EXPANSION OF IN VITRO DRUG METABOLISM ASSAY CAPACITY AND EFFICIENCY TOWARDS HIGH-THROUGHPUT SCREENING USING AUTOMATED LIQUID HANDLING

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Introduction

- High-throughput screening (HTS) can be used to quickly and effectively move drug candidates through development. An automated liquid handling platform offers a programmable system for increasing the capacity and turnaround of assays routinely conducted manually.
- Here we describe our efforts to develop methods on the Hamilton Microlab® STAR automated liquid handling platform. Our first goal was to evaluate and place into production an automated method for a basic drug discovery assay. We chose metabolic stability for our first method. We would then build upon this basic program, more sophisticated enzyme kinetics schemes.
- We designed a web-based portal with a user-friendly design to generate parameters for the Hamilton program for each in vitro assay. We executed the method for metabolic stability with several commercially available compounds; the results were consistent with our manual procedure. These results suggest that the Hamilton Microlab STAR is an appropriate platform to develop HTS methods for our routine in vitro assays.

Methods

- The web portal was used to generate a tailored experimental procedure and input file.

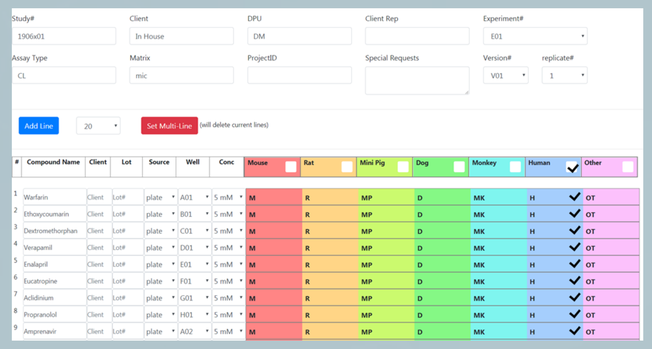

Figure 1: Drug Metabolism Information Management Web Portal Input

- Compounds were tested for metabolic stability in mouse and human liver microsomes, *n*=3. Compounds were spiked into liver microsomes purchased from BioIVT for an incubation concentration of 0.5 µM. Microsomes were prepared at a protein concentration of 0.5 mg/mL in potassium phosphate buffer. An NADPH-regenerating system was used as a cofactor. Time points were quenched with acetonitrile containing internal standard at 0, 5, 15, 30, and 45 minutes.

Methods (Cont.)

Figure 3: Hamilton MicrolabSTAR deck configuration for microsome clearance assay

- Samples were analyzed by LC-MS/MS with results reported as peak area ratios of each analyte to internal standard.
- Half-life ($t_{1/2}$) and intrinsic clearance (CL_{int}) were determined from the first-order kinetics by nonlinear regression using the following equations, where *k* is the elimination rate constant:

$$k = 0.693 \div t_{1/2}$$

$$CL_{int} = \frac{k}{min} \times \frac{mL}{0.5\ mg} \times \frac{mg\ protein}{g\ liver}$$

Results

Table 1: Elimination rate constant, half-life, and intrinsic clearance of test compounds in mouse and human liver microsomes

