Electronic Supplementary Material (ESI) for Analytical Methods.

SUPPORTING INFORMATION FOR:

Rapid Differentiation of Xylazine Metabolites using SLIM IM-MS

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SUPPORTING TABLES

Table S1. Instrumental parameters for the Agilent 6560 IM-QTOF.

Instrument Region	Instrumental Parameter	Experimental Value
Source	Gas Temp	325 °C
	Drying Gas	12 L/min
	Nebulizer	20 psi
	Sheath Gas Temp	275 °C
	Sheath Gas Flow	10 L/min
	VCap	4000 V
	Nozzle Voltage (Expt)	1000 V
	Fragmentor	400 V
	Oct 1 RF Vpp	750 V
High Pressure Funnel	High Pressure Funnel Delta	150 V
	High Pressure Funnel RF	200 V
Trapping Funnel	Trap Funnel Delta	180 V
	Trap Funnel RF	200 V
	Trap Funnel Exit	10 V
	Entrance Grid Delta	10 V
	Entrance Grid Low	96 V
	Entrance Grid High	107 V
	Trap Entrance	91 V
	Trap Exit	90 V
	Trap Fill Time	3900 µs
	Trap Release Time	150 µs
	Trap Exit Grid 1 Delta	4 V
	Trap Exit Grid 1 Low	86.3 V
	Trap Exit Grid 1 High	90.3 V
	Trap Exit Grid 2 Delta	8.5 V
	Trap Exit Grid 2 Low	85.8 V
	Trap Exit Grid 2 High	94.3 V
Drift Tube	Drift Tube Entrance Voltage	1700 V
	Drift Tube Exit Voltage	250 V
	Drift Tube Field Strength	18.6 V/cm

Table S2. Instrumental parameters for the MOBILion MOBIE SLIM & Agilent 6546 QTOF.

Instrument Region	Instrumental Parameter	Experimental Value
Ionization Source	Polarity	Positive
	Gas Temp	325 °C
	Drying Gas	12 L/min
	Nebulizer	20 psi
	Sheath Gas Temp	275 °C
	Sheath Gas Flow	10 L/min
	VCap	4000 V
	Nozzle Voltage (Expt)	1000 V
	Fragmentor	400 V
	Oct 1 RF Vpp	750 V
SLIM Conditions	Funnel In	165 V
	Funnel Out	100 V
	Funnel Conductance Limit (CL)	95 V
	SLIM Bias	90 V
	SLIM Mode	HRIM (13 m)
	SLIM Wave Shape	Sine
	Fill Time	16 ms
	Trap Time	0.1 ms
	Release Time	3.2 ms
	IMS Frame Length	600 ms
	Fill TW Frequency	15 kHz
	Fill TW Amplitude	5 V_{pp}
	Release TW Frequency	15 kHz
	Release TW Amplitude	30 V_{pp}
	Separation TW Frequency	25 kHz
	Separation TW Speed	225 m/s
	Separation TW Amplitude	30 V_{pp}
	SLIM Exit CL	50 V
	Quad Bias	45 V
	Quad Pressure (Rough Vac)	2.5 Torr
	SLIM RF Amplitude	270 V
	SLIM RF Frequency	1200 kHz

Table S3. Chromatographic parameters. All experiments performed on an Agilent 1290 Infinity II UHPLC system.

Parameter	**Value**	
Column	Agilent ZORBAX Extend-C18 column (2.1 × 50 mm, 1.8 µm)	
Injection Volume	10 µL	
Column Temperature	50 °C	
Flow Rate	0.500 mL/min	
Gradient	Mobile Phase A: Water (0.1% Formic Acid)	Mobile Phase B: Methanol
0.00	90%	10%
2.00	90%	10%
3.00	40%	60%
8.00	0%	100%
9.00	0%	100%
9.01	90%	10%

Table S4. Instrumental parameters for the Agilent 6560 IM-QTOF.

