Discovery Research

In-vivo Pharmacokinetics Capabilities

Discovery Research
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Overview

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In-Vivo ADME Studies

- Rodent bioavailability studies (Mouse, Rat, Guinea pig & Hamster)
- Non Rodent bioavailability study (Rabbit & Beagle Dog)
- Site specific absorption studies in rats (Single pass In situ intestinal perfusion studies)
- In-vivo approach for evaluation of MBI of CYP3A in rats
- CNS penetration studies:

  Total, discreet brain regions, Steady state brain penetration, In situ brain perfusion and CSF penetrations studies

  Free drug estimation in brain using microdialysis

  Mouse brain uptake assay (MBUA)
- Disposition studies (Bile, Urine and Feces) using [3H] or [14C] ligands using LSA
- PK/PD studies (exposure in plasma and brain)/ Allometric Scaling to predict human PK

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Surgical Models

- Jugular vein Cannulated Rats, Guinea pigs & Hamsters
- Femoral vein Cannulated Rat & Guinea pigs
- Portal vein Cannulated Rat and Guinea pigs
- Carotid artery Cannulated Rats
- Abdominal aorta Cannulated Rats
- Bile Duct Cannulated Rats
- Duodenal, Jejunum, Ileum and Colon Cannulated Rats
- CSF collection from rats

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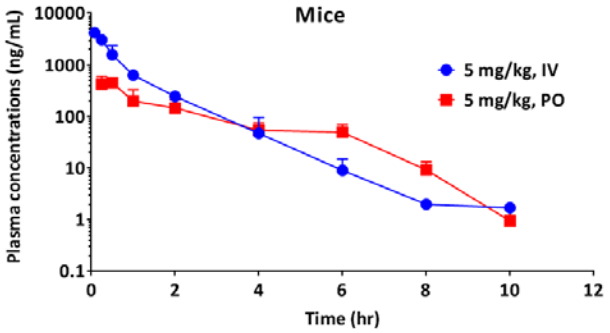
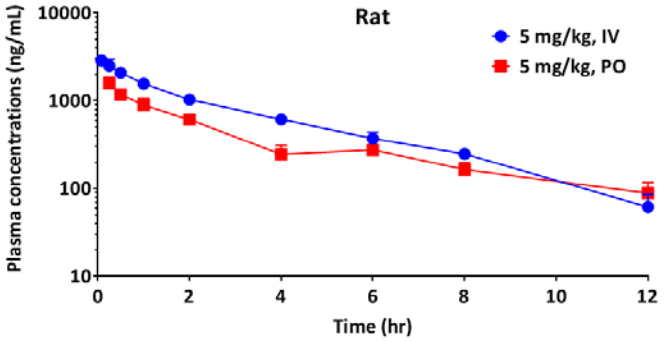
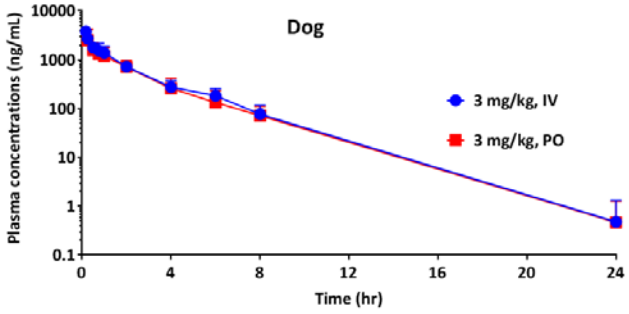
Bioavailability assessment in rodents and non-rodents







- Species: Mice, Rat, Guinea pigs, Rabbits and Dogs
- Single dose/ Repeated dose
- Parallel or Cross-over study design
- Solution, Suspension, Tablet or Capsule Dosage form administration.
- Extravascular / parenteral route of administration
- Biological matrices: Blood/ plasma / Serum
- Blood sampling for PK and PD analysis
- PK analysis using Phoenix WinNonlin

