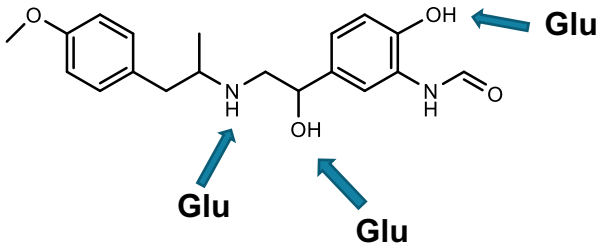
Febuxostat



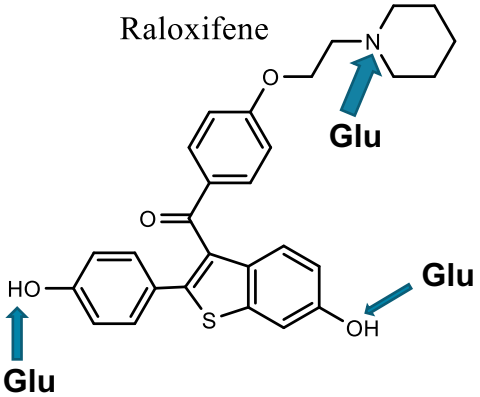
Propranolol



Formoterol

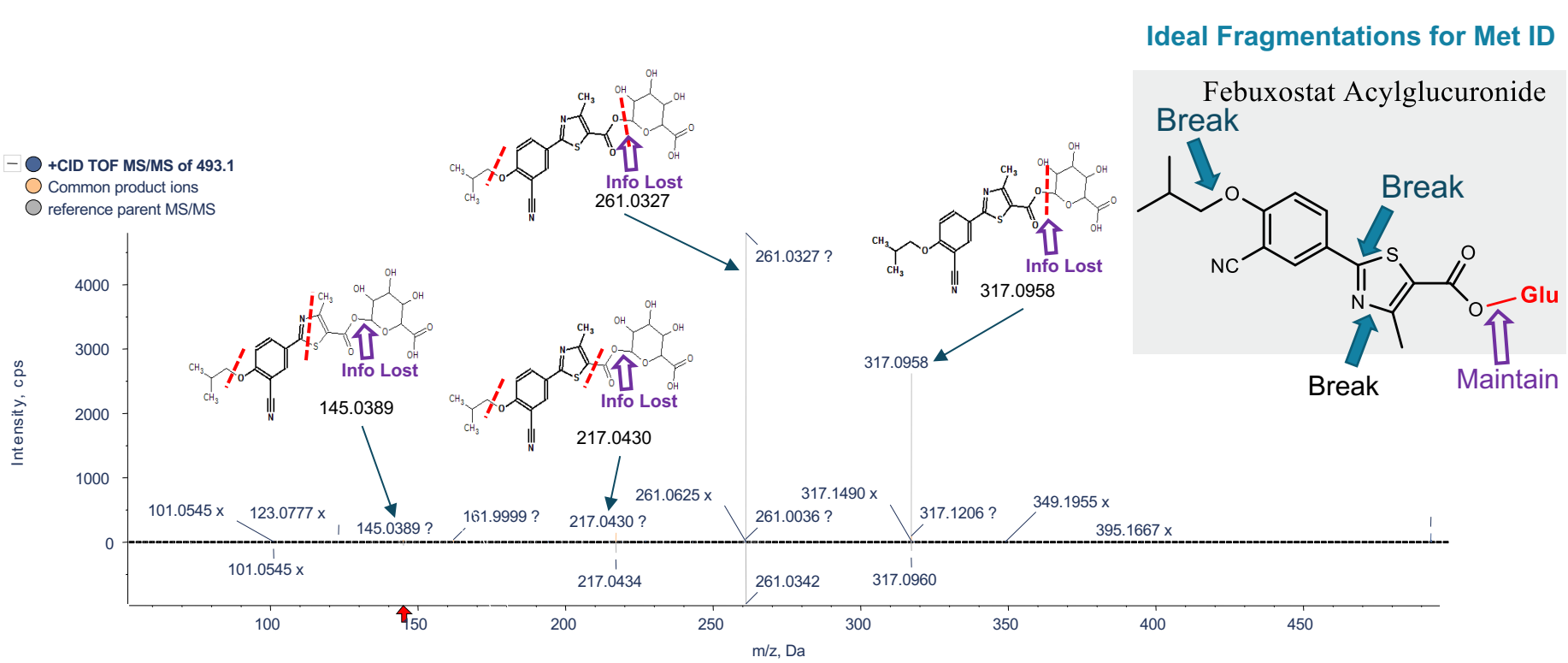


Raloxifene



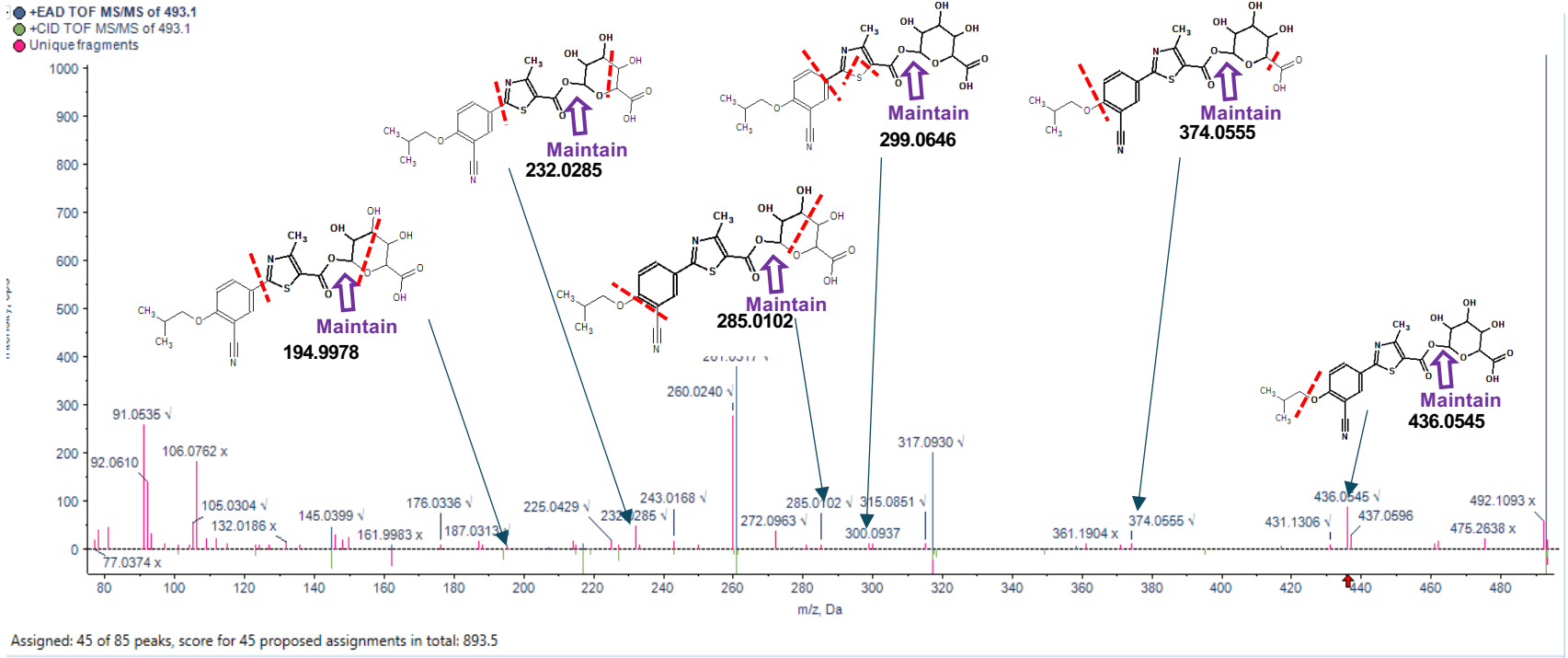
- Each drug has multiple potential conjugation sites.
- One main objective of MetID is to locate the bonding sites of conjugation metabolites by analyzing MS/MS spectra.

