

# Cloe Screen Chemical Stability with Non Specific Binding Assessment

**cyprotex**experts in **ADME**

## Background Information



'At the drug discovery stage, when drug candidates are screened against biological targets, compounds need to have sufficient stability in the assay buffers for enzyme, receptor, or cell-based assays to reliably measure biological activity.'

<sup>1</sup>Di L, Kerns E.H, Chen H, and Petusky SL. (2006) *J Biomol Screen* 2006; 11 (1) 40-47.

- A compound is chemically unstable when it is degraded by non-enzymatic processes. Degradation may be caused by several mechanisms, the most common being hydrolysis, oxidation, or light-catalysed degradation.
- Compounds that are highly unstable may not be suitable as drug candidates since it may be difficult to maintain a therapeutically effective formulation.
- Compounds designed for oral administration must be chemically stable at the low pH values observed in the stomach in order for this to be an acceptable route. A range of different pH values are available.
- Cloe Screen Chemical Stability assay is combined with an assessment of non specific binding by performing the assay in plates prepared from two different materials with different binding characteristics, polypropylene and PTFE (otherwise known as polytetrafluoroethylene or Teflon®).
- Both non specific binding to apparatus such as plates or tips and chemical instability can be responsible for inaccurate *in vitro* data.

### Protocol

**Test Compound Concentration**  
1  $\mu$ M (different concentrations available)

**DMSO Concentration**  
0.33 %

**Time Points**  
0, 5, 15, 30, 45, 120 min

**Number of Replicates**  
2

**Incubation Plate Material**  
PTFE (all time points) and polypropylene (2 hr only)

**Compound Requirements**  
50  $\mu$ L of 10 mM solution

**Analysis method**  
LC-MS/MS

**Data Delivery**  
% Parent compound remaining at each time point  
% Parent compound bound to polypropylene compared to PTFE

Oral administration is the route of choice for new drugs. Compounds must be chemically stable at the low pH values observed in the stomach in order for this to be an acceptable route.

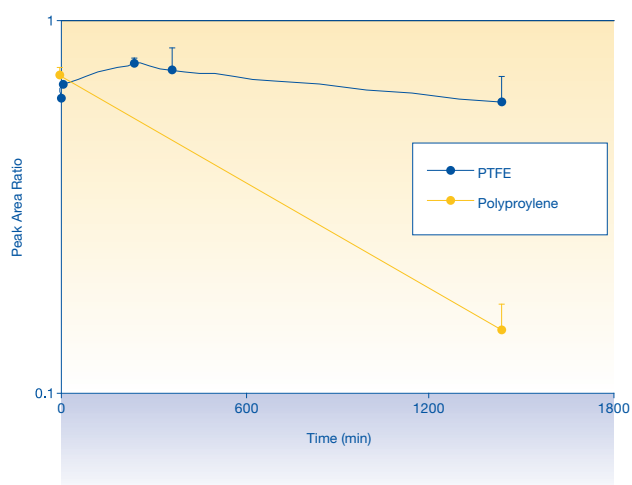


### Cloe Screen Chemical Stability with Non Specific Binding Assessment

A series of compounds, with potential issues with non-specific binding, was screened through the Cloe Screen Chemical Stability. The stability was assessed in PTFE at a five time points up to 24 hr by monitoring the peak area ratio. The peak area ratio was also monitored after incubating the test compound in polypropylene over 24 hr. Using this method, the stability of the test compound and the extent of binding to polypropylene as compared to PTFE could be assessed.

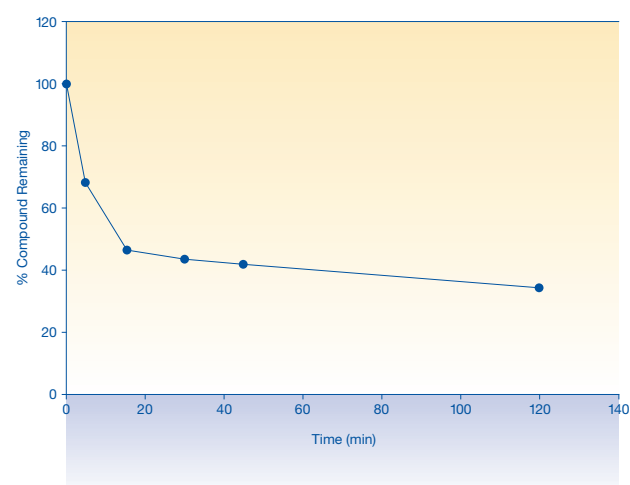
**Figure 1**

Graph showing the peak area ratio (mean  $\pm$  standard deviation) of a compound following incubation in buffer at pH7.4 in PTFE and polypropylene over 24 hr.



**Figure 2**

Illustration of a Compound which exhibits pH dependent degradation over 120 minutes at pH 2.



#### References

<sup>1</sup> Di L *et al.* (2006) *J Biomol Screen* **11** (1); 40-47.