



Supplementary material

# Validation of an LC-MS/MS method to quantify the new TRPC6 inhibitor SH045 (larixyl *N*-methylcarbamate) and its application in an exploratory pharmacokinetic study in mice

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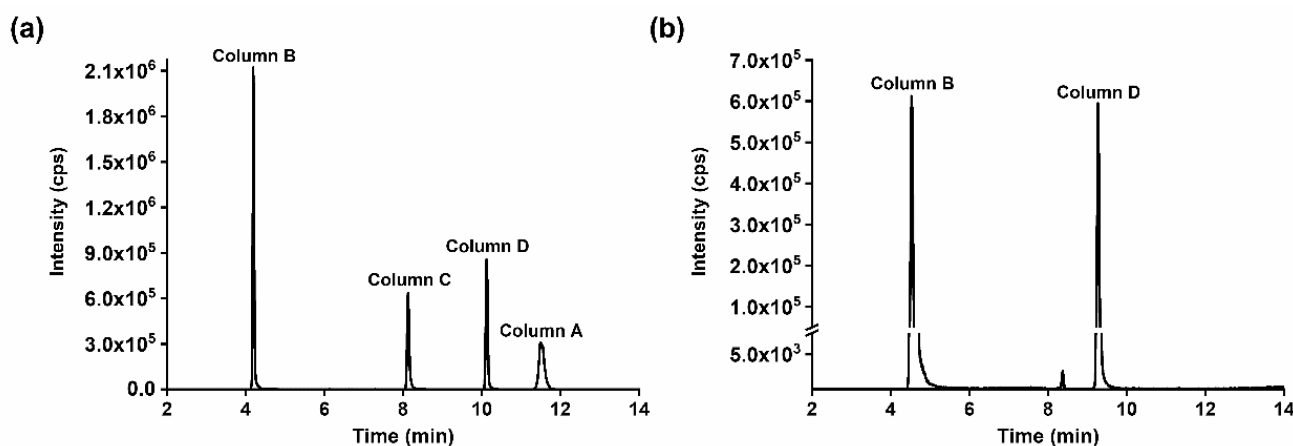
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**Figure S1.** Peak shapes of SH045 in (a) pure solvent and in (b) plasma after separation at different columns: A - Luna C18(2) 100 Å (100 × 3.0 mm, 3 μm); B - Luna C18 100 Å (50 × 2.1 mm, 5 μm); C - Zorbax Eclipse XDB-CN 80 Å (150 × 3.0 mm, 3.5 μm); D - Zorbax Eclipse XDB-C8 80 Å (100 × 3.0 mm, 3.5 μm), see also Table S1.

**Table S1.** Features of considered columns, LC method, peak shapes for SH045 in solvent and mice plasma.

Column <sup>c</sup>	Solvent <sup>a</sup>				Plasma <sup>b</sup>	
	A	B	C	D	B	D
Size (mm)	100 × 3.0	50 × 2.1	150 × 3.0	100 × 3.0		
Particle Size (µm)	3	5	3.5	3.5	see left	see left
Stationary phase	C18	C18	Cyano	C8		
Gradient (ACN %)	0-13 min, 20-90%; 13-16 min, 90%; 16-20 min, 20%	0-5 min, 40-90%; 5-6 min, 90%; 6-8 min, 40%	see A	see A	0-5 min, 49-90%; 5-6 min, 90%; 6-8 min, 49%	see left
Injection volume (µL)	0.4	0.4	0.4	0.4	10	10
Flow rate (mL/min)	0.7	0.4	0.7	0.7	0.4	0.7
SH045 retention time (min)	11.52	4.20	8.13	10.12	4.54	9.27
Peak area (cps × min)	3.17×10 <sup>6</sup>	5.33×10 <sup>6</sup>	2.58×10 <sup>6</sup>	2.90×10 <sup>6</sup>	2.51×10 <sup>6</sup>	2.01×10 <sup>6</sup>
Height (cps)	3.26×10 <sup>5</sup>	2.21×10 <sup>6</sup>	6.65×10 <sup>5</sup>	8.36×10 <sup>5</sup>	6.86×10 <sup>5</sup>	6.14×10 <sup>5</sup>
FWHM (min)	0.15	0.03	0.06	0.05	0.05	0.05
Theoretical plates ( <i>N</i> ) <sup>d</sup>	32,676	108,068	101,716	226,950	45,675	190,427
Asymmetry	1.308	1.017	1.200	1.422	0.985	1.216
Tailing factor	1.200	1.045	1.250	1.226	1.009	1.124

<sup>a</sup> 8.58 µg/mL SH045 in water/ ACN 1:1 (*v/v*). <sup>b</sup> 909 ng/mL SH045 in mice plasma. <sup>c</sup> column A: Luna C18(2) 100 Å (Phenomenex, Aschaffenburg, Germany), column B: Luna C18 100 Å (Phenomenex), column C: Zorbax Eclipse XDB-CN 80 Å (Agilent Technologies, Waldbronn, Germany), and column D: Zorbax Eclipse XDB-C8 80 Å (Agilent Technologies) <sup>d</sup>Theoretical plates was calculated as:  $N = 5.54 \times (R.t./FWHM)^2$  where *R.t.* is the retention time of SH045 and FWHM is the Full Width at Half Maximum of peak height.

**Table S2.** Calibration validation for the inter-day accuracy for SH045 of three subsequent days.

Calibration sample nominal concentration (ng/mL)	Concentrations found, mean ± SEM (ng/mL)	Accuracy (%)	Precision (%)
2	2.0 ± 0.1	98.2	8.7
5	5.1 ± 0.2	101.8	5.3
10	9.8 ± 0.3	97.8	5.8
100	101 ± 5	100.6	7.7
200	193 ± 6	96.8	5.0
800	796 ± 1	99.5	0.2
1200	1228 ± 17	102.3	2.4
1600	1588 ± 53	99.3	6.1