IDENTIFYING ACCURATE METABOLISM SITES OF VEPDEGESTRANT （ARV-471） USING NOVEL ELECTRON-ACTIVATED DISSOCIATION HIGH-RESOLUTION MASS SPECTROMETRY (EAD-HRMS)

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Introduction

Drug metabolite identification (MetID) is an integrated part of DMPK studies of lead compounds and drug candidate in drug discovery and development. Accurate characterization of metabolite structures is needed in some cases, such as metabolic soft-spot determination, reactive metabolite screening, and supporting metabolite synthesis. However, product ion spectra generated by commonly used collision-induced disassociation (CID) often fail to provide useful fragment ions for the task, which is indeed a bottleneck of MetID by LC-HRMS. Recently, a ZenoTOF 7600 mass spectrometer equipped with electron-activated dissociation (EAD-HRMS) was introduced.

Objectives of the study

- Applied EAD-HRMS to identify metabolic modification sites of vepdegestrant (ARV-471) in dog liver microsomes incubation
- Compared effectiveness of EAD and CID spectra in metabolite structural elucidation

Experimental

- ARV-471 (10 μM) was incubated in dog liver microsomes in the presence of NADPH and UDPGA for 60 min.
- EAD and CID spectra of ARV-471 metabolites were acquired by ZenoTOF 7600 mass spectrometer.
- Detection of metabolites was carried out via processing LC-HRMS dataset using SCIEX OS software and SCIEX Molecule Profiler software.
- Structural elucidation of ARV-471 metabolites was accomplished based on EAD spectral interpretation.
- For comparison, CID spectra were utilized for structural characterization of ARV-471 metabolites.

Conclusions

- Metabolic modification sites of all 12 ARV-471 metabolites were fully identified based on EAD spectral interpretation, including phenyl glucuronidation, pieridine mono-oxidation and dehydrogenation, glutarimide hydrolysis, piperazine *N*-dealkylation, and piperidinemethanol oxidation.
- CID spectral interpretation was not able to the determine metabolic modification sites of seven metabolites generated from phenyl glucuronidation, glutarimide hydrolysis, and/or piperidinemethanol oxidation.
- The study demonstrates that the orthogonal fragmentations via EAD can reveal labile fragments enabling more definitive identification of metabolite structures or metabolic modification sites.

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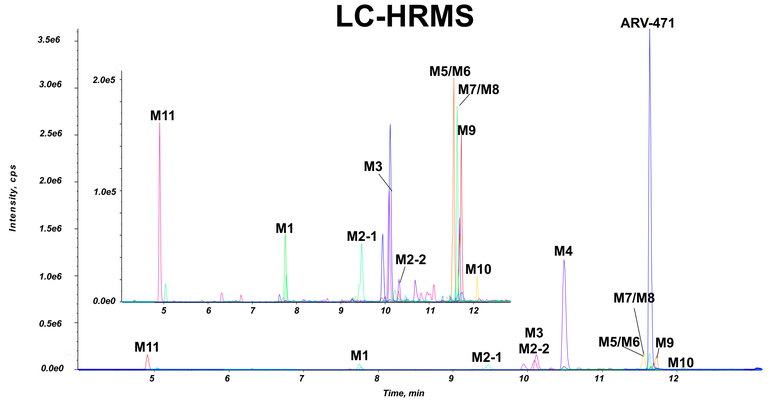
Results

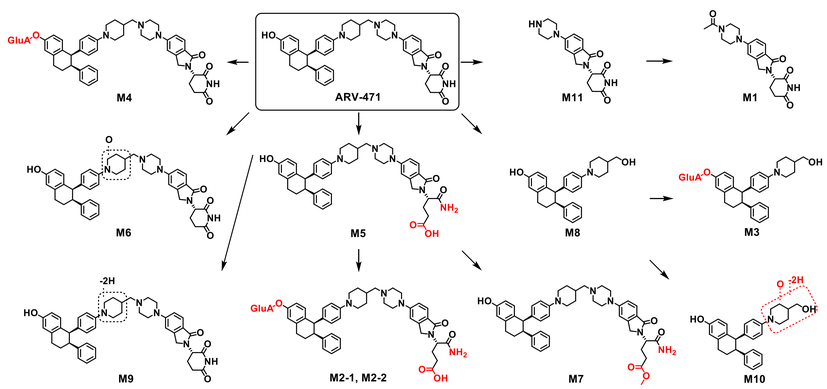
Detection of ARV-471 metabolites in dog liver microsomes by LC-HRMS



