

Estimation of Rat Brain Free Fraction by Equilibrium Dialysis

Background

Assessment of brain unbound concentration ($C_{u,brain}$) is crucial in understanding the pharmacokinetics and pharmacodynamics of CNS targeted drug candidates, as only the unbound drug is available to elicit a pharmacological effect. The drug unbound fraction ($f_{u,brain}$) determined by *In Vitro* experiments correlates well with pharmacodynamic endpoints across a range of CNS targets. Brain tissue binding experiments using equilibrium dialysis with rat brain homogenates is a widely accepted method for determining the free fraction of drug candidates. NoAb BioDiscoveries has developed its brain tissue binding assay using a 96-well equilibrium dialysis unit. Combined with accurate LC-MS/MS analysis, the 96-well format offers a rapid throughput method for quickly and accurately determining the free fraction of drug candidates in brain tissue.

Assay Outline

The assay is performed in a 96-well Teflon dialysis unit. Each well consists of two chambers separated by a vertically aligned dialysis membrane of pre-determined pore size. Rat brain homogenate spiked with the test drug candidate of interest is added to one chamber and blank buffer to the opposing chamber. Over time, free drug candidate that is not bound to brain homogenate diffuses from the brain homogenate chamber to the buffer chamber and attains equilibrium. The drug unbound fraction ($f_{u,brain}$) is then calculated as the concentration in the buffer divided by the total concentration in the brain homogenate. Buffer spiked with drug candidate is also dialyzed against blank buffer in order to determine whether equilibrium was reached.

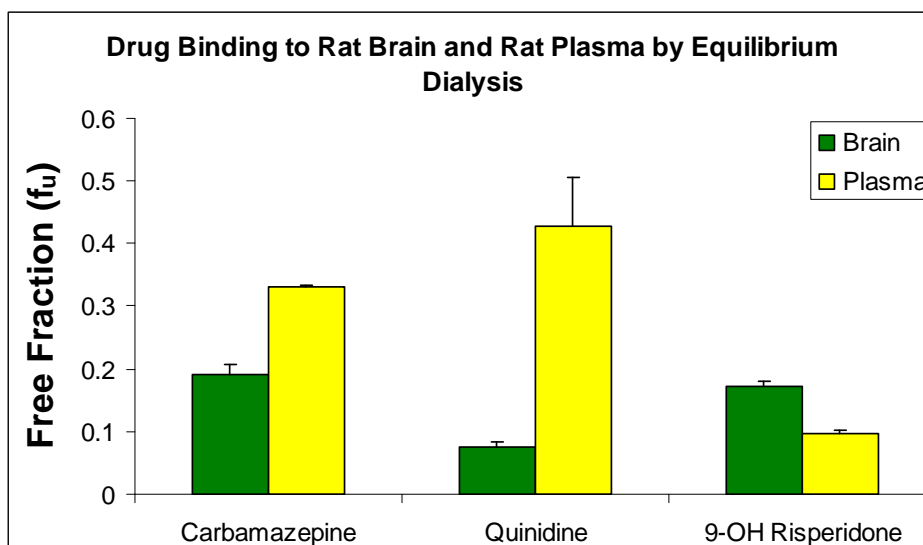
Typical Results

Binding to rat plasma and rat brain by the equilibrium dialysis method
Data represent the mean \pm S.D. of 3 replicates.

Compound ID	Matrix	Concentration ^a	Unbound ^b	Recovery
		(μ M or μ moles/g)	(%)	(%)
Carbamazepine	Plasma	2.17 \pm 0.21	33.1 \pm 0.32	96.3 \pm 9.5
	Brain	3.15 \pm 0.078	19.2 \pm 1.6	95.6 \pm 1.8
Quinidine	Plasma	3.27 \pm 0.18	42.8 \pm 7.7	92.9 \pm 1.8
	Brain	3.95 \pm 0.43	7.55 \pm 0.92	92.6 \pm 8.5
9-OH Risperidone	Plasma	3.54 \pm 0.050	9.78 \pm 0.38	75.5 \pm 1.0
	Brain	3.52 \pm 0.17	17.2 \pm 0.77	88.0 \pm 3.4

^a Concentration (bound & unbound) measured at equilibrium. ^b Observed values are consistent with values reported in the literature.¹

¹ Liu X, Natta KV, Yeo, H, Vilenski O, Weller PE, Worboys PD, and Monshouwer M (2009). Unbound drug concentration in brain homogenate and cerebral spinal fluid at steady state as a surrogate for unbound concentration in brain interstitial fluid. *Drug Metab Dispos.* **37**:787-793.



Other Blood Brain Barrier Models

In addition to determining the free fraction of a drug candidate in brain tissue, researchers must obtain a PK/PD relationship for development of their drug candidates. To this end, NoAb BioDiscoveries offers a full suite of discovery research services aimed at evaluating the ability of drug candidates to cross the blood brain barrier.

Whole Brain Collection in Rodents

In Vivo terminal sampling of dosed rodent brain tissue for screening brain permeability of drug candidates, which can be combined with equilibrium dialysis to determine drug unbound fraction

In Cerebro™ Rat Brain Endothelial Cell Culture Permeability Assay

In Vitro Blood Brain Barrier Permeability model which correlates well with *In Vivo* results and can be used with *In Silico* Physiologically Based Pharmacokinetic modelling

Serial Cerebrospinal Fluid (CSF) and Plasma Collection in Freely Moving Rats

In Vivo model providing simultaneous plasma and CSF kinetics for drug candidates in the same animal, where CSF is useful as a surrogate for unbound drug brain concentrations

Quantitative Microdialysis

In Vivo model providing the most direct measurement of unbound drug candidate and physiological biomarker kinetics in a specific region of the brain

For more information please contact us at:

Toll free: 866.729.6622

Email: info@noabbiodiscoveries.com

www.noabbiodiscoveries.com

