SUPPORTING INFORMATION

Species differences in the *in vitro* metabolism of lasiocarpine

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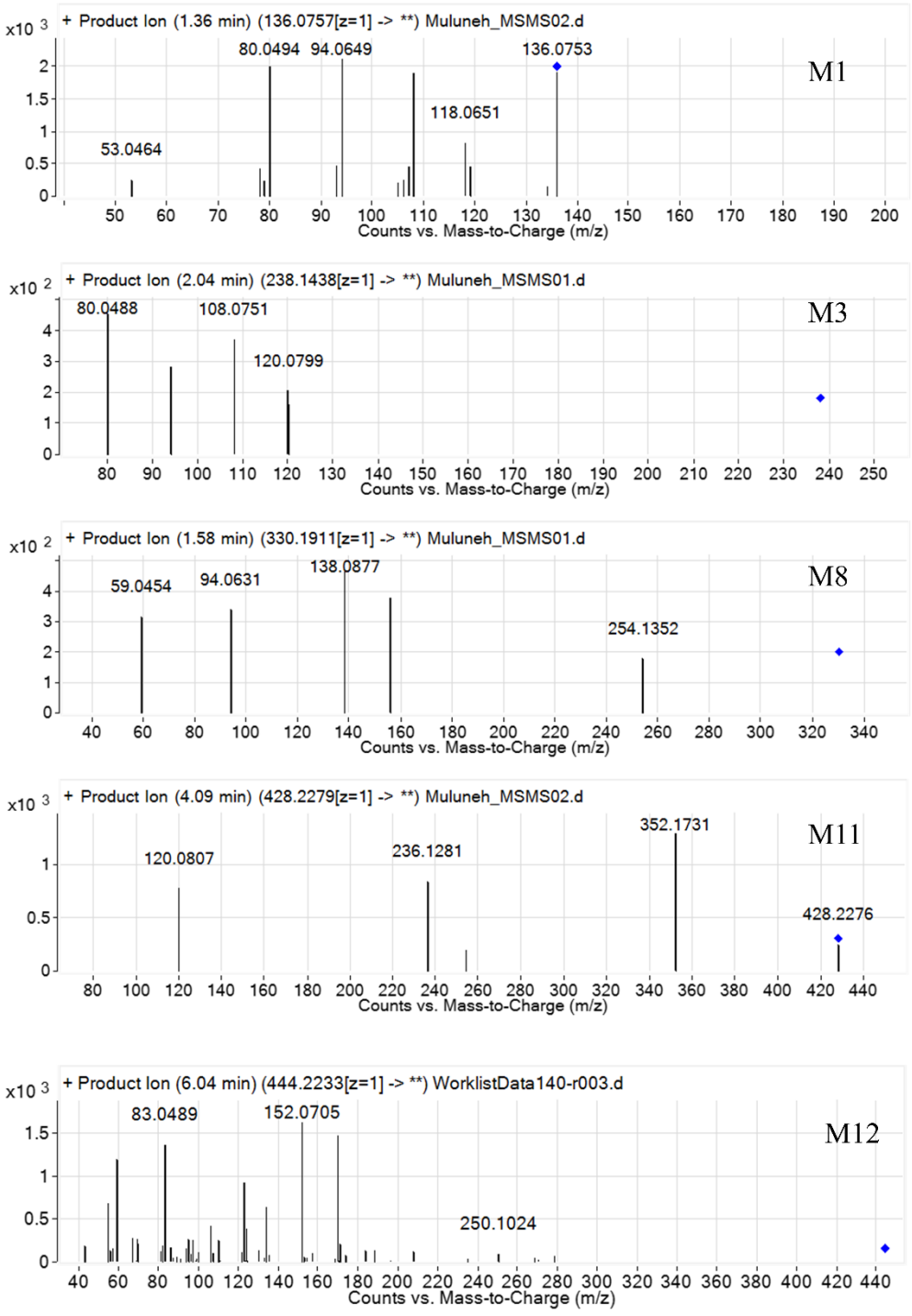


Figure S1. ESI-MS/MS spectra of M1, M3, M8, M11, and M12. The MS/MS data were acquired by Q-ToF MS.