CID: Febuxostat Glucuronide



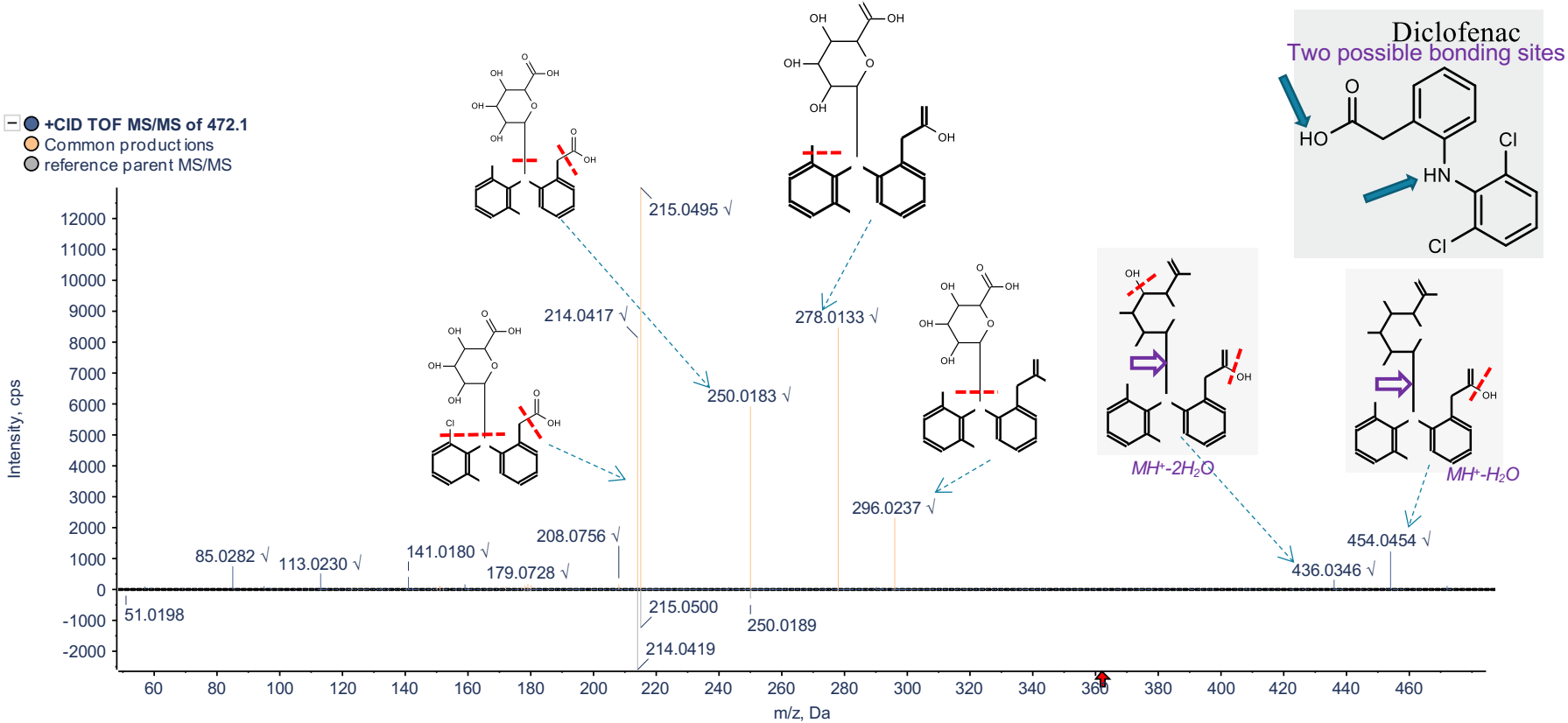
CID broke the weak glucuronidation bond before fragmenting the other stronger bonds of the parent drug, posing challenges in narrowing down the glucuronidation site.

EAD: Febuxostat Glucuronide



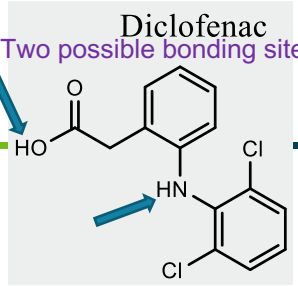
EAD can fragment the parent drug but keep the glucuronidation bond intact, providing valuable structural information to identify the glucuronidation site.

