

Supplementary Materials: Investigating the Central Nervous System Disposition of Actinomycin D: Implementation and Evaluation of Cerebral Microdialysis and Brain Tissue Measurements Supported by UPLC-MS/MS Quantification

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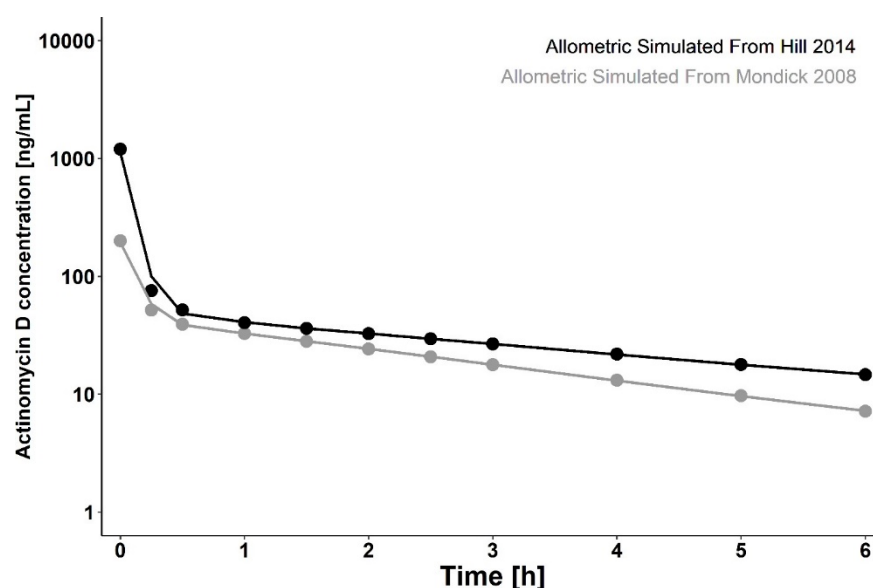


Figure S1. Allometric simulation of actinomycin D's pharmacokinetic profile in mice according to published data in humans [1,2] by allometrically scaling the clearance and volumes parameters using classic exponents (0.75 for clearances and 1 for volumes) from mouse to human [3].

Table S1. Recovery data.

		Low QC	Medium QC	High QC	IS
		0.150 ng/mL	3.00 ng/mL	60.0 ng/mL	1.50 ng/mL
Plasma	Recovery [%]	94.1	91.0	95.0	99.7
	Precision [% CV]	6.43	0.35	1.61	3.58
Brain tissue	Recovery [%]	103.9	104.0	96.6	111.4
	Precision [% CV]	2.92	9.10	8.74	2.07

CV: Coefficient of variation. N = 3 replicates at each QC concentration.

Table S2. Calibration curves of validation batches calculated with linear regression and $1/x^2$ weighting.

Batch	Formula of calibration curve	Coefficient of regression (r^2)
Validation plasma 1	$0.0580 x + 0.0014$	0.9964
Validation plasma 2	$0.0227 x + 0.0015$	0.9920
Validation plasma 3	$0.0338 x + 0.0008$	0.9973
Validation brain tissue 1	$0.6332 x + 0.0002$	0.9916
Validation brain tissue 2	$0.4975 x - 0.0034$	0.9904
Validation brain tissue 3	$0.4092 x - 0.0091$	0.9926

Table S3. Summary of quality control results for plasma actinomycin D.

		Nominal actinomycin D concentrations [ng/mL]			
		LLOQ	Low QC	Mid QC	High QC
		0.050	0.150	3.00	60.0
Within-batch					
1	Mean [ng/mL]	0.047	0.141	3.10	60.2
	Accuracy [%]	94.0	93.4	103.2	100.3
	Precision [% CV]	13.2	4.95	4.94	3.49
2	Mean [ng/mL]	0.045	0.154	3.23	63.5
	Accuracy [%]	90.0	102.8	107.7	105.8
	Precision [% CV]	1.57	6.98	5.68	3.19
3	Mean [ng/mL]	0.050	0.162	3.14	60.4
	Accuracy [%]	99.0	108.0	104.7	100.7
	Precision [% CV]	8.74	5.79	8.12	6.71
Batch-to-batch					
	Mean [ng/mL]	0.048	0.152	3.16	61.3
	Accuracy [%]	95.9	101.6	105.2	102.2
	Precision [% CV]	8.10	8.17	6.27	5.10

N = 5 replicates at each LLOQ and QC level. CV: coefficient of variation; LLOQ: lower limit of quantification; QC: quality control homogenate.

