Supplementary information

Interspecies differences in stability kinetics and plasma esterases involved in hydrolytic activation of curcumin diethyl disuccinate, a prodrug of curcumin

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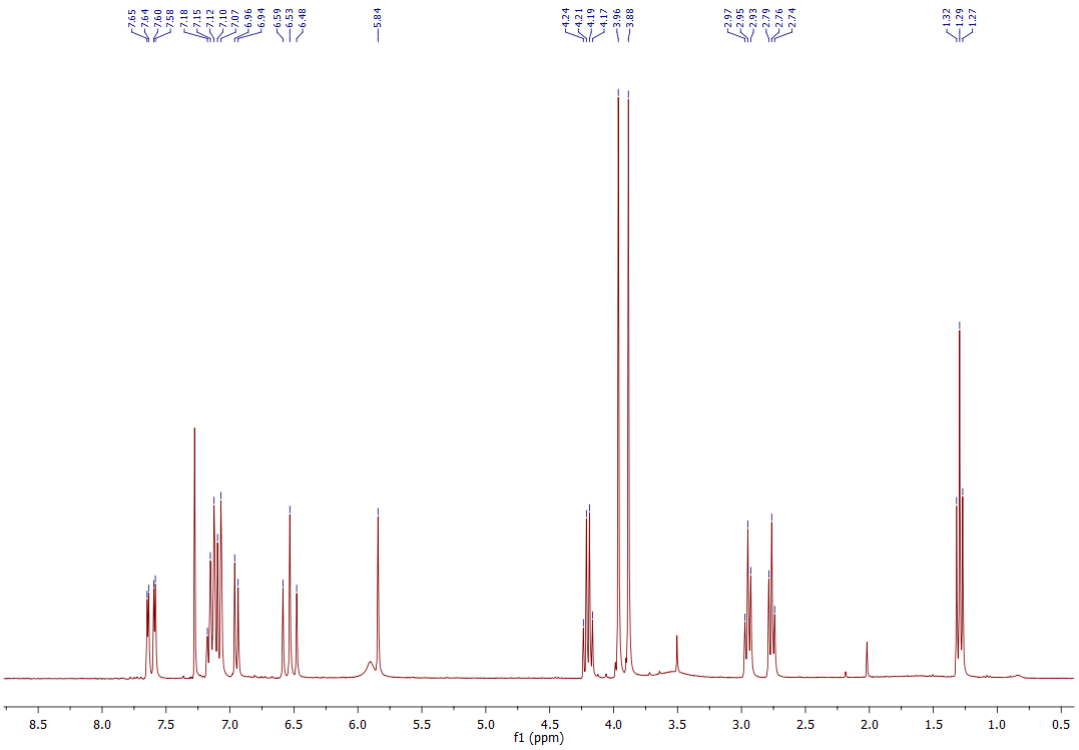
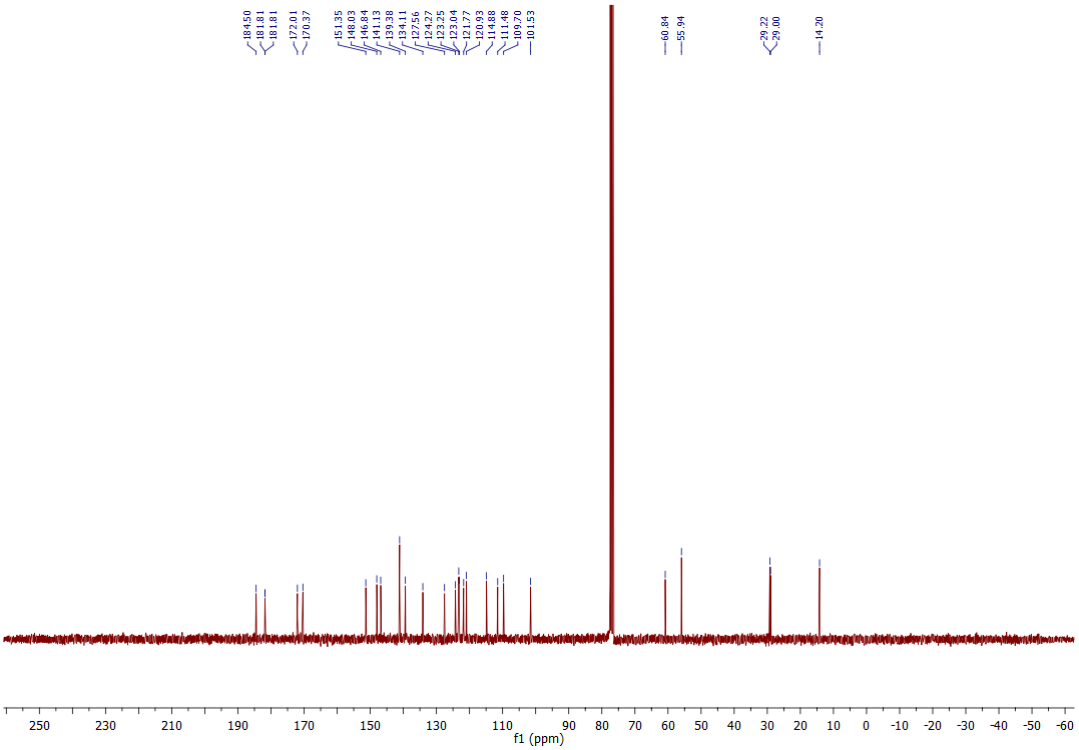
Synthesis of monoethylsuccinyl curcumin (MSCUR)

Curcumin (2g, 5.43 mmol) and 4-(*N*,*N*-dimethylamino) pyridine (DMAP, 0.663 mg, 5.43 mmol) were dissolved in 500 mL of dichloromethane. The mixture was stirred at room temperature until both compounds dissolved completely. Then, the reaction mixture was slowly added with ethyl-4-chloro-4-oxobutyrate (95% assay, 814.3 µL, 5.43 mmol) at room temperature. After stirring for 2 h, 100 mL of 0.1 N HCl was added and the mixture was stirred for 5 min. The organic layer was washed with deionized water three times to remove residual acids. The residual water in the organic phase was removed using anhydrous sodium sulfate and the organic layer was concentrated by rotary evaporator. The concentrated mixture was purified by reverse phase C-18 column chromatography eluted with 80% methanol and 20% water. Subsequently, the combined eluate was dried by rotary evaporator and crude product was crystallized in methanol yielding ethyl (4-((1*E*,3*Z*,6*E*)-3-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-5-oxohepta-1,3,6-trien-1-yl)-2-methoxyphenyl) succinate or monoethyl succinyl curcumin (MSCUR; 239.7 mg; 0.48 mmol; 8.84%). MS: $[M+H]^+$ m/z 497.2; $[M+Na]^+$ m/z 519.1. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 15.8, 3.6 Hz, 2H), 7.21 – 7.00 (m, 5H), 6.95 (d, *J* = 8.2 Hz, 1H), 6.53 (t, *J* = 16.1 Hz, 2H), 5.84 (s, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.96 (s, 3H), 3.88 (s, 3H), 2.95 (t, *J* = 6.8 Hz, 2H), 2.76 (t, *J* = 6.9 Hz, 2H), 1.29 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 184.50, 181.81, 181.81, 172.01, 170.37, 151.35, 148.03, 146.84, 141.13, 139.38, 134.11, 127.56, 124.27, 123.25, 123.04, 121.77, 120.93, 114.88, 111.48, 109.70, 101.53, 60.84, 55.94, 29.22, 29.00, 14.20.