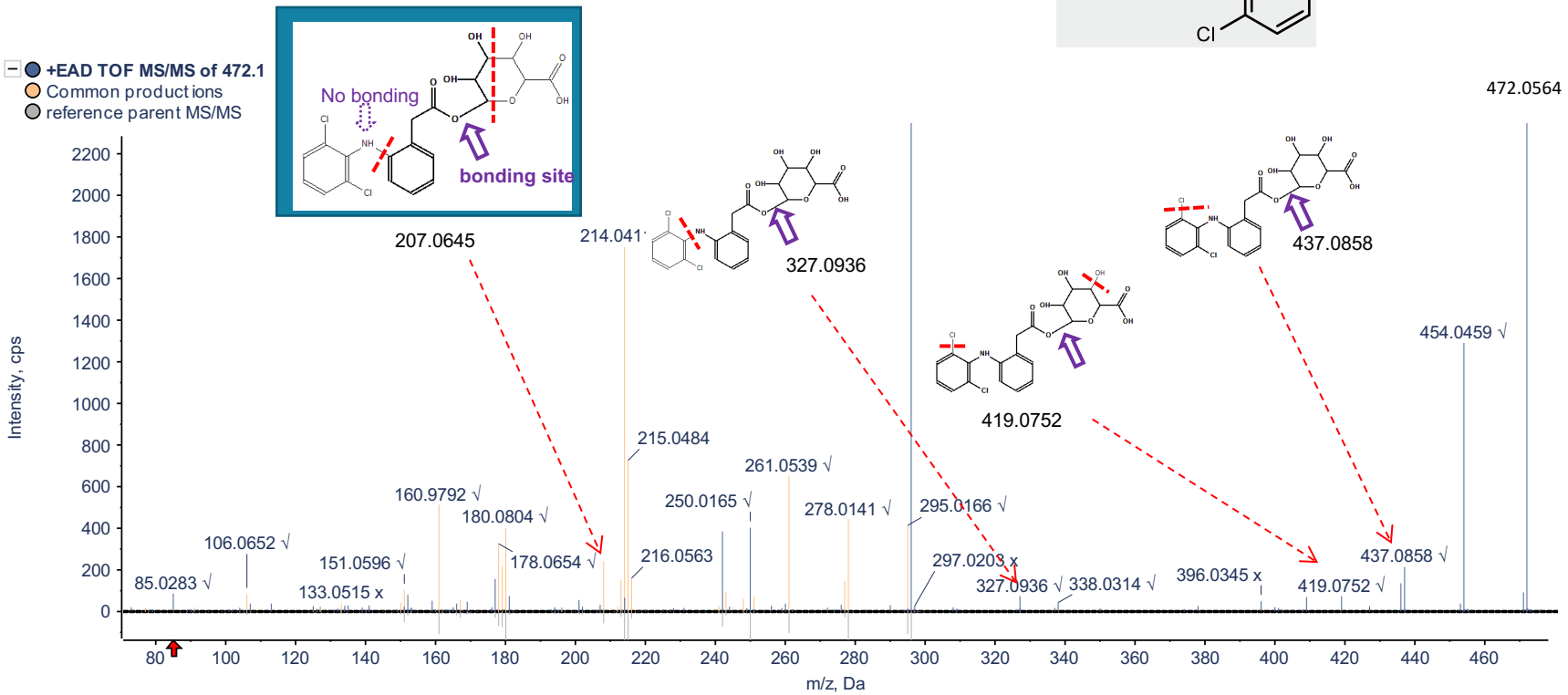
CID : Diclofenac Glucuronide



- **CID could not provide definitive information on the glucuronide bonding site.**
- **The software predicted the wrong bonding site (N-glucuronidation).**