(A combined extracted ion chromatogram of ARV-471 metabolites in dog liver microsomes incubation sample. The insert is a combined extract chromatogram of all minor metabolites)

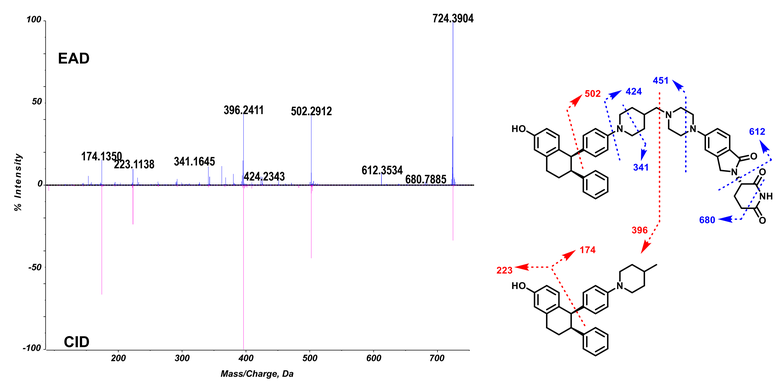
Proposed ARV-471 metabolite structures based on EAD spectral interpretation

(Metabolic modification sites of the metabolites in red were not determined by CID)

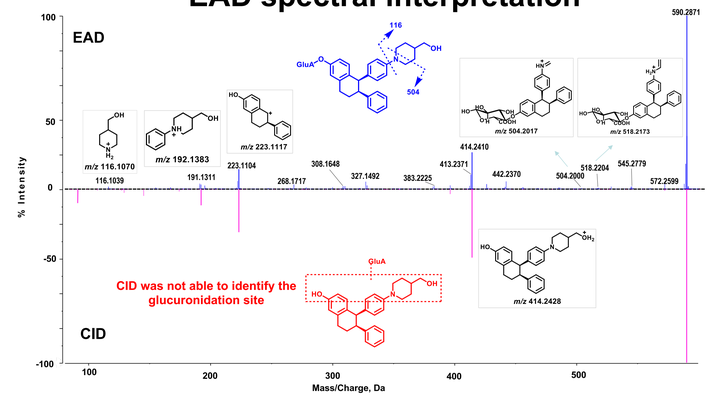


Comparison of EAD and CID spectra of ARV-471

(Product ions only produced via EAD are presented in blue).

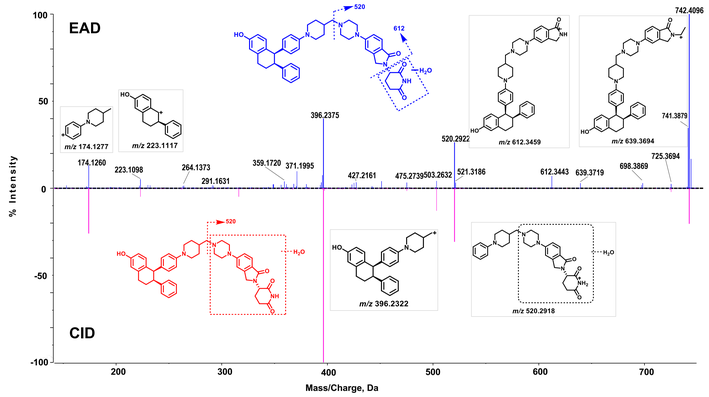


Elucidating glucuronidation site of M3 via EAD spectral interpretation



Elucidating the hydrolysis site of M5 via EAD spectral interpretation

(CID was not able to determine the metabolism site)



Elucidating metabolic modification sites of M2 via EAD spectral interpretation

(CID was not able to determine the metabolism sites)