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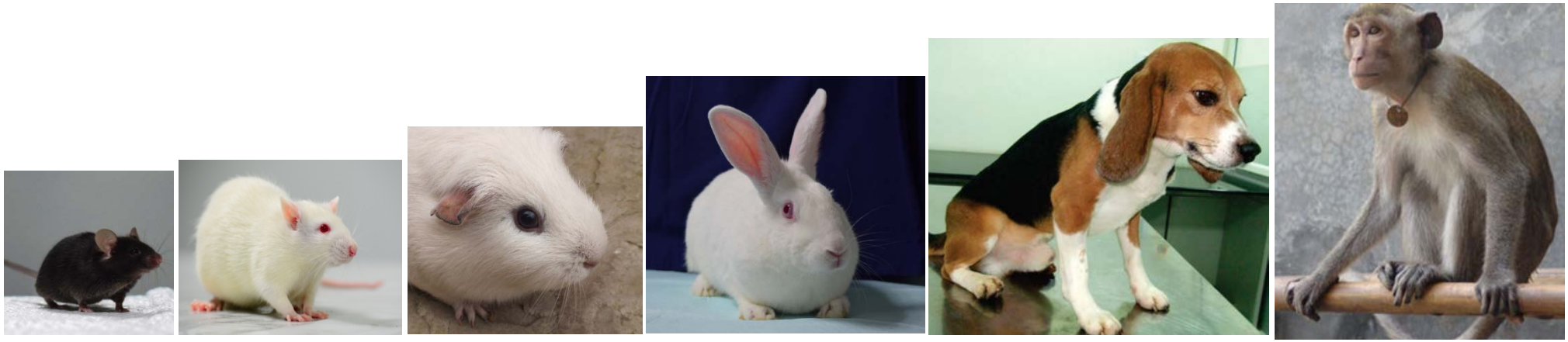
Animal Species for Pharmacokinetics



Mouse Rat Guinea pig Rabbit Dog Monkey

Small animals

Large animals

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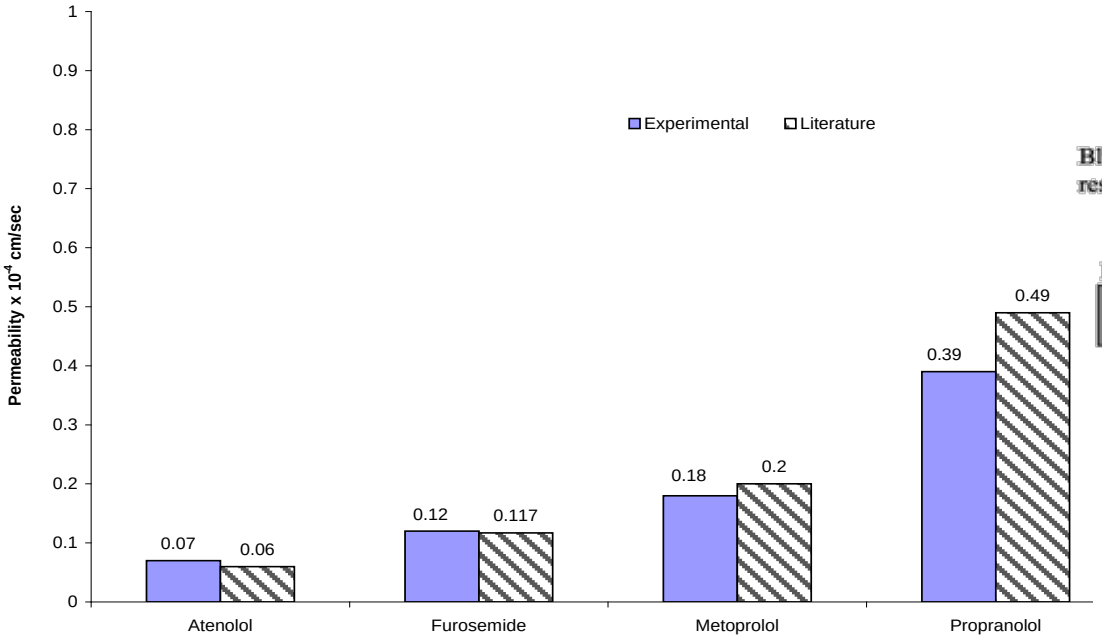
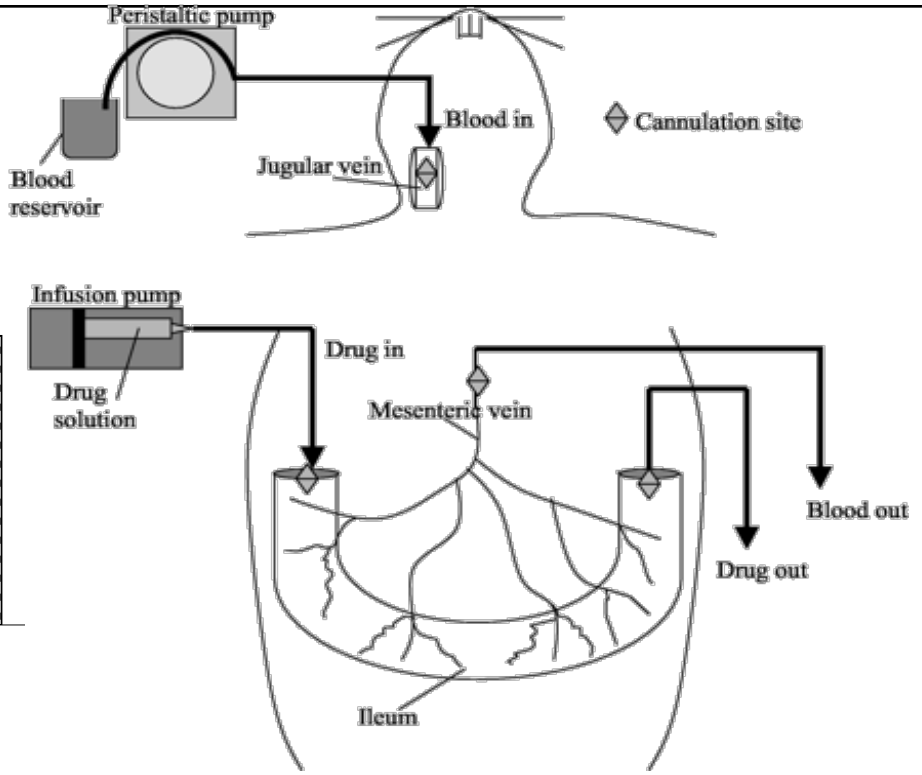
Site Specific Absorption Studies (Single pass in situ intestinal perfusion)





