

Interaction of cucurbit[7]uril with oxime K027, atropine, and paraoxon: Is this delivery system risk or advantage?

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Supplementary Material

We carried out a full validation for plasma and partial validation for brain homogenate samples according to the EMA and FDA guidelines on Bioanalytical method validation - EMA 2011, FDA 2018. All the evaluated parameters met the required criteria; detailed validation results are presented in **Table 1-4**.

Table 5 is a legend for Functional observatory battery (FOB) scoring.

Table 1. Reproducibility of calibration curves (n=6 for plasma, n=4 for the brain)

		PARAOXON	4-NITROPHENOL	ATROPINE
PLASMA	Calibration model	Linear fit	Linear fit	Linear fit
	Weighting analysis	1/X ²	1/X ²	1/X ²
	Coefficient of determination R ²	>0.997	>0.997	>0.995
	Regression coefficient <i>a</i>	0,0174	0,0208	0,0167
	Standard deviation SD(<i>a</i>)	0,0015	0,0007	0,0010
	Relative standard deviation RSD(<i>a</i>)	8,7%	3,4%	5,9%
BRAIN	Calibration model	Linear fit	Linear fit	Linear fit
	Weighting analysis	1/X ²	1/X ²	1/X ²
	Coefficient of determination R ²	>0.996	>0.995	>0.997
	Regression coefficient <i>a</i>	0,0165	0,0219	0,0152
	Standard deviation SD(<i>a</i>)	0,0011	0,0012	0,0013
	Relative standard deviation RSD(<i>a</i>)	6,6%	5,4%	8,5%

Table 2. Summary of validation results (precision and accuracy) in mouse plasma for four different concentrations - limit of quantitation(LLOQ), low (QC A), mid (QC B) and high (QC C) level, and diluted level (QC D)

		PARAOXON					4-NITROPHENOL					ATROPINE				
		LLOQ	QC A	QC B	QC C	QC D	LLOQ	QC A	QC B	QC C	QC D	LLOQ	QC A	QC B	QC C	QC D
ng/mL		0,50	1,25	20,80	192,00	19,20	0,50	1,25	20,80	192,00	19,20	2,50	5,00	83,20	768,00	76,80
PLASMA	Within-batch 1															
	Mean (ng/mL)	0,45	1,25	21,29	204,85	17,83	0,51	1,32	22,62	209,49	18,45	2,25	5,28	84,63	786,64	71,12
	Precision (%CV)	11,7%	8,3%	2,3%	2,5%	2,5%	7,1%	2,5%	0,8%	1,4%	2,6%	8,3%	6,1%	1,6%	4,3%	4,0%
	Accuracy (%)	-10,3%	0,1%	2,4%	6,7%	-7,2%	1,4%	5,8%	8,8%	9,1%	-3,9%	-9,9%	5,6%	1,7%	2,4%	-7,4%
	Within-batch 2															
	Mean (ng/mL)	0,48	1,22	20,79	205,93	18,37	0,52	1,28	22,22	205,89	18,66	2,31	5,26	80,94	702,16	71,23
	Precision (%CV)	10,4%	6,1%	1,7%	1,2%	1,3%	4,7%	0,9%	1,7%	1,9%	2,5%	8,3%	5,3%	2,8%	3,5%	3,8%
	Accuracy (%)	-3,5%	-2,3%	-0,1%	7,3%	-4,3%	4,4%	2,7%	6,8%	7,2%	-2,8%	-7,7%	5,2%	-2,7%	-8,6%	-7,2%
	Within-batch 3															
	Mean (ng/mL)	0,46	1,22	20,00	193,87	18,12	0,48	1,31	22,18	206,62	18,57	2,70	5,54	88,11	786,77	74,24
	Precision (%CV)	5,9%	2,2%	2,0%	1,4%	3,4%	4,1%	4,3%	1,3%	1,8%	1,7%	3,9%	3,0%	1,1%	2,9%	3,7%
	Accuracy (%)	-8,4%	-2,6%	-3,8%	1,0%	-5,6%	-4,1%	4,6%	6,6%	7,6%	-3,3%	8,2%	10,8%	5,9%	2,4%	-3,3%
	Batch-to-batch															
	Mean (ng/mL)	0,46	1,23	20,73	201,76	18,09	0,50	1,30	22,34	207,33	18,56	2,42	5,36	84,56	758,52	72,20
	Precision (%CV)	9,5%	5,8%	3,3%	3,2%	2,7%	6,2%	3,0%	1,5%	1,8%	2,2%	10,7%	5,2%	4,0%	6,4%	4,1%
	Accuracy (%)	-7,4%	-1,6%	-0,3%	5,1%	-5,8%	0,6%	4,4%	7,4%	8,0%	-3,3%	-3,1%	7,2%	1,6%	-1,2%	-6,0%