^{1}H NMR spectrum of MSCUR



^{13}C NMR spectrum of MSCUR

Kinetics of CDD hydrolysis

The hydrolysis of CDD follows the consecutive pseudo-first order reaction as shown in the following scheme:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

where A is CDD; B is the intermediate metabolite or MSCUR; C is CUR.

The rate constants were obtained by plotting the peak area ratios between the analytes and the internal standard (DMC). The following equations were used to extract rate constants (k_1 and k_2) using nonlinear regression analysis.

$$[A]_t = [A]_0 \, e^{-k_1 t}$$

$$[B]_t = [A]_0 \left(\frac{k_1}{k_2 - k_1}\right)\left(e^{-k_1 t} - e^{-k_2 t}\right)$$

$$[C]_t = [A]_0 \left[1 + \frac{k_2 e^{-k_1 t} - k_1 e^{-k_2 t}}{k_1 - k_2}\right]$$

Besides the rate constants, half-lives ($t_{1/2}$) of CDD and time to reach the maximum of MSCUR (t_{max}) were also calculated using following equations:

$$t_{1/2} = \frac{\ln 2}{k_1}$$

$$t_{max} = \frac{1}{k_1 - k_2} \cdot \ln \frac{k_1}{k_2}$$

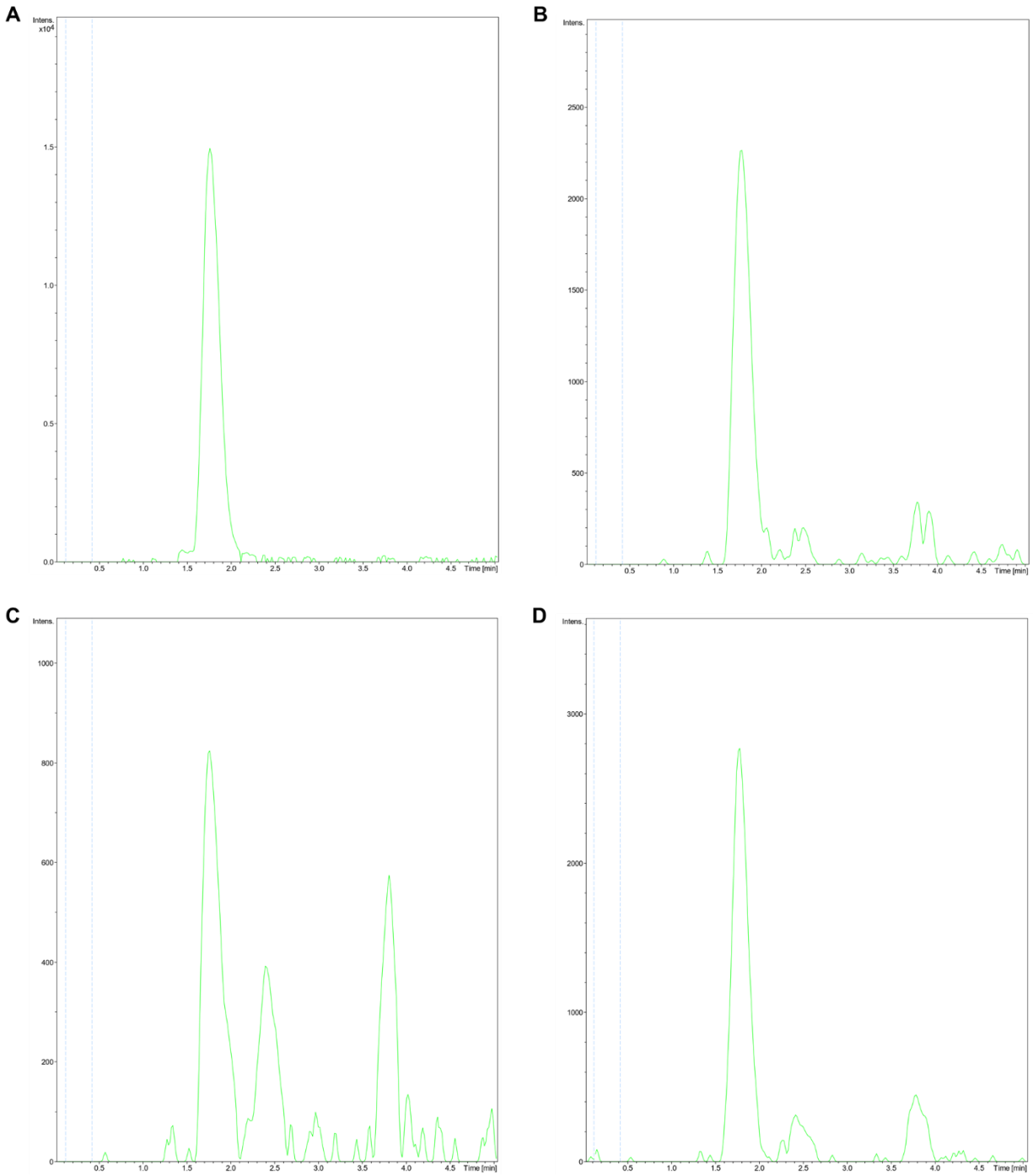


Fig. S1 Extracted-ion chromatograms (XIC) of (A) standard CUR (B) M1 in rat plasma (C) M1 in dog plasma and (D) M1 in human plasma.