Cummins et al. JPET 305:306–314, 2003

Animal : Male Wistar Rat (Or as specified by Sponsor)

Number of animals : 5 per compound

Intestinal Region : Jejunum & Ileum or as specified by sponsor

Test concentration : 10 µM

Internal permeability reference standard (e.g. Metoprolol 100 µM)

Non absorbable marker Phenol red 100 µg.

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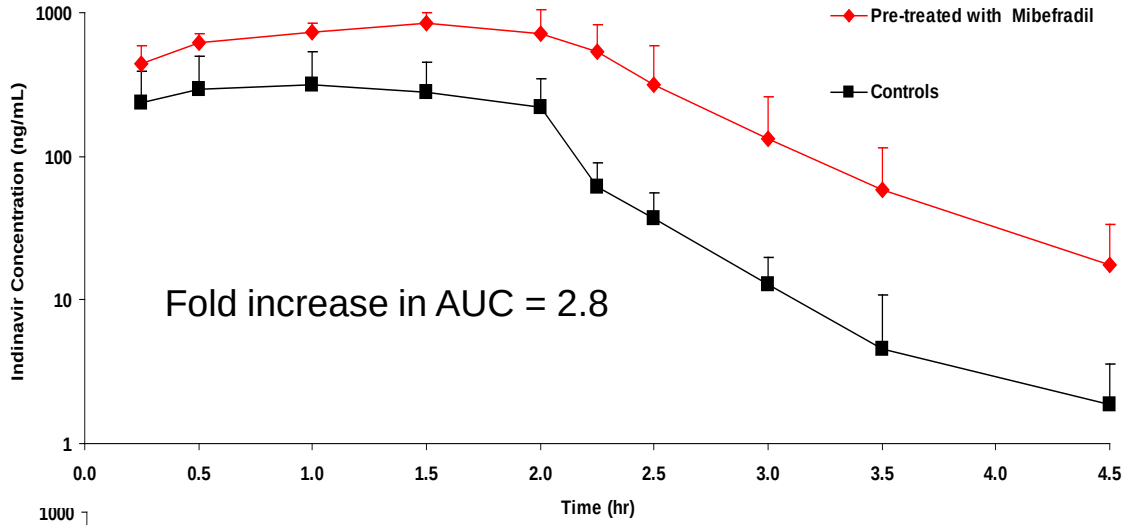
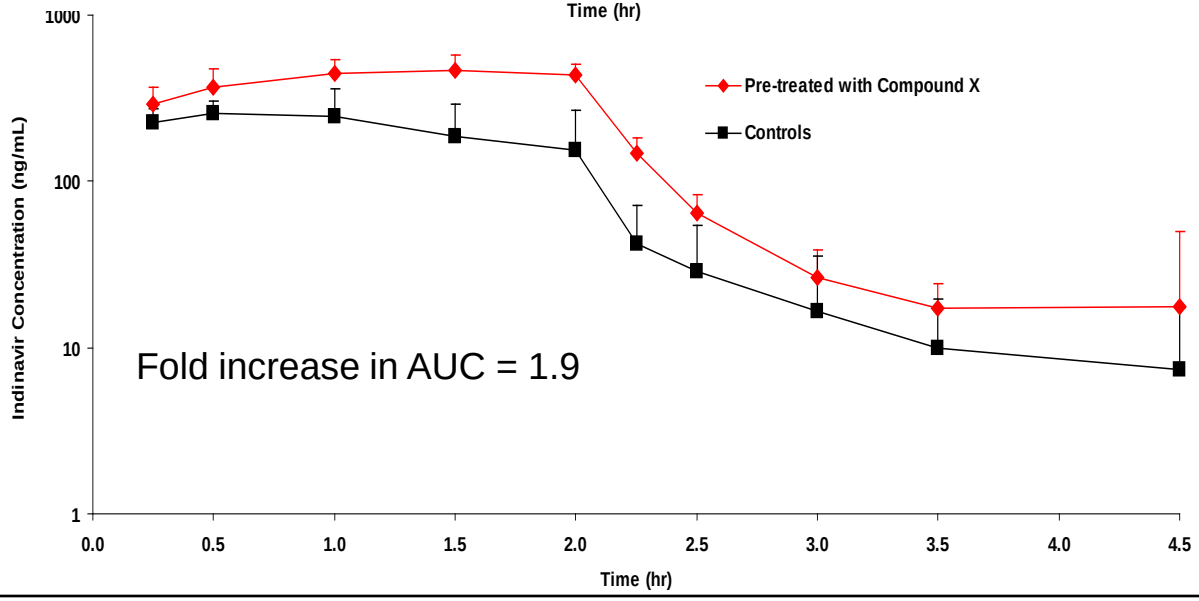
Evaluation of MBI of CYP3A in Rats





