

### Supplementary File

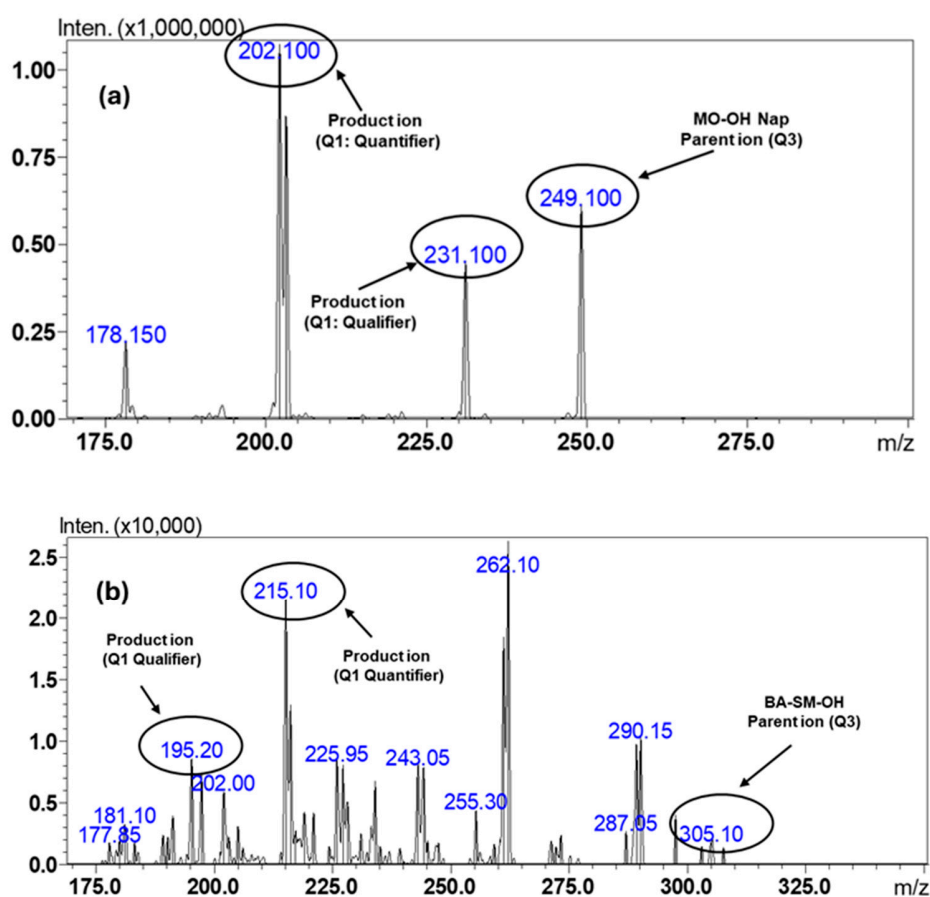
#### **Development and validation of a LC-MS/MS assay for the quantitation of MO-OH-Nap Tropolone in mouse plasma: Application to in-vitro and in-vivo pharmacokinetic studies**

