SUPPLEMENTARY INFORMATION

Quantification of Hordenine in a Complex Plant Matrix by Direct Analysis in Real Time – High-Resolution Mass Spectrometry: Application to the "Plant of Concern" *Sceletium tortuosum*

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This document contains: (1) Figures S1A-B which show the DART high-resolution mass spectra of plant material collected under CID conditions (60 V) in comparison to that collected for the analysis of the authentic standards of hordenine and mesembrine under identical conditions, rendered as head-to-tail plots; (2) Figure S2 which shows the DART mass spectra for the fourth and fifth 5 mL extracts of the indicated *S. tortuosum* materials; (3) Figure S3 which shows the DART mass spectra for the blank samples; (4) Table S1 which shows that peak area ratios for the recovery and matrix effect experiments; (5) Table S2 which shows the results for the calibration curve; (6) Tables S3 a-d which show the results for the QCs; and (7) Table S4 which shows the moisture content determination calculations and results.

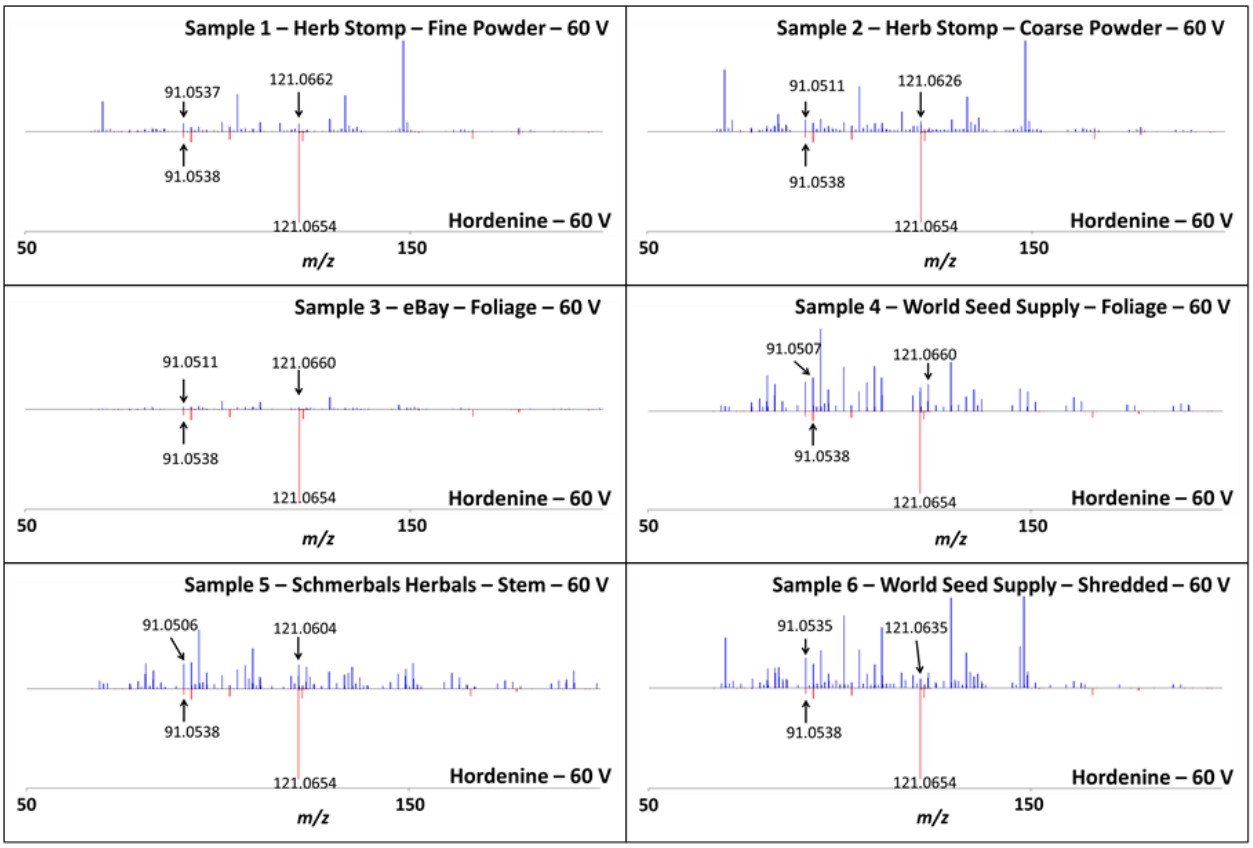

Figure S1-A. DART-HRMS analysis of bulk material (top spectrum – blue) and the hordenine authentic standard (bottom spectrum – red) acquired under CID conditions (with an orifice 1 voltage of 60 V). Fragment peaks consistent with the presence of hordenine in the bulk material were observed in all cases.