Test compound administration

(Mifibradil - 12 mg/kg, p.o. or

Compound X -12 mg/kg, p.o.)

24 hr after Mifibradil or

12 hr after Compound X

Infusion of Indinavir (4 mg/kg) Via

Portal vein for 2 hr

Blood sampling via Jugular vein at

0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5

from the start of infusion

Concentration of Indinavir was measured using LC-MS/MS and calculated fold change in AUC of CYP3A substrate Indinavir

(AUC_i / AUC_0)

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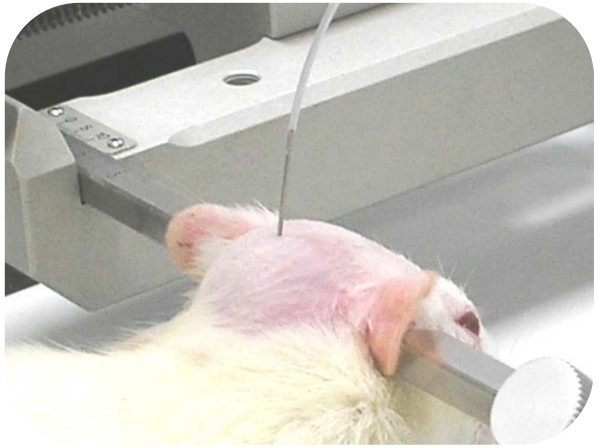
Assessment of CNS Penetration



Brain Penetration Studies

- Single dose
- Steady state

Collection of CSF

- Direct puncture of cisterna magna
- Species: Rat, Mouse, Rabbit, Dog

Brain Microdialysis

- Estimation of unbound concentration
- PK/PD assessment
- Species: Rat, Mouse, Rabbit

