

Discovery Research

In-vivo Pharmacokinetics Capabilities



Discovery Research
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In-Vivo ADME Studies

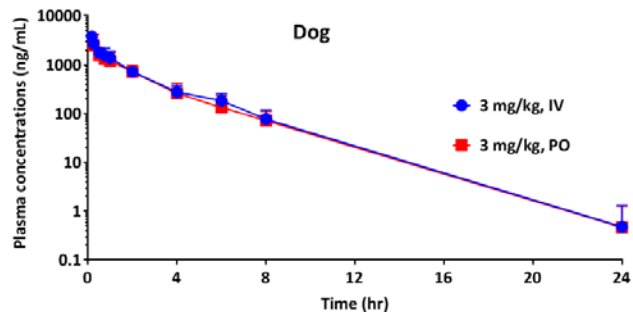
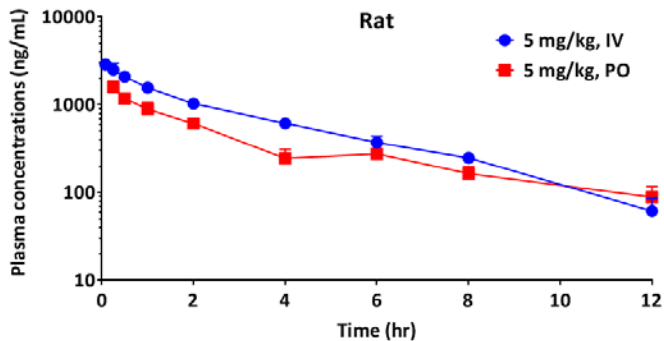
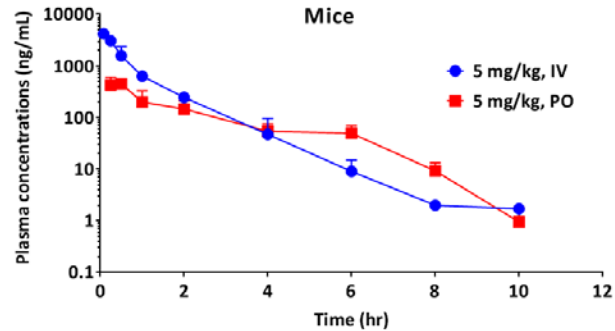
- Rodent bioavailability studies (Mouse, Rat, Guinea pig & Hamster)
- Non Rodent bioavailability study (Rabbit & Beagle Dog)
- Site specific absorption studies in rats (Single pass In situ intestinal perfusion studies)
- In-vivo approach for evaluation of MBI of CYP3A in rats
- CNS penetration studies: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