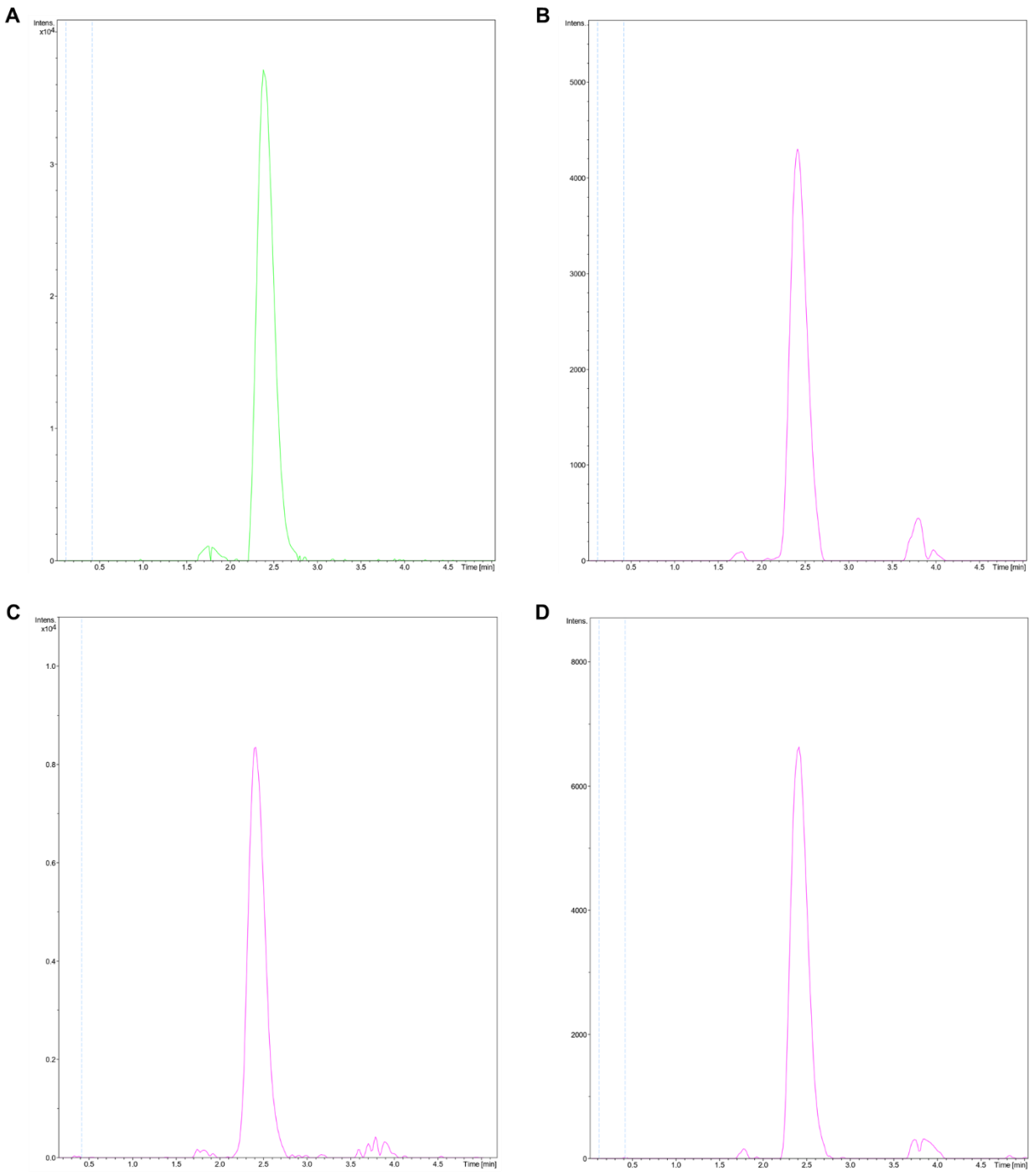


Fig. S2 Extracted-ion chromatograms (XIC) of (A) Standard MSCUR (B) M2 in rat plasma (C) M2 in dog plasma and (D) M2 in human plasma.

