Supplemental Data 1

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In Vitro Metabolism of Irosustat, a Novel Steroid Sulphatase Inhibitor: Inter-Species Comparison, Metabolite Identification and Metabolic Enzyme Identification.

Drug Metabolism and Disposition (DMD #38315)

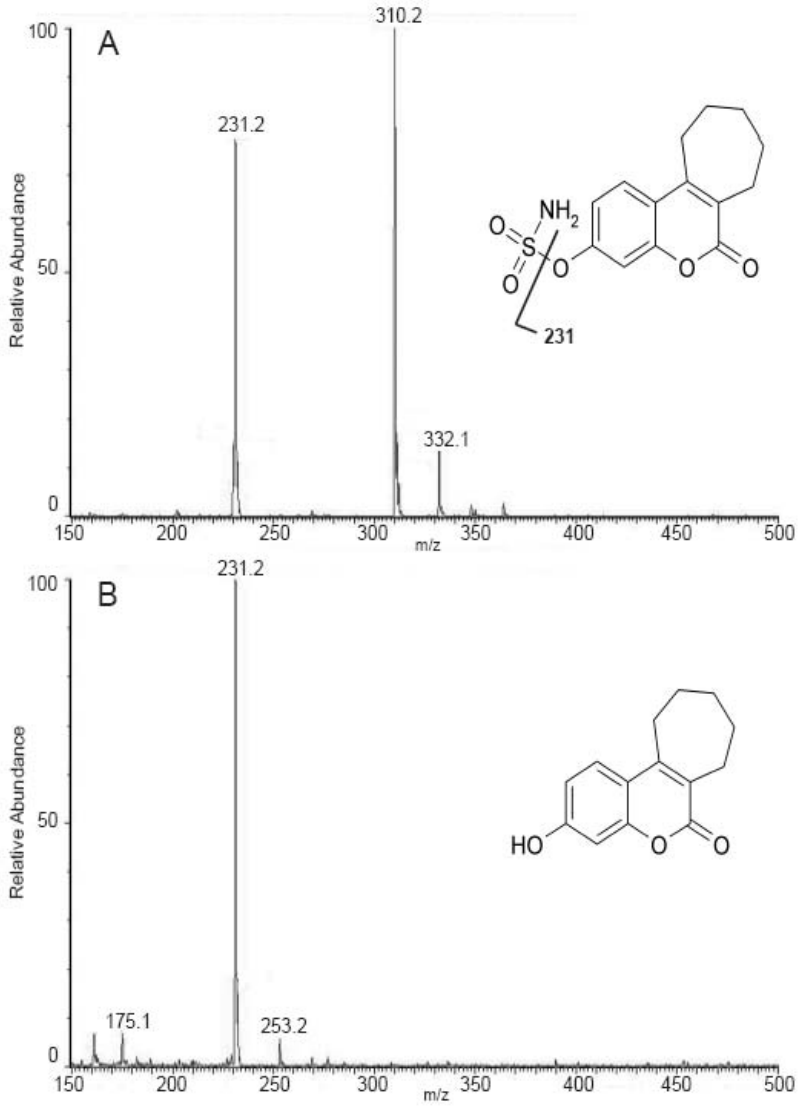

Fig. 1. MS Spectra (ESI positive ion mode) of Irosustat (A) and 667-Coumarin (B) at 60 V cone voltage.