- 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



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



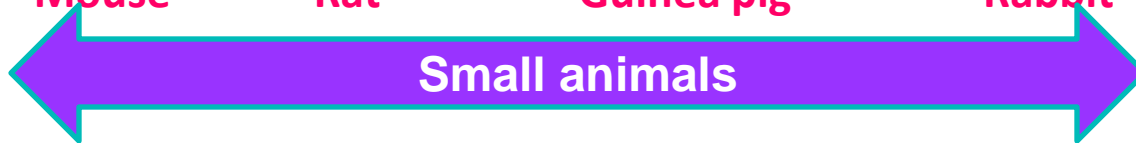
Rabbit



Dog

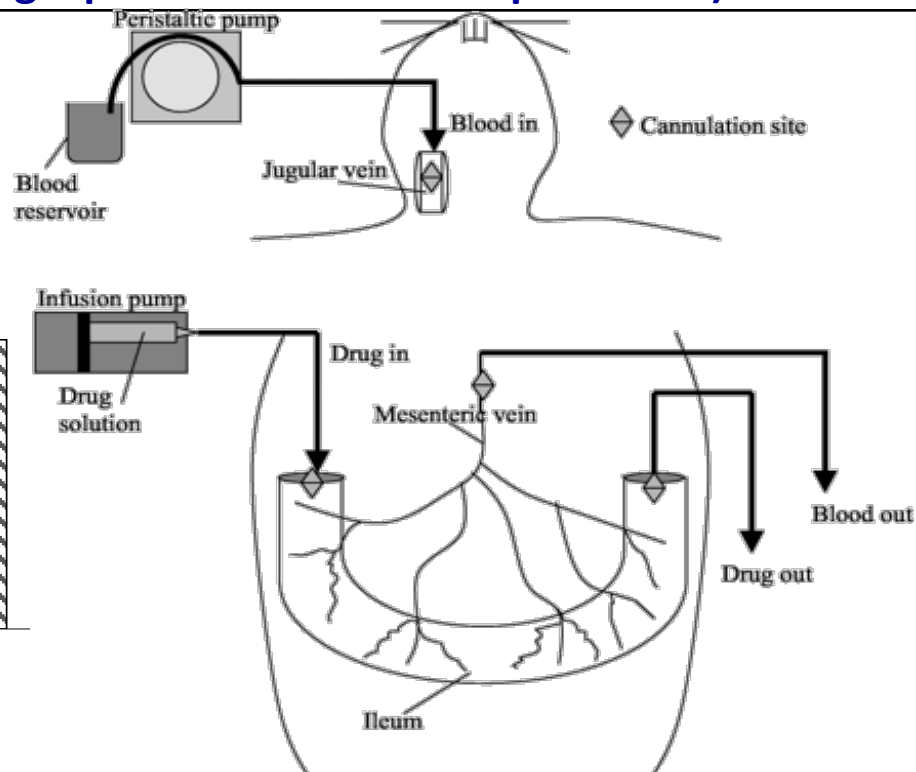
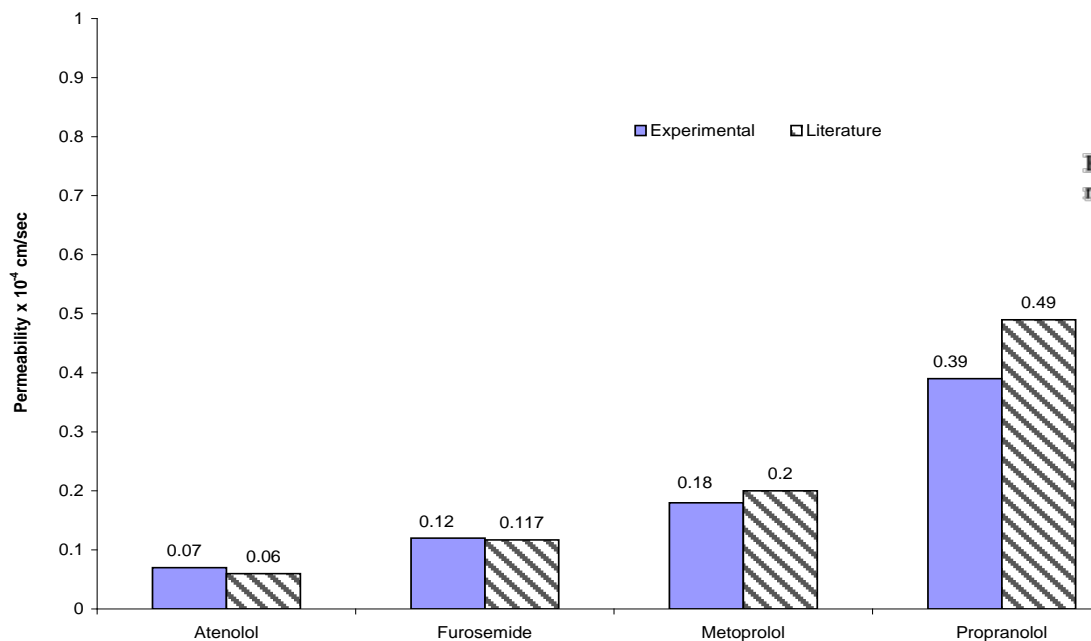


