Metabolic disposition of triazolam and clobazam in humanized CYP3A mice with a double knockout background of mouse *Cyp2c* and *Cyp3a* genes

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Drug Metabolism and Disposition
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Supplemental Table 3. Conditions used for LC-MS/MS analysis.

The LC-MS/MS system consisted of either Shimadzu LCMS8050 equipped with Nexera series HPLC system (System 1) or SCIEX QTRAP4500 equipped with a Shimadzu Prominence series HPLC system (System 2). Data from System 1 and 2 were processed using Lab Solution software (version 5.97 SP1, Shimadzu) and MultiQuant software (version 1.3, SCIEX), respectively. Mobile phases consisted of 0.1% formic acid in distilled water (Mobile phase 1), 0.1% formic acid in acetonitrile/methanol=1/1 (v/v) (Mobile phase 2), 10% methanol in distilled water (Mobile phase 3) and methanol (Mobile phase 4). The column was a COSMOSIL 3C18-MS-II (2.0 mm x 50 mm; Nacalai Tesque). Temperature of the autosampler and column was maintained at 4°C and 40°C, respectively.

<table>
<tr><th>Sample</th><th>drug</th><th>MRM transition</th><th>System</th><th>retention time (min)</th><th>Flow rate (ml/min) Time; rate</th><th>Injection volume (μL)</th><th>Mobile phase (A/B)</th><th>Gradient condition Time; B concentration</th></tr>
<tr><td rowspan="3">incubation mixture</td><td>1'-hydroxytriazolam</td><td>359.3 > 176.1</td><td rowspan="3">1</td><td>1.92</td><td rowspan="5">0.6</td><td rowspan="3">1</td><td rowspan="5">1/2</td><td rowspan="3">0-0.6 min; 15%
0.6-2.0 min;15%→90%
2.0-3.8 min; 90%
3.8-3.81 min; 90%→15%
3.81-4.51 min; 15%</td></tr>
<tr><td>4-hydroxytriazolam</td><td>359.3 > 314.0</td><td>1.93</td></tr>
<tr><td>propranolol</td><td>260.1 > 116.2</td><td>1.59</td></tr>
<tr><td rowspan="2">plasma</td><td>triazolam</td><td>343.0 > 308.1</td><td rowspan="2">2</td><td>2.40</td><td rowspan="2">5</td><td rowspan="2">0-2.0 min; 15%→90%
2.0-3.7 min; 90%</td></tr>
<tr><td>1'-hydroxytriazolam</td><td>359.0 > 175.9</td><td>2.31</td></tr>
</table>

	4-hydroxytriazolam	359.0 > 313.9		2.32				3.7-3.71 min; 90%→15%
	propranolol	260.1 > 116.0		1.89				3.71-5.0 min; 15%
plasma	CLB	301.1 > 259.3	1	3.30	0-2.4 min; 0.4→0.6 2.4-7.5 min; 0.6	2	3/4	0-1.0 min; 5% 1.0-2.4 min; 5%→98% 2.4-7.0 min; 98% 7.0-7.01 min; 98%→5% 7.01-7.5 min; 5%
	NCLB	287.1 > 245.2		3.23				
	oxazepam	287.1 > 241.2		3.35				