Instrument Region	Instrumental Parameter	Experimental Value
Source	Gas Temp	325 °C
	Drying Gas	12 L/min
	Nebulizer	20 psi
	Sheath Gas Temp	275 °C
	Sheath Gas Flow	10 L/min
	VCap	4000 V
	Nozzle Voltage (Expt)	1000 V
	Fragmentor	400 V
	Oct 1 RF Vpp	750 V
High Pressure Funnel	High Pressure Funnel Delta	150 V
	High Pressure Funnel RF	200 V
Trapping Funnel	Trap Funnel Delta	180 V
	Trap Funnel RF	200 V
	Trap Funnel Exit	10 V
	Entrance Grid Delta	10 V
	Entrance Grid Low	96 V
	Entrance Grid High	107 V
	Trap Entrance	91 V
	Trap Exit	90 V
	Trap Fill Time	3900 µs
	Trap Release Time	150 µs
	Trap Exit Grid 1 Delta	4 V
	Trap Exit Grid 1 Low	86.3 V
	Trap Exit Grid 1 High	90.3 V
	Trap Exit Grid 2 Delta	8.5 V
	Trap Exit Grid 2 Low	85.8 V
	Trap Exit Grid 2 High	94.3 V
Drift Tube	Drift Tube Entrance Voltage	1700 V
	Drift Tube Exit Voltage	250 V
	Drift Tube Field Strength	18.6 V/cm

Table S5. Comparison of $^{DT}CCS_{N2}$ and $^{SLIM}CCS_{N2}$ measurements of xylazine and its metabolites, including as singly and doubly dansylated products; also includes associated chemical formula/theoretical mass.

Compound	Formula	$[M+H]^+$ *m/z*	$^{DT}CCS_{N2}$ (Å^2)	$^{SLIM}CCS_{N2}$ (Å^2)	ΔCCS
Xylazine	$C_{12}H_{16}N_2S$	221.111	148.1 ± 0.1	148.5 ± 0.1	+0.3%
3-Hydroxy Xylazine	$C_{12}H_{16}N_2OS$	237.106	154.4 ± 0.1	153.4 ± 0.1	-0.6%
4-Hydroxy Xylazine	$C_{12}H_{16}N_2OS$	237.106	154.4 ± 0.1	153.1 ± 0.1	-0.8%
Dansylated 3-Hydroxy Xylazine	$C_{24}H_{27}N_3O_3S_2$	470.157	214.4 ± 0.1	213.5 ± 0.1	-0.4%
Dansylated 4-Hydroxy Xylazine	$C_{24}H_{27}N_3O_3S_2$	470.157	218.4 ± 0.1	218.4 ± 0.1	0%
Doubly Dansylated 3-Hydroxy Xylazine	$C_{36}H_{38}N_4O_5S_3$	703.208	261.8 ± 0.1	258.0 ± 0.1	-1.5%
				264.4 ± 0.1	+1.0%
Doubly Dansylated 4-Hydroxy Xylazine	$C_{36}H_{38}N_4O_5S_3$	703.208	264.4 ± 0.1	260.9 ± 0.1	-1.3%
			273.6 ± 0.1	271.1 ± 0.1	-0.9%

