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In vitro **ADME & PK**

Brain Tissue Binding

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.'

[1]Reichel 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 concentrations[1].
- 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 matrices[2].
- For compounds which undergo drug transport, difference 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 mechanism[2].
- Cyprotex's Brain Tissue Binding assay is performed using equilibrium dialysis, one of the most widely accepted methods for assessing protein and tissue binding.
- Cyprotex's Brain Tissue Binding assay delivers a value of fraction of compound unbound to brain tissue (fu_{brain}).

Protocol

Method
Equilibrium dialysis using brain homogenate

Typical Test Article Concentration
5 µM (different concentrations available)

Number of Replicates
2

Test Article Requirements
150 µL of 10 mM DMSO solution

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

Data Delivery
Fraction unbound in brain
Recovery

To find out more contact **enquiries@cyprotex.com**

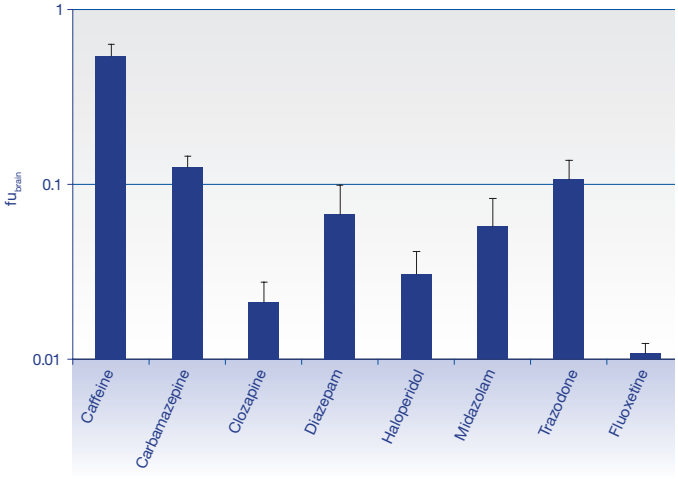
High specific binding at the pharmacological target in the CNS and greater free fractions in brain can counterbalance poor BBB permeation and/or extensive plasma protein binding[1].

Brain Tissue Binding

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

Figure 1

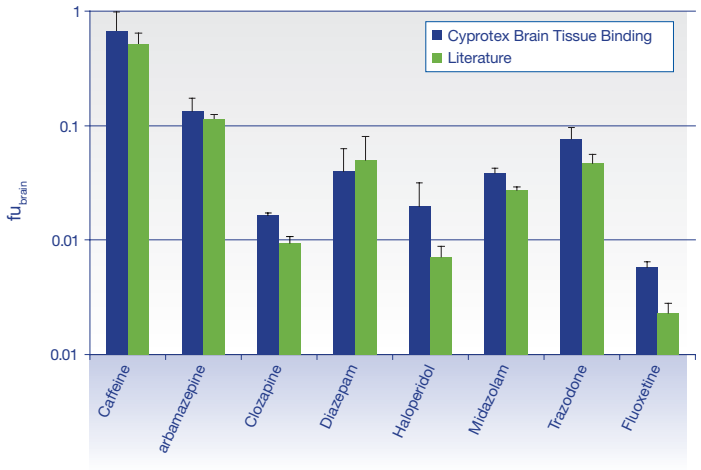
Graph showing Cyprotex's Rat Brain Tissue Binding data for a set of eight compounds over three separate assays.



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 Cyprotex's Brain Binding data (mean ± standard deviation; n=3) and literature[3] data for a set of eight compounds.



Cyprotex's 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.