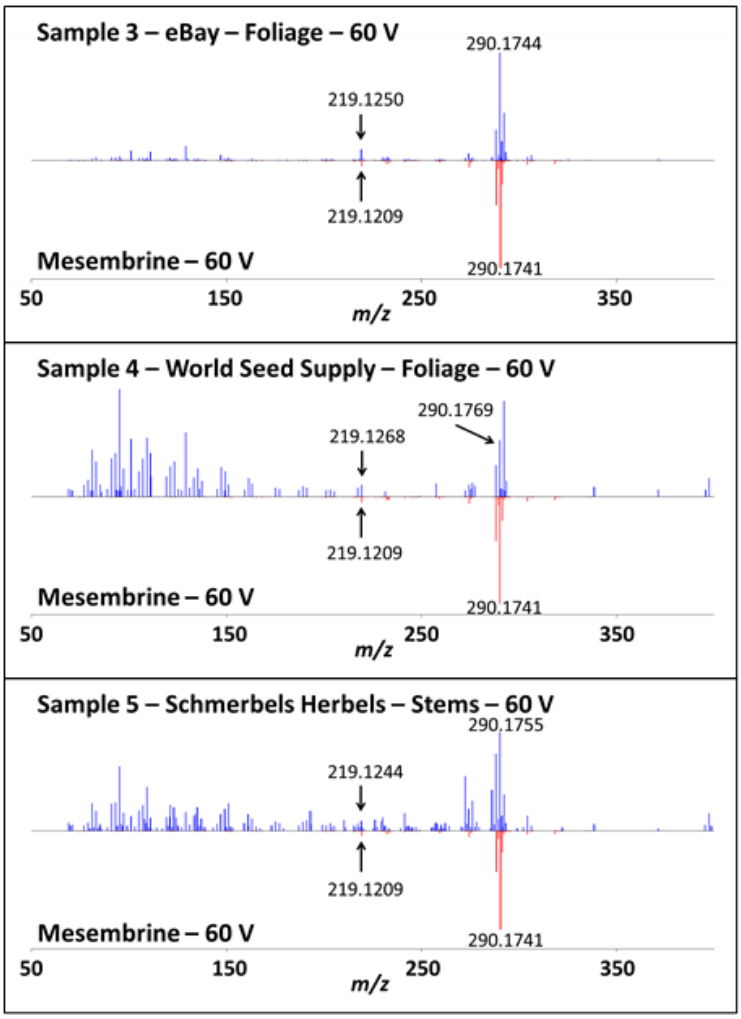

Figure S1-B. DART-HRMS analysis of bulk material (top spectrum – blue) and the mesembrine authentic standard (bottom spectrum – red) acquired under CID conditions (with an orifice 1 voltage of 60 V). Fragment peaks consistent with the presence of mesembrine in the bulk material were observed in all cases.