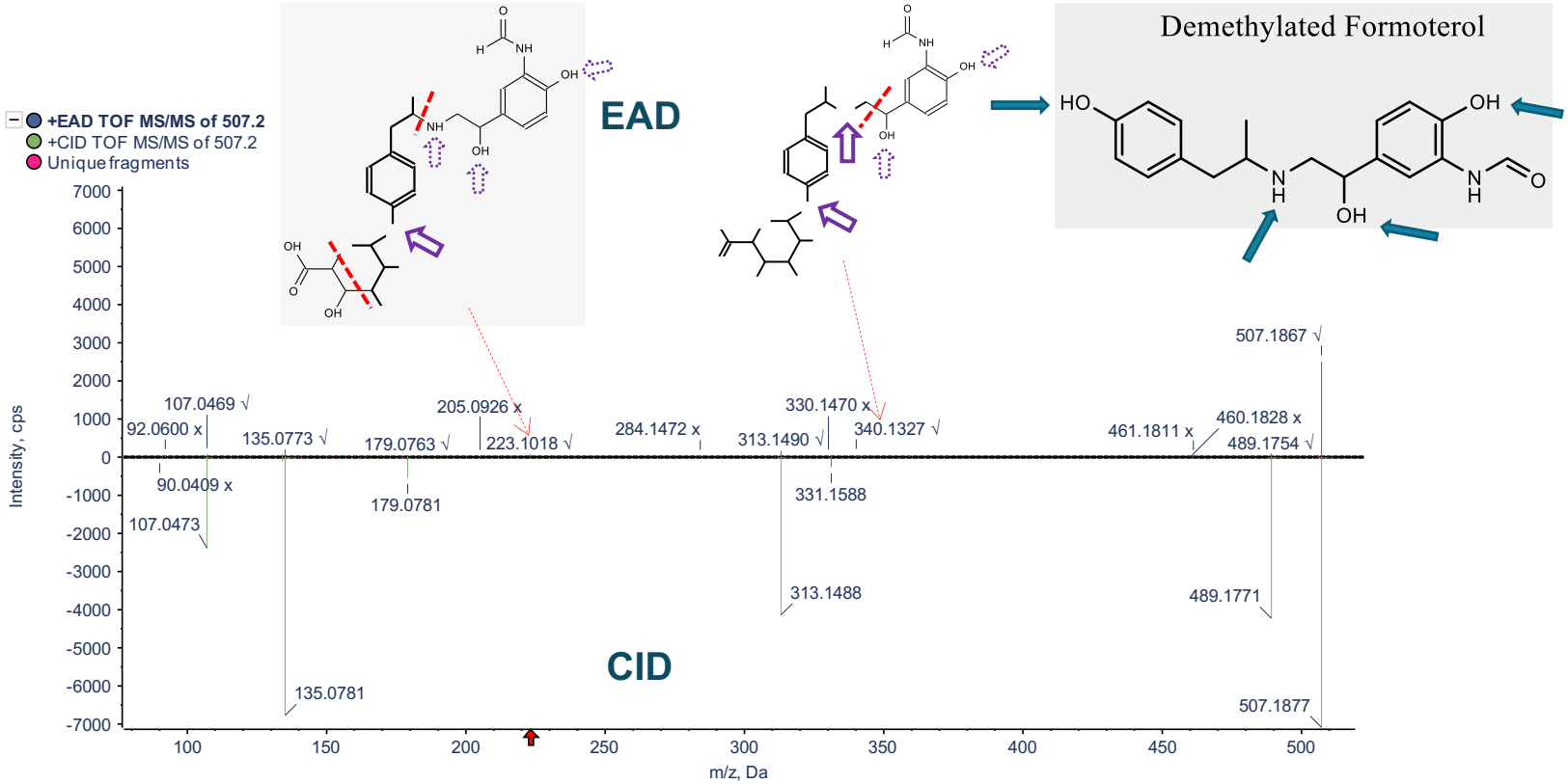
EAD: Diclofenac Glucuronide





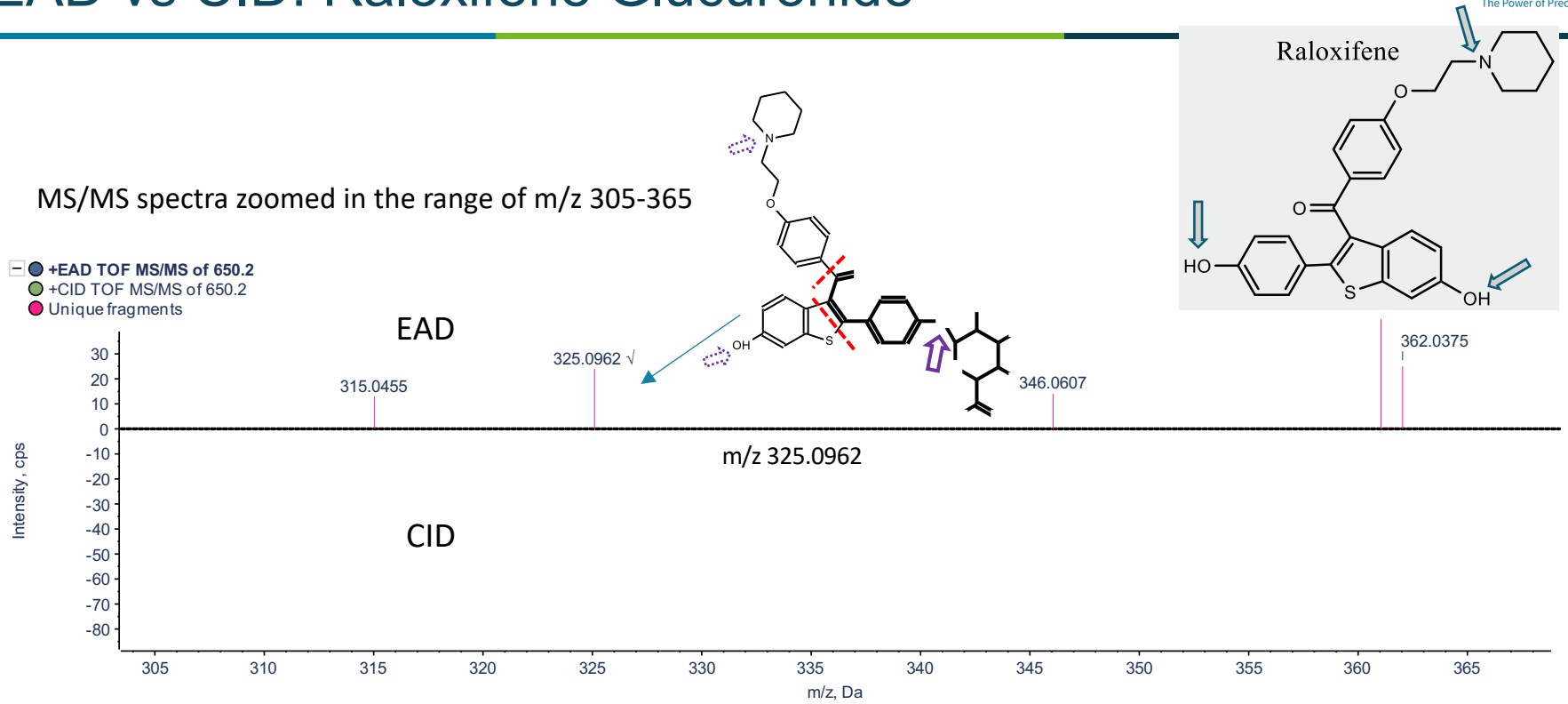
EAD fragmentation indicated the right glucuronidation bonding site (O-glucuronidation).

EAD vs CID: Formoterol Demethylated Glucuronide



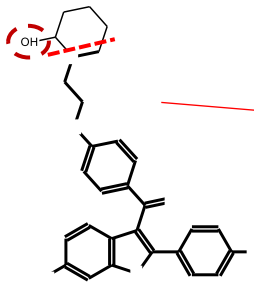
EAD generated more informative fragmentations and assisted in identification of the glucuronidation site.