Monkey





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

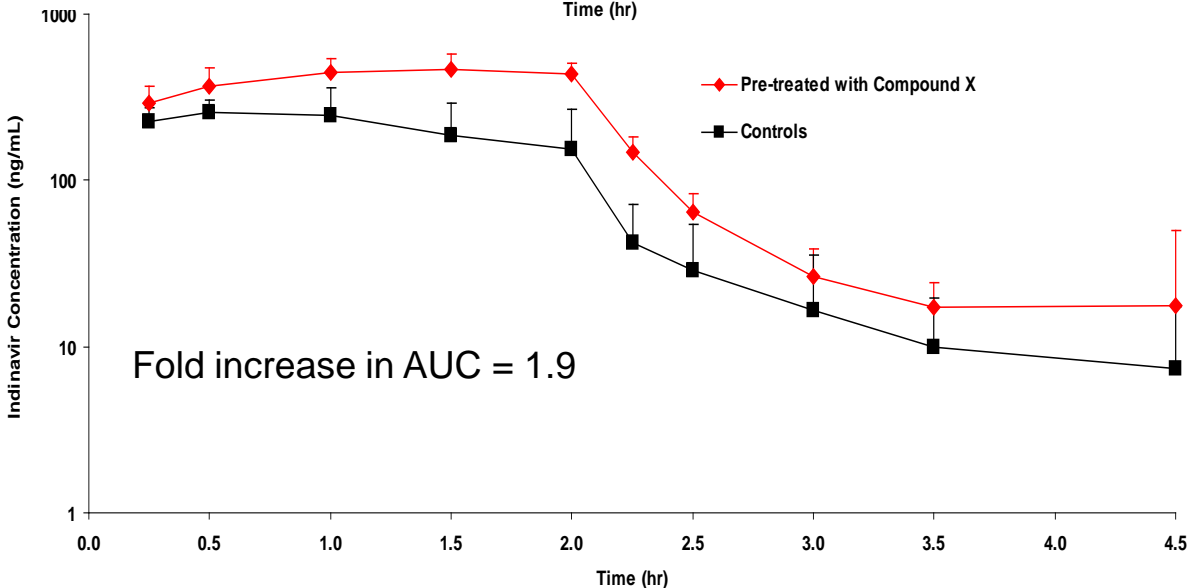
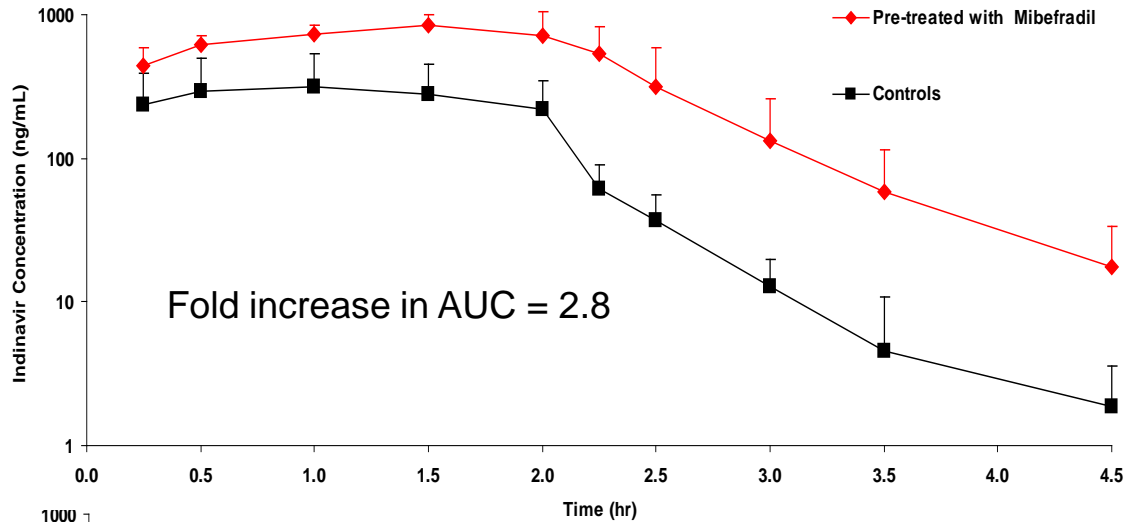
Test concentration : 10 μ M

Internal permeability reference standard (e.g. Metoprolol 100 μ M)

Non absorbable marker Phenol red 100 μ g.