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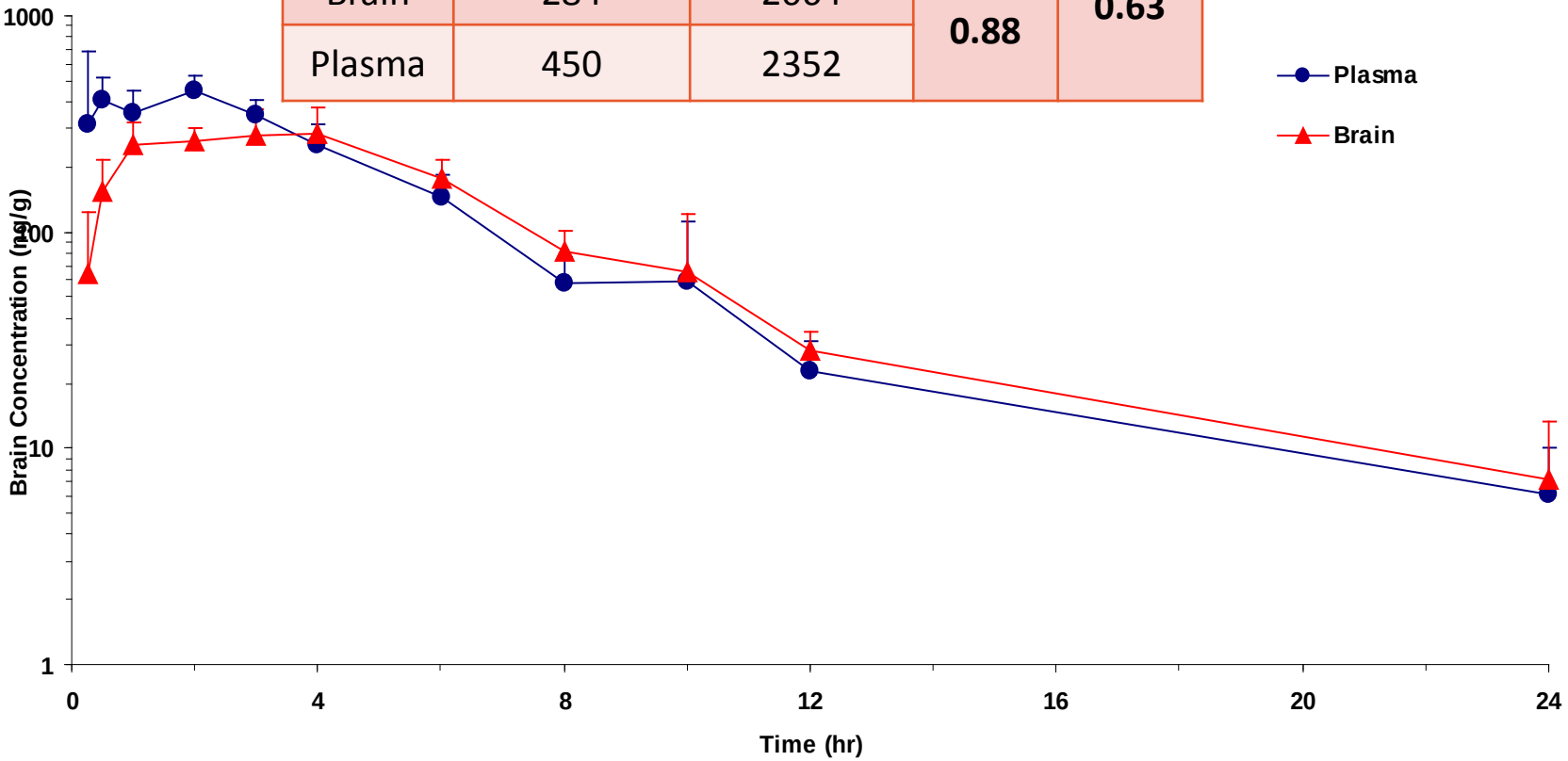
CNS Penetration: Single dose brain penetration in rats

Matrix	C_{max} (ng/mL or g)	AUC (ng.hr/mL)	AUC_b/AUC_P	C_b/C_p
Brain	284	2064	**0.88**	**0.63**
Plasma	450	2352		



Mean Plasma, Brain Profile of SB-742457, 10 mg/kg, *p.o.*

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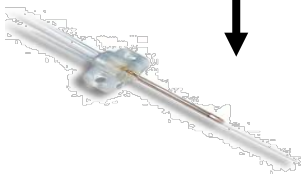
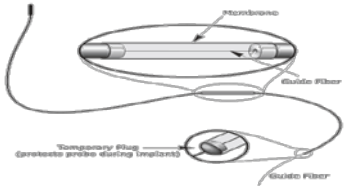
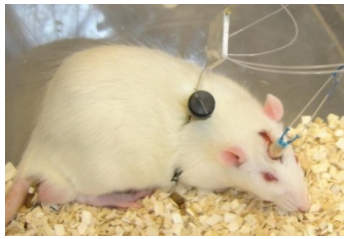
CNS Penetration: Microdialysis – Target Site Concentrations

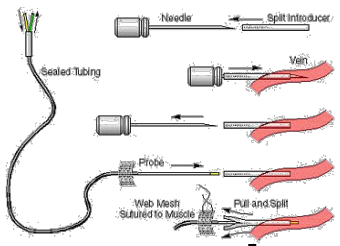
Microdialysis, a minimally-invasive sampling technique used for continuous measurement of free, unbound analyte concentrations in the extracellular fluid of virtually any tissue

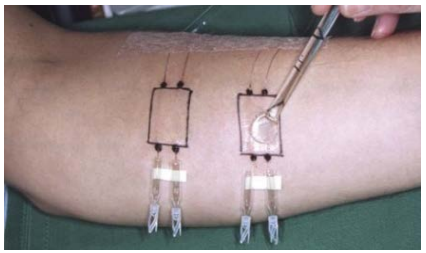
Infusion of physiological fluid





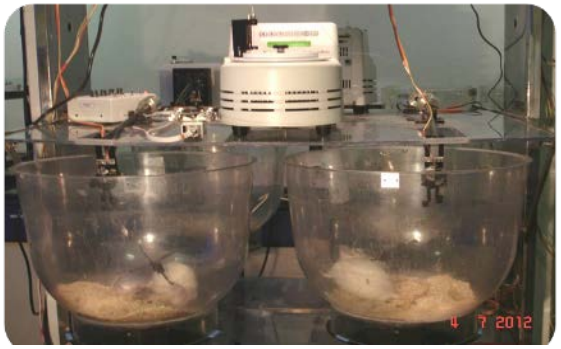






Picture taken from presentation
for PQRI Workshop March 2013 (Benfeldt E)