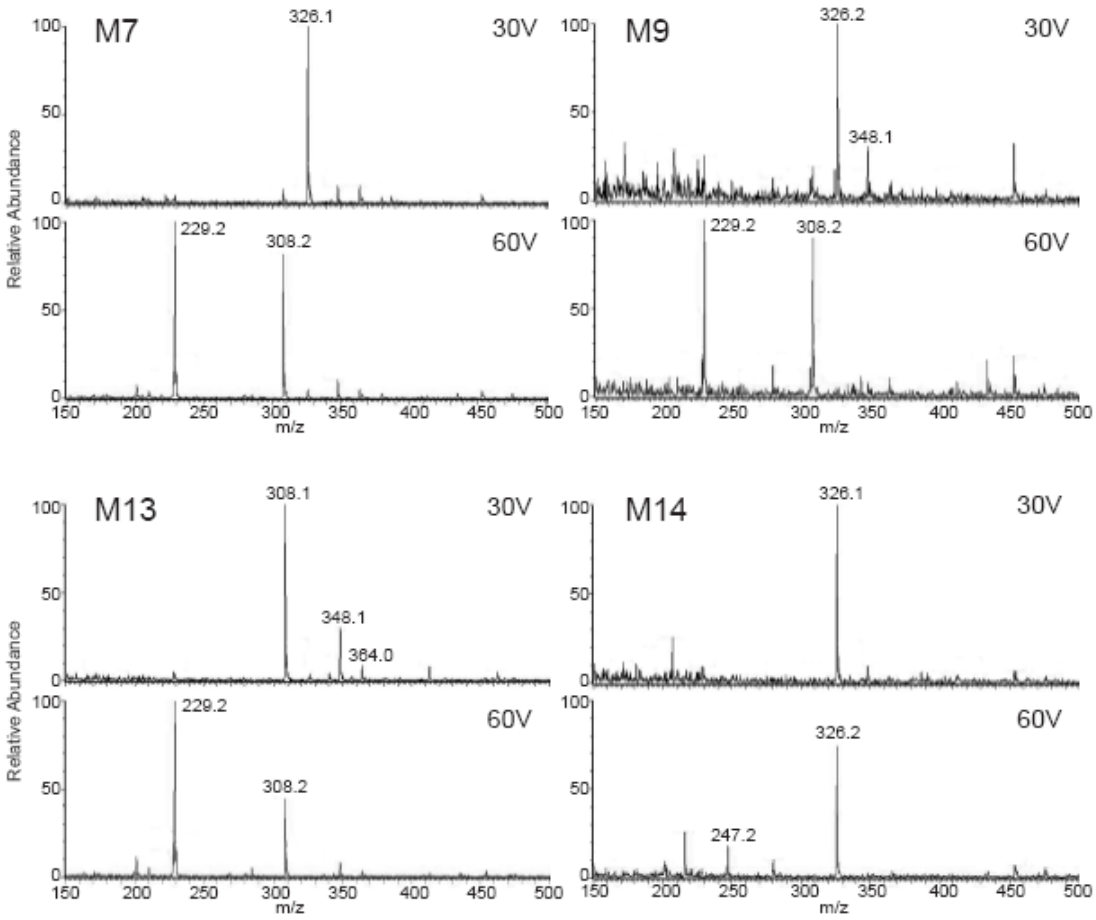

Fig. 2. MS Spectra (ESI positive ion mode) of the mono-oxidized metabolites of Irosustat: M7, M9, M13 and M14. Upper panel: MS spectra obtained at 30 V cone voltage; Lower panel: MS spectra obtained at 60 V cone voltage. M7, M9 and M14 showed a protonated molecular ion of *m/z* 326, representing an increase of 16 mass units in its parent compound Irosustat. Although M13 showed a protonated molecular ion of *m/z* 308, the formation of ions showing *m/z* consistent with the Na^+ and K^+ adducts of *m/z* 326, suggested that M13 was also a mono-oxidized metabolite of Irosustat, and that probably was dehydrated in the MS source (consequently losing 18 mass units).