Table S4. Summary of quality control results for actinomycin D in brain tissue homogenate.

		Nominal actinomycin D concentrations [ng/mL]			
		LLOQ	Low QC	Mid QC	High QC
		0.050	0.150	3.00	60.0
Within-batch					
1	Mean [ng/mL]	0.053	0.169	3.35	57.8
	Accuracy [%]	106.3	112.7	111.6	96.3
	Precision [%CV]	4.82	1.18	4.10	5.30
2	Mean [ng/mL]	0.051	0.164	3.28	56.8
	Accuracy [%]	101.7	109.2	109.4	94.7
	Precision [%CV]	6.97	3.42	2.27	2.80
3	Mean [ng/mL]	0.052	0.158	3.03	53.7
	Accuracy [%]	104.0	105.6	100.9	89.6
	Precision [%CV]	12.7	2.66	6.68	4.03
Batch-to-batch					
	Mean [ng/mL]	0.050	0.164	3.23	56.4
	Accuracy [%]	99.6	109.4	107.7	94.0
	Precision [% CV]	9.81	3.59	5.96	4.90

N = 5 replicates at each LLOQ and QC level. CV: coefficient of variation; LLOQ: lower limit of quantification; QC: quality control.

Table S5. Summary of quality control results for actinomycin D in micro-dialysate.

		Nominal actinomycin D concentrations [ng/mL]			
		LLOQ	Low QC	Mid QC	High QC
		0.050	0.150	3.00	60.0
Mean [ng/mL]		0.048	0.162	2.78	52.5
Accuracy [%]		96.0	108.1	92.7	87.5
Precision [% CV]		11.7	3.34	6.12	3.41

N = 5 replicates at each LLOQ and QC level. CV: coefficient of variation; LLOQ: lower limit of quantification; QC: quality control.

Table S6. Summary of quality control results for actinomycin D in cell homogenate.

	Nominal actinomycin D concentrations [ng/mL]			
	LLOQ 0.050	Low QC 0.150	Mid QC 3.00	High QC 60.0
Mean [ng/mL]	0.050	0.156	3.20	66.4
Accuracy [%]	100.3	103.9	106.7	110.6
Precision [% CV]	12.2	6.21	7.51	3.75

N = 5 replicates at each LLOQ and QC level. CV: coefficient of variation; LLOQ: lower limit of quantification; QC: quality control.

Table S7. Stability data of the plasma validation.

	Low QC 0.150 ng/mL	Medium QC 3.00 ng/mL	High QC 60.0 ng/mL
Freeze-and-thaw stability			
Accuracy [%]	93.6	92.0	87.6
Precision [% CV]	5.80	7.59	2.65
1-month plasma stability at -25 °C			
Accuracy [%]	95.6	98.3	100.7
Precision [% CV]	14.7	2.44	2.00
2 days buffer stability at RT			
Accuracy [%]	108.7	113.5	104.7
Precision [% CV]	5.21	1.69	8.49
6-month stability in solution at 4°C			
Accuracy [%]	88.3	86.4	87.2
Precision [% CV]	1.60	5.54	3.34

CV: Coefficient of variation. N = 3 replicates at each QC concentration. RT: room temperature.

Table S8. Results of the incurred sample reanalysis.

Mouse #	Time of sampling [h]	Actinomycin D concentration [ng/mL] Original analysis	Actinomycin D concentration [ng/mL] Incurred reanalysis	Deviation from mean [%]
5	22	1.40	1.19	16.2
6	6	1.31	1.17	11.3
6	22	1.36	1.16	15.9
7	6	5.51	5.23	5.21
7	22	4.48	5.17	14.3

Table S9. Summary of quality control results for actinomycin D quantification of minimally diluted QC samples with calibration samples spiked with regular solutions.