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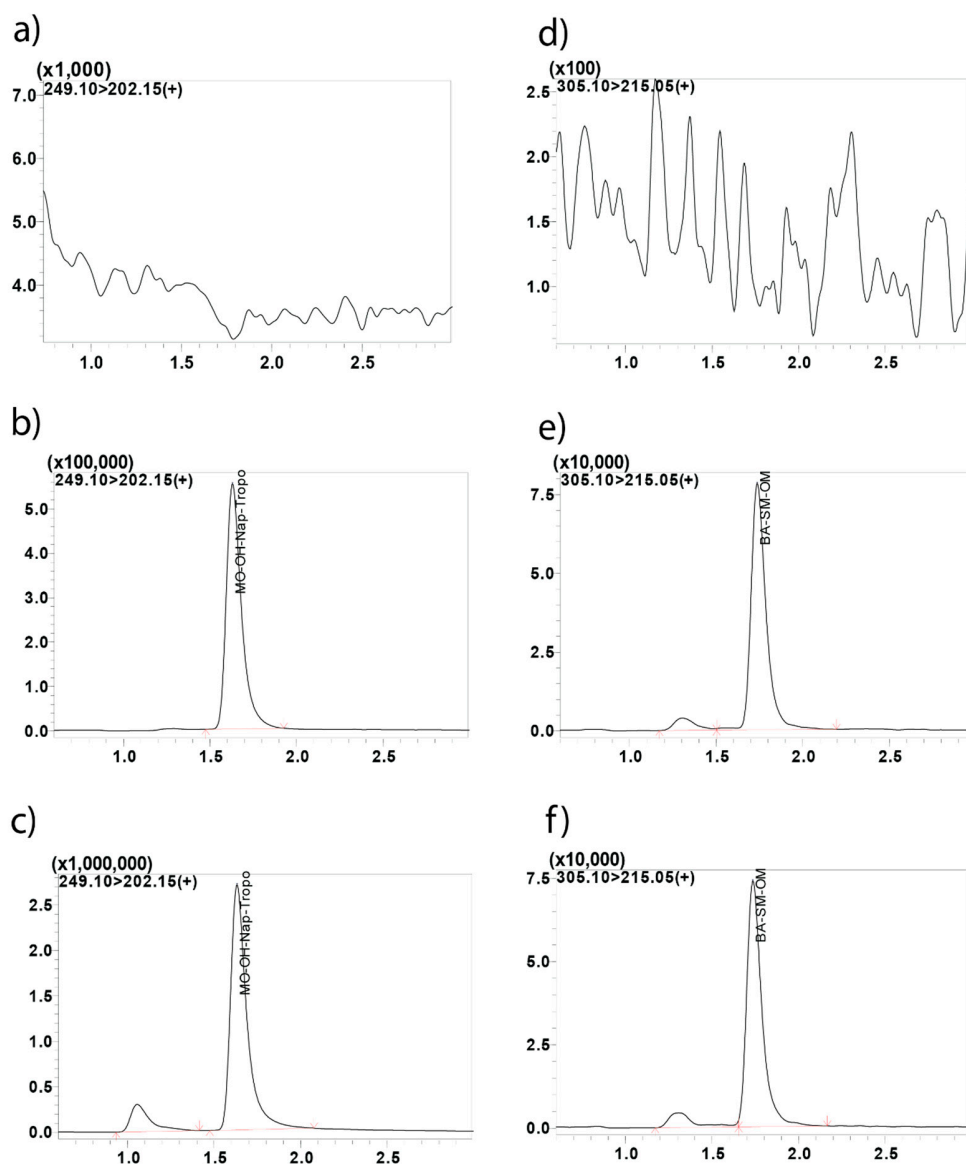
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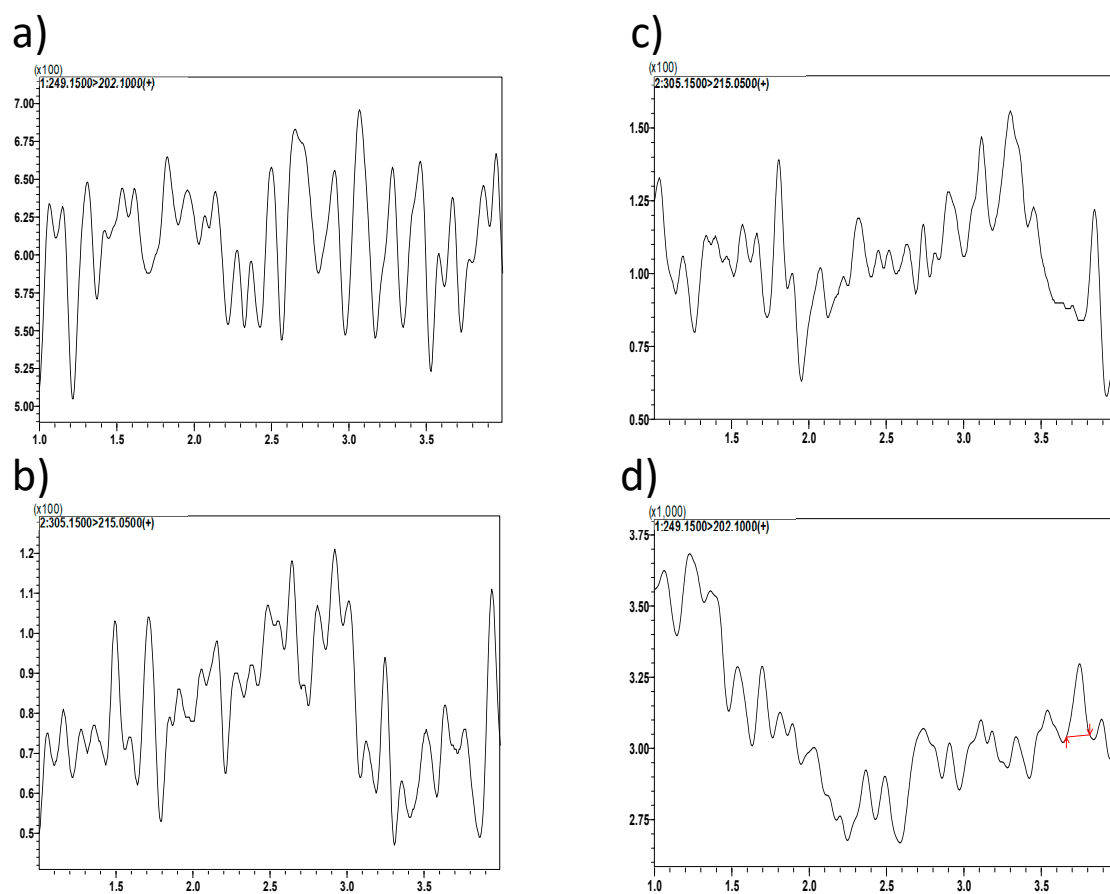