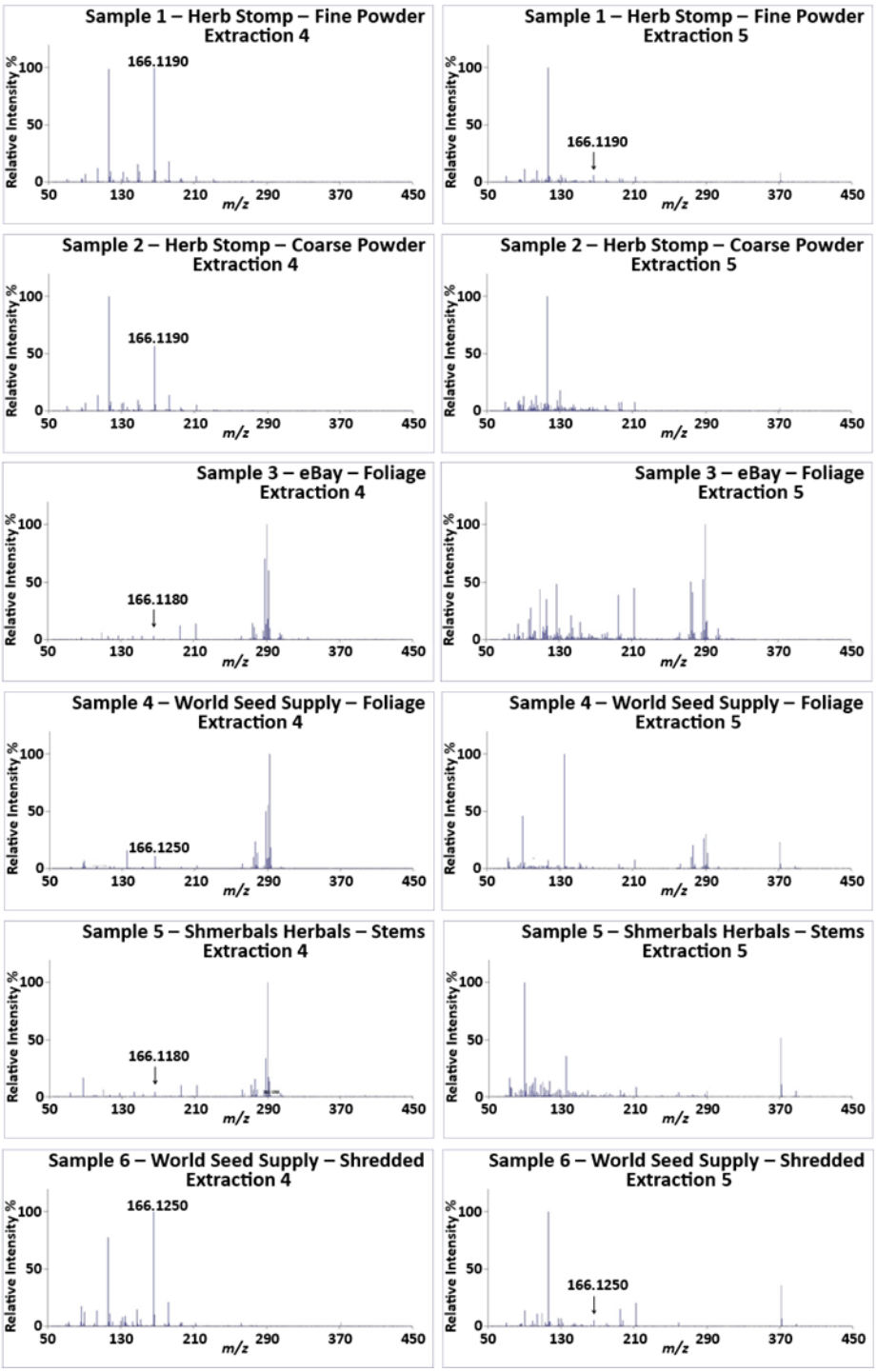

Figure S2: Hordenine detection as a function of number of extraction rounds. Left panels: DART mass spectra of the fourth 5 mL extract of the indicated *S. tortuosum* materials; Right panels: DART mass spectra of the fifth 5 mL extract of the indicated *S. tortuosum* materials. For Samples 2 – 5, no hordenine was detected in the fifth extraction round. For Samples 1 and 6, trace hordenine was detected in the fifth extraction round.