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

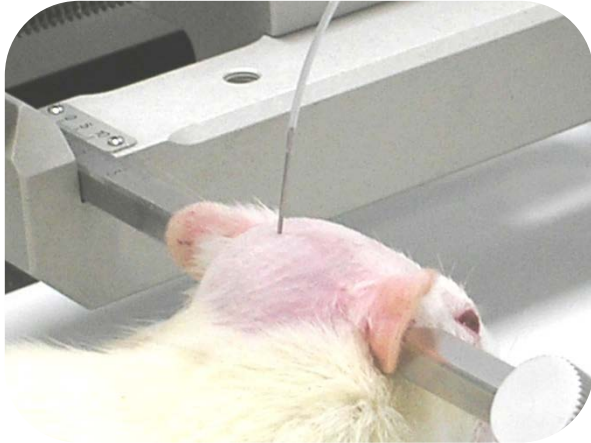
Infusion of Indinavir (4 mg/kg) Via
Portal vein for 2 hr

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)



Assessment of CNS Penetration



Brain Penetration Studies

- Single dose
- Steady state

Collection of CSF

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

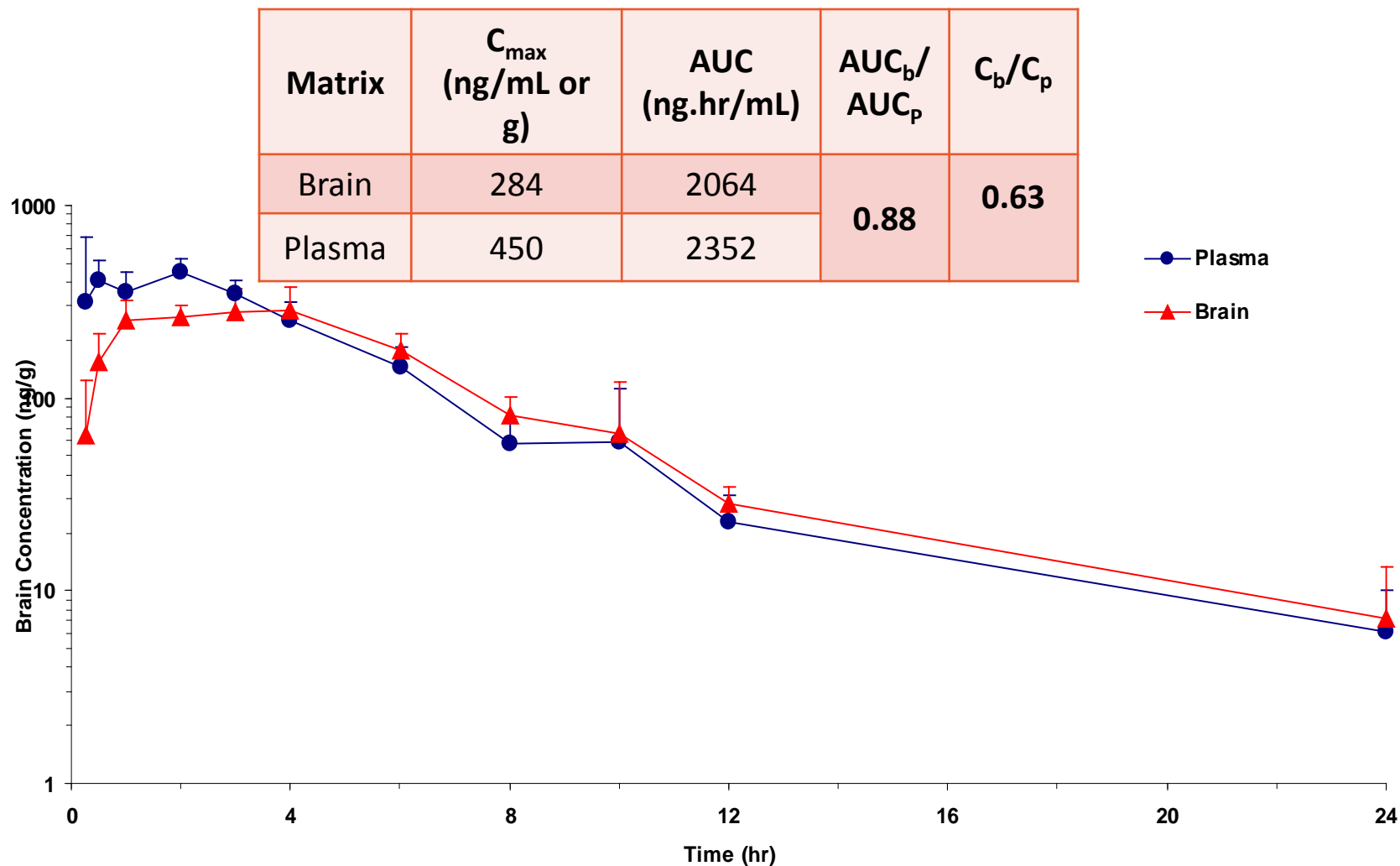
Brain Microdialysis

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





CNS Penetration: Single dose brain penetration in rats

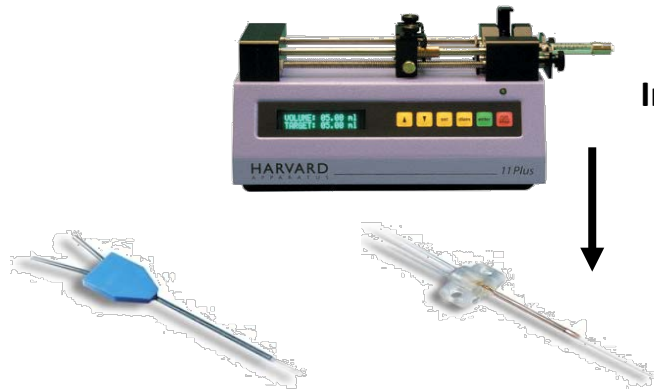


Mean Plasma, Brain Profile of SB-742457, 10 mg/kg, *p.o.*

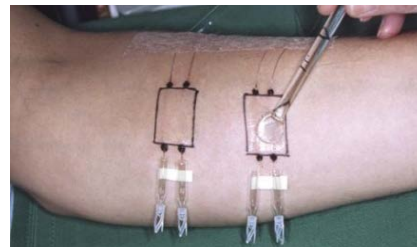
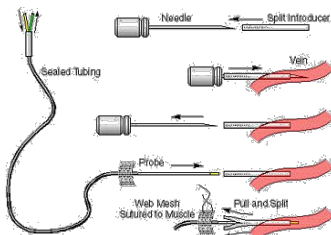
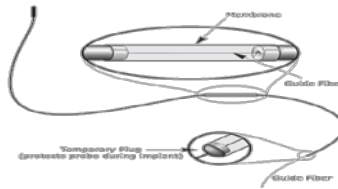