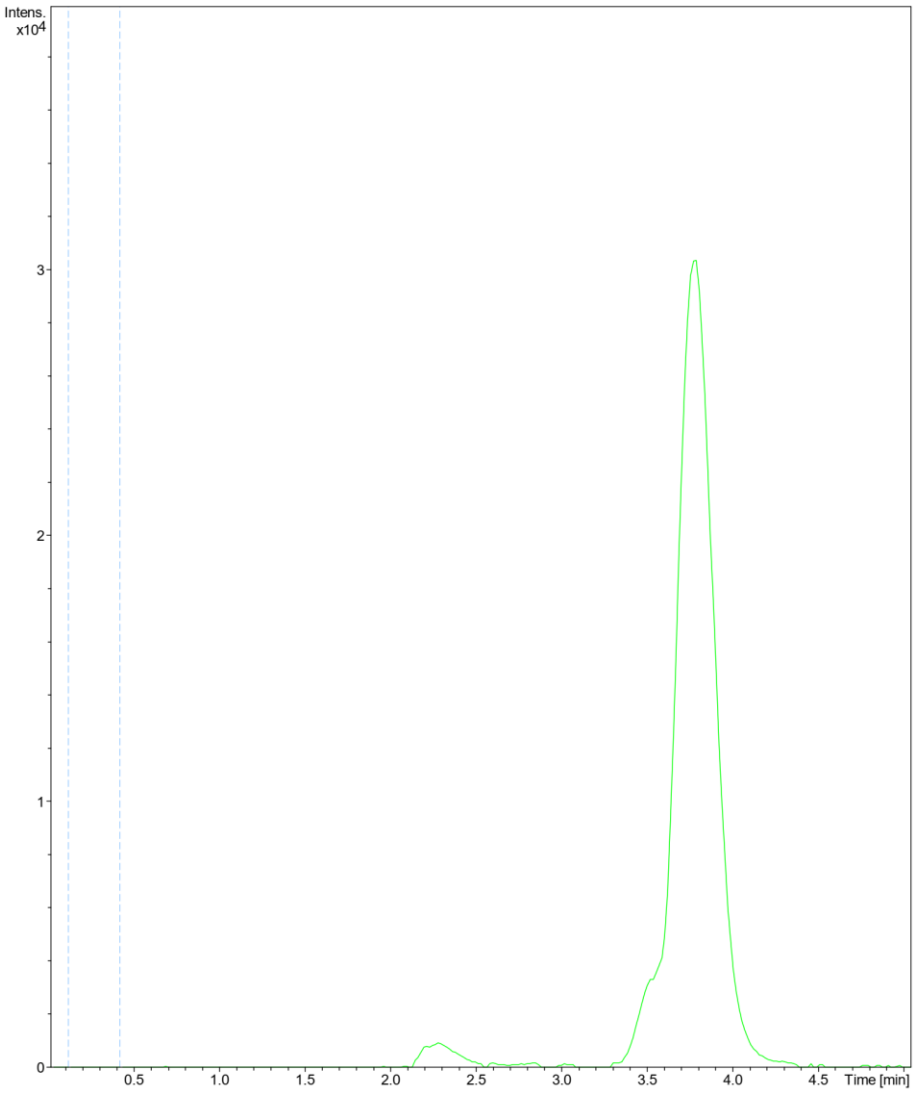


Fig. S3 Extracted-ion chromatograms (XIC) of Standard CDD

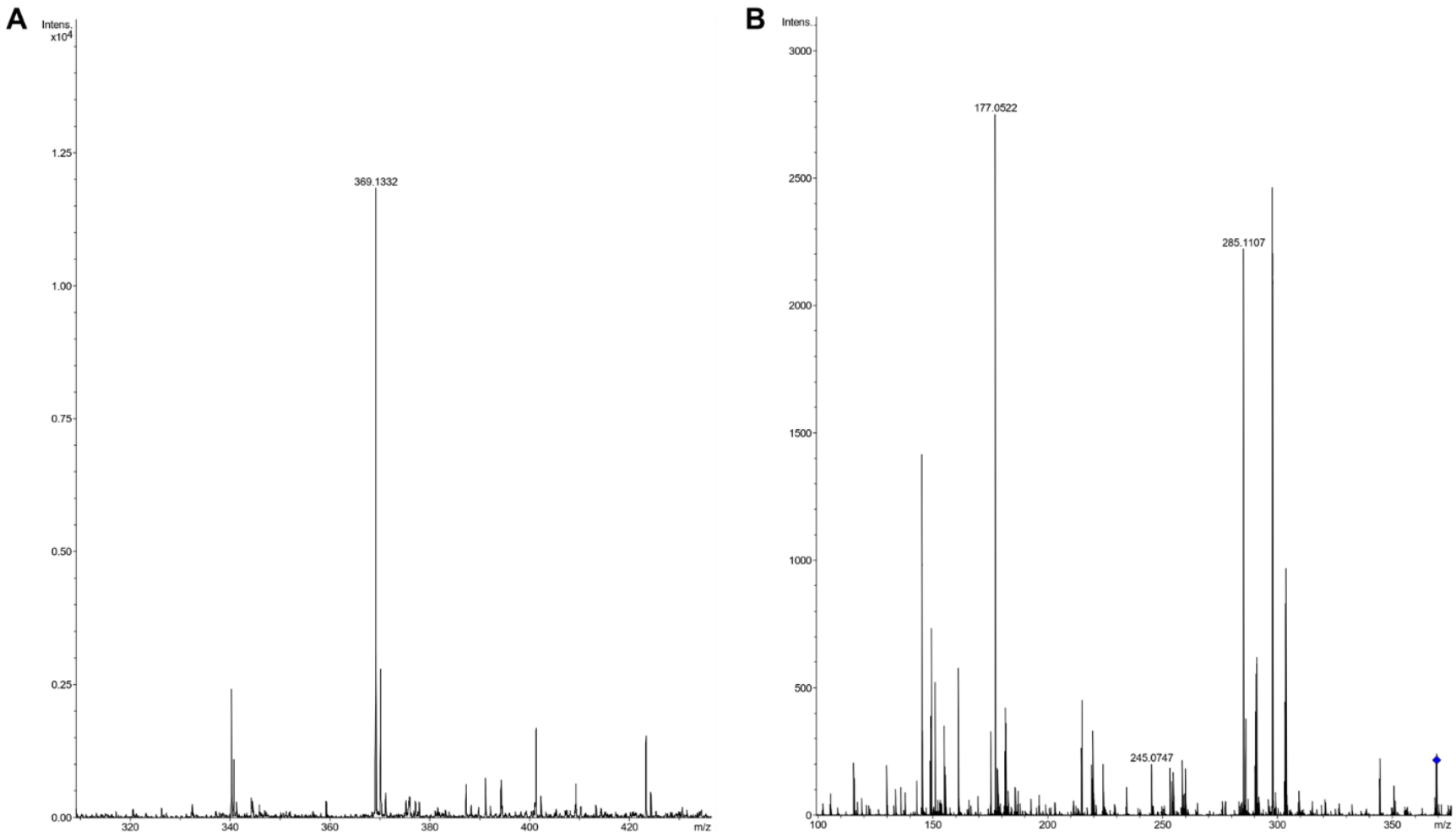


Fig. S4 (A) MS spectrum of CUR and (B) MS^2 spectrum of CUR.

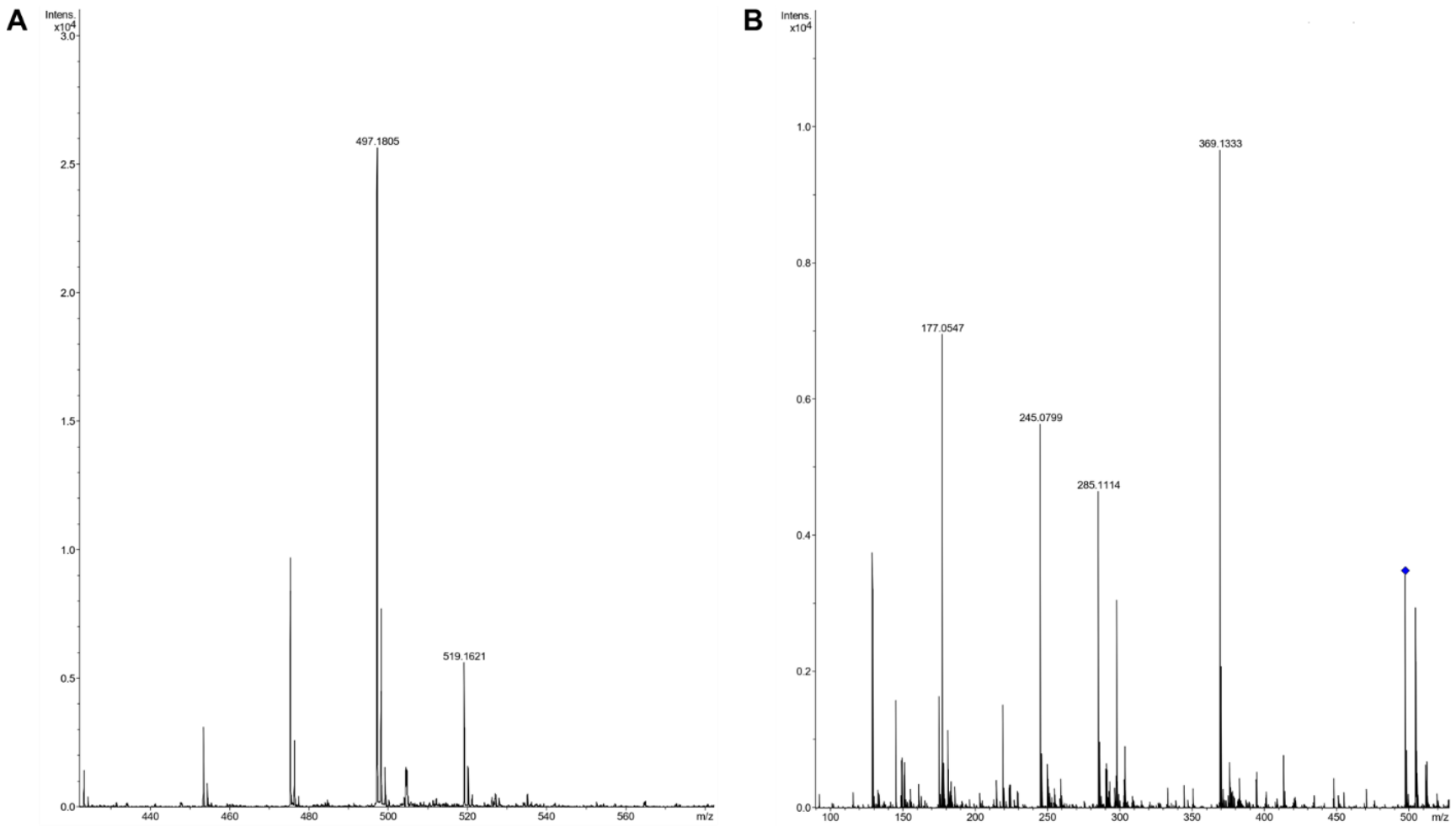


Fig S5 (A) MS spectrum of MSCUR and (B) MS^2 spectrum of MSCUR.