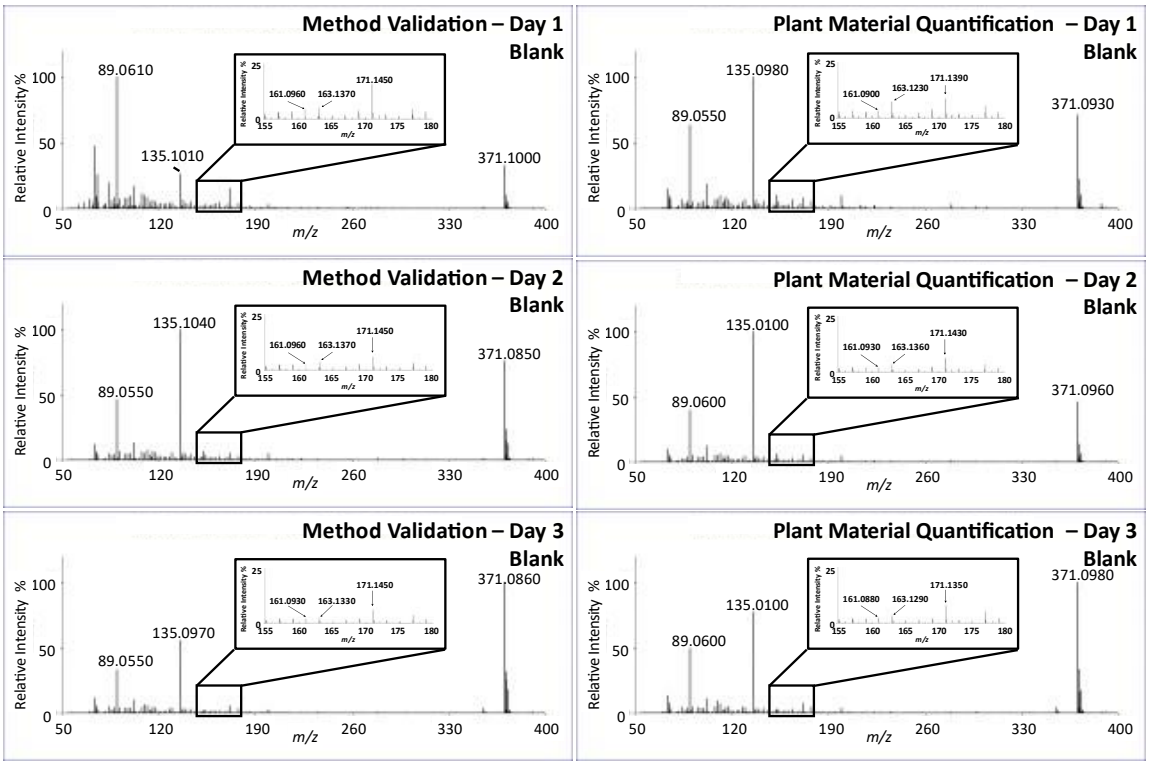

Figure S3: DART mass spectra for blank samples analyzed alongside validation curve and plant material samples to determine if diagnostic masses (hordenine at *m/z* value 166.1231 ±5 mmu and hordenine-d_6 at *m/z* value 172.1608 ±5 mmu) were detected. Left panels: DART mass spectra of the blank samples analyzed during the method validation experiments for each day; Right panels: DART mass spectra of the blank samples analyzed during the plant material quantification experiments for each day. None of the spectra contained *m/z* values consistent with compounds of interest (±5 mmu).

Table S1: Results of the recovery and matrix effect experiments. Peak area ratios for extracts of *Sceletium tortuosum* spiked with 10 µg/mL hordenine-d_6 were consistent with the peak area ratio of a 10 µg/mL standard solution of hordenine-d_6.

Sample	Concentration	Replicate	Analyte Peak Area	IS Peak Area	Peak Area Ratio	Average Peak Area Ratio
Standard	10 µg/mL	1	217984	430432	0.506	0.449
		2	115113	261275	0.441	
		3	136089	340978	0.399	
Extract 1	10 µg/mL	1	100676	231222	0.435	0.443
		2	100222	198220	0.506	
		3	211322	545754	0.387	
Extract 2	10 µg/mL	1	480757	869359	0.553	0.442
		2	269457	701233	0.384	
		3	131687	339101	0.388	
Extract 3	10 µg/mL	1	105795	239458	0.442	0.458
		2	328688	736091	0.447	
		3	398124	821690	0.485	

Table S2: Results for the calibration curve re-calculations with the relative error percentage for each concentration.

		Run 1		Run 2		Run 3	
	Nominal Conc.	Recalc. Conc.	Relative Error %	Recalc. Conc.	Relative Error %	Recalc. Conc.	Relative Error %
LLOQ	1	1.11	-11.13	0.93	6.89	1.13	-12.77
Point 1	2	1.90	4.89	1.88	5.99	2.12	-5.79
Point 2	4	3.81	4.66	3.90	2.47	4.01	-0.19
Point 3	6	6.14	-2.42	6.16	-2.71	5.92	1.36
Point 4	8	8.10	-1.26	8.29	-3.59	7.90	1.28
Point 5	12	12.09	-0.73	12.06	-0.46	12.15	-1.22
Point 6	16	15.67	2.06	15.72	1.74	15.14	5.36
ULOQ	20	20.17	-0.84	20.06	-0.31	20.64	-3.22

Table S3a: QC calculations for the high point including mean, relative error percentage and coefficient of variation for between runs and within runs. QC1 (A1-A5) and QC2 (B1-B5).