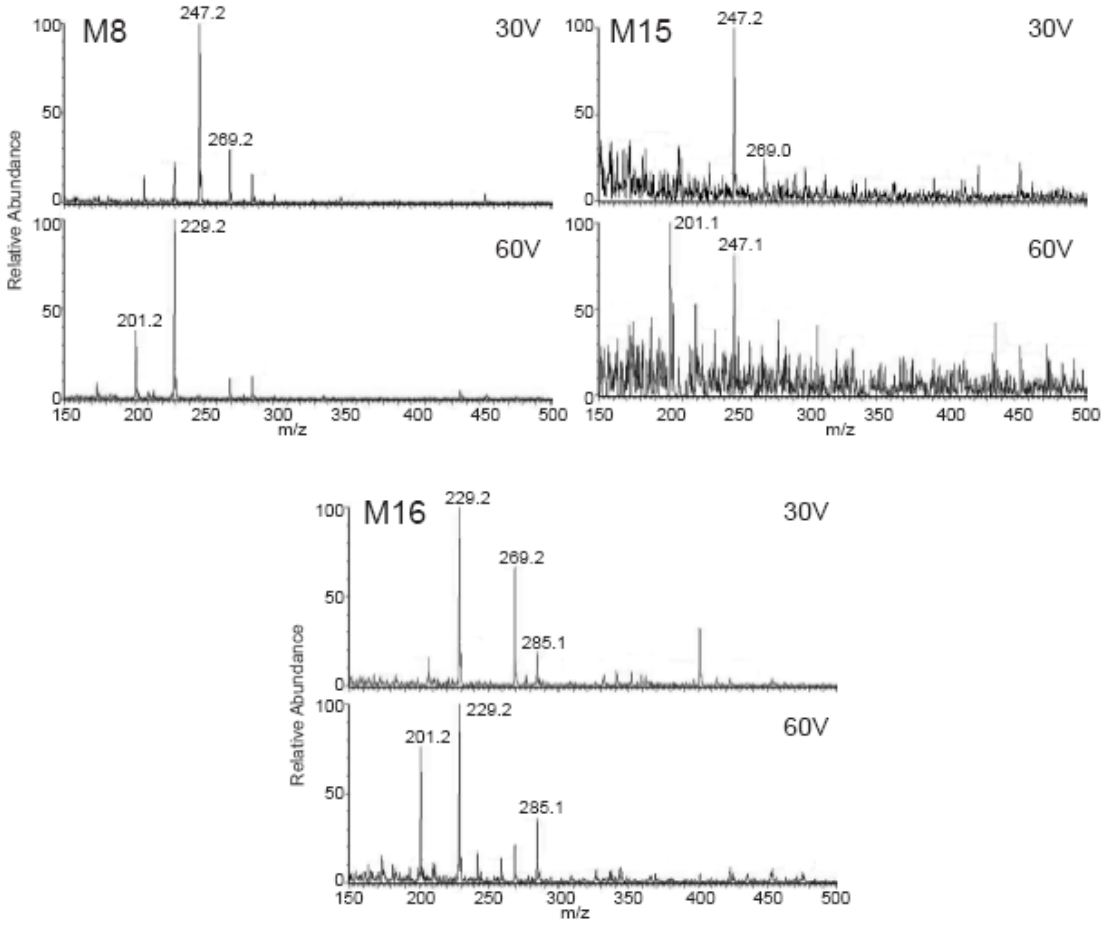

Fig. 3. MS Spectra (ESI positive ion mode) of the mono-oxidized metabolites of 667-Coumarin: M8, M15 and M16. Upper panel: MS spectra obtained at 30 V cone voltage; Lower panel: MS spectra obtained at 60 V cone voltage. M8 and M15 showed a protonated molecular ion of *m/z* 247, representing an increase of 16 mass units in its parent compound 667-Coumarin. Although M16 showed a protonated molecular ion of *m/z* 229, the formation of ions showing *m/z* consistent with the Na^+ and K^+ adducts of *m/z* 247, suggested that M16 was also a mono-oxidized metabolite of 667-Coumarin, and that probably was dehydrated in the MS source (consequently losing 18 mass units).