EAD vs CID: Raloxifene Glucuronide

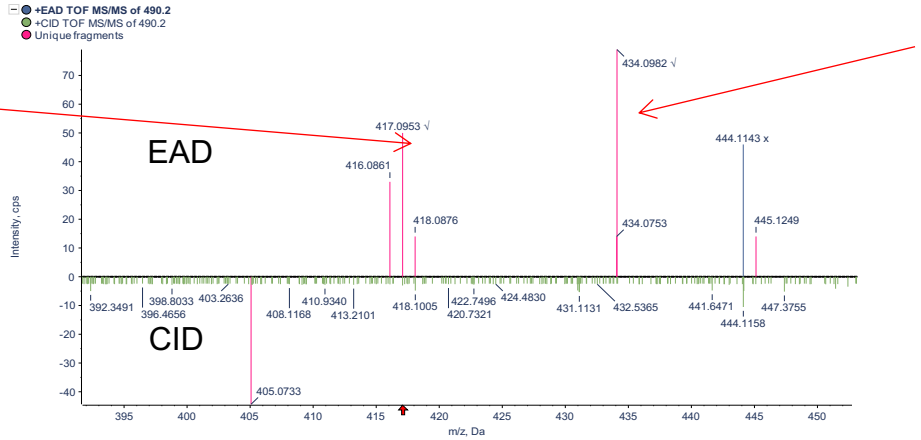


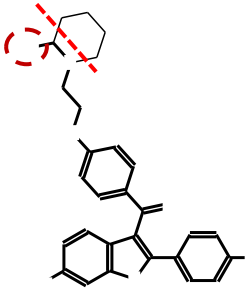
EAD generated more informative fragmentations and assisted in identification of the glucuronidation site.