Collection of dialysates at 4° c using Fraction collector



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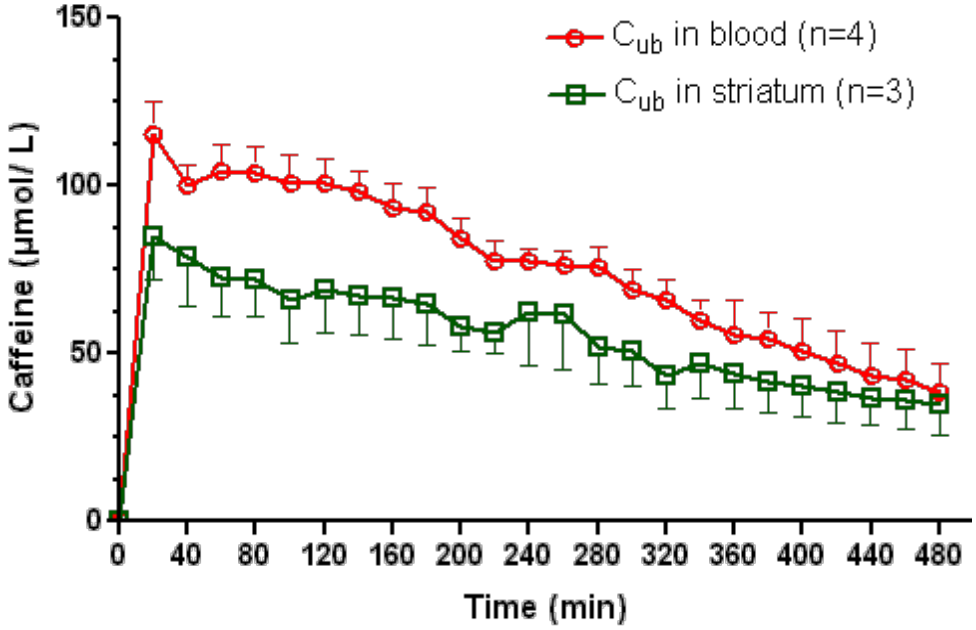
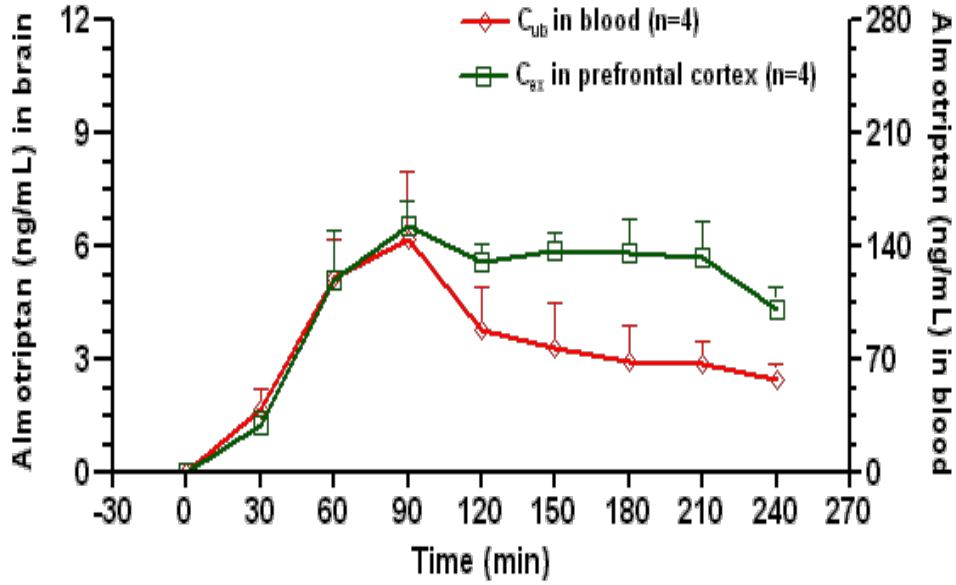
CNS Penetration: Unbound concentration in plasma and brain





Data expressed as mean ± SEM.