CNS Penetration: Microdialysis – Target Site Concentrations

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



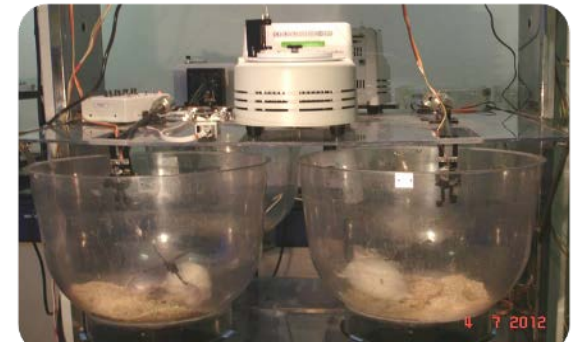
Infusion of physiological fluid



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

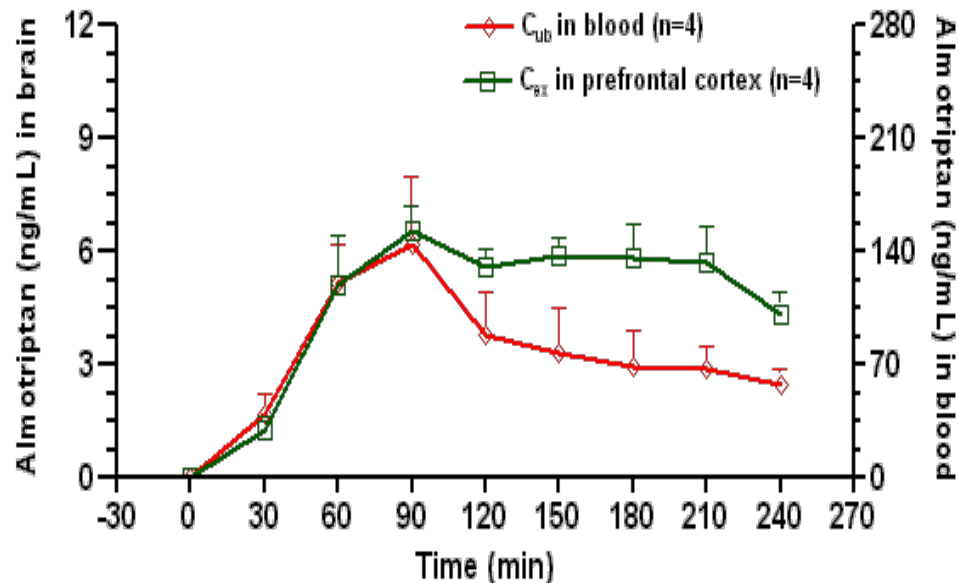
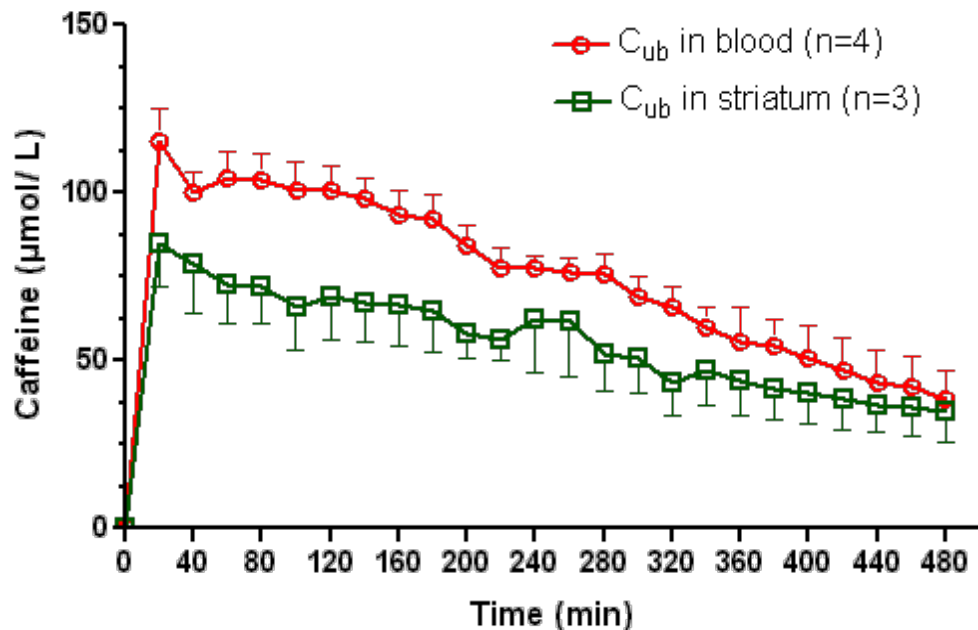


Collection of dialysates at 4° c using Fraction collector





CNS Penetration: Unbound concentration in plasma and brain



Data expressed as mean \pm SEM.