EAD vs CID: Raloxifene Oxidation Metabolite

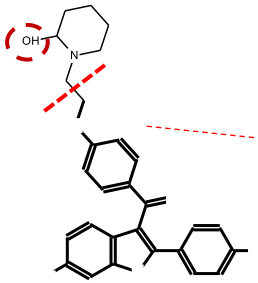


m/z 417.0953

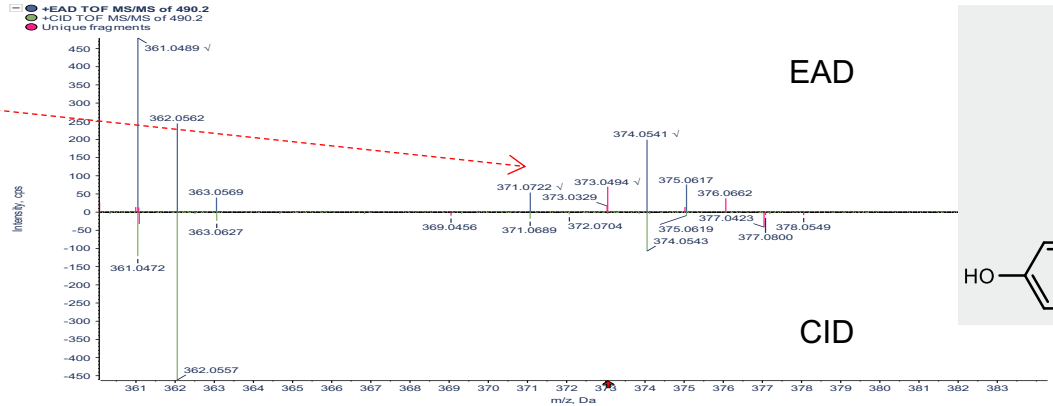


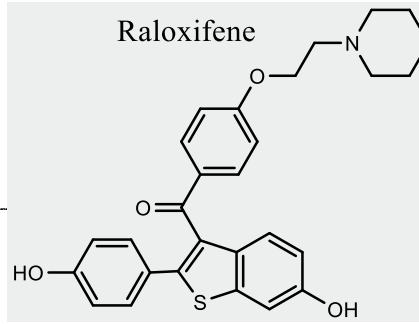


m/z 434.0982



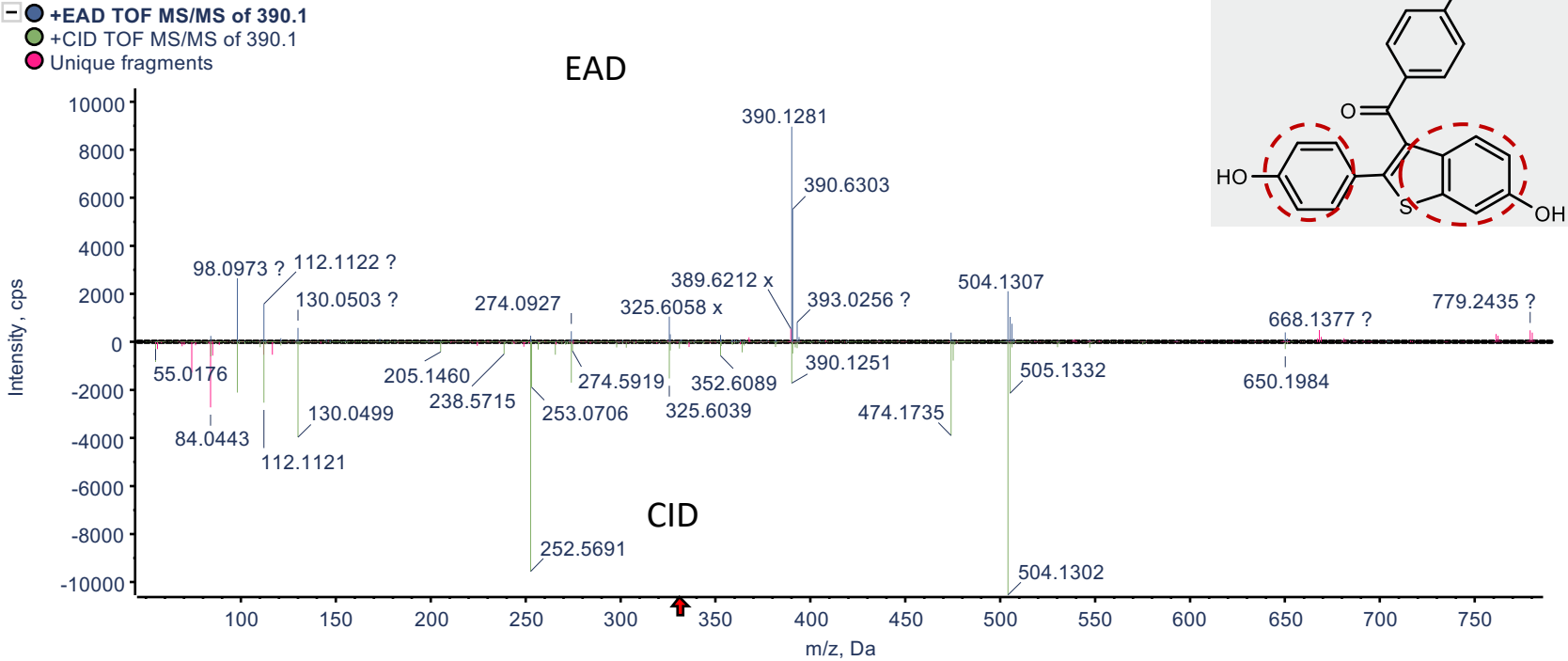
m/z 373.0494





