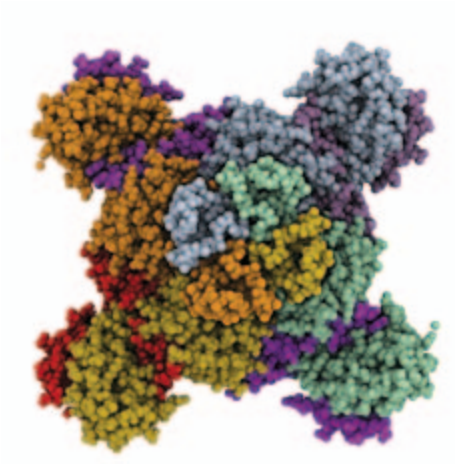
Automated equilibrium dialysis for plasma protein binding analysis

Johnson & Johnson has used a Freedom EVO® workstation to develop a semi-automated high throughput equilibrium dialysis assay for plasma protein binding



Protein complex.

At Johnson and Johnson's Pharmaceutical Research and Development facility in Beerse, Belgium, the ADME-Tox department runs a range of in-house designed *in vitro* and *in vivo* screening assays of varying complexity, generating data to support drug discovery across several therapeutic areas. A significant part of the department's workflow uses the Freedom EVO platform to deliver high quality ADME data.

The use of physiologically-based models to predict human pharmacokinetics and pharmacodynamics is becoming increasingly prevalent within the pharmaceutical industry. The binding affinity of candidate compounds for plasma proteins is a key parameter in these studies, requiring an accurate method for determination of plasma protein binding (PPB). Equilibrium dialysis is the most automation-friendly PPB technique, and the Beerse ADME-Tox group has developed, validated and implemented a high throughput PPB assay using the Pierce 48-well format Rapid Equilibrium Dialysis (RED) device. This microdialysis platform uses disposable inserts, and has two side-by-side chambers separated by a vertical cylinder of dialysis membrane. Following initial set-up, all the liquid handling steps of the semi-automated assay are performed on a Freedom EVO 200 workstation equipped with a liquid handling (LiHa) arm and a MultiChannel Arm™ 96 (MCA 96), and has a capacity of 32 plasma protein binding measurements per screen. Each assay uses triplicate incubations per measurement, and is fully flexible in terms of the number of compounds and plasma types that it can accommodate.

Claire Mackie, Department Head for ADME-Tox, explained: "We have a variety of Tecan platforms which are used to automate our *in vitro* assays for high throughput ADME purposes. Our assays are typically based on a 96-well microplate format, screening from 10 or 12 compounds up to 100 compounds at a time, depending on the complexity of the assay. The Freedom EVO 200s, which we use for 90 % of our work, are employed as standalone platforms for PPB analysis with the RED device, preparing samples for quantitative bioanalysis by liquid chromatography coupled with tandem mass spectrometry (LC- MS/MS). The Freedom EVO transfers spiked plasma and buffer to the RED device, which is then sealed and incubated for four and a half hours in an off-line incubator. During the incubation period, further automated procedures are carried out to prepare the calibration curves and quality control standards. After incubation, the RED device is returned to the Freedom EVO, where plasma and buffer sampling and protein precipitation are performed. The plate is then centrifuged prior to analysis by LC-MS/MS."

"The performance of the semi-automated assay is comparable with manual techniques and published literature values, and we have also implemented a test for measuring protein content in the buffer compartment, to confirm the integrity of each insert. From data produced in our laboratory over a six month period we estimate that, compared to a manual equilibrium dialysis assay, this semi-automated method can generate four times as much data with approximately half the resource costs."

Claire concluded: "We have invested a lot in staff training and the Tecan training courses have played a significant part in our achievements, helping us to take full advantage of all the features of the instrument when developing new protocols. Tecan offers a good local service and is very responsive, providing engineering support very quickly when it is needed."

To find out more on Tecan's Protein Science solutions, visit **www.tecan.com/proteinscience**