Supplementary materials

Potential Pharmacokinetic Drug-Drug Interaction Between Harmine, a Cholinesterase Inhibitor, and Memantine, a Non-Competitive N-Methyl-D-Aspartate Receptor Antagonist

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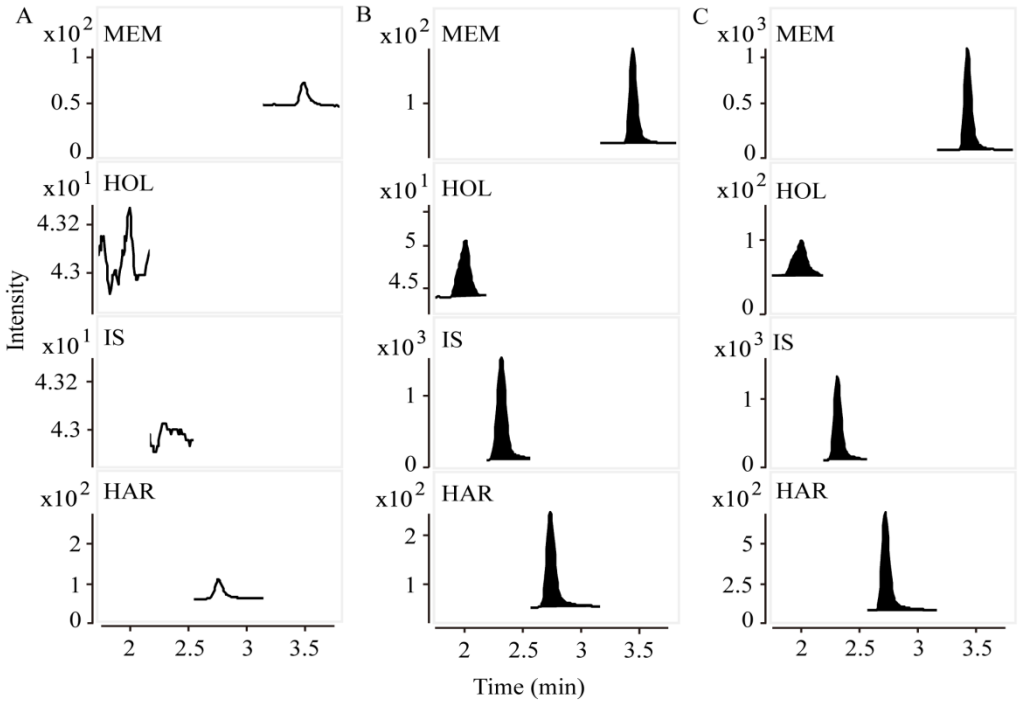

Supplementary materials Figure S1. Representative MRM chromatograms of HAR, MEM, HOL and IS in rat plasma: (A) a blank plasma; (B) a blank sample spiked with the analytes (with LLOQ) and IS (40.0 ng/ml); and (C) a plasma sample (2.0 min) from a rat after the co-administration of medium dose (40.0 mg/kg) HAR with 5.0 mg/kg MEM.

Table S1. MS/MS conditions for multiple reaction monitoring of MEM, HAR, HOL and IS.

Analytes	Precursor Ion	Product Ion	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
MEM	180.2	163.1	120	90	16	1	Positive
HAR	213.2	170.0	120	125	35	1	Positive
HOL	199.2	131.1	120	155	36	1	Positive
IS	199.2	144.1	120	170	45	1	Positive

Table S2. LLOD, LLOQ and representative calibration curves of each standard substance (n=5).

Analytes	LLOD (ng/mL)	LLOQ (ng/mL)	Linear range (ng/mL)	Slope	Intercept	R^2
MEM	0.10	0.40	0.40-400	0.550	0.00638	0.997
HAR	0.10	0.40	0.40-400	3.23	0.0294	0.998
HOL	0.10	0.40	0.40-400	4.05	-0.00185	0.998

Table S3. Summary of within-run and between-run precision for the UPLC-MS/MS method (n=6).