Animal : Male Wistar rats

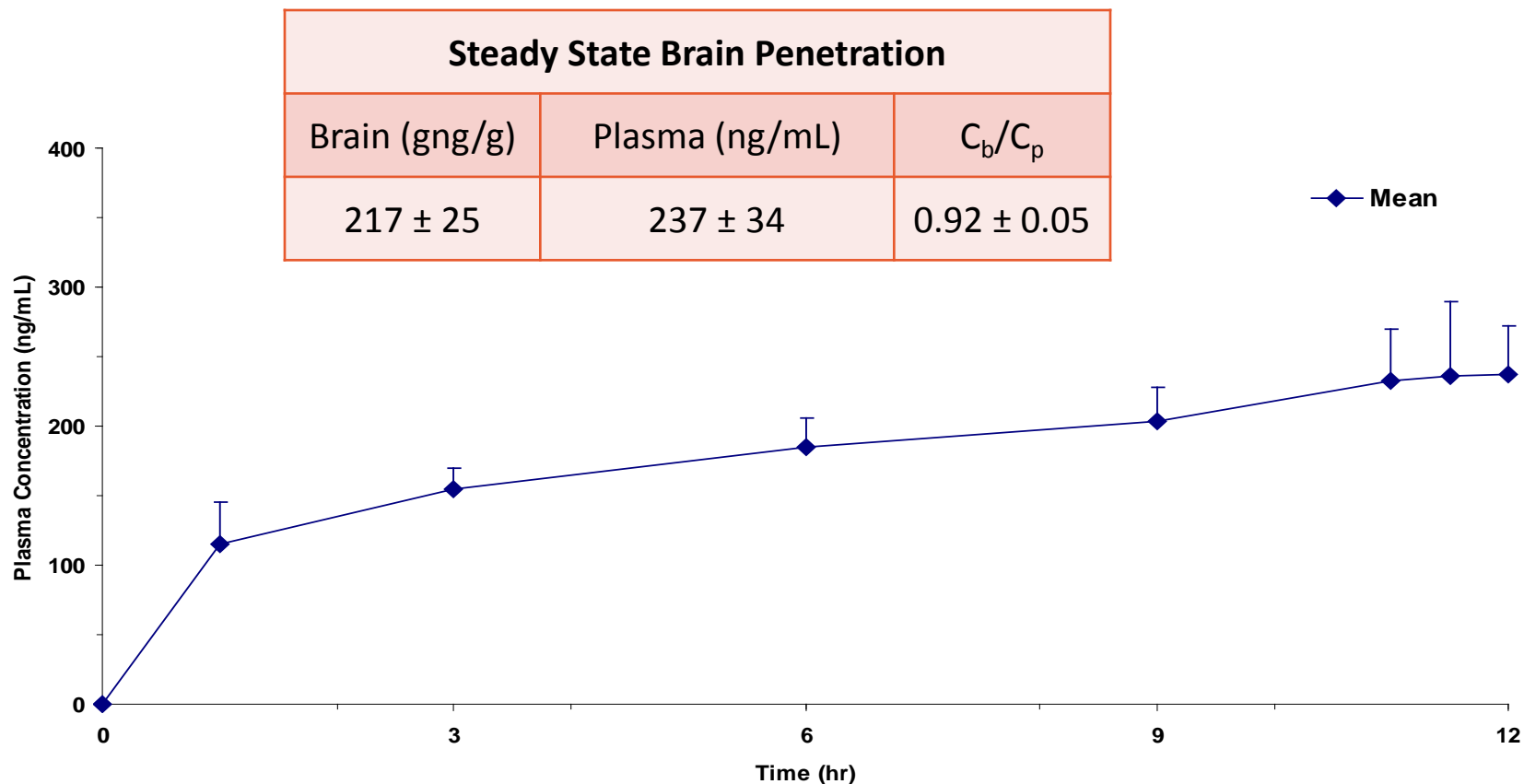
Probe : CMA/20, 10 mm (blood), CMA/12, 4 mm (striatum) and CMA/11, 4 mm (prefrontal cortex)