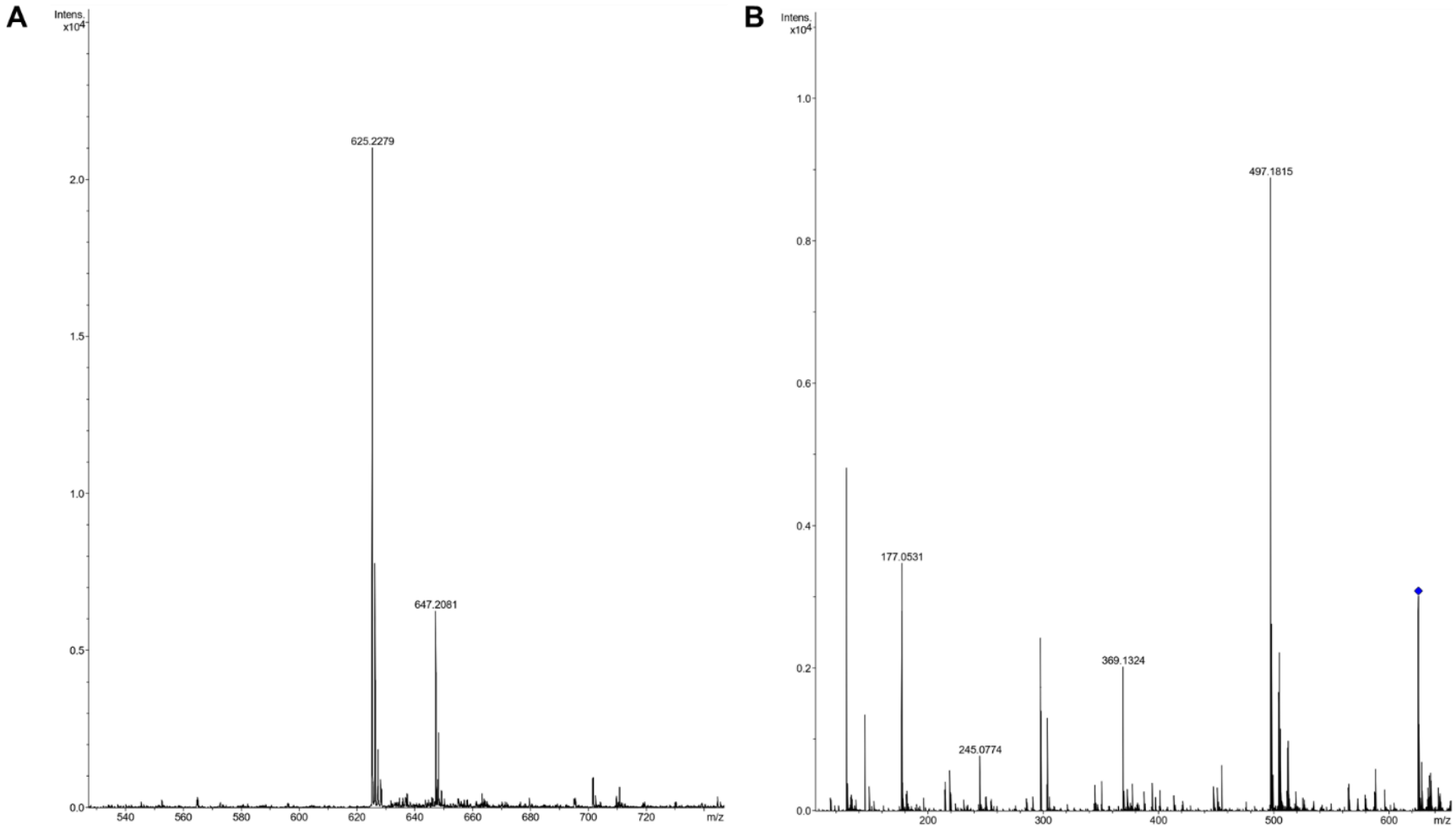


Fig S6 (A) MS spectrum of CDD and (B) MS^2 spectrum of CDD.

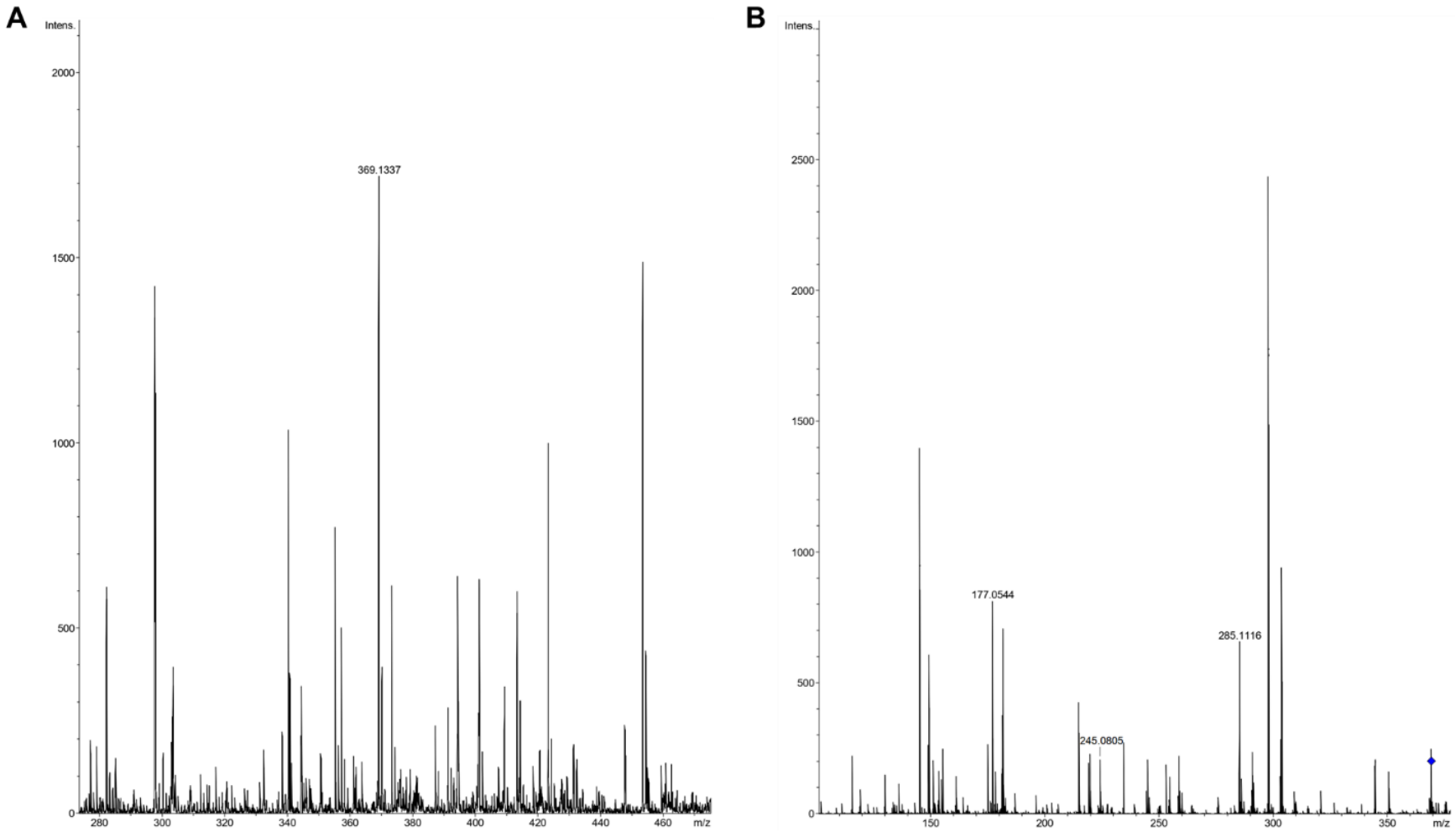


Fig. S7 (A) MS spectrum of M1 metabolite and (B) MS^2 spectrum of M1 metabolite in rat plasma.

