High-Throughput Assessment For Drug Protein Binding Using Magnetized Silica Beads Method In Human Liver Microsomes

Hongguang Shao, Gerard X Smith, Jr., Libin Li, Kunru Wang, Taeseok Oh, Matthew McCoy, Jun Sun

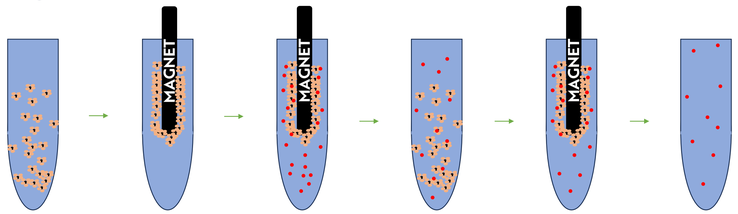
RESOLIAN BIOANALYTICS

Background

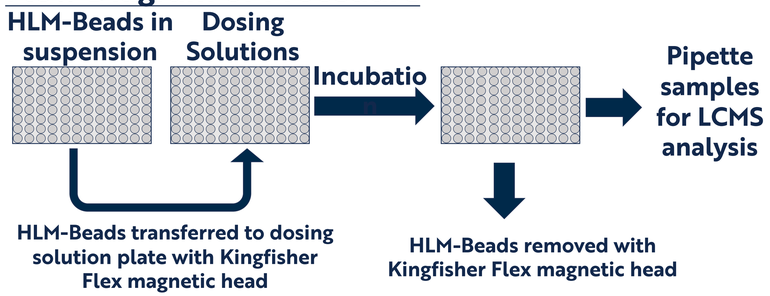
- Protein binding assays determine the unbound fraction (f_u) of novel drugs
- f_u is key parameter for pharmacokinetic modelling and is related to drug efficacy, toxicity, and DDI risk
- Current methods to determine f_u are limited by long incubations and limited application for larger drugs
- Novel protein binding assessments are needed to more efficiently determine f_u and assess the protein binding of larger molecules, such as oligonucleotide drugs

Methods

Magnetized silica beads

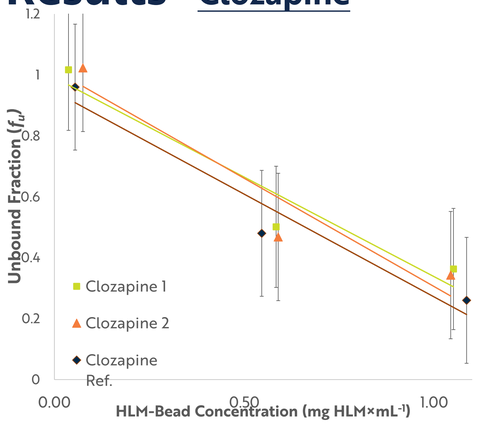


Flow Diagram of Procedure



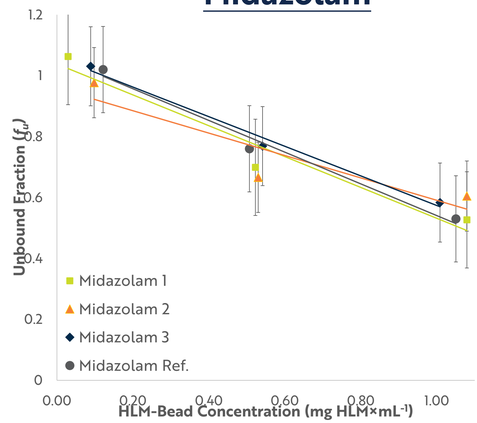
Results

Clozapine



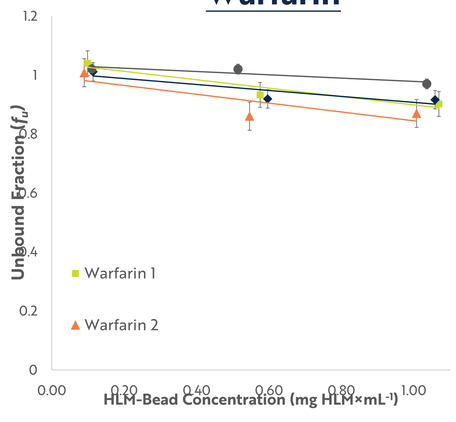
Test Compound	HLM Bead Concentration (mg HLM×mL⁻¹) 0.025	0.50	1.0	Correlation to reference
Clozapine 1	1.02	0.50	0.36	0.994
Clozapine 2	1.02	0.47	0.34	0.990
Clozapine ref.	0.96	0.48	0.26	1.00