Animal : **Male Wistar rats**

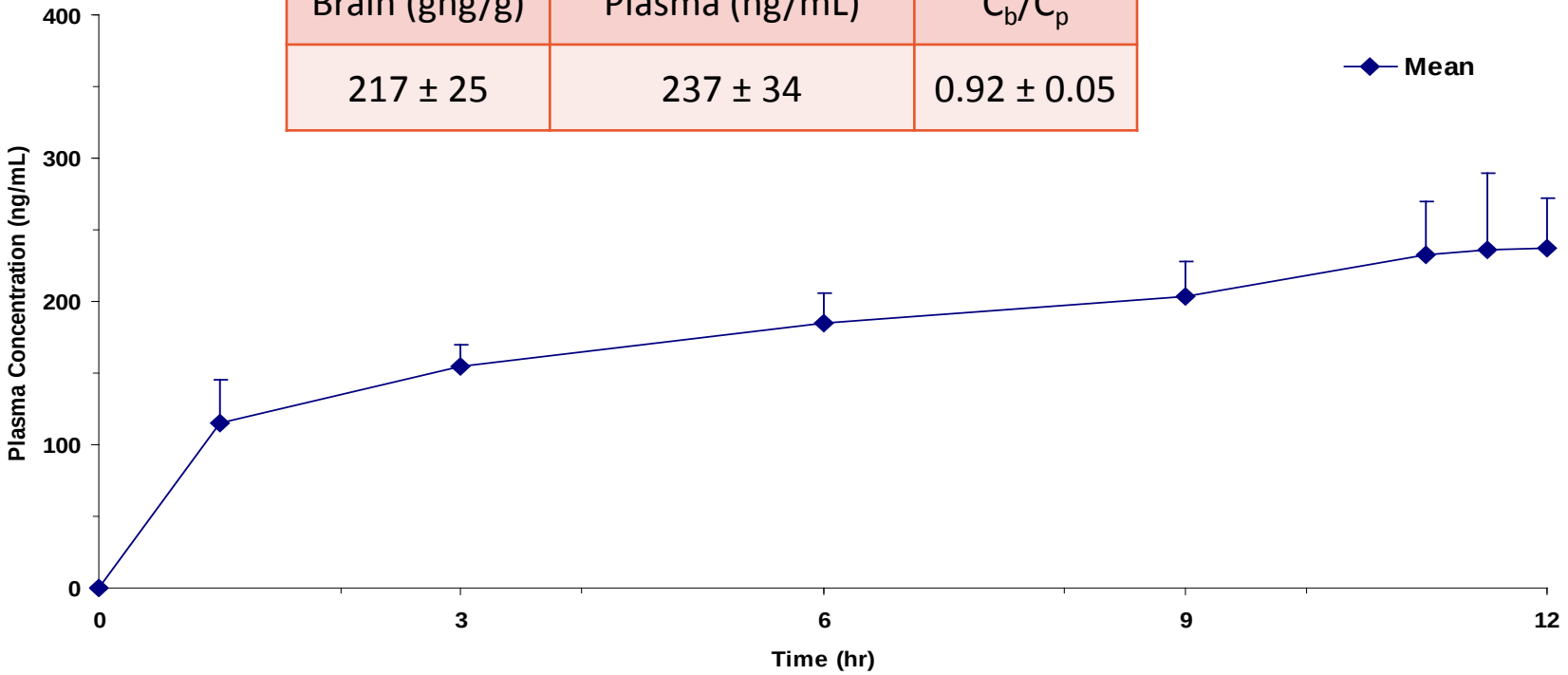
Probe : **CMA/20, 10 mm (blood), CMA/12, 4 mm (striatum) and CMA/11, 4 mm (prefrontal cortex)**

Quantification : **LC-MS/MS**

Cub= dialysate concentration X % in vivo recovery

CNS Penetration: Steady State Brain Penetration in rats

Steady State Brain Penetration		
Brain (gng/g)	Plasma (ng/mL)	C_b/C_p
217 ± 25	237 ± 34	0.92 ± 0.05



Mean Plasma Profile of SB-742457, 1 mg/kg, *i.v* constant infusion

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CNS Penetration: Exposures in CSF

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A simple and rapid method to collect the cerebrospinal fluid of rats and its application for the assessment of drug penetration into the central nervous system