Analytes	Nominal level (ng/mL)	Within-run precision			Between-run precision		
		Mean±SD	CV (%)	RE (%)	Mean±SD	CV (%)	RE (%)
MEM	0.40(LLOQ)	0.426±0.0184	4.32	6.59	0.424±0.00272	0.640	6.15
	1.00(QCL)	1.09±0.0319	2.93	8.87	1.06±0.0241	2.27	6.24
	100(QCM)	102±2.69	2.63	2.08	101±3.71	3.67	1.01
	300(QCH)	311±5.79	1.86	3.79	303±12.1	4.00	1.06
	400(ULOQ)	414±26.8	6.48	3.41	401±13.6	3.40	0.147
HAR	0.40(LLOQ)	0.471±0.0153	3.26	17.6	0.441±0.0265	6.02	10.1
	1.0(QCL)	1.10±0.0408	3.71	9.90	1.08±0.0267	2.47	8.18
	100(QCM)	106±3.99	3.75	6.45	103±4.66	4.54	2.67
	300(QCH)	302±8.02	2.66	0.606	298±8.49	2.85	-0.750
	400(ULOQ)	398±29.8	7.50	-0.530	396±5.08	1.28	-1.02
HOL	0.40(LLOQ)	0.449±0.0398	8.86	12.3	0.434±0.0170	3.92	8.59
	1.0(QCL)	1.08±0.0621	5.73	8.39	1.07±0.0192	1.80	6.68
	100(QCM)	97.7±2.54	2.60	-2.30	98.7±3.95	4.00	-1.29
	300(QCH)	276±5.08	1.84	-8.15	286±13.8	4.83	-4.80
	400(ULOQ)	366±8.68	2.37	-8.59	377±11.4	3.02	-5.83

Table S4. Summary of extraction yield and matrix effect for the UPLC-MS/MS method (n=6).

Analytes	Add conc. (ng/mL)	Measured conc. (ng/mL, mean±SD)	Matrix effect (%, mean±SD)	Recovery (%, mean±SD)
MEM	1	1.05 ± 0.0440	96.4±3.20	105±4.40
	100	104 ± 6.34	102±7.50	104±6.34
	300	298 ± 7.09	98.2±1.96	99.5±2.36
HAR	1	1.05±0.0321	98.0±4.63	105±3.21
	100	102±5.63	101±6.99	102±5.63
	300	299±4.48	99.2±1.77	99.6±1.49
HOL	1	1.06±0.0382	102±2.17	106±3.82
	100	102±3.94	102±5.03	102±3.94
	300	292±6.06	98.7±2.74	97.2±2.02
IS	40	41.1±0.265	99.0±1.68	103±0.663

Table S5. Stability of each standard substance in rat plasma under different storage conditions (n=5).

Conditions	Nominal levels	MEM		HAR		HOL	
		Mean±SD	CV%	Mean±SD	CV%	Mean±SD	CV%
AT	QCL	1.06±0.0138	1.31	1.10±0.0236	2.15	1.07±0.0111	1.04
	QCM	104±3.90	3.75	104±3.90	3.74	103±2.77	2.69
	QCH	309±4.65	1.50	303±5.16	1.70	301±9.79	3.25
4˚C	QCL	1.09±0.0319	2.93	1.10±0.0408	3.71	1.08±0.0621	5.73
	QCM	102±2.69	2.63	106±3.99	3.75	97.7±2.54	2.60
	QCH	311±5.79	1.86	302±8.02	2.66	276±5.08	1.84
-20˚C	QCL	1.04±0.0552	5.30	1.05±0.0466	4.43	1.05±0.0446	4.27
	QCM	96.9±4.32	4.45	97.5±6.51	6.68	95.4±3.80	3.98
	QCH	289±16.5	5.71	288±14.9	5.17	270±11.1	3.97
Freeze/thaw	QCL	1.05±0.0440	4.20	1.05±0.0321	3.04	1.06±0.0382	3.58
	QCM	104±6.34	6.09	102±5.63	5.51	12.0±3.94	3.87
	QCH	298±7.09	2.38	299±4.48	1.50	292±6.06	2.08