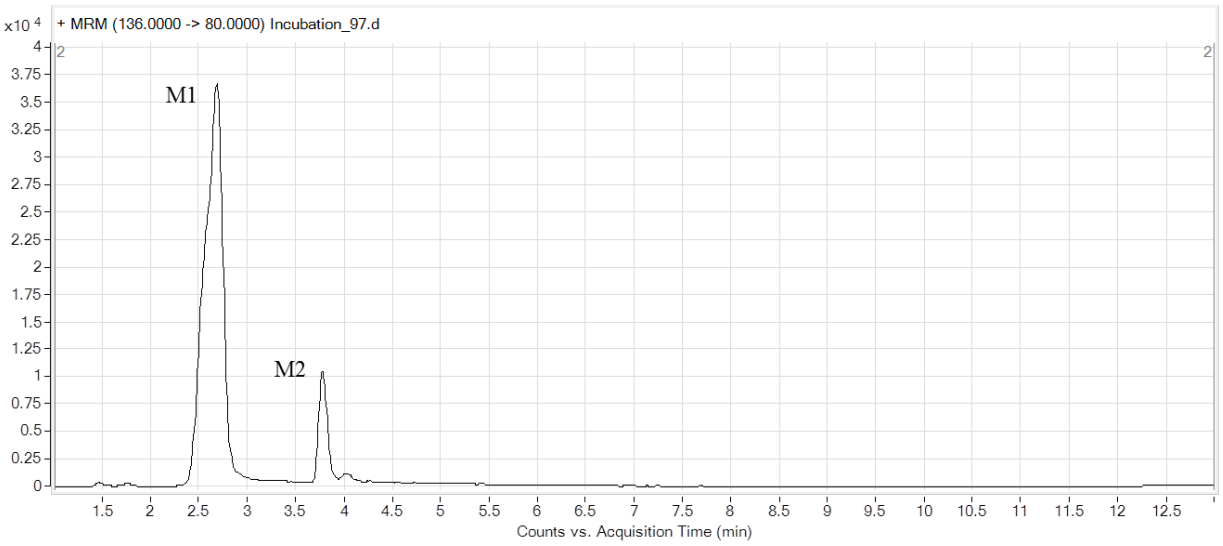


Figure S2. LC/MS chromatogram of (3H-pyrrolizin -7-yl)methanol) M1 and its minor isomer M2. 100 µM lasiocarpine was incubated in a 100 mM potassium phosphate buffer (pH 7.4) with the human liver microsomes in the presence of NADPH and liver microsomes of human origin for 30 min.