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ABSTRACT

Many central nervous system (CNS) drug discovery programs require the successful collection of cerebrospinal fluid (CSF) for assessing CNS penetration and distribution of new chemical entities. The objective of the present investigation was to simplify the technique for collecting maximum CSF from cisterna magna of the rats. Rat was anesthetized with 5% halothane and positioned in a stereotaxic frame. The rat head was flexed downward at approximately 45°, a depressible surface with the appearance of a rhomb between occipital protuberances and the spine of the atlas becomes visible. The 23 G needle was punctured into the cisterna magna for CSF collection without making any incision at this region. The blunt end of the needle was inserted into a 10 in. length of PE-50 tubing and other end of the tubing was connected to a collection syringe. The non-contaminated sample was drawn into the syringe by simple aspiration. This technique is simple and can be performed by one person. The technique has a greater than 95% success rate of CSF collection and it was free of red blood cell contamination. In addition, it yielded 100–120 μL of CSF per rat. This method is simple, effective, and easy to perform and has been successfully applied in preclinical screening of novel chemical entities in neuropharmacotherapy for CNS use. The present method is demonstrated by studying the CSF concentrations of carbamazepine and raclopride.

Fig. 2. Collection of CSF from cisterna magna.

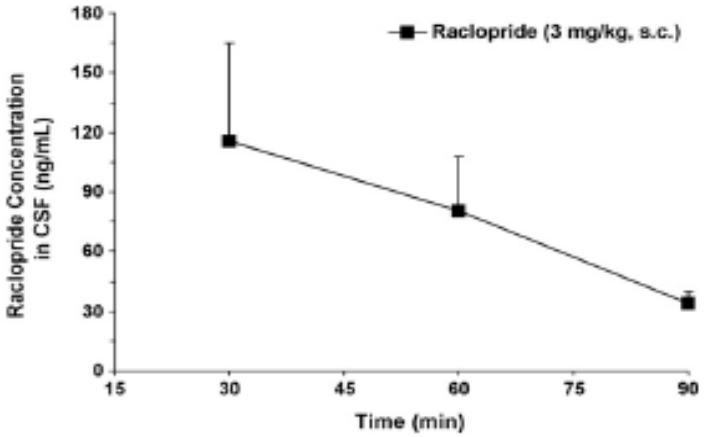

Fig. 4. CSF concentration time profile of raclopride following 3 mg/kg subcutaneous administration to Wistar rats.

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Mass Balance Studies using ^{14}C Test compounds

^{14}C labeled test compound dosing

Pharmacokinetics	Mass Balance	Tissue distribution
(Blood/ plasma)	(Blood/ plasma/ Bile Urine/ Feces)	(Blood/ plasma/ Other organs)

Plasma/ Tissue digestion

↓

Addition of Scintillation liquid, Equilibration

↓

Beta counts Recording
(Tricarb LSC)

Species: Mice, Rat and Guinea pig

Tricarb 3110TR : Lquid scintillation analyzer for detecting small amounts of alpha, beta and gamma radioactivity .

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Mass Balance Studies using ^{14}C Test compounds

Species: Male Sprague-Dawley rats

Route of Admin: Oral (Gavage)

Dose level (Free base): 20 mg/kg or µCi/kg, Dose Volume: 5 mL/kg

Collection Intervals: 0-8, 8 -24, 24 - 48, 48 - 72 and 72 - 96 h post dose, Daily Cage rinse and cage wash at terminus

Matrices	Interval (hours)	% Administered dose recovery			Mean	SD
		Rat #1	Rat #2	Rat #3		
Urine	**0-8**	79.86	78.71	81.34	79.97	1.32
	8-24	4.86	5.08	4.91	4.95	0.11
	24-48	1.2	1.0	0.24	0.80	0.49
	48-72	0.32	0.19	0.26	0.26	0.06
	72-96	0.34	0.15	0.08	0.19	0.14
	Subtotal	86.54	85.13	86.83	86.17	0.91
Feces	**8-24**	4.7	2.9	7.9	5.15	2.51
	24-48	4.7	7.2	2.8	4.93	2.21
	48-72	0.95	0.97	0.37	0.77	0.34
	72-96	0.42	0.67	0.30	0.47	0.18
	Subtotal	10.83	11.76	11.36	11.31	0.47
Cage rinse		0.34	1.02	0.75	0.71	0.34
Total		**97.71**	**97.91**	**98.94**	**98.19**	**0.66**

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Quality Assurance

- Independent Quality Assurance team
- Quality System Procedures (QSP's) for Quality System Management and Standard Operating Procedures (SOP's) for Operation, Calibration, Maintenance of Equipment's
- Accredited by ISO/IEC 17025:2005 Quality System since 2005
- Document and Data Control, Conducting Internal Audits, Study Specific Audits
- Dedicated Archive facility for the retention of the records
- Facility audited and approved by many global pharmaceutical companies and majority of Indian Pharma Companies
- Integrity of studies is maintained by following critical components of GLP compliance like SOP, test and control article handling, data handling, training documents, archives etc.

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