

Cloe Select Brain Tissue Binding

cyprotexexperts in **ADME**

Background Information



'Neither total brain levels nor BBB permeability can be taken without considering the binding capacity of the brain tissue, when a link between exposure and efficacy is needed.'

¹Reichel A (2009) *Chemistry and Biodiversity* **6**, 2030-2049

- The extent of partitioning into brain tissue influences CNS penetration which in turn influences the efficacy and / or toxicological effects of a drug.
- The composition of brain and plasma are very different, with plasma having twice as much protein and brain having 20 fold more lipids, therefore free fraction in plasma is not a suitable surrogate for unbound brain concentrations¹.
- Assuming passive equilibrium, it is expected that brain to plasma drug exposure levels for any species will be predicted by the relative ratio of free fractions in these matrices².
- For compounds which undergo drug transport, differences between the unbound plasma-to-brain fraction ratios and brain-to-plasma exposure can be used to examine the net influence of active efflux processes on CNS exposure independent of the exact cellular mechanism².
- The Cloe Select Brain Tissue Binding assay is performed using equilibrium dialysis, one of the most widely accepted methods for assessing protein and tissue binding.
- Cloe Select Brain Tissue Binding assay delivers a value of fraction of compound unbound to brain tissue ($f_{u,brain}$).

Protocol

Method

Equilibrium dialysis using brain homogenate

Typical Test Compound Concentration5 μ M (different concentrations available)**Number of Replicates**

2

Compound Requirements150 μ L of a 10mM DMSO solution**Analysis Method**

LC-MS/MS quantification (both brain homogenate and buffer standards prepared)

Data DeliveryFraction unbound in brain
Recovery

High specific binding at the pharmacological target in the CNS and greater free fractions in brain can counterbalance poor BBB permeation and/or extensive plasma protein binding¹.

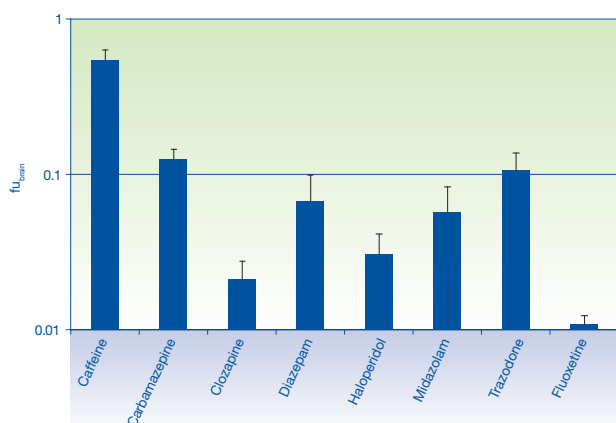


Cloe Select Brain Tissue Binding

For the validation, eight compounds were screened in the Cloe Select Brain Tissue Binding assay (rat and mouse) on three separate occasions. Data were compared with literature data (figure 2).

Figure 1

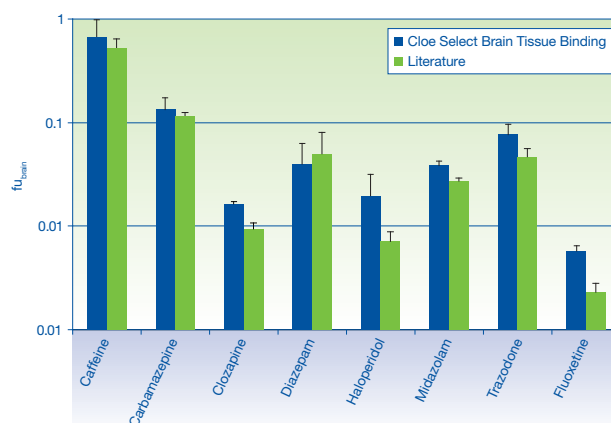
Graph showing Cloe Select Rat Brain Tissue Binding data for a set of eight compounds over three separate assays.



These data illustrate good consistency is achieved over a number of different days for compounds with a range of binding values.

Figure 2

Graph showing a comparison of fraction unbound in mouse brain between Cloe Select Brain Binding data (mean \pm standard deviation; n=3) and literature³ data for a set of eight compounds.



The Cloe Select data correlate well with literature data for compounds with a range of different binding values.

References

- ¹ Reichel A (2009) *Chem Biodiv* **6**, 2030-2049.
- ² Kalvass JC and Maurer TS. (2002). *Biopharm Drug Dispos* **23**; 327-338.
- ³ Maurer TS et al. (2005) *Drug Metab Dispos* **33**; 175-181.