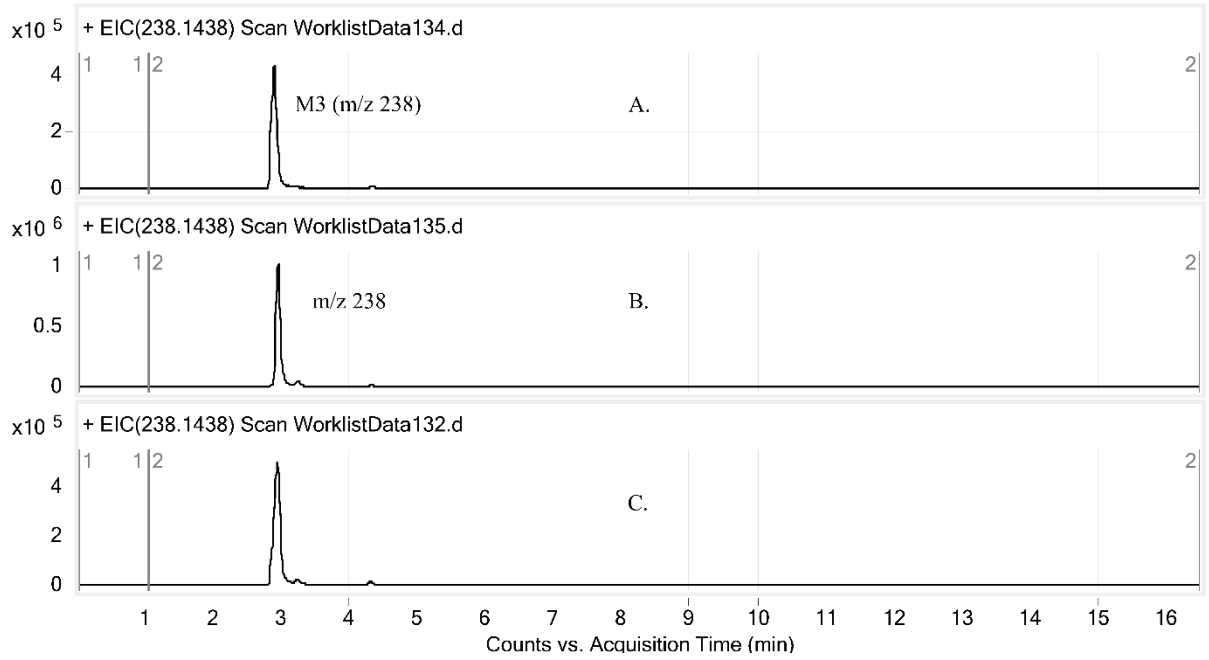


Figure S3. Metabolism of M9 to M3. A metabolite with identical LC/MS spectra was produced after incubation of lasiocarpine (A) or echimidine (B) in human liver microsomes for 60 min at 37 °C. Panel C shows a mixture of the two incubation samples (1:1).

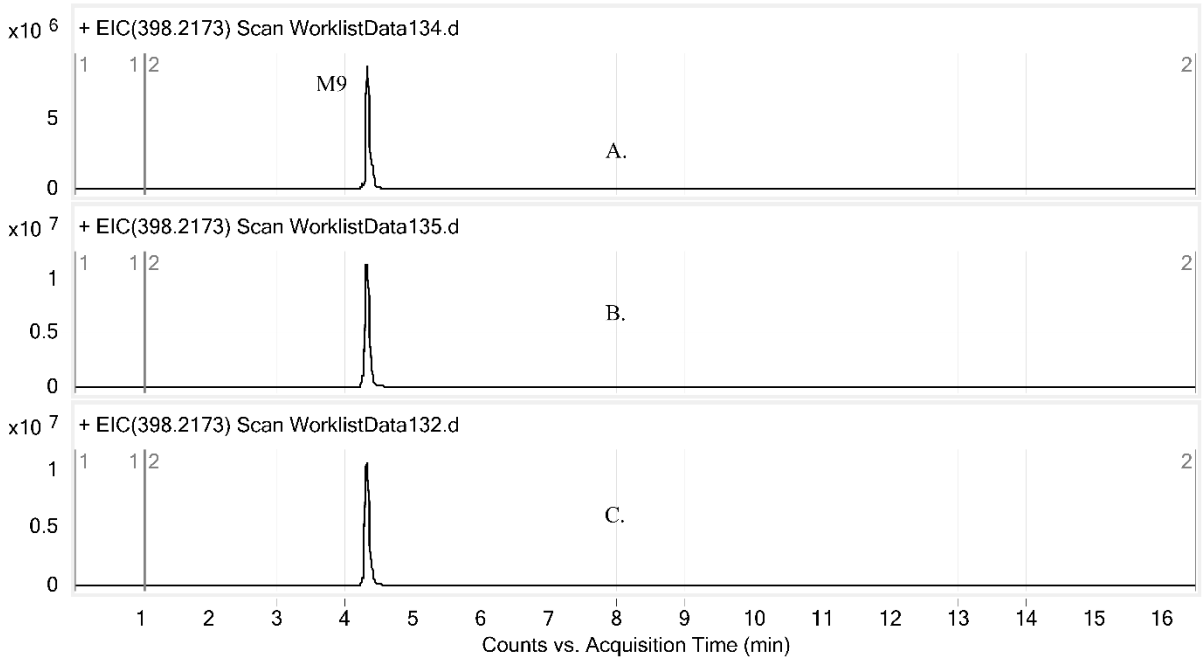


Figure S4. Confirmation of molecular structure of M9. The molecular structure of M9 was confirmed by comparing the LC/MS chromatograms of M9 (A), echimidine (B) and the mixture of M9 and echimidine (C). M9 was produced by incubating lasiocarpine in human liver microsomes in the presence of NADPH regenerating system for 60 min at 37 °C.