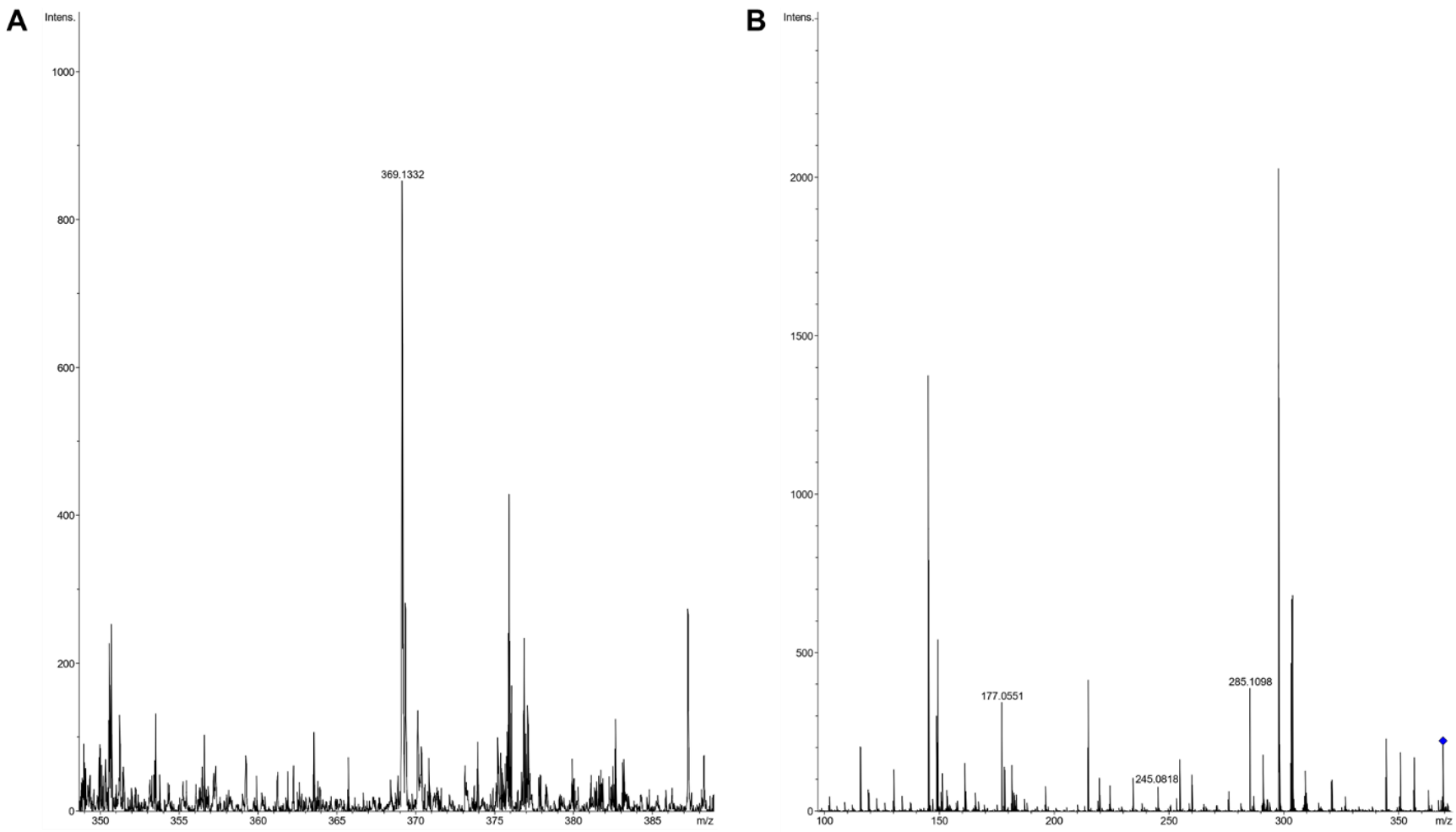


Fig. S8 (A) MS spectrum of M1 metabolite and (B) MS^2 spectrum of M1 metabolite in dog plasma.