	Nominal actinomycin D concentrations [ng/mL]		
	Low QC 0.150	Mid QC 3.00	High QC 60.0
Mean [ng/mL]	0.142	2.80	53.8
Accuracy [%]	94.7	93.5	89.8
Precision [% CV]	10.0	9.03	1.43

N = 3 replicates at each LLOQ and QC level. CV: coefficient of variation; QC: quality control.

Table S10. Matrix effect data.

		Low QC	Medium QC	High QC	IS
		0.150 ng/mL	3.00 ng/mL	60.0 ng/mL	1.50 ng/mL
Plasma	Matrix effect [%]	66.2	74.2	73.1	66.2
	Matrix effect IS-normalized [%]	104.6	109.8	102.0	
	Precision [% CV]	13.7	1.05	6.91	1.08
Brain tissue	Matrix effect [%]	56.0	63.2	62.3	52.0
	Matrix effect IS-normalized [%]	110.3	114.5	112.8	
	Precision [% CV]	8.04	2.75	4.89	2.46

CV: Coefficient of variation. N = 3 replicates at each QC concentration.

Table S11. *In vitro* probe performance comparison.

Probe	CAM7 (Cat.No.:8012411) n = 2	CMA8 (Cat.No.:8012201) n = 1	MD-2211 n = 5
Material	Polyethersulfone (PES)	Polyacrylethersulfone (PAES)	Cellulose
MWCO	55kDa	20kDa	35kDa
RD recovery rate (%)	81.6 ± 9.9	88.83	79.4 ± 2.8

RD: Retrodialysis.

Table S12. Actinomycin D in vitro retrodialysis results.

	Probe 1	Probe 2	Probe 3	Probe 4	Mean
1 h (ng/mL)	11.2	20.0	3.82	5.37	
2 h (ng/mL)	9.78	13.6	7.54	16.9	
3 h (ng/mL)	16.4	17.0	9.84	13.3	
Stock (ng/mL)†	95.6	73.6	67.5	76.6	
Recovery rate (%)	87.0	77.1	89.5	84.5	84.5 ± 5.4

† Expected concentration of 100 ng/mL.

Table S13. Actinomycin C in vitro retrodialysis results using 1 % BSA.

	Probe 1	Probe 2	Mean
1 h (ng/mL)	6.27	4.14	
2 h (ng/mL)	6.38	9.59	
3 h (ng/mL)	11.8	13.6	
Stock (ng/mL)†	67.4	71.08	
Recovery rate (%)	87.9	87.2	87.6 ± 0.5

† Expected concentration of 100 ng/mL.

Table S14. Summary of quality control results for actinomycin C in micro-dialysate.

	Nominal actinomycin C concentrations [ng/mL]			
	LLOQ 0.100	Low QC 0.300	Mid QC 6.00	High QC 75.0
Mean [ng/mL]	0.083	0.313	6.67	80.1
Accuracy [%]	83.2	104.3	111.2	106.8
Precision [% CV]	3.65	5.78	4.95	11.09

N = 5 replicates at each LLOQ and QC level. CV: coefficient of variation; LLOQ: lower limit of quantification; QC: quality control.

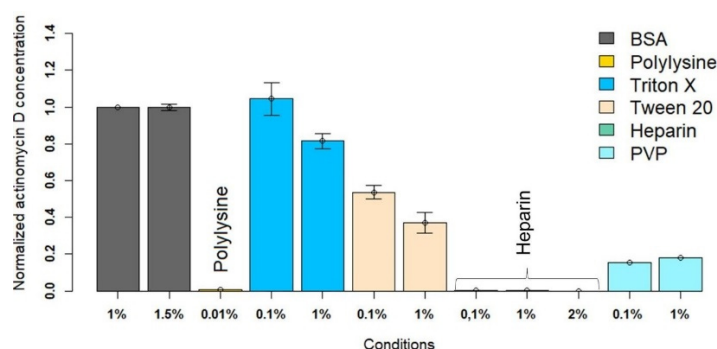


Figure S2. Coating strategies to avoid non-specific binding of actinomycin D in case of BSA and Tritonx100. Microdialysis tubes were coated for 1 h with indicated chemicals and drug concentrations normalized to the nominal value. Data is expressed as mean \pm standard deviation. Coatings were not stable for the whole duration of microdialysis procedure (data not shown).

References

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