			Calculated			Between runs		
		Nominal	Run 1	Run 2	Run 3	Mean	RE%	CV
High	A1	18	18.19	17.92	17.79	18.36	-2.01	5.72
	A2		17.56	19.52	17.55			
	A3		18.62	19.65	17.24			
	A4		19.22	18.54	17.76			
	A5		19.52	19.28	17.80			
	B1		15.43	20.17	17.79			
	B2		17.39	20.31	17.94			
	B3		18.51	18.63	17.46			
	B4		18.09	19.16	17.28			
	B5		18.70	20.06	17.77			
Within-run		mean	18.12	19.32	17.64			
		RE%	-0.68	-7.35	2.01			
		CV	6.05	3.82	1.30			

Table S3b: QC calculations for the medium point including mean, relative error percentage and coefficient of variation for between runs and within runs. QC1 (A1-A5) and QC2 (B1-B5).

			Calculated			Between runs		
		Nominal	Run 1	Run 2	Run 3	Mean	RE%	CV
Medium	A1	9	8.32	9.73	8.99	9.21	-2.37	6.89
	A2		9.37	10.34	8.82			
	A3		9.13	9.94	9.51			
	A4		9.22	10.93	8.79			
	A5		9.07	9.34	9.83			
	B1		8.07	10.55	8.59			
	B2		8.57	9.43	8.76			
	B3		8.70	9.70	9.21			
	B4		9.02	8.89	9.06			
	B5		8.51	9.06	8.95			
Within-run		mean	8.80	9.79	9.05			
		RE%	2.23	-8.77	-0.57			
		CV	4.60	6.37	3.94			

Table S3c: QC calculations for the low point including mean, relative error percentage and coefficient of variation for between runs and within runs. QC1 (A1-A5) and QC2 (B1-B5).

			Calculated			Between runs		
		Nominal	Run 1	Run 2	Run 3	Mean	RE%	CV
Low	A1	3	2.84	2.98	3.02	2.93	2.37	6.19
	A2		2.88	3.03	3.33			
	A3		2.84	2.92	3.00			
	A4		2.75	3.07	3.05			
	A5		2.82	3.05	2.84			
	B1		2.66	3.02	3.04			
	B2		2.63	2.85	3.12			
	B3		2.69	2.85	3.37			
	B4		2.70	2.88	2.91			
	B5		2.67	2.87	3.19			
Within-run		mean	2.75	2.95	3.09			
		RE%	8.41	1.61	-2.90			
		CV	3.12	2.83	5.21			

Table S3d: QC calculations for the LLOQ point including mean, relative error percentage and coefficient of variation for between runs and within runs. QC1 (A1-A5) and QC2 (B1-B5).

			Calculated			Between runs		
		Nominal	Run 1	Run 2	Run 3	Mean	RE%	CV
LLOQ	A1	1	1.07	0.93	1.09	1.02	-1.64	10.26
	A2		1.14	0.90	1.11			
	A3		1.09	0.90	1.15			
	A4		1.07	0.87	1.09			
	A5		1.10	0.87	1.13			
	B1		1.04	0.85	1.12			
	B2		0.99	0.86	1.11			
	B3		1.04	0.84	1.13			
	B4		1.04	0.86	1.05			
	B5		1.03	0.90	1.12			
Within-run		mean	1.06	0.88	1.11			
		RE%	-6.04	12.13	-10.99			
		CV	3.71	3.16	2.35			

Table S4. Moisture content determination for 20 and 100 min times intervals for each plant material type. The final hordenine concentrations computed were based on the mass of plant material determined after evaporation of moisture from a ~0.2 g sample

Heating duration	Vendor	Herb Stomp	Herb Stomp	eBay	World Seed Supply	Schmerbals Herbals	World Seed Supply
	Material Type	Fine Powder	Coarse Powder	Foliage	Foliage	Stems	Shredded
20 min	Weight before heating (g)	0.20150	0.19749	0.19930	0.20188	0.20061	0.13377
	Weight after heating (g)	0.18751	0.18358	0.18285	0.18348	0.18981	0.13106
	Weight loss (g)	0.01399	0.01391	0.01645	0.01840	0.01080	0.00271
	Weight loss (%)	6.943	7.043	8.254	9.114	5.384	2.026
	Hordenine concentration (mg/g)*	1.1514	0.4776	0.0438	0.0511	0.0289	0.0838
100 min	Weight before heating (g)	0.20150	0.19749	0.19930	0.20188	0.20061	0.13377
	Weight after heating (g)	0.18756	0.18211	0.18321	0.18354	0.19000	0.13134
	Weight loss (g)	0.01394	0.01538	0.01609	0.01834	0.01061	0.00243
	Weight loss (%)	6.918	7.788	8.073	9.085	5.289	1.817
	Hordenine concentration (mg/g)*	1.1510	0.4814	0.0437	0.0511	0.0289	0.0836

*Based on the sample weight following moisture loss on heating.