

# Supplementary Materials: Identification and Distribution of Novel Metabolites of Lolitrem B in Mice by High-Resolution Mass Spectrometry

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**Table S1.** Parameters used to identify lolitrem B metabolites with Compound Discoverer™

	Lolitrem B (C <sub>42</sub> H <sub>55</sub> NO <sub>7</sub> )
phase I transformations	<b>Oxidation, reduction, dehydration, dehydrogenation, hydration and reduction</b>
phase II transformations	<b>acetylation, glucuronide conjugation, methylation and sulfation</b>
maximum number of dealkylation steps	<b>1</b>
maximum number of phase II reactions	<b>1</b>
maximum number of reactions	<b>5</b>

**Table S2.** Data-dependent acquisition during analysis of liver samples with LCMS on Thermo QExactive.

<b>Mass [m/z]</b>	<b>Polarity</b>	<b>Start [min]</b>	<b>End [min]</b>	<b>Mass [m/z]</b>	<b>Polarity</b>	<b>Start [min]</b>	<b>End [min]</b>
602.34692	Positive	6.38	8.38	716.41278	Positive	7.77	9.77
618.34125	Positive	5.47	7.47	718.39233	Positive	5.75	7.75
620.35864	Positive	5.99	7.99	718.39368	Positive	6.22	8.22
622.37103	Positive	7.68	9.68	718.39386	Positive	6.76	8.76
636.35034	Positive	5.44	7.44	718.39423	Positive	5.47	7.47
636.35406	Positive	4.83	6.83	718.42853	Positive	7.79	9.89
650.38513	Positive	7.96	9.96	718.42926	Positive	7.68	9.68
654.41608	Positive	7.71	9.71	718.42938	Positive	7.85	9.85
655.40021	Positive	7.72	9.72	722.42444	Positive	7.88	9.88
662.37012	Positive	7.79	9.79	726.39746	Positive	7.88	9.88
666.37689	Positive	7.55	9.55	726.40179	Positive	7.77	9.77
666.37909	Positive	7.71	9.71	730.39178	Positive	7.63	9.63
668.39374	Positive	7.71	9.71	730.39844	Positive	7.52	9.52
686.40308	Positive	7.56	9.56	732.37341	Positive	5.82	7.82
688.41754	Positive	7.85	9.85	732.37616	Positive	5.41	7.41
699.38922	Positive	7.77	9.77	732.40814	Positive	7.03	9.03
699.39215	Positive	7.85	9.85	734.38873	Positive	4.83	6.83
700.38251	Positive	6.78	8.78	734.38885	Positive	5.90	7.90
700.38361	Positive	6.97	8.97	734.38989	Positive	5.36	7.36
702.39832	Positive	6.74	8.74	734.39020	Positive	5.28	7.28
702.39948	Positive	6.81	8.81	734.39056	Positive	5.09	7.09
702.39954	Positive	6.39	8.39	734.39062	Positive	5.79	7.79
702.39960	Positive	6.86	8.86	734.42273	Positive	7.85	9.85
702.40222	Positive	7.09	9.09	736.40515	Positive	7.82	9.82
714.40143	Positive	7.61	9.61	736.40649	Positive	7.57	9.57
714.40308	Positive	7.90	9.90	744.40967	Positive	7.58	9.58
716.37708	Positive	6.08	8.95	746.4259	Positive	7.77	9.77
716.37781	Positive	6.68	8.68	748.36908	Positive	5.59	7.59
716.37866	Positive	6.80	8.80	762.41846	Positive	7.57	9.57
716.37982	Positive	5.27	7.27	762.4245	Positive	7.68	9.68
716.38098	Positive	6.18	8.18	720.41116	Positive	7.00	9.00

**Table S3.** The mean relative abundance of lolitrem B biotransformation products distributed in the body and brain tissue of mice exposed to lolitrem B at high (2.0 mg/kg b.wt) and low (0.5 mg/kg b.wt) doses at time points 6 h and 24 h post treatment.

