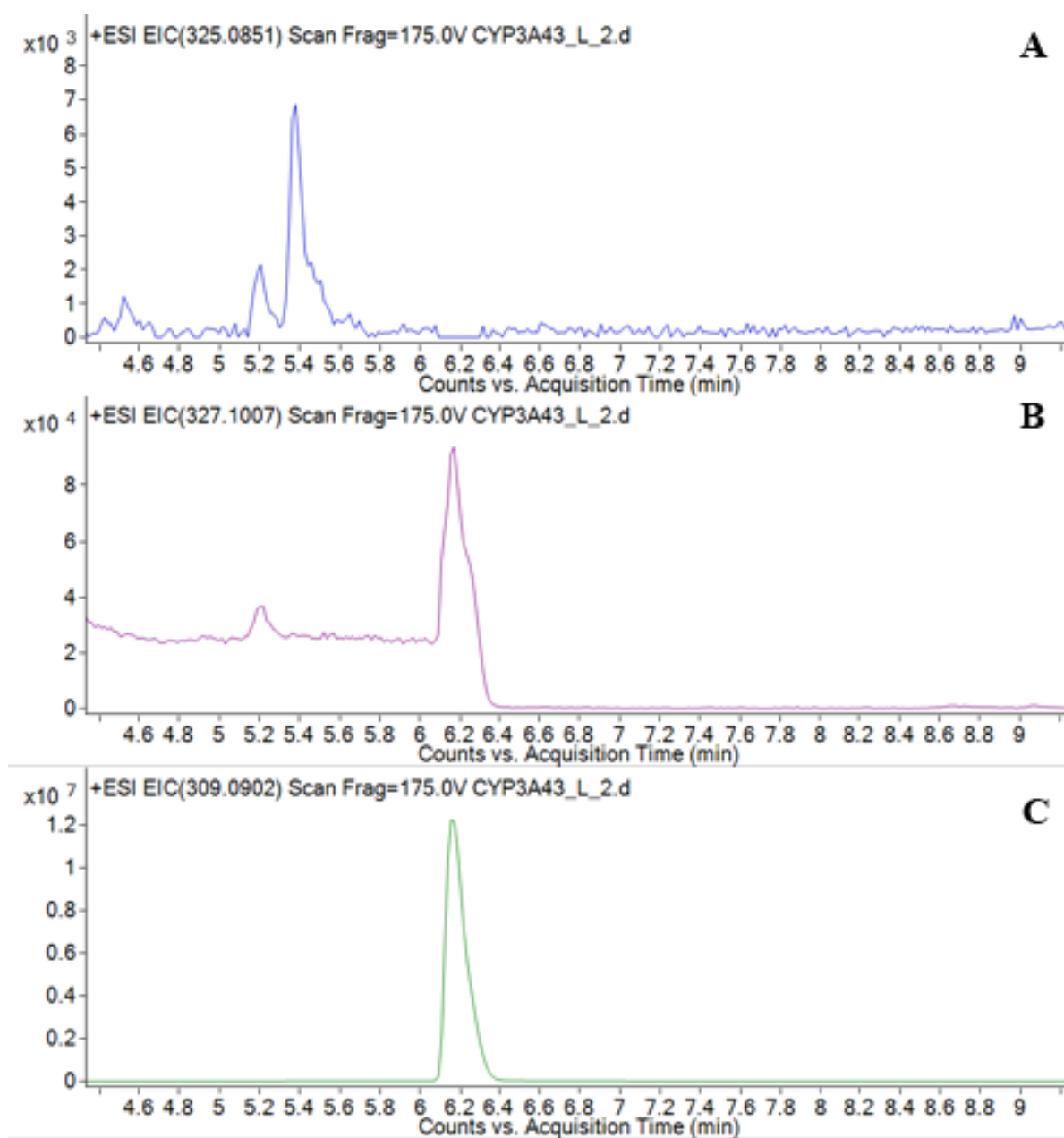
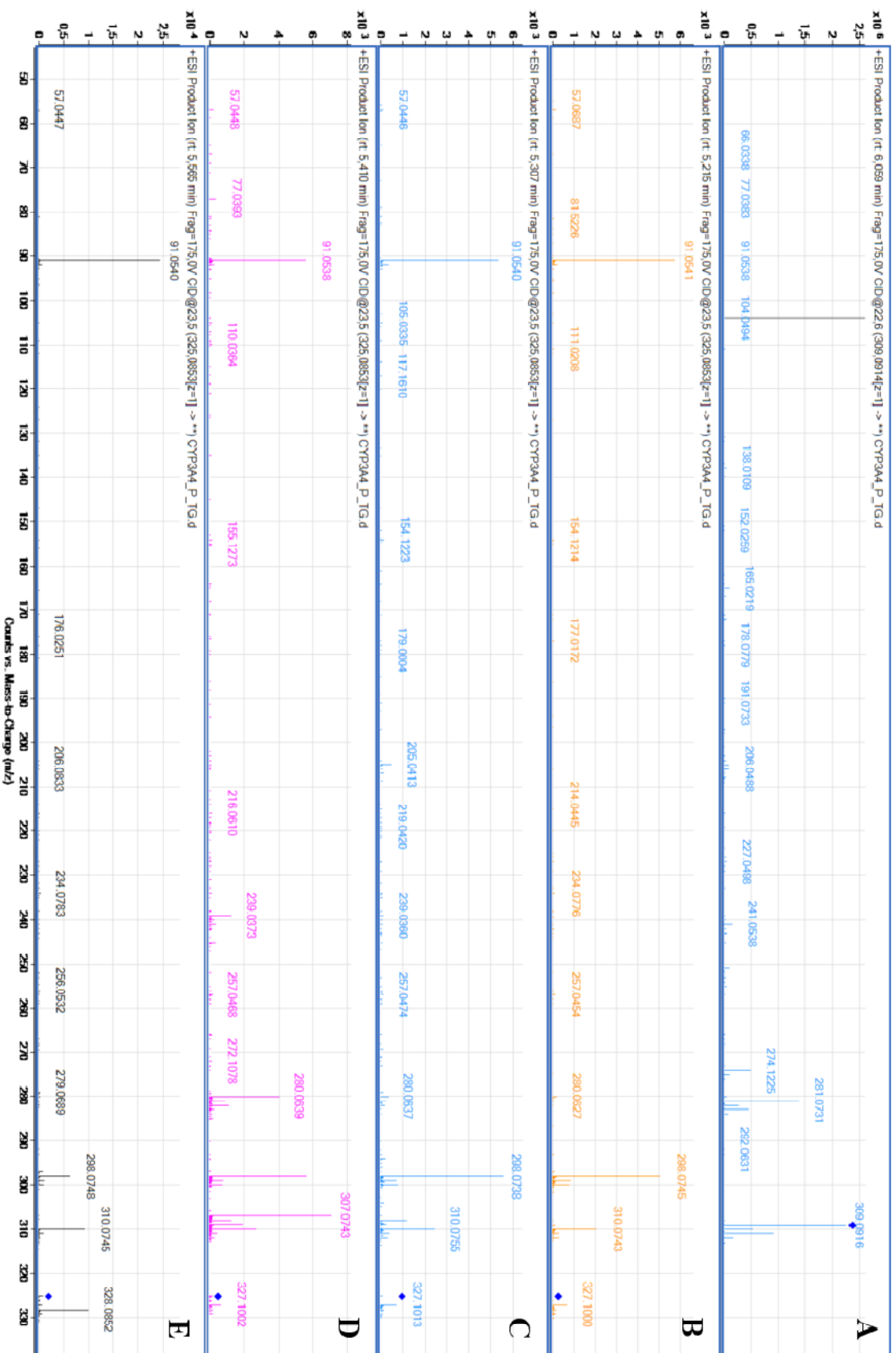