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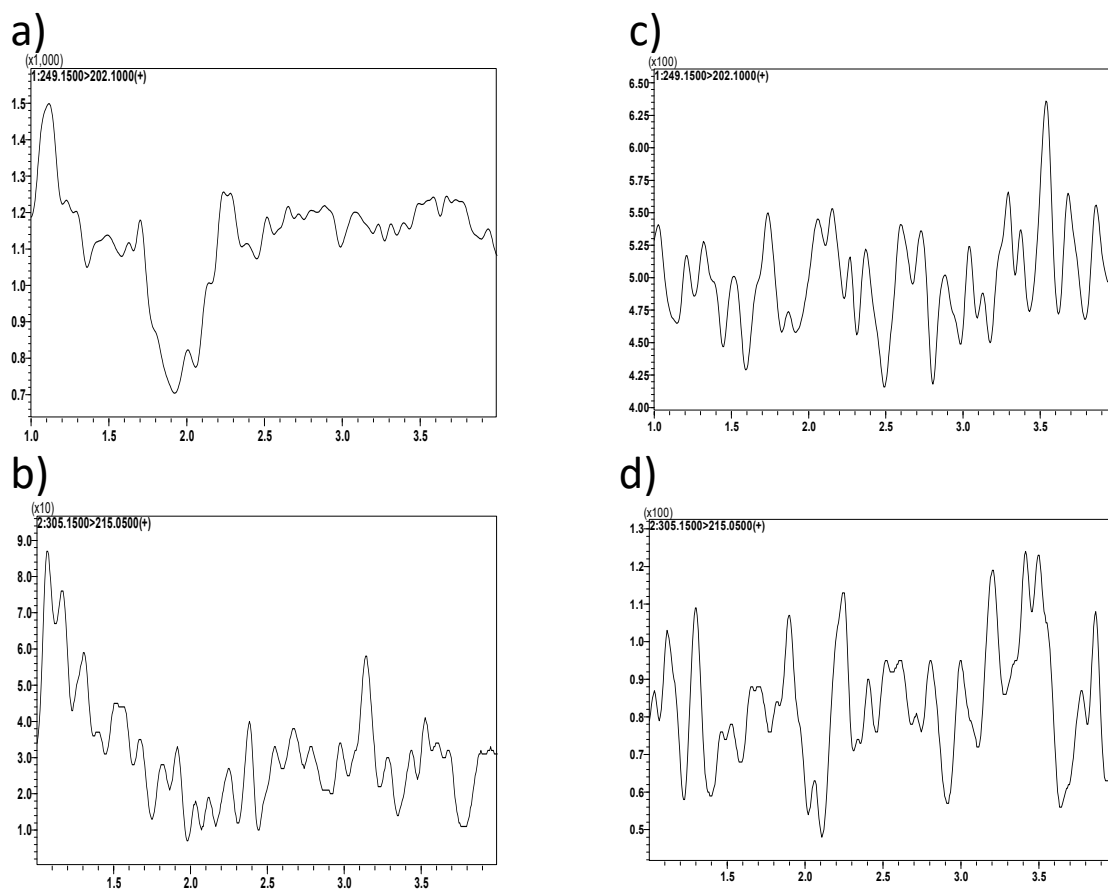
**Figure S1.** MS/MS spectra of MO-OH-Nap tropolone (a) and BA-SM-OH (IS) (b).



