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In vitro **ADME & PK**

Plasma Stability

Background Information

'Plasma stability assay has many applications in drug discovery: to alert teams to labile structural motifs, to prioritize compounds for *in vivo* studies and to screen prodrugs and antedrugs.'

[1]Di L ,Kerns E.H., Hong Y and Chen H. (2005) *International Journal of Pharmaceutics* **297**; 110-119.

- Determination of the stability of new chemical entities in plasma is important as compounds (with the exception of pro-drugs) which rapidly degrade in plasma generally show poor *in vivo* efficacy.
- Instability in plasma can result in misleading *in vitro* data which can be difficult to interpret (e.g., plasma protein binding data). Storing and analysing clinical samples from *in vivo* pharmacokinetic studies may also prove challenging.
- Compounds with the following functional groups tend to be more susceptible to hydrolysis in plasma: esters, amides, lactones, lactams, carbamides, sulphonamides, and peptic mimetics[1].
- Compounds may exhibit interspecies differences in their stability in plasma.
- Plasma stability is very useful for screening of prodrugs and antedrugs, where rapid conversion in plasma is desirable.

Protocol

Assay Matrix
Plasma from multiple donors (other matrices available on request)

Test Article Concentration
1 µM (different concentrations available)

DMSO Concentration
0.25%

Incubation Time
0, 5, 15, 30, 60 and 120 min

Test Article Requirements
30 µL of 10 mM DMSO solution

Analysis Method
LC-MS/MS

Assay Controls
Positive control compound which undergoes degradation in plasma

Data Delivery
Half-life of compound
Percent parent compound remaining at each time point

Follow on metabolite profiling studies

Cyprotex's plasma stability assay can be extended to profile the metabolites that are formed. Cyprotex's biotransformation services are supported by high resolution, accurate mass spectrometry. These services can provide information on an individual species' metabolite profile, or a cross-species comparison to identify potential differences in metabolism which could in turn help to interpret pharmacology and toxicity data. Structural elucidation can also be performed on the potential metabolites' MS/MS fragmentation data. All biotransformation studies are performed by a dedicated team of experts.

Please refer to Cyprotex's Metabolite Profiling and Identification section for further details.

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

Plasma stability has several applications: to understand data where compounds are unexpectedly rapidly cleared; to screen for prodrugs and antedrugs; and to determine the liability of drugs with susceptible structural motifs.

Cyprotex's Plasma Stability identifies compounds which are unstable in plasma.

The test compound (final incubation concentration = 1µM) is incubated with plasma (final DMSO concentration = 0.25%) at six different time points (0, 5, 15, 30, 60 and 120 min). The reaction is terminated by acetonitrile containing internal standard. Following centrifugation, the disappearance of test compound is monitored by LC-MS/MS. The half-life and the percentage of test compound remaining at the individual time points relative to the 0 minute sample are then reported. A control compound known to be metabolised by plasma esterases is also incubated alongside each batch of test compounds.

Figure 1

Graphs displaying disappearance of benfluorex in human and rat plasma, and propantheline in mouse and dog plasma over 120 minutes (data is an average from n≥19 across multiple assays and different pooled plasma batches).

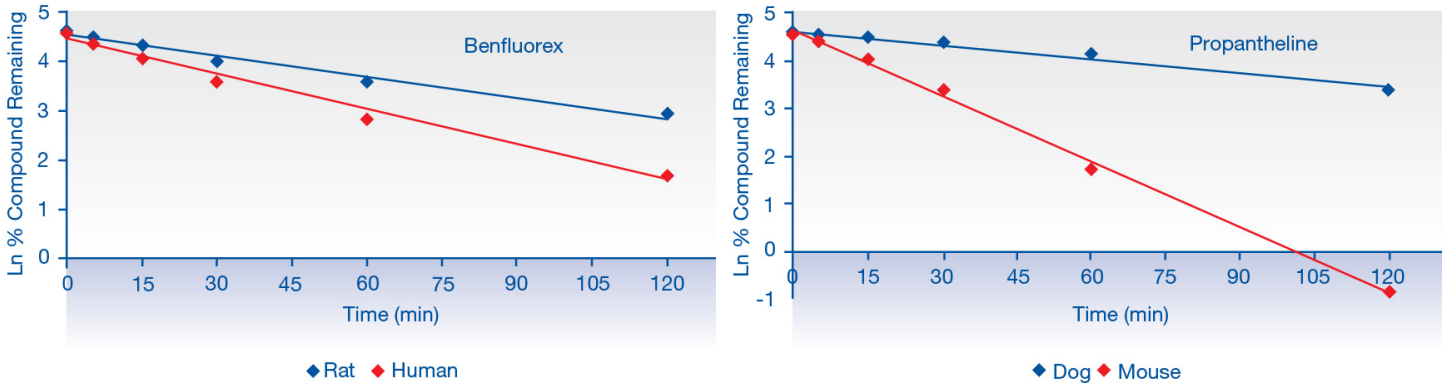

Table 1

Average half-life for benfluorex and propantheline in plasma (average obtained from multiple assays utilising different batches of pooled plasma).

Species	Positive Control	t1/2 (min)	Standard Deviation	n
Human	Benfluorex	23.4	4.87	19
Rat	Benfluorex	47.8	11.6	19
Mouse	Propantheline	14.9	1.18	20
Dog	Propantheline	69.7	7.29	20

References

[1] Di L *et al*. (2005) *International Journal of Pharmaceutics* **297**; 110-119.

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