Drug Metabolism and Disposition

CPY3A4-mediated lopinavir bioactivation and its inhibition by ritonavir

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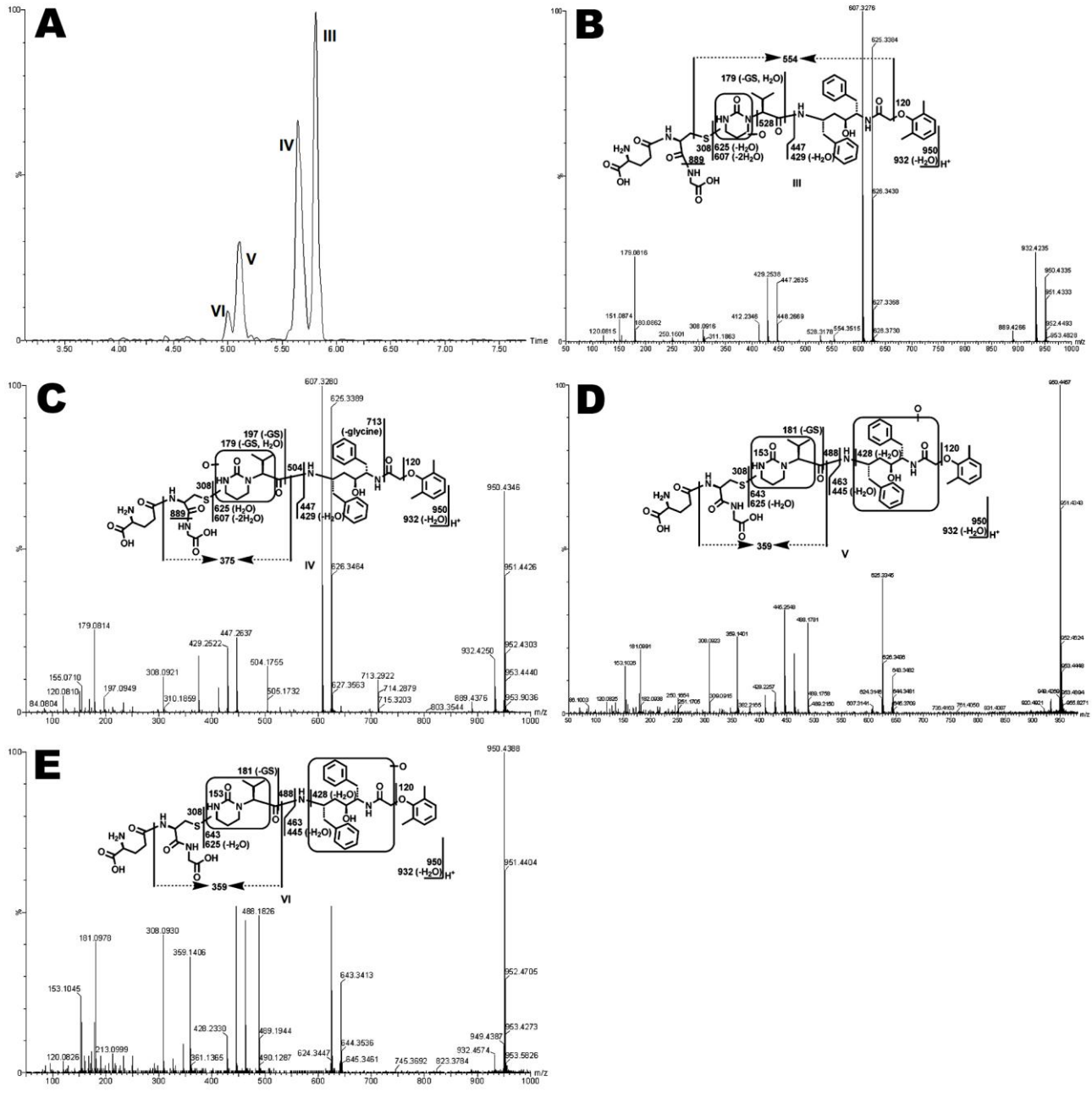
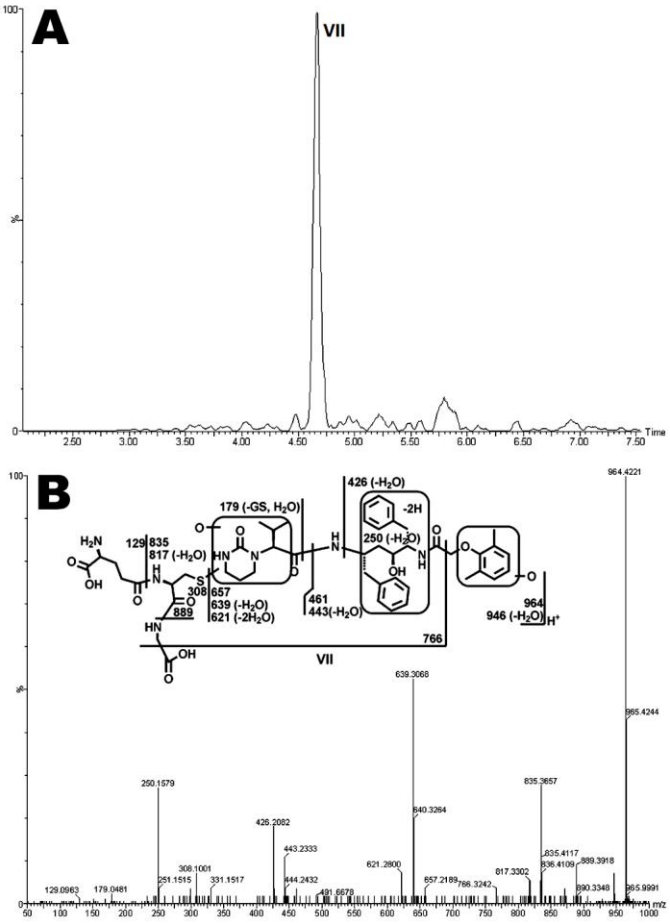
Supplemental Figure 1