EAD generated more signature fragments for identification of the oxidation site.

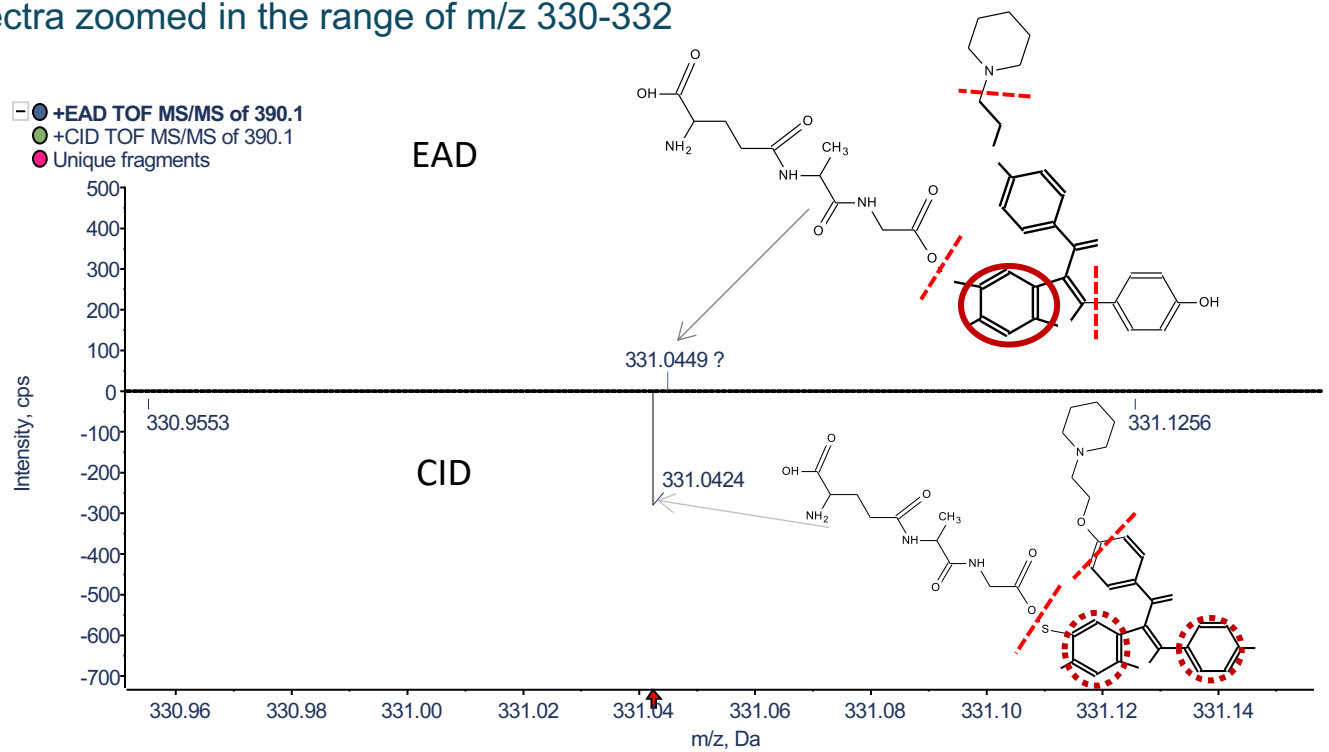
EAD vs CID: Raloxifene GSH Conjugation Metabolite



EAD and CID showed different fragmentation patterns of the GSH conjugation metabolite.