Compound	Lolitrem B <sup>1</sup>	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
<b>[M+H]<sup>+</sup> m/z</b>	686.4036	702.4014	702.3971	618.3426	602.3470	602.3466	716.3779	716.3792	718.3946	718.3954	734.3923
<b>Retention time, min</b>	8.56	7.34	7.83	6.46	7.38	7.72	7.77	7.66	7.73	6.45	6.89
<b>Kidney</b>											
<b>High Dose 6 h</b>	10275508 (n=7)	325609 (n=7)	82340 (n=6)	6297 (n=3)	83863 (n=7)	8715 (n=2)	27560 (n=4)	114476 (n=5)	37896 (n=7)	91987 (n=7)	ND
<b>High Dose 24 h</b>	7923119 (n=8)	38878 (n=8)	19268 (n=1)	4552 (n=1)	10393 (n=7)	10220 (n=1)	7464 (n=1)	ND	ND	5706 (n=3)	ND
<b>Low Dose 6 h</b>	1714329 (n=8)	35471 (n=7)	15580 (n=1)	6152 (n=3)	9248 (n=5)	15148 (n=1)	9633 (n=1)	ND	ND	6336 (n=3)	ND
<b>Low Dose 24 h</b>	1342210 (n=8)	7044 (n=3)	ND	5732 (n=2)	6515 (n=2)	8067 (n=1)	9460 (n=1)	ND	8661 (n=1)	ND	ND
<b>Liver</b>											
<b>High Dose 6 h</b>	3510325 (n=7)	1381557 (n=8)	1647784 (n=8)	146661 (n=8)	447949 (n=8)	41949 (n=6)	451932 (n=7)	2182552 (n=8)	4184258 (n=8)	3254011 (n=8)	209101 (n=7)
<b>High Dose 24 h</b>	888700 (n=8)	141226 (n=8)	657443 (n=8)	20237 (n=6)	39645 (n=8)	ND	139136 (n=8)	90107 (n=6)	270427 (n=8)	783628 (n=8)	55205 (n=8)
<b>Low Dose 6 h</b>	517071 (n=8)	358957 (n=8)	381751 (n=8)	21101 (n=6)	113841 (n=6)	11835 (n=2)	45921 (n=5)	359662 (n=6)	1047318 (n=8)	927846 (n=8)	40885 (n=6)
<b>Low Dose 24 h</b>	180935 (n=5)	41020 (n=4)	100081 (n=5)	28173 (n=2)	9337 (n=3)	ND	14829 (n=3)	ND	20144 (n=4)	190591 (n=5)	11475 (n=1)
<b>Cerebral cortex</b>											
<b>High Dose 6 h</b>	35979 (n=7)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<b>High Dose 24 h</b>	40751 (n=8)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<b>Low Dose 6 h</b>	12822 (n=3)	ND	ND	ND	3858 (n=1)	ND	ND	ND	ND	ND	ND
<b>Low Dose 24 h</b>	14008 (n=4)	ND	ND	ND	ND	6418 (n=1)	ND	ND	ND	ND	ND

Thalamus											
<b>High Dose 6 h</b>	21943 (n=6)	5080 (n=1)	6085 (n=1)	ND	ND	6402 (n=1)	ND	ND	ND	ND	ND
<b>High Dose 24 h</b>	27861 (n=6)	ND	7151 (n=1)	ND	ND	ND	ND	6097 (n=1)	5350 (n=2)	ND	ND
<b>Low Dose 6 h</b>	18211 (n=3)	ND	5442 (n=1)	ND	ND	ND	ND	ND	5498 (n=2)	ND	ND
<b>Low Dose 24 h</b>	22645 (n=7)	ND	4729 (n=1)	ND	ND	ND	ND	5692 (n=1)	5708 (n=2)	ND	ND
Cerebellum											
<b>High Dose 6 h</b>	ND	6476 (n=3)	5515 (n=1)	ND	ND	ND	ND	7227 (n=1)	5526 (n=3)	ND	ND
<b>High Dose 24 h</b>	ND	6755 (n=1)	ND	ND	ND	ND	ND	ND	7325 (n=1)	6324 (n=1)	ND
<b>Low Dose 6 h</b>	ND	ND	6373 (n=1)	10010 (n=1)	ND	ND	5192 (n=1)	6859 (n=2)	ND	ND	ND
<b>Low Dose 24 h</b>	ND	ND	ND	ND	7931 (n=1)	ND	ND	5643 (n=2)	ND	8517 (n=1)	ND
Brainstem											
<b>High Dose 6 h</b>	ND	ND	5294 (n=1)	ND	ND	5793 (n=1)	ND	6617 (n=2)	ND	6334 (n=4)	ND
<b>High Dose 24 h</b>	ND	6197 (n=1)	ND	ND	ND	ND	ND	6299 (n=1)	ND	7016 (n=3)	ND
<b>Low Dose 6 h</b>	ND	ND	6015 (n=2)	ND	ND	ND	ND	7421 (n=2)	6201 (n=1)	ND	ND
<b>Low Dose 24 h</b>	ND	ND	6277 (n=1)	7966 (n=2)	ND	ND	ND	5946 (n=1)	ND	ND	ND

ND= Not detected, n = number of observations within the treatment group.

<sup>1</sup> Lolitrem B quantitative analysis is described in Reddy et al [13].