## Supplementary Informations



**Figure S1** EIC spectra of CYP3A43 L293P biotransformed alprazolam samples  $m/z$  325.0851 ( $[(C_{17}H_{13}ClN_4O+H)^+]$ , **A**),  $m/z$  327.1007 ( $[(C_{17}H_{15}ClN_4O+H)^+]$ , **B**) and  $m/z$  309.0902 ( $[(C_{17}H_{13}ClN_4+H)^+]$ , **C**).



**Figure S2.** MS<sup>2</sup> spectra with different retention time of alprazolam and mono oxygen metabolites of alprazolam in the CYP3A biotransformed alprazolam sample. The first spectrum (**A**) represents alprazolam (precursor  $m/z$  309.0902 ( $[C_{17}H_{13}ClN_4+H]^+$ ), RT = 6.1 min, fragments of  $m/z$  274, 281, and 292). The third (**C**) and fourth spectra (**D**) are the MS<sup>2</sup> spectra of two isomers of 4-hydroxy alprazolam (precursor  $m/z$  325.0851 ( $[C_{17}H_{13}ClN_4O+H]^+$ ), RT<sub>C</sub> = 5.3 min, RT<sub>D</sub> = 5.4 min, which are most similar showing fragment ions at  $m/z$  310, 307, 298, 280, 257 and 239, especially the 307 (-H<sub>2</sub>O), 280 (-NHCHOH) and 239 (-C<sub>3</sub>H<sub>6</sub>N<sub>2</sub>O). The second MS<sup>2</sup> spectrum (**B**) is assigned to 5-N-O alprazolam (precursor  $m/z$  325.0851 ( $[C_{17}H_{13}ClN_4O+H]^+$ ), RT<sub>B</sub> = 5.2 min, closely eluting to the compounds at RT = 5.3 min/5.4 min) showing the common fragment ions at 310, 298, 280 and 257, and a new fragment with the MS at 214 (-C<sub>4</sub>N<sub>3</sub>O) and the absence of 307 can help to distinguish it from the spectra at 5.3 min/5.4 min. For the fifth spectrum (**E**, precursor  $m/z$  325.0851 ( $[C_{17}H_{13}ClN_4O+H]^+$ ), RT<sub>E</sub> = 5.6 min) the product ions at  $m/z$  310, 298, 279, 256 and 206 represent the MS<sup>2</sup> spectrum of  $\alpha$ -hydroxy alprazolam.

**Table S1.** Sites of Metabolism (SoM) prediction for alprazolam with CYP3A4 on SMARTCyp.

Atom identifier	Energy estimate [kcal/mol]	Corresponding metabolite
C.4	48.5	M <sub>1</sub> /M <sub>2</sub> (4 $\alpha$ / $\beta$ hydroxy alprazolam)
C. $\alpha$	59.9	M <sub>3</sub> ( $\alpha$ -hydroxy alprazolam)
N.5	61.9	M <sub>4</sub> (5N-O alprazolam)