EAD vs CID: Raloxifene GSH Conjugation Metabolite

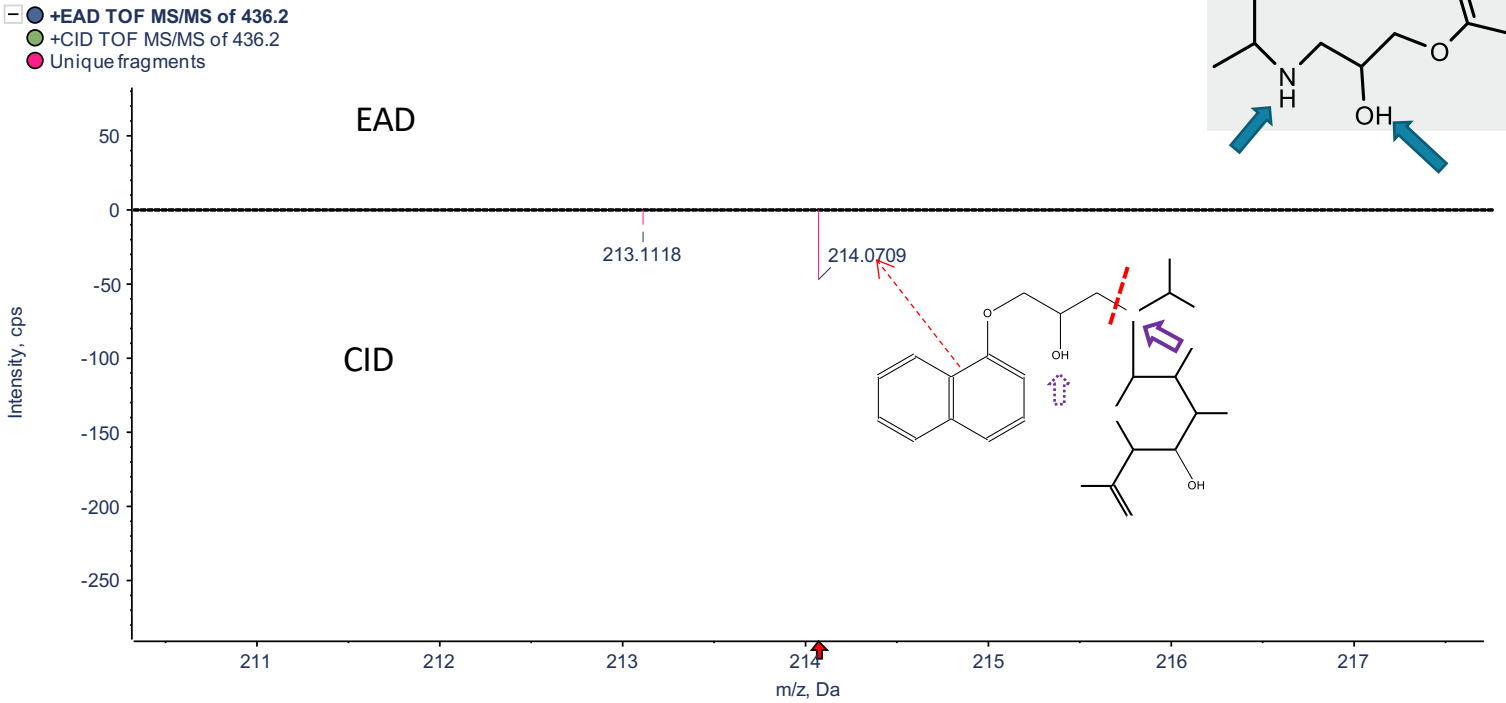
MS/MS spectra zoomed in the range of m/z 330-332

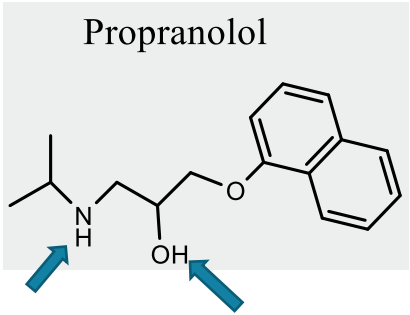


EAD provided more confirmative information on the GSH bonding motif

EAD vs CID: Propranolol Glucuronide

MS/MS spectra zoomed in the range of m/z 210-217





One unique CID fragment indicated that Glu-1 is an N-glucuronide.

Conclusion

- EAD-enabled MS/MS spectra offer a greater variety of fragments compared to CID, resulting in more comprehensive structural information for metabolic modification analysis
- The implementation of EAD in metabolite profiling studies enables the generation of unique fragments not produced by CID. This breakthrough technology significantly improves the elucidation of conjugation drug metabolites, such as glucuronide and GSH conjugates.
- EAD and CID are complementary techniques for fragmentation, making them valuable tools for metabolite identification. By combining both technologies, more accurate and comprehensive results can be achieved.

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