Table 3. Summary of matrix effect and recovery

		PARAOXON			4-NITROPHENOLE			ATROPINE		
		QC A	QC B	QC C	QC A	QC B	QC C	QC A	QC B	QC C
PLASMA	ME % (no IS)	60,3 ± 3,6%	69,9 ± 2,1%	69,9 ± 4,0%	97,9 ± 5,7%	94,1 ± 3,4%	99,1 ± 3,2%	84,3 ± 2,2%	78,4 ± 6,7%	82,8 ± 2,9%
	ME %	90 ± 1,1%	99,1 ± 0,8%	99,9 ± 0,7%	109,9 ± 4,0%	100,4 ± 0,6%	96,4 ± 1,2%	92,5 ± 1,2%	88,9 ± 2,7%	94,9 ± 1,7%
	RE %	48,8 ± 4,8%	42,6 ± 1,2%	53,5 ± 1%	99,5 ± 3,7%	93,1 ± 1,6%	97,7 ± 0,9%	87,7 ± 1,3%	88,5 ± 1,6%	81,8 ± 0,8%
BRAIN	ME % (no IS)	54,3 ± 6,6%	58,8 ± 4,7%	61,0 ± 4,2%	95,8 ± 6,5%	96,1 ± 5,7%	92,1 ± 2,0%	75,4 ± 5,3%	73,2 ± 4,0%	78,8 ± 3,5%
	ME %	91,3 ± 3,3%	99,7 ± 0,9%	101,1 ± 0,5%	103,9 ± 3,6%	101,4 ± 1,5%	96,5 ± 0,8%	94,4 ± 1,5%	93,6 ± 1,1%	99,4 ± 1,5%
	RE %	42,1 ± 6,2%	45,9 ± 3,2%	48,2 ± 4,9%	94,2 ± 4,7%	95,8 ± 3,2%	90,2 ± 3,9%	80,4 ± 4,1%	85,8 ± 2,2%	79,9 ± 4,7%

ME % (no IS) - Matrix effect without IS normalization; ME % - Matrix effect normalized with IS ; RE % - Recovery

Table 4. Stability evaluation in mouse plasma as the average deviation from the reference value

		PARAOXON		4-NITROPHENOL		ATROPINE	
		QC B	QC C	QC B	QC C	QC B	QC C
PLASMA	Autosampler stability	-2,6%	-2,5%	-1,4%	-1,5%	-3,9%	-7,0%
	Freeze-Thaw stability	NE	NE	-4,9%	-4,3%	-2,5%	-4,7%
	Long-term stability	NE	NE	-8,6%	-6,4%	-10,5%	-5,6%

NE - not evaluated

Table 5. Legend for FOB scoring

Marker	Scored values									
	-2	-1	0	1	2	3	4	5	6	7
posture				<i>sitting or standing</i>	<i>rearing</i>	<i>asleep</i>	flattened	lying on side	crouched over	head bobbing
catch difficulty				passive	<i>normal</i>	defense	flight	escape	aggression	
ease of handling				very easy	<i>easy</i>	moderately difficult	difficult			
muscular tonus	atonic	hypotonic	<i>normal</i>	hypertonic	rigidity	fasciculation				
lacrimation			<i>none</i>	slight	severe	crusts	colored crusts			
eye closed				<i>open</i>	slightly dropping	half-way dropping	completely shut	ptosis		
endo/exophthalmos		endophthalmos	<i>normal</i>	exophthalmos						
fur abnormalities			<i>normal</i>	colored	disheveled	colored + disheveled	bald spot	injury	other changes	piloerection
skin abnormalities			<i>normal</i>	pale	erythema	cyanosis	pigmentation	cold	injury	
salivation			<i>none</i>	slight	severe					
nose secretion			<i>none</i>	slight	severe	colored				
hyperkinesia (hyperkinetic disorders)			<i>normal</i>	repetitive movements of mouth and jaws	non-rhythmic quivers	mild tremors	severe tremors	myoclonic jerks	clonic convulsion	
tremors			<i>none</i>	only during stimulation	mild/local	mild/overall	affecting movement	impeding movement		
clonic movements			<i>none</i>	twitches	non-rhythmic movement					
tonic movements			<i>normal</i>	contraction of extensors	opisthotonus	emprostotonus	explosive jumps	tonic convulsions		
gait			<i>normal</i>	ataxia	overcompensation of hind limb movements	feet point outwards from the body	forelimbs are extended	walks on tiptoes	hunched body	the body is flattened against the surface
ataxia (movement coordination disorder)			<i>none</i>	mild	strong					
paresis (disturbance of free movement)			<i>none</i>	mild	strong					
gait score				<i>normal</i>	slightly impaired	somewhat impaired	impaired			
mobility score				<i>normal</i>	slightly impaired	somewhat impaired	impaired			
activity				very low	sporadic	reduced	<i>normal</i>	enhanced	permanent	
tension			<i>none</i>	partial (ears)	stupor					
vocalization (sounds expressions)			<i>none</i>	provoked	spontaneous	expressive				
stereotypy			<i>none</i>	head weaving	body weaving	grooming	circling	others		
bizarre behavior			<i>none</i>	head	body	self-mutilation	abnormal movements	others		
approach response				no reaction	<i>normal</i>	increased reaction	energetic reaction	exaggerated reaction		
touch response				no reaction	<i>normal</i>	increased reaction	energetic reaction	exaggerated reaction		
click response				no reaction	<i>normal</i>	increased reaction	energetic reaction	exaggerated reaction		
tail-pinch response				no reaction	<i>normal</i>	increased reaction	energetic reaction	exaggerated reaction		
pupil size	miosis	slight narrowing	<i>normal</i>	slight dilation	mydriasis					
pupil response			no reaction	slow reaction	<i>normal reaction</i>					
air-righting reflex from the vertical position				<i>normal</i>	slightly uncoordinated	lands on side	lands on back			
respiration	breathless	slow breathing	<i>normal</i>	accelerated breathing	stuffiness					