Midazolam



Test Compound	HLM Bead Concentration (mg HLM×mL⁻¹) 0.025	0.50	1.0	Correlation to reference
Midazolam 1	1.06	0.699	0.527	0.986
Midazolam 2	0.977	0.666	0.605	0.945
Midazolam 3	1.03	0.769	0.583	0.998
Midazolam ref.	1.02	0.76	0.53	1.00

Warfarin



Test Compound	HLM Bead Concentration (mg HLM×mL⁻¹) 0.025	0.50	1.0	Correlation to reference
Warfarin 1	1.04	0.933	0.902	0.672
Warfarin 2	1.01	0.860	0.870	0.451
Warfarin 3	1.01	0.919	0.916	0.523
Warfarin ref.	1.02	1.02	0.97	1.00

Amitriptyline

Test Compound	HLM Bead Concentration (mg HLM×mL⁻¹) 0.025	0.50	1.0	Percent of in-house reference
Amitriptyline 1	1.08	0.392	0.258	108
Amitriptyline 2	0.955	0.315	0.257	87.0
Amitriptyline ref.	NA	0.362	NA	100

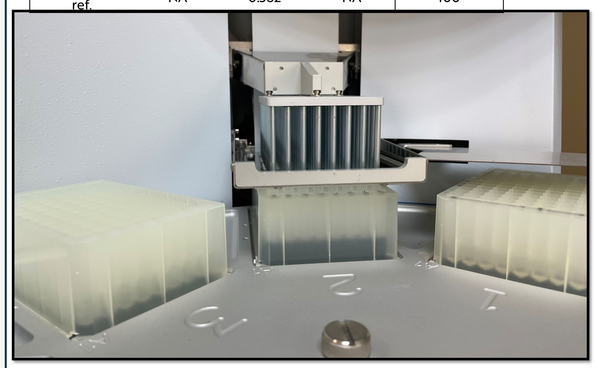
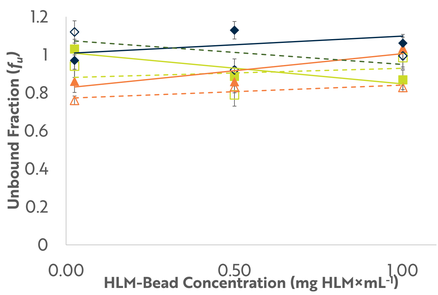


Image of Kingfisher Flex setup with three plates and magnetic pipetting head

Oligonucleotides



Test Compound	HLM Bead Concentration (mg HLM/mL) 0.025	0.5	1.0
AOP1	1.03	0.886	0.868
AOP1	0.94	0.789	0.985
AOP2	0.86	0.858	1.034
AOP2	0.76	0.831	0.828
AOP3	0.97	1.129	1.061
AOP3	1.12	0.920	0.994

Conclusions

- HLM-coated magnetic silica beads showed concentration dependent protein binding four small molecules.
- Strong correlations between f_u values determined in the current beads method and the values published validate the beads method.
- Automation with the Kingfisher flex greatly increases the throughput of protein binding with magnetic beads.
- Oligonucleotide test compounds did not show concentration dependent protein binding under current assay conditions

References

Horspool, A. M., Wang, T., Scaringella, Y. S., Taub, M. E., & Chan, T. S. (2020). Human liver microsomes immobilized on magnetizable beads: A novel approach to study in vitro drug metabolism. *Drug Metabolism and Disposition, 48*(8), 645–654. https://doi.org/10.1124/DMD.120.090696

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Acknowledgements

We thank Hoora Shanghaghi, Timmothy Schwemler, and David Young for providing LC/MS support for this project