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Purpose
Accurate measurement of plasma protein binding (PPB) of certain compounds can be challenging, e.g., highly bound, high molecular weight, highly lipophilicity and low solubility. As a result, the regulatory agencies have issued drug-drug interaction (DDI) draft guidelines on the lower reportable limit of 1% for plasma protein binding. Setting a lower limit for fraction unbound (fu) at 1% is somewhat arbitrary, does not necessarily reflect an assay’s capability or performance, and it can lead to over-prediction of DDI for highly bound compounds and resulted in the conduct of unnecessary and expensive clinical studies. It is, therefore, important to understand the limitations of a plasma protein binding assay and to set realistic expectations on reportable fu values based on scientific data.

Methods
Three different equilibrium dialysis methods (standard, dilution and pre-saturation) were used to evaluate the accuracy and precision of fu measurement for a set very challenging compound (e.g., itraconazole, amiodarone and UCN-01). The data were compared with literature fu values using other approaches (erythrocyte portioning method and ultracentrifugation method). Statistical analysis was applied to evaluate intraday and interday assay variability.

Results
Data analysis of over 2000 PPB fu values (ranging from 0.00005 to ~1) for Pfizer drug discovery compounds with diverse structures and physicochemical properties in five different species (human, rat, mouse, dog, and monkey) showed that there is no bias of fu being less precise for highly bound compounds than weakly bound ones. Using the pre-saturation method and the dilution method, fu values of the very challenging compounds can be measured accurately and the data is comparable with what is reported in the literature using very different methods (see table below).

Conclusion
The study show that PPB below 1% can be measured accurately when appreciate assay conditions are used and these values should be used for DDI prediction. Setting 1% reportable lower limit of PPB is overly conservative and can lead over-prediction of DDI.

*Some of the material has been published in J Pharm Sci and presented at DDI (June 19 - July 1) and Gordon (July 13-16) conferences.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>fu (Dilution Method)</th>
<th>fu (Pre-Saturation Method)</th>
<th>fu (Literature Values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itraconazole</td>
<td>0.0029</td>
<td>0.022</td>
<td>0.052 (equilibrium dialysis)</td>
</tr>
<tr>
<td>UCN-01</td>
<td>0.0019</td>
<td>0.0031</td>
<td>0.0022 (ultracentrifugation)</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>0.00011</td>
<td>0.0021</td>
<td>0.0002 (erythrocyte partition)</td>
</tr>
</tbody>
</table>