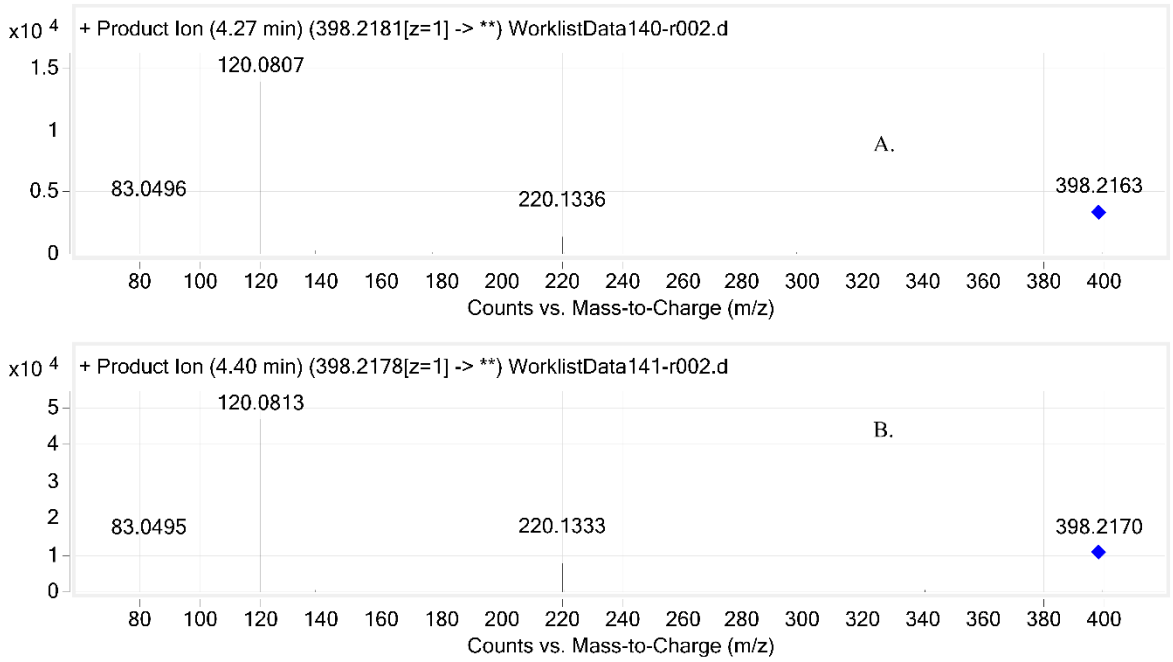


Figure S5. ESI-MS/MS spectra of M9 (A) and echimidine (B).

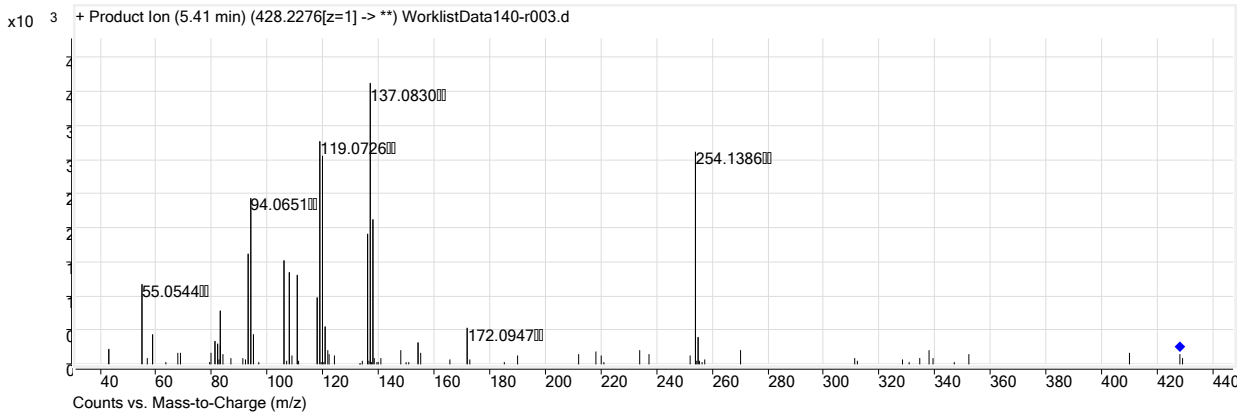


Figure S6. ESI-MS/MS spectra of lasiocarpine N-oxide. The ESI-MS/MS ion clusters at the lower mas units are characteristic to the N-oxides of pyrrolizidine alkaloids and are often used in the identification of these metabolites (i.e. pyrrolizidine N-oxides).[1]