SUPPORTING FIGURES

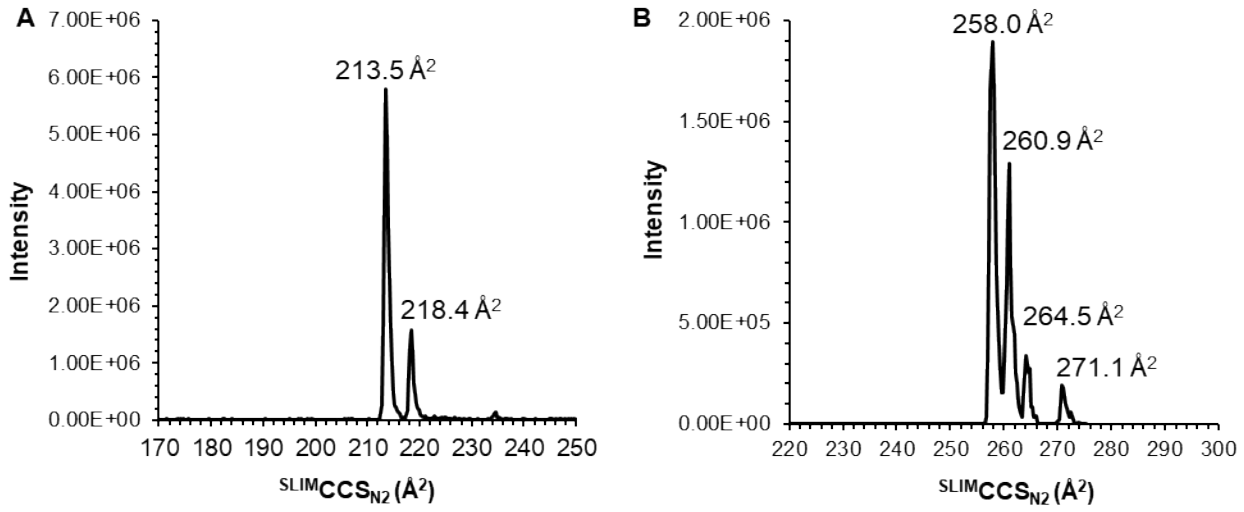


Figure S1. SLIM CCS distribution for (A) a mixture of singly dansylated 3-hydroxy and 4-hydroxy xylazine, and (B) a mixture of doubly dansylated 3-hydroxy and 4-hydroxy xylazine.

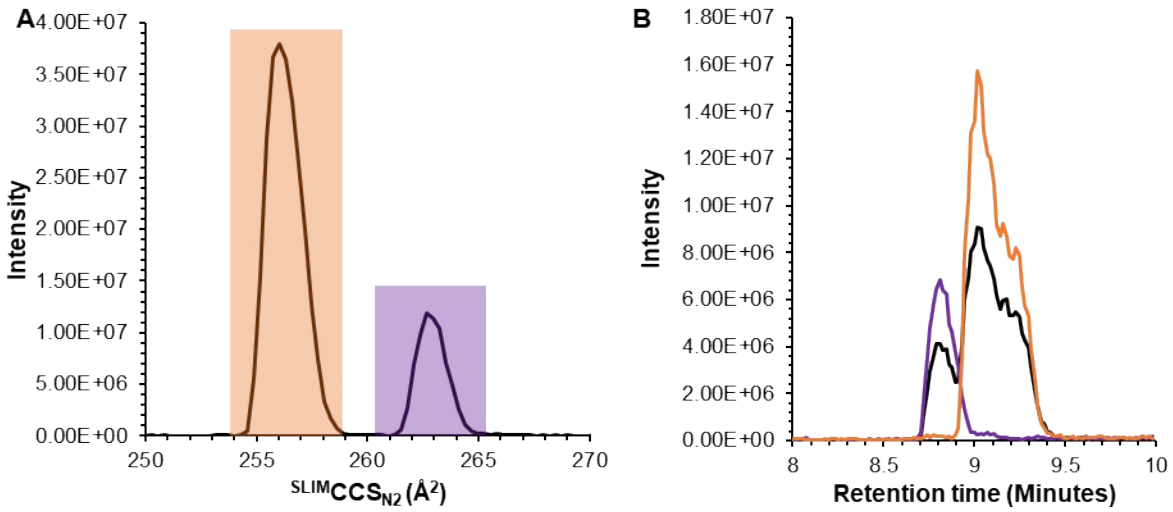


Figure S2. (A) SLIM CCS distribution for atropisomers of doubly dansylated 3-hydroxy xylazine. (B) Extracted ion chromatogram for each of the mobility peaks, demonstrating their chromatographic separation; mixture of the two is shown in the black trace.