**Figure S2.** MRM ion-chromatograms of MO-OH and IS following PPT extraction of (a) blank mouse plasma using the same conditions for MO-OH-Nap detection, (b) MO-OH-Nap spiked in mouse plasma at 10 ng/mL (retention time 1.7 min), (c) MO-OH-Nap mouse plasma study sample (retention time 1.7 min.), (d) blank mouse plasma using the conditions for BA-SH-OH (IS) detection.(e) BA-SH-OH spiked at 200 ng/mL in mouse plasma (retention time 1.85 min,) and (f) BA-SH-OH spiked at 200 ng/mL in mouse plasma study sample (retention time 1.85 min).



**Figure S3.** Chromatograms of blank sample using the same conditions for MO-OH-Nap detection after running the SPE HQC spiked (a) MO-OH-Nap in plasma, (b) BA-SH-OH in plasma, (c) MO-OH-Nap in liver tissue homogenate, (b) BA-SH-OH in liver tissue homogenate.



**Figure S4.** Chromatograms of blank sample using the same conditions for MO-OH-Nap detection after running the PPT HQC spiked (a) MO-OH-Nap in plasma, (b) BA-SH-OH in plasma , (c) MO-OH-Nap in liver tissue homogenate, (b) BA-SH-OH in liver tissue homogenate.

**Table S1.** Accuracy and precision. Intra- and inter-assay accuracy and precision of MO-OH-Nap tropolone in liver tissue homogenates (n = 6).

Extraction techniques	Nominal Conc. (ng/mL)	Accuracy		Precision	
		%Bias intra-assay	%Bias inter-assay	%RSD intra-assay	%RSD inter-assay
SPE	LLOQ (1.0 ng/mL)	9.34	12.20	1.61	1.12
	LQC (3.0 ng/mL)	1.83	2.79	0.49	4.52
	MQC (100 ng/mL)	13.03	-14.77	13.78	9.19
	HQC (375 ng/mL)	11.36	-4.06	8.00	13.48

**Table S2:** Mean matrix effect of MO-OH-Nap tropolone mice liver tissue homogenates following SPE extraction (n=3).

Extraction techniques	Nominal Conc. (ng/mL)	MO-OH-Nap Tropolone	BA-SM-OH (IS)
		Mean Matrix effect (%)	Mean Matrix effect (%)
SPE	LQC (3.0 ng/mL)	102.45 ±2.24	99.22 ±2.22
	MQC (100 ng/mL)	111.06 ± 17.71	
	HQC (375 ng/mL)	84.00 ±12.43	