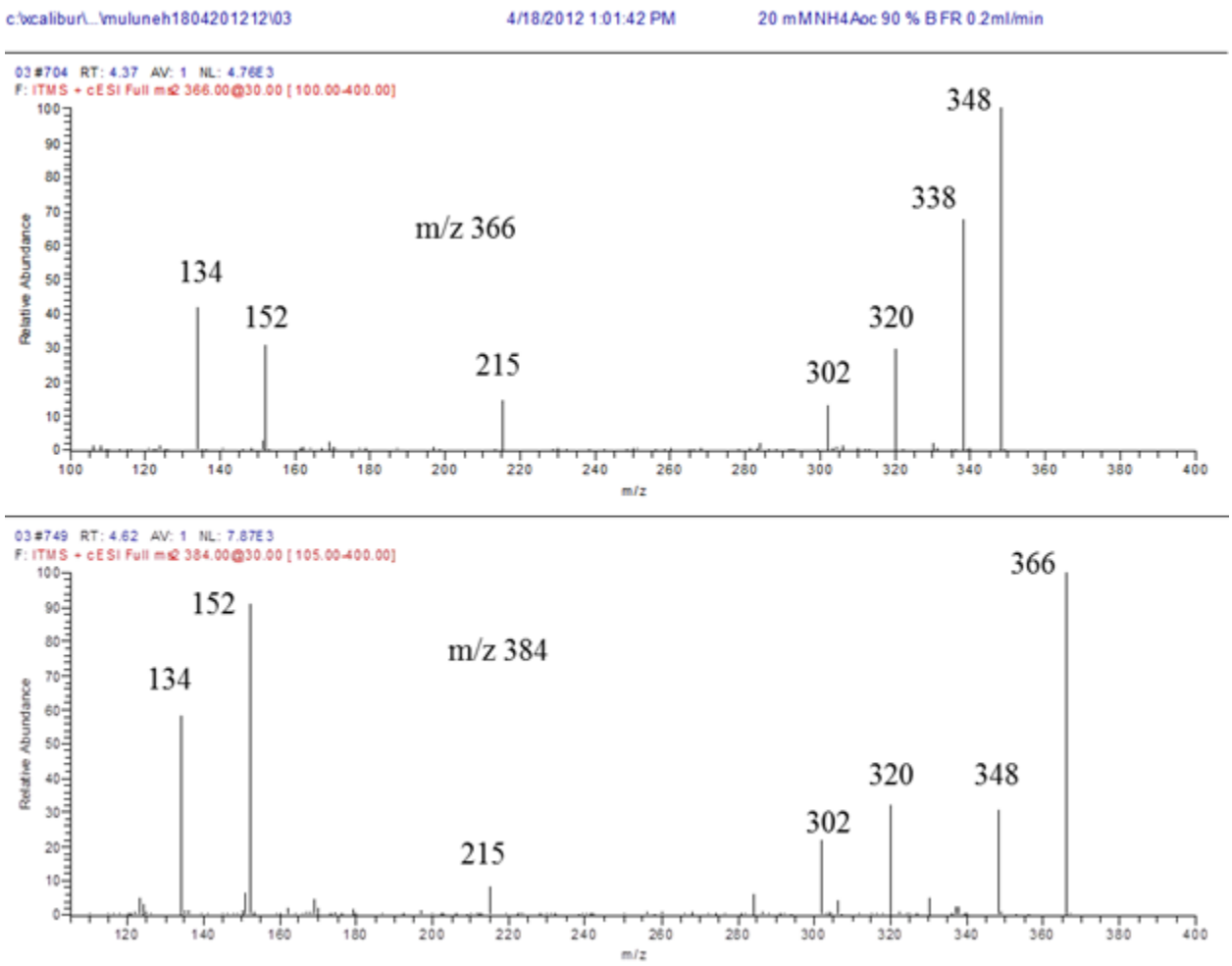


Figure S7. ESI-MS/MS fragment ions of m/z 366 and m/z 384. These two metabolites were observed after online electrochemical oxidation of retrorsine (*m/z* 352) in 20 mM ammonium acetate buffer at +800 mV. However, we did not observe these metabolites after incubation of retrorsine with human liver microsomes in the presence of NADPH at 37 ^{o}C.[2]