Species	Test Compound	Manual			Average Hamilton			
		Elimination Rate Constant (k) (min-1)	Half-life (t1/2) (min)	Intrinsic Clearance (CLint) (mL/min/g liver)	Elimination Rate Constant (k) (min-1)	Elimination Rate Standard Deviation	Half-life (t1/2) (min)	Intrinsic Clearance (CLint) (mL/min/g liver)
Mouse	Warfarin	<0.0038	>180	<0.3	<0.0038	NA	>180	<0.3
	Ethoxycoumarin	0.206	3.365	19.771	0.644	0.080	1.093	61.778
	Dextromethorphan	0.051	13.707	4.854	0.092	0.007	7.610	8.794
	Verapamil	0.116	5.995	11.097	0.254	0.033	2.785	24.345
	Enalapril	0.007	96.441	0.690	<0.0038	NA	>180	<0.3
	Eucatropine	0.013	54.992	1.210	0.027	0.005	26.437	2.609
	Aclidinium	0.243	2.852	23.328	0.585	0.040	1.190	56.188
	Propranolol	0.298	2.322	28.655	0.310	0.023	2.244	29.797
	Amprenavir	0.335	2.069	32.150	0.231	0.019	3.014	22.219
	Fluvoxamine	0.031	22.710	2.929	0.084	0.017	8.603	8.018
	Diclofenac	0.045	15.401	4.320	0.043	0.007	16.444	4.152
	Amitriptyline	0.079	8.792	7.567	0.149	0.009	4.682	14.264
	Methazolamide	<0.0038	>180	<0.3	<0.0038	NA	>180	<0.3
	Benzydamine	0.165	4.190	15.878	0.299	0.016	2.328	28.660
	Ramipril	0.008	88.452	0.752	0.004	0.002	>180	0.406
	Metoprolol	0.011	65.119	1.022	0.011	0.003	70.140	1.019
	Chlorthalidone	0.011	61.209	1.087	0.011	0.002	66.804	1.030
	Acetaminophen	<0.0038	>180	<0.3	<0.0038	NA	>180	<0.3
	Digoxin	<0.0038	>180	<0.3	<0.0038	NA	>180	<0.3
	Testosterone	0.167	4.143	16.057	0.357	0.060	2.001	34.266
	Midazolam	0.288	2.405	27.665	0.357	0.061	2.003	34.307
Human	Warfarin	<0.0038	>180	<0.3	<0.0038	NA	>180	<0.3
	Ethoxycoumarin	0.066	10.557	5.212	0.109	0.012	6.450	8.636
	Dextromethorphan	0.019	37.055	1.485	0.027	0.002	25.466	2.178
	Verapamil	0.058	11.949	4.605	0.134	0.028	5.379	10.665
	Enalapril	<0.0038	>180	<0.3	<0.0038	NA	>180	<0.3
	Eucatropine	<0.0038	>180	<0.3	<0.0038	NA	>180	<0.3
	Aclidinium	0.084	8.258	6.663	0.158	0.009	4.409	12.527
	Propranolol	0.021	33.708	1.632	0.017	0.000	41.025	1.342
	Amprenavir	0.172	4.021	13.683	0.444	0.082	1.608	35.265
	Fluvoxamine	<0.0038	>180	<0.3	<0.0038	NA	>180	<0.3
	Diclofenac	0.079	8.806	6.248	0.114	0.018	6.222	9.068
	Amitriptyline	0.006	122.213	0.450	<0.0038	NA	>180	<0.3
	Methazolamide	<0.0038	>180	<0.3	<0.0038	NA	>180	<0.3
	Benzydamine	0.007	105.941	0.519	0.006	0.001	118.753	0.474
	Ramipril	0.020	35.150	1.565	0.017	0.002	42.413	1.310
	Metoprolol	<0.0038	>180	<0.3	<0.0038	NA	>180	<0.3
	Chlorthalidone	<0.0038	>180	<0.3	<0.0038	NA	>180	<0.3
	Acetaminophen	<0.0038	>180	<0.3	<0.0038	NA	>180	<0.3
	Digoxin	<0.0038	>180	<0.3	<0.0038	NA	>180	<0.3
	Testosterone	0.042	16.480	3.339	0.033	0.007	21.723	2.635
	Midazolam	0.147	4.719	11.660	0.190	0.042	3.834	15.072

Results (Cont.)

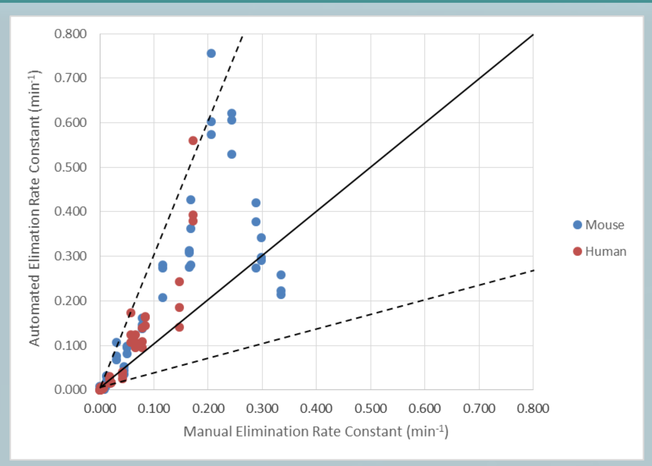

Figure 4: Comparison of automated elimination rate constant and manual elimination rate constant

Table 2: Average half-life and standard deviation of historical data for midazolam, *n*=10

Platform	Average Half-life (min)	SD	Relative SD
Manual	5.201	0.944	0.181
Automated	4.914	1.057	0.215

Conclusions

- The elimination rate constants calculated from data generated by the automated method for metabolic stability are similar to those calculated from our manual procedure
- The newly developed automated method allows for more efficient execution of studies. The method used in this study supports the incubation of up 300 conditions per instrument per day.
- After demonstrating that the Hamilton Microlab® STAR is suitable for reactions involving liver microsome metabolism, we will develop more complicated methods using liver microsomes, such as the IC_{50} and time-dependent inhibition assays. Additionally we plan on developing methods utilizing other matrices, such as stability and protein binding in plasma and blood.

Acknowledgements

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