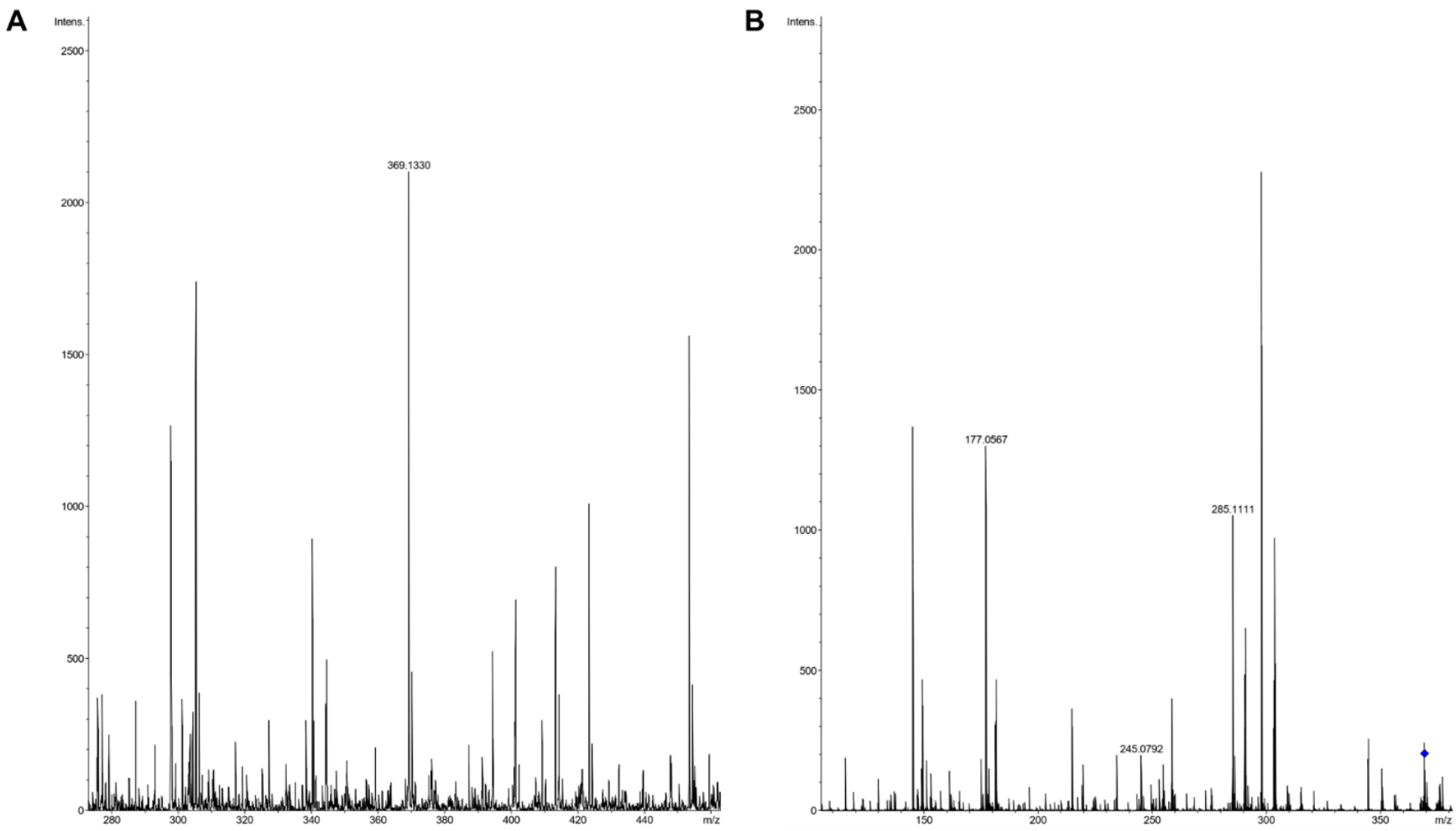


Fig. S9 (A) MS spectrum of M1 metabolite and (B) MS^2 spectrum of M1 metabolite in human plasma.

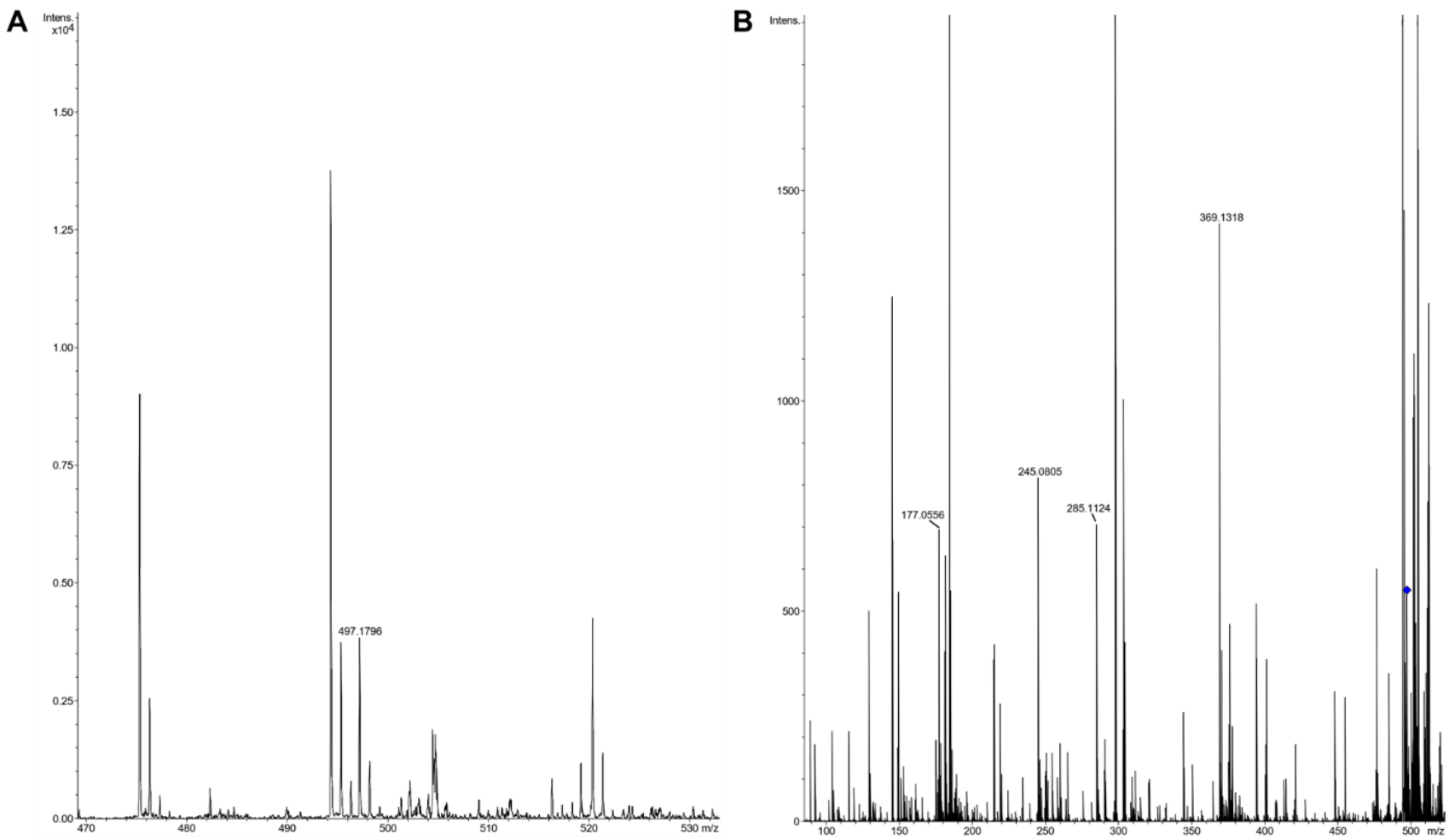


Fig. S10 (A) MS spectrum of M2 metabolite and (B) MS^2 spectrum of M2 metabolite in rat plasma.