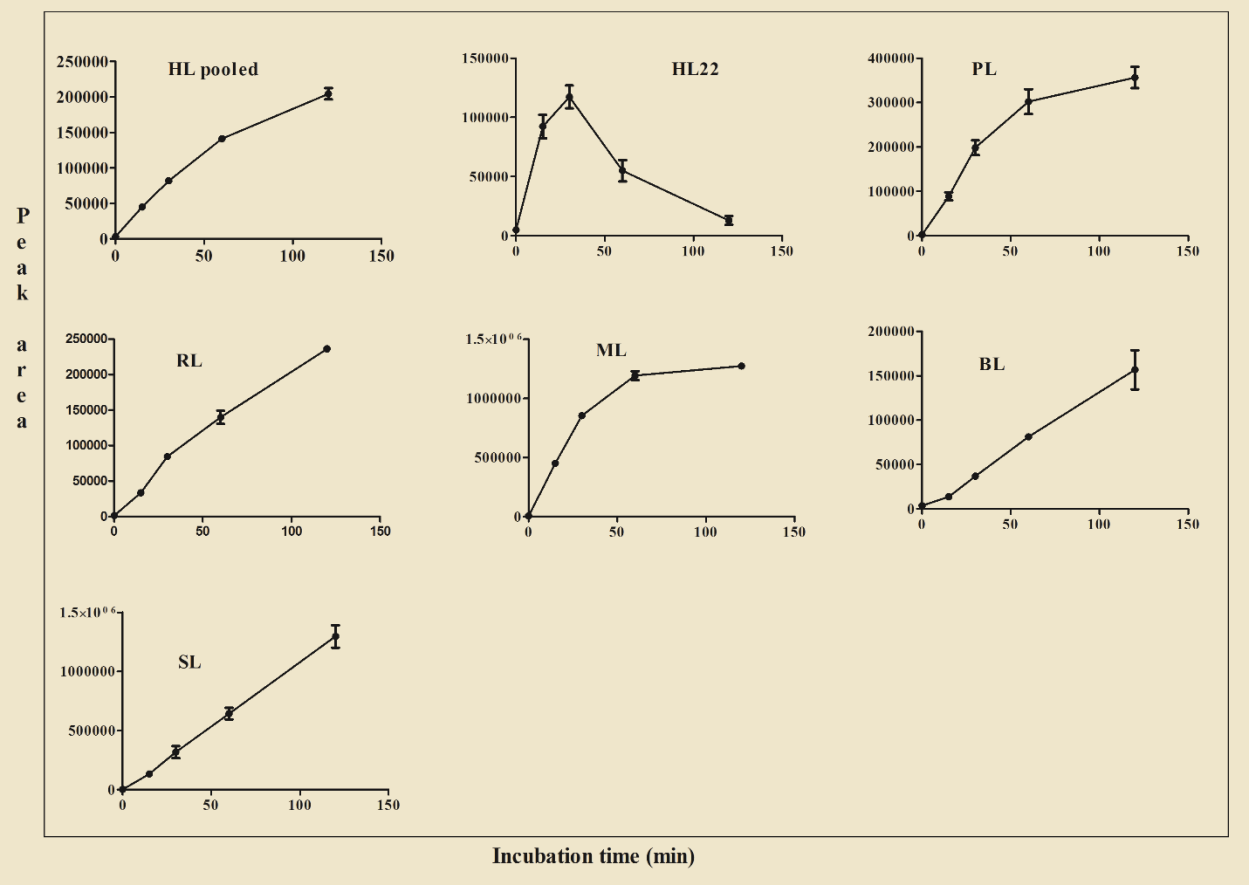
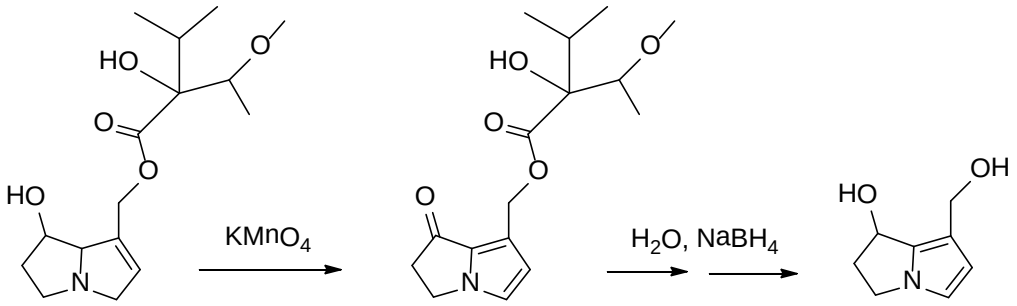


Figure S8. Time dependent formation of M9 in the liver microsomes of human, pig, rat, mouse, rabbit and sheep. 10 μM lasiocarpine was incubated at 37 ^{0}C in the presence of NADPH and samples were withdrawn at selected time points between 0 and 120 minutes. HL pooled: pooled human liver microsomes; HL22: individual human liver microsomes; PL: pooled pig liver microsomes; RL: pooled rat liver microsomes; ML: mice liver microsomes; BL: pooled rabbit liver microsomes; SL: pooled sheep liver microsomes.



Scheme S1. Preparation of 7-oxo derivatives of dehydroheliotrine. [3,4]

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