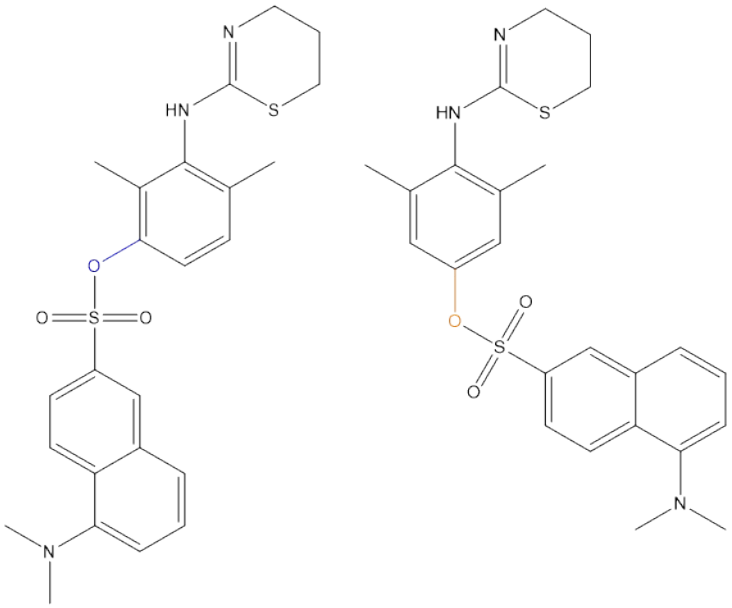
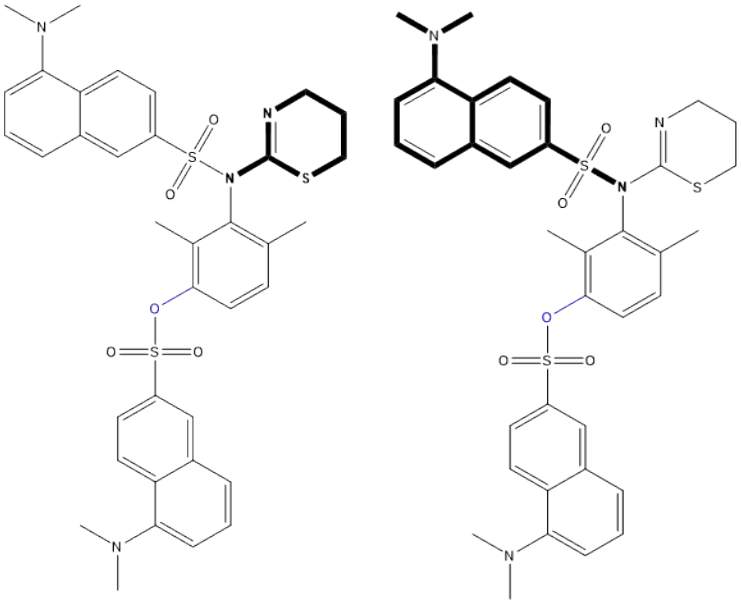
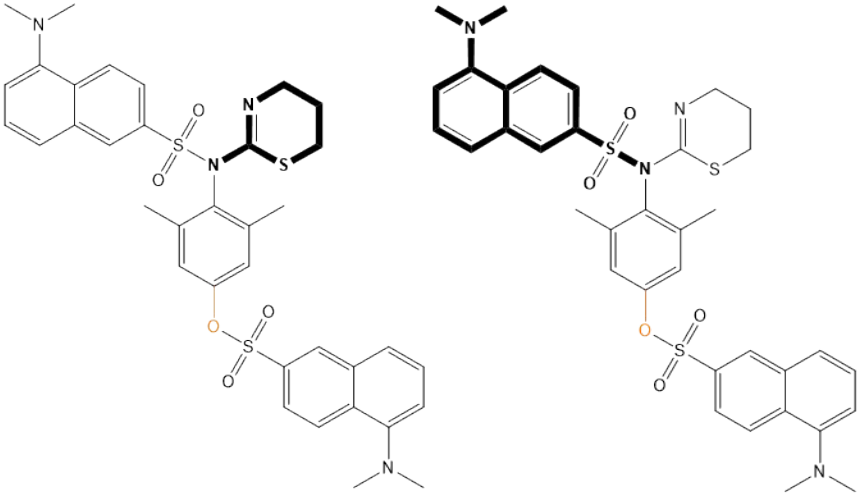


Figure S3. Proposed structures for dansylation reaction products of 3-hydroxy xylazine and 4-hydroxy xylazine.



Atropisomers of Doubly Dansylated 3-Hydroxy Xylazine



Atropisomers of Doubly Dansylated 4-Hydroxy Xylazine

Figure S4. Proposed structures for the atropisomers of the double dansylation reaction of 3-hydroxy xylazine (top) and 4-hydroxy xylazine (bottom).