Quantification : LC-MS/MS

Cub= dialysate concentration X % in vivo recovery



CNS Penetration: Steady State Brain Penetration in rats



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



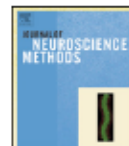
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A simple and rapid method to collect the cerebrospinal fluid of rats and its application for the assessment of drug penetration into the central nervous system

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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.

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Fig. 2. Collection of CSF from cisterna magna.

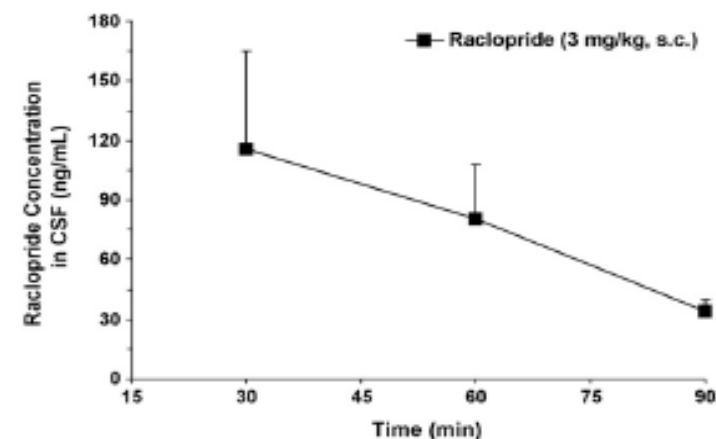


Fig. 4. CSF concentration time profile of raclopride following 3 mg/kg subcutaneous administration to Wistar rats.



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)

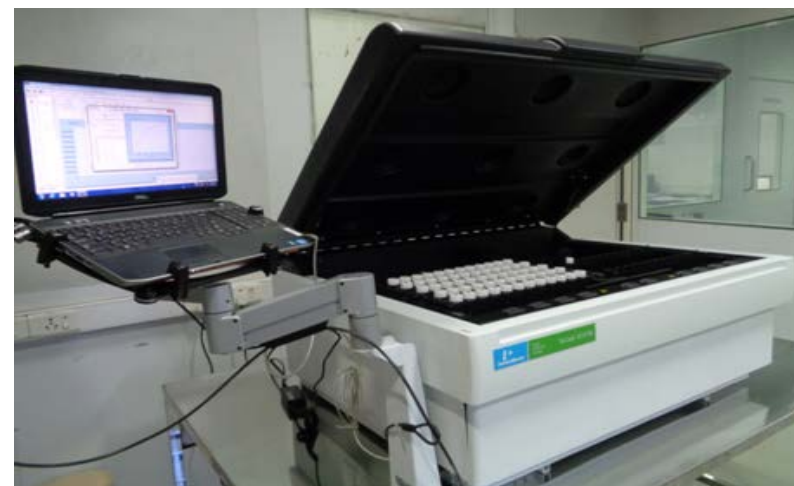
Plasma/ Tissue digestion

Addition of Scintillation liquid, Equilibration

Beta counts Recording
(Tricarb LSC)

Species: Mice, Rat and Guinea pig

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





Mass Balance Studies using ¹⁴C Test compounds

Species: Male Sprague-Dawley rats

Route of Admin: Oral (Gavage)

Dose level (Free base): 20 mg/kg or μ Ci/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
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
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
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- Facility audited and approved by many global pharmaceutical companies and majority of Indian Pharma Companies
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