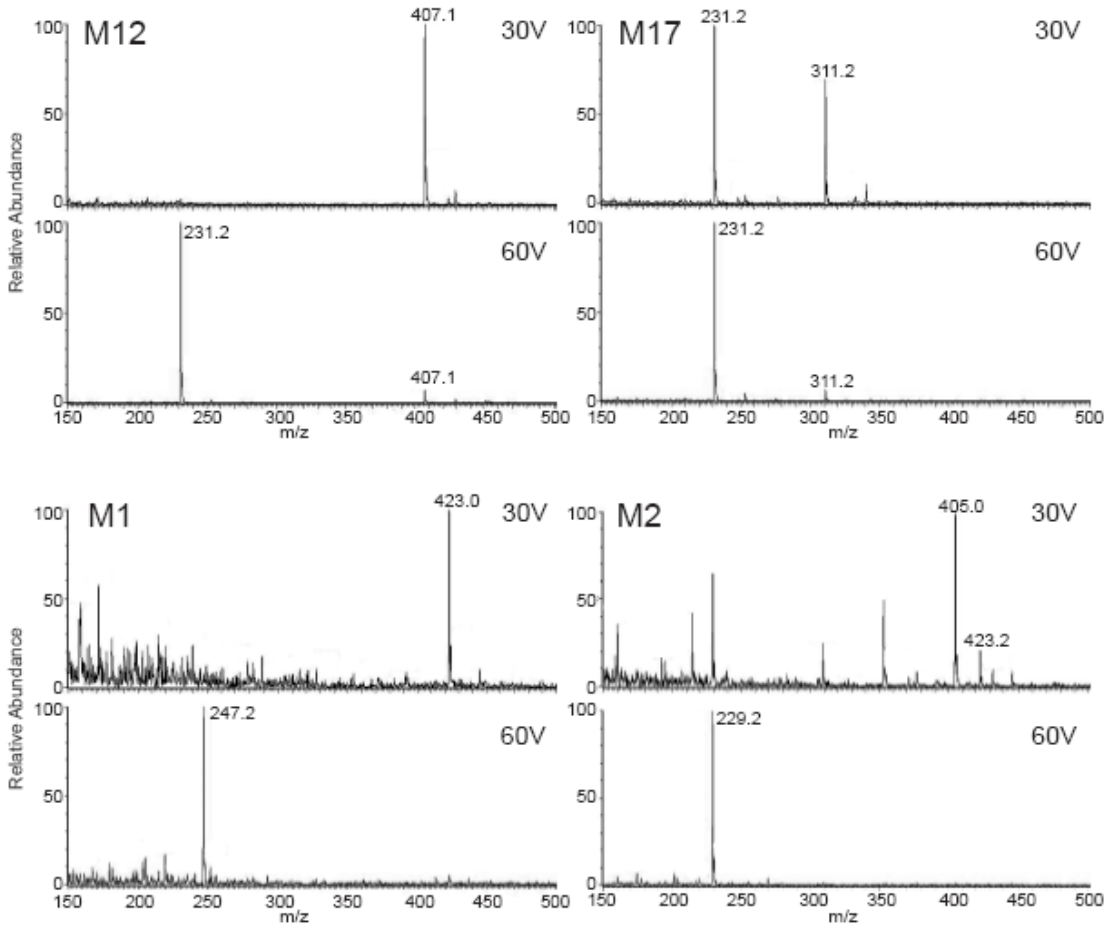

Fig. 4. MS Spectra (ESI positive ion mode) of the 667-Coumarin glucuronide: M12; the 667-Coumarin sulphate: M17; and two glucuronides of mono-oxidized 667-Coumarin metabolites: M1 (glucuronide of metabolite M11), and M2 (glucuronide of metabolite M16). Upper panel: MS spectra obtained at 30 V cone voltage; Lower panel: MS spectra obtained at 60 V cone voltage. At the cone voltage of 60 V, M1 showed a fragmentation product of *m/z* 247 corresponding to the mono-oxidized 667-Coumarin residue after the neutral loss of the glucuronide, whereas M2 showed a fragmentation product of *m/z* 229 corresponding to a typical fragmentation product of some mono-oxidized 667-Coumarin derivatives. Metabolites M12 and M17 showed a fragmentation product of *m/z* 231 corresponding to the *m/z* of 667-Coumarin residue after the neutral loss of the glucuronide or the sulphate, respectively.