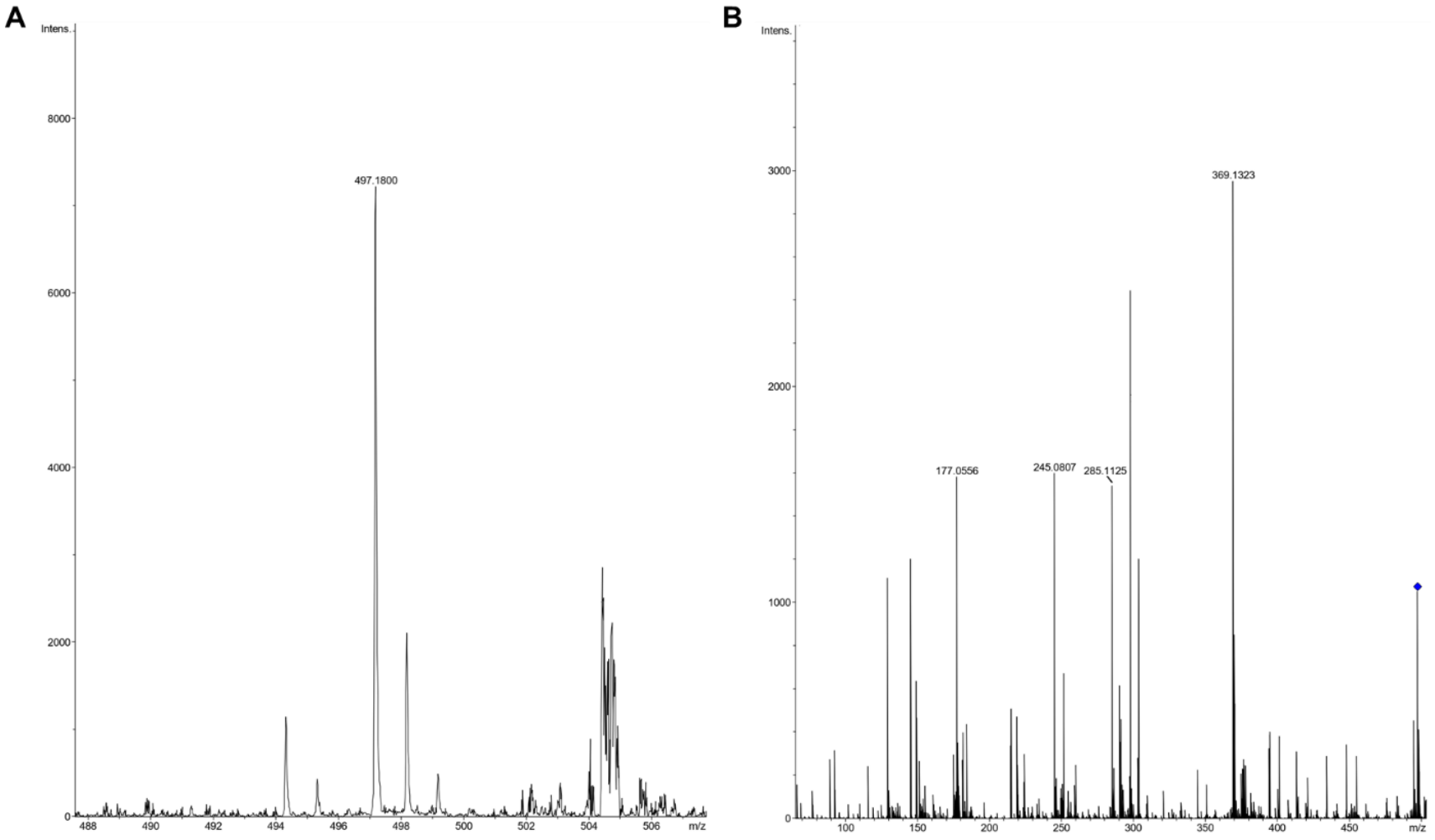


Fig. S11 (A) MS spectrum of M2 metabolite and (B) MS^2 spectrum of M2 metabolite in dog plasma.

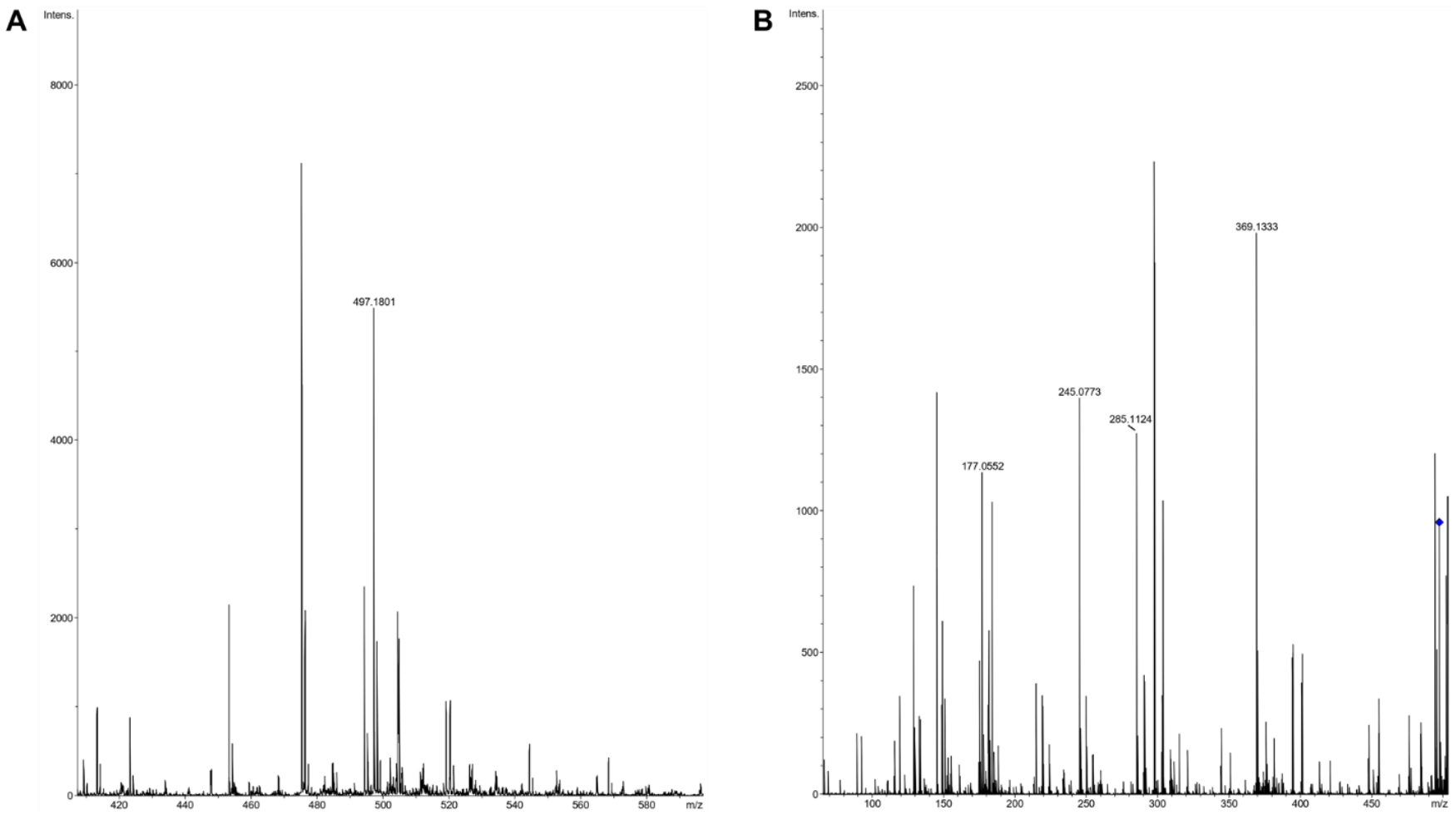


Fig. S12 (A) MS spectrum of M2 metabolite and (B) MS^2 spectrum of M2 metabolite in human plasma.

Fig. S13 Proposed mass fragmentations of (A) CUR (B) MSCUR and (C) CDD.