Supplemental Figure 1. The extracted ion chromatograms (A) and MS/MS spectra of LPV adducts III (B), IV (C), V (D), and VI (E). LPV was incubated in HLM containing GSH and analyzed by UPLC-TOFMS. Four monohydroxylated LPV_GSH adducts were identified. Adduct III **(B)**, eluted at 5.81 min, had a protonated molecule

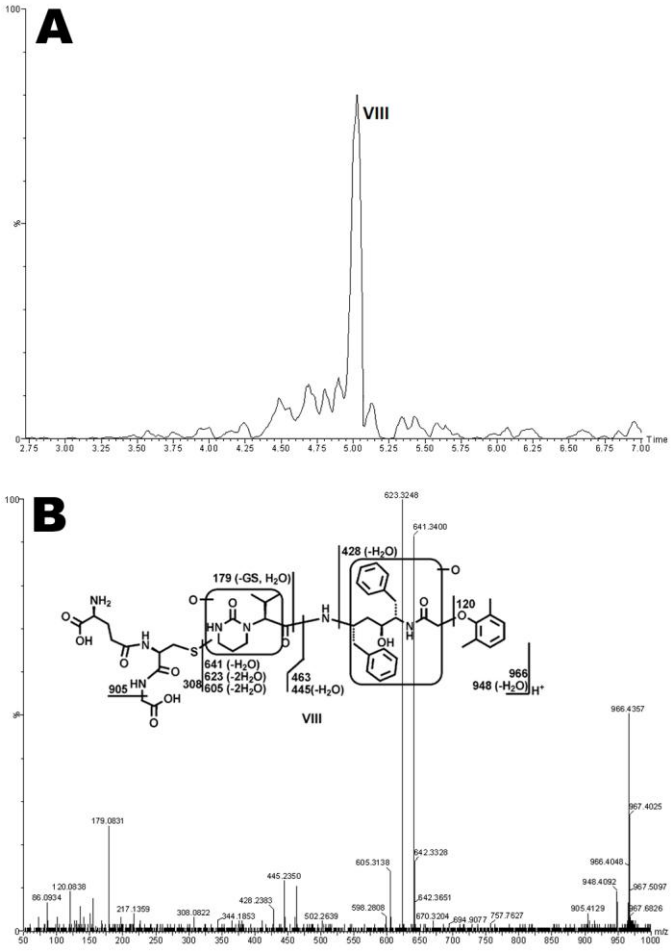
$[M+H]^+$ at *m/z* 950.4335, 16 Daltons higher than that of LPV_GSH adduct. The fragment ions at *m/z* 554.3515 and 528.3178 suggest that the GSH moiety was attached to the encircled unit. The fragment ion at *m/z* 179.0816 suggests that hydroxylation took place in the encircled unit of the molecule. Adduct IV (at a retention time of 5.65 min) corresponded to a protonated molecule $[M+H]^+$ at *m/z* 950.4346, 16 mass units higher than that of LPV_GSH adduct. Adduct IV **(C)** had the same major fragment ions at *m/z* 625.3384, 607.3276, 447.2635, and 429.2538 as those of adduct III. The formation of fragment ions at *m/z* 504.1755 and 375.1332 indicate that the GSH moiety was linked to the encircled unit. The fragment ion at *m/z* 179.0814 suggests that hydroxylation took place in the encircled unit of the molecule. Compared to mass spectra of adducts I and II, the ion at *m/z* 375.1329 is 16 Daltons higher than the ions (*m/z* 359.1408) generated from adducts I and II, which further demonstrates the oxidized position. Adduct V **(D)** was detected at 5.11 min with a protonated molecule $[M+H]^+$ at *m/z* 950.4457. The MS/MS of adduct V produced the fragment ions at *m/z* 643.3482 (loss of GSH), 488.1781, 463.2630, and 359.1410. The fragment ions at *m/z* 488.1781 and 359.1410 indicate that GSH was coupled to the left encircled panel, which consists with the MS/MS spectra of adducts I and II (Figures 2C and 2D). The ion at *m/z* 463.2630 is 16 mass units higher than the ion at *m/z* 447.2668 generated from LPV adducts I and II (Figure 2). The formation of fragment ions at *m/z* 463.2634 and 120.0825 suggest that the oxidation occurred in the right encircled unit. Adduct VI **(E)** was eluted at 5.00 min, having a protonated molecule $[M+H]^+$ at *m/z* 950.4388. Adduct VI had similar mass spectra fragmentation patterns to that of adduct V. The formation of ions at *m/z* 488.1826, 463.2612, and 359.1406 suggest that the GSH adduction and oxidation happened in the same encircled panels like adduct V, but at a different position.

Supplemental Figure 2



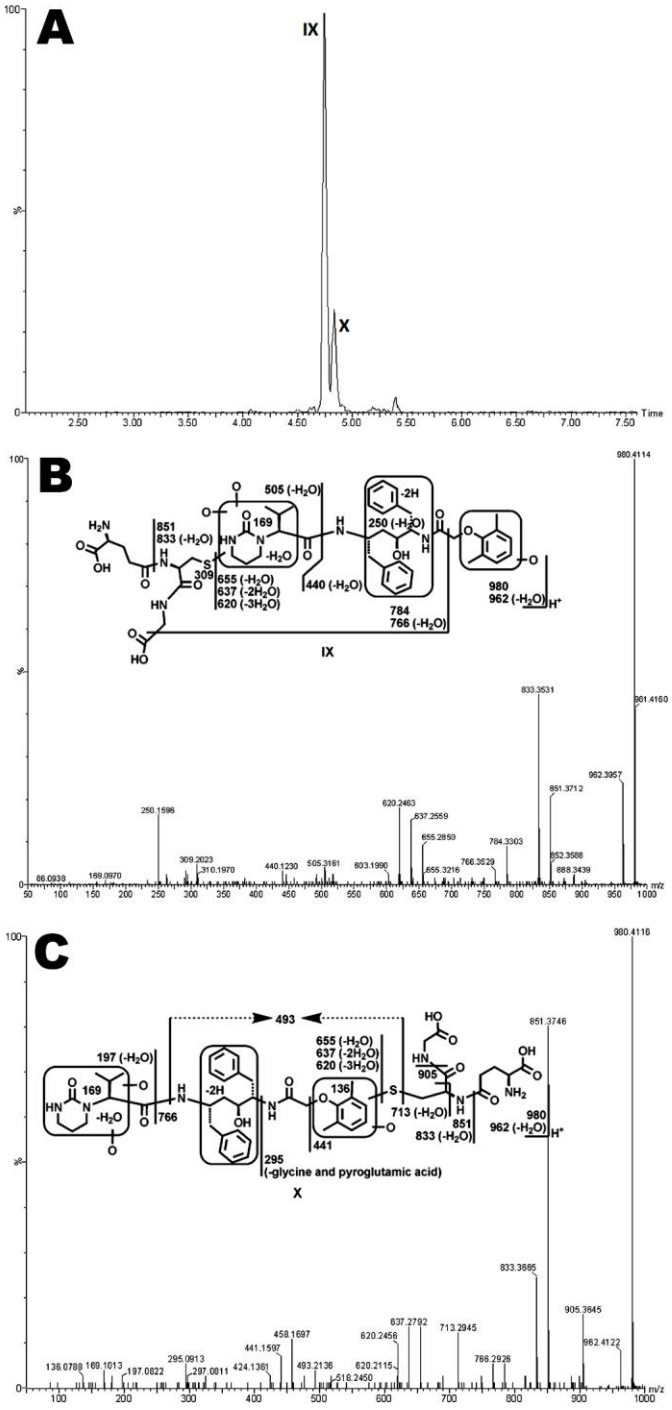
Supplemental Figure 2. The extracted ion chromatogram (A) and MS/MS spectra (B) of LPV adduct VII. LPV was incubated in HLM containing GSH and analyzed by UPLC-TOFMS. LPV adduct VII was detected at 4.66 min, having a mass of $[M+H]^+$ at *m/z* 964.4221. The MS/MS of LPV adduct VII produced fragment ions at *m/z* 835.3657 (loss of pyroglutamic acid), and 639.3068 (loss of GSH and H_2O). The fragmental ions at *m/z* 766.3242 and 179.0481 suggest that one oxidation took place on the 2,6-dimethylphenol moiety and the other one on left encircled panel. In addition, compared to the ions at *m/z* 463.2607 and 445.2548 generated from adducts V and VI, the ions at *m/z* 463.2607 and 443.2333 indicate that the dehydrogenation occurred on the middle encircled panel.

Supplemental Figure 3



Supplemental Figure 3. The extracted ion chromatogram (A) and MS/MS spectra (B) of LPV adduct VIII. LPV was incubated in HLM containing GSH and analyzed by UPLC-TOFMS. LPV adduct VIII (retention time at 5.03 min) had a mass of $[M+H]^+$ at *m/z* 966.4357. The corresponding MS/MS analysis showed the major fragment ions at *m/z* 948.4092 (loss of H_2O), 641.3400 (loss of GSH and H_2O), and 120.0838 (2,6-dimethylphenol). The fragment ion at *m/z* 463.2536, 428.2350, 179.0831, and 120.0838 suggest that one oxidation happened on the left encircled panel and the other one on the right encircled panel.

Supplemental Figure 4



Supplemental Figure 4. The extracted ion chromatograms (A) and MS/MS spectra of IX (B) and X (C). LPV was incubated in HLM containing GSH and analyzed by UPLC-TOFMS. LPV adduct IX **(B)**, eluted at 4.70 min, had a protonated molecule

$[M+H]^+$ at *m/z* 980.4114. MS/MS analysis of LPV adduct V produced daughter ions at *m/z* 962.3957 (loss of H_2O), 851.3712 (loss of pyroglutamic acid), 655.2850 (loss of GSH and H_2O). The fragment ions at *m/z* 784.3303 and 505.3161 suggest that one oxidation occurred in the 2,6-dimethylphenol moiety and dioxidation happened on the left encircled panel. Adduct X **(C)**, eluted at 4.78 min, had a mass of $[M+H]^+$ at *m/z* 980.4116. MS/MS analysis of X produced daughter ions at *m/z* 962.4122 (loss of H_2O), 851.3746 (loss of pyroglutamic acid), 655.2849 (loss of GSH and H_2O). The fragment ions at *m/z* 197.0822 and 136.0788 suggest that dioxidation happened on the left encircled panel and one oxidation occurred in the 2,6-dimethylphenol moiety. The ions at *m/z* 493.2136 and 441.1597 indicate that the GSH attached to the oxidized 2,6-dimethylphenol moiety.