Determining Plasma Protein Binding of Liraglutide with the new *EScalate* Equilibrium Shift Assay

J. Ungewiss[1], S. Gericke[1], H.Boriss[2]

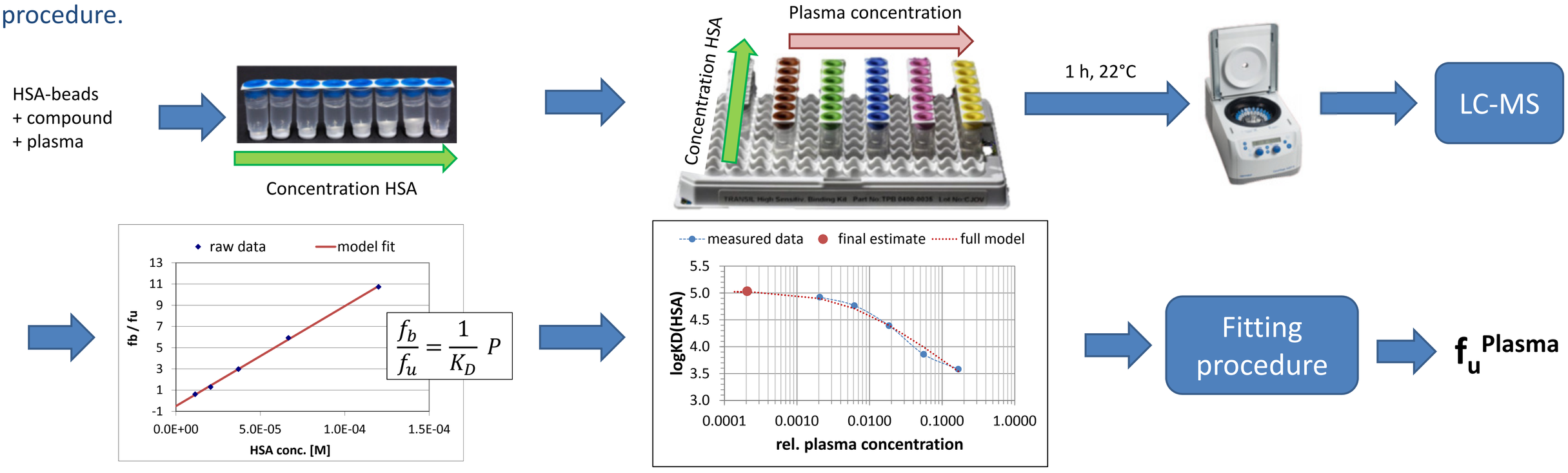
[1]3B Pharmaceuticals GmbH, Berlin, Germany; [2]Sovicell GmbH, Leipzig, Germany

Background

The accurate determination of plasma protein binding of larger molecules represents a significant challenge, especially if their plasma protein binding is very high. The commonly used equilibrium dialysis and ultrafiltration methods are often not applicable for peptides and oligonucleotides due to low migration velocities through the semipermeable membrane. Moreover, substantial non-specific binding to the membrane and limited solubility in protein-free aqueous buffer further limit the application of those assays. In order to overcome these limitations, we developed the bead-based *EScalate* equilibrium shift *in vitro* assay. The applicability of the assay was shown for the peptidic drug liraglutide and the small molecule drugs carbamazepine, desipramine, pyrimethamine and warfarin.

EScalate assay principle

The *EScalate* assay is based on the determination of the binding of the compound to HSA immobilized on beads. By adding plasma to the supernatant over the HSA beads the compound also binds to plasma proteins. The equilibrium of the bead binding is shifted resulting in a lower apparent K_D for the bead binding. By independent variation of both the amount of HSA coated beads and the plasma concentration in the supernatant the unbound fraction of the compound in plasma without HSA beads can be calulated using a two-dimensional fitting procedure.



Results

First, the accuracy of the *EScalate* assay was confirmed with the four well-studied small molecule drugs, carbamazepine, desipramine, pyrimethamine and warfarin (table 1).
Then, the plasma protein binding data for the high serum albumin binding peptidic drug liraglutide was determined five-fold on 3 different days (table 2).

Table 1: *Comparison of the unbound fraction f_u in human plasma determined by EScalate with data obtained by ultrafiltration (UF) oder equilibrium dialysis (ED)*

Compound	f_u *EScalate*	f_u Literature
Carbamazepine	28.8%	28.0% (UF)[1]
Desipramine	17.3%	14.0% (ED)[2]
Pyrimethamine	12.5%	11.7% (ED)[3]
Warfarin	0.55%	0.69% (ED)[4]

Table 2: *Unbound fraction f_u of Liraglutide in human plasma determined by EScalate*

Measurement	f_u Liraglutide
Day 1 run 1	0.66%
Day 1 run 2	0.58%
Day 2 run 1	0.40%
Day 2 run 2	0.44%
Day 3 run 1	0.47%
Average	**0.51%**
Literature[5]	0.53%

Advantages of the Escalate assay:

- **No dialysis membrane** used
- **Rapid equilibration** at room temperature
- **Presence of plasma in all samples** to prevent non-specific binding to vessels and compound precipitation
- **Capability to analyze strong plasma protein binders** due to indirect determination of f_u Plasma

Literature:
1. Fortuna A, Alves G, Soares-da-Silva P, Falcão A 2013. Epilepsy Research 107(1):37-50.
2. Zhang F, Xue J, Shao J, Jia L 2012. Drug Discovery Today 17(9–10):475-485
3. Rudy AC, Poynor WJ 1990. Pharmaceutical Research 7(10):1055-1060.
4. Mungall D, Wong YY, Talbert RL, Crawford MH, Marshall J, Hawkins DW, Ludden TM 1984. Journal of 5. Pharmaceutical Sciences 73(7):1000-1001.
5. Flint, A.; Nazzal, K.; Jagielski, P.; Hindsberger, C.; Zdravkovic, M., British Journal of Clinical Pharmacology 2010, 70 (6), 807-814.

Conclusion

The newly developed *EScalate* assay is providing accurate results across a broad range of plasma protein binding levels. It is unique in providing reliable data for mid-sized organic molecules with high affinity to plasma proteins.

Corresponding author: Dr. Jan Ungewiss, jan.ungewiss@3b-pharma.com
3B Pharmaceuticals GmbH, Magnusstr. 11, 12489 Berlin, Germany.
www.3b